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**Cover art:** D1 and D2 dopamine receptor-expressing medium spiny projection neurons of the striatum and their terminal fields, labeled by bacterial artificial chromosome (BAC) vector-driven expression of enhanced green fluorescent protein. Illustration is a composite of images from Chapter 6 by Surmeier et al., "D1 and D2 dopamine receptor modulation of glutamatergic signaling in striatal medium spiny neurons", and Chapter 28 by Gerfen, "D1 dopamine receptor supersensitivity in the dopamine-depleted striatum: Aberrant ERK1/2 signaling". Overlaid in red is an activity trace of a medium spiny neuron recorded intracellularly *in vivo* in a dopamine-depleted rat by Kuei Tseng.

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## Dedication



We dedicate this Volume to Prof. Stephen T. Kitai, friend, mentor and colleague to many of the contributors and one of the founders of modern basal ganglia research.

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## Preface

The basal ganglia, a group of forebrain nuclei that are interconnected with the cerebral cortex, thalamus and brainstem, belong to the phylogenetically oldest parts of the brain. Since the first anatomical description of the corpus striatum in 1664 by the English anatomist Thomas Willis, the modern view of the basal ganglia was slow in arising. For more than 250 years, the term "corpus striatum" (striated body, due to the many passing fiber bundles) was used to group several subcortical nuclei, including structures that were eventually found to be functionally unconnected. The principal parts of the corpus striatum comprised the caudate nucleus and the lenticular nucleus (nucleus lentiformis, named for its lensshape), which itself consisted of the putamen and the external and internal segments of the globus pallidus. The substantia nigra (locus niger crurum cerebri; discovered by Vicq d'Azir, 1786, and Soemmerring, 1788) and the subthalamic nucleus ("body of Luys"; Luys, 1865) were included in the functional organization of the basal ganglia only after the introduction of tract tracing techniques in the 1960s.

Regarding the function of the basal ganglia, Willis (1664) recognized the central position in the hemisphere of the corpus striatum and its association with prominent ascending and descending fiber bundles, and he believed that this structure was related to important sensory and motor functions of the brain. However, after Willis, anatomists and neurologists of the 18th and 19th centuries shifted their attention towards the cerebral cortex and cerebellum, in part due to the emerging attractiveness of the cytoarchitectural organization of these structures. Especially, the cerebral cortex with its presumed association with higher mental functions quickly obscured the position of the corpus striatum for another 100-200 years. A turning point in the history of the basal ganglia was reached at the beginning of the 20th century with the publication of several pathophysiological reports showing that brain lesions involving the corpus striatum resulted in movement disorders (e.g., Vogt, 1911; Wilson, 1912; Vogt and Vogt, 1920). These findings returned attention to the basal ganglia, which then began to gain importance once again. Modern basal ganglia terminology such as "striatum" (for caudate nucleus and putamen) and "pallidum" was introduced by Cécile and Oskar Vogt (1941) in their attempt to simplify forebrain anatomical nomenclature.

Decades later, two massive expansions of scientific knowledge propelled the basal ganglia to the prominence

they hold today. The first was made possible by a revolution in neuroanatomical methodologies that included the development of tract tracing techniques to delineate neuronal pathways and connections, and of histochemical methods to localize neurotransmitters, enzymes and receptor binding sites. This progress in our understanding of the structural organization of the basal ganglia is documented in the eloquent monograph by Parent (1986). The second expansion of knowledge was enabled by the advances in neurophysiological recording techniques that went hand in hand with the molecular revolution that swept the biological sciences during the last decade of the 20th century. Together, these technical innovations further clarified the molecular and functional characteristics of individual neuron types, including their interactions in basal ganglia circuits and related networks. From a mere 23-line paragraph in an exemplary early review 200 years ago (Bell, 1809), our knowledge on basal ganglia structure and function has expanded to the volume at hand.

The present volume provides and integrates basal ganglia knowledge from molecular to behavioral and clinical levels. The first, introductory, part presents an overview of the neuroanatomical organization of the basal ganglia, offering the general organizational principles and serving as a guide and reference tool for the remainder of the book. This part also reviews the cell types and neurotransmitter receptors present in the different nuclei of the basal ganglia and provides a detailed account of the evolution of this brain system. The second part provides chapters on anatomical and physiological aspects of the striatum, including reviews of the various neuronal types and the regulation of striatal activity by the different neurotransmitter and neuromodulator systems. The third part addresses anatomy and physiology of the other basal ganglia nuclei, globus pallidus, subthalamic nucleus and substantia nigra, including their cellular composition, neurotransmitters and connections. The fourth part provides reviews on the network integration of the basal ganglia, especially on the organization of inputs from cortex, thalamus and other brain regions, as well as the various basal ganglia outputs to thalamus and brainstem. The fifth part offers accounts of advances in second-messenger signaling and gene regulation by neurotransmitter receptors in the basal ganglia, with an emphasis on the striatum. The sixth and last part provides reviews on various aspects of basal ganglia function and dysfunction. These include chapters on dopamine function and learning and memory processes, as well as papers addressing the role of the basal ganglia in movement disorders and the emerging involvement in drug addiction. Finally, this part also discusses advances in the treatment of such disorders, including new insights in pharmacotherapies and deep-brain stimulation.

#### **FURTHER READING**

- Bell C (1809) The Anatomy of the Human Body. Vol 3: Nervous System. New York: Collins and Perkins.
- Parent A (1986) Comparative Neurobiology of the Basal Ganglia. New York: Wiley.
- Vogt C, Vogt O (1941) Thalamusstudien I-III. J Psychol Neurol 50: 31–154.

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We were very fortunate to receive tireless support from our Developmental Editor at Elsevier, Renske van Dijk, and our editorial assistant, Peter Campbell. Last but not least, speaking with Frank Preston Stearns, ambition is a plant that requires the sunshine of opportunity; we would like to thank the Series Editor, Joseph P. Huston, for inviting us to edit this volume.

# The Neuroanatomical Organization of the Basal Ganglia

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#### I. INTRODUCTION

The basal ganglia connect the cerebral cortex with neural systems that effect behavior. Most cortical areas provide inputs to the basal ganglia, which in turn provide outputs to brain systems that are involved in the generation of behavior. Among the behavior effector systems targeted are thalamic nuclei that project to those frontal cortical areas involved in the planning and execution of movement; midbrain regions including the superior colliculus, which is involved in the generation of eye movements; the pedunculopontine nucleus, which is involved in orienting movements; and hypothalamic systems involved in autonomic functions. Two points concerning the function of the basal ganglia are emphasized. First, while the basal ganglia connect the cerebral cortex with a wide range of behavior effector systems, the basal ganglia operates in parallel with other output systems of the cerebral cortex. These other corticofugal systems may have a more primary role in the actual generation of behavior. For example, the frontal cortical areas involved in the planning and execution of movement behavior provide direct projections to the spinal cord that are responsible for the generation of movement. Thus, the exact role of the basal ganglia in affecting cortically generated behavior remains a matter of debate (see Part F of this volume). Second, while the basal ganglia are connected with a wide range of behavior effector systems, not all regions of the basal ganglia are connected with all of the output systems. In other words, there is a conservation of regional functional organization of the cerebral cortex in the connections of the basal ganglia. In considering the neuroanatomical organization of the basal ganglia there are differing views. On the one hand, the basal ganglia have been proposed to provide for interactions between disparate functional circuits, for example, between socalled "limbic" and "nonlimbic" functions. Another view holds that there are parallel functional circuits, in which distinct functions are for the most part maintained, or segregated, one from the other. This review is biased toward the view that there is maintenance of parallel functional circuits in the organization of the basal ganglia, with considerable interactions between adjacent circuits.

Most details of the neuroanatomical and neurophysiological organization of basal ganglia circuits have first been established in rodents and confirmed in primates. Accordingly, the present review is mainly based on studies in rodents (as are the schemes used to illustrate the organizational principles). Several of the following chapters provide detailed information on the functional organization of the primate basal ganglia.

What are the most significant differences in the organization between rodents and other mammals, notably primates? The most obvious differences between rodents and primates are those involving the gross anatomy of the nuclei of the basal ganglia. There are two major examples. The first is the striatum, which in the primate is subdivided into caudate nucleus and putamen by the internal capsule that provides a structural separation between these two nuclei. This structural separation does provide a gross separation of functional regions in the striatum in that the caudate nucleus is mainly the target of prefrontal cortical inputs, whereas the putamen is the target of motor and somatosensory inputs. As the cortical input to the striatum is in a large part responsible for its function, the caudate and putamen in the primate are to a major extent functionally distinct. However, the internal capsule does not provide a precise divider of functional zones and there is some overlap of inputs from prefrontal cortex to the putamen. In the rodent, which lacks such a distinct structural separation, there are nonetheless regional differences in the striatum which are comparable to those of the caudate and putamen, again determined by the regional distribution of inputs from different cortical areas.

The second major gross anatomical difference between rats and primates involves the internal segment of the globus pallidus. In primates, this nucleus is situated immediately adjacent to the external segment of the globus pallidus, whereas in rodents, the homologous nucleus is separated from the external segment of the globus pallidus and is embedded in the fiber tract of the internal capsule. In rodents, this nucleus has historically been termed the entopeduncular nucleus, which reflects its location. However, as this nucleus is functionally comparable to the internal segment of the globus pallidus in primates, this nomenclature is adopted for the present review. Both nuclei represent, along with the substantia nigra pars reticulata, which is nearly identical in both rodents and primates, the output structures of the basal ganglia.

Despite the gross anatomical differences noted, the major connectional organization of the basal ganglia in rodents and primates is remarkably similar. Three of the major features of basal ganglia organization that will be dealt with in some depth in this review, the organization of direct and indirect output pathways of the striatum, the patch-matrix compartmental organization of the striatum and the dual projections of individual striatal neurons, have been demonstrated in both rodents and primates, and appear in the main, nearly identical in organization.

Differences in the organization of the basal ganglia between rodents and primates may for the most part be attributed to the expanded cortex in primates. In primates, cortical fields are considerably elaborated and more precisely defined in terms of functional segregation of different cortical areas. While the organization of cortico-striatal patterns appears to follow the same general principles in rodents and primates, the elaboration of more detailed precise mapping patterns appear to predominate in the primate. Thus, in summary, the major organizational principles of the basal ganglia appear for the most part nearly identical in rodents and primates.

#### II. OVERVIEW OF BASAL GANGLIA ORGANIZATION

The organization of the basal ganglia is intimately linked to that of the cerebral cortex, with distinct differences between those regions of the basal ganglia that receive inputs from neocortical, six-layered cortex, compared with those receiving inputs from allocortical areas. This review focuses primarily on the neocortical part of the basal ganglia. A general canonical organizational plan of the neocortical-related basal ganglia is described (Fig. 1.1). The components of this canonical basal ganglia system include the cortex, the striatum, including caudate-putamen and nucleus accumbens, the external segment of the globus pallidus, the subthalamic nucleus, the internal segment of the globus pallidus, and the substantia nigra. The major input to



FIGURE 1.1 Connections of the basal ganglia with the cerebral cortex shown on a sagittal diagram of the rat brain. A. The major input and output connections of the basal ganglia. Layer 5 pyramidal neurons of most areas of the cerebral cortex provide a major input to the striatum, which comprises the caudate-putamen (CPu) and the nucleus accumbens. The output of the basal ganglia arises from GABA neurons in the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). These neurons provide inhibitory inputs to thalamic nuclei, including the ventral lateral (vl), mediodorsal (md), ventromedial (vm) nuclei and intralaminar/parafascicular (pf) complex, as well as to the superior colliculus (SC) and pedunculopontine nucleus (PPN). B. The direct and indirect striatal projection pathways arising from two subsets of striatal medium spiny neurons. Direct projecting neurons provide an axon with collaterals to the external segment of the globus pallidus (GPe) and to the GPi and SNr. Indirect striatal projection neurons project to the GPe. These neurons are indirectly connected to the GPi and SNr through connections that involve the GPe and subthalamic nucleus (STN). C. Feedback pathways of the basal ganglia include the nigro-striatal dopamine (DA) pathway from the substantia nigra pars compacta (SNc), a thalamo-striatal pathway from the pf complex to the striatum, and thalamo-cortical pathways from the vl, md and vm thalamic nuclei to the frontal cortex.

this system comes from layer 5 glutamatergic neurons from nearly all areas of the neocortex (Fig. 1.1A). The output of this system are the projections of GABAergic neurons in the internal segment of the globus pallidus and the substantia nigra pars reticulata (Fig. 1.1A). The basal ganglia output targets thalamic nuclei, which project to frontal cortical areas involved in the planning and execution of movement behavior; the intralaminar thalamic nuclei, which provide inputs to the cortex and the striatum; the intermediate layers of the superior colliculus, which are involved in the generation of eye and head movements; and the pedunculopontine nucleus, which is involved in orienting movements of the body. In between the cortical inputs and the GABAergic output systems are the neuroanatomical circuits that comprise the prototypical basal ganglia (Fig. 1.1B). The main input structure of the basal ganglia is the striatum. Those regions of the striatum that receive inputs from neocortical areas are the caudate-putamen and core of the nucleus accumbens. The targets of the cortical input are medium-sized spiny GABAergic projection neurons, which account for about 95% of neurons in the striatum. These neurons are divided into two types, which give rise to the two main components of the prototypical basal ganglia circuit, the "direct" and "indirect" striatal projection systems. The direct striatal projection system is so-named because these neurons provide direct inputs to the output neurons of the basal ganglia in the internal segment of the globus pallidus and the substantia nigra pars reticulata. Indirect striatal projection neurons provide inputs to the external segment of the globus pallidus, which together

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with the subthalamic nucleus compose the major components of the indirect basal ganglia circuit. GABAergic neurons in the external segment of the globus pallidus project back to the striatum; to the output neurons of the basal ganglia in the internal segment of the globus pallidus and substantia nigra; and to the subthalamic nucleus. The subthalamic nucleus, which itself receives inputs from the cortex, provides excitatory projections to the output neurons of the basal ganglia.

The cerebral cortex and thalamus provide excitatory inputs to the striatum, whose output, through the direct and indirect projection systems, provides both inhibitory and excitatory regulation of the output of the basal ganglia. The output neurons of the basal ganglia, GABAergic neurons in the internal segment of the globus pallidus and substantia nigra pars reticulata, display a relatively high level of tonic activity. In a long held model of basal ganglia function, the excitatory input from the cortex has been demonstrated to function through a disinhibitory mechanism. Thus, activation of the direct striatal output neurons by excitatory input from the cortex results in inhibition of the tonic inhibitory output of the basal ganglia. The role of the indirect circuit is more complex. On the one hand, the target of the indirect striatal output neurons are GABAergic neurons in the external segment of the globus pallidus, which project to the output neurons of the basal ganglia and to the subthalamic nucleus. Thus, cortical excitation of the indirect pathway inhibits the GABAergic pallidal output, resulting in disinhibition of the output neurons of the basal ganglia and the subthalamic nucleus. The subthalamic nucleus, which also receives direct excitatory inputs from the cerebral cortex, provides excitatory inputs to the output neurons of the basal ganglia. Additionally, it has been demonstrated that the interconnections between the external segment of the globus pallidus and subthalamic nucleus generate an oscillatory pattern of activity that is conveyed to the output neurons of the basal ganglia. Given the complexities of the organization of these circuits, at this time the exact mechanisms responsible for regulating the output of the basal ganglia remain to be established. However, in general terms, activity in the direct and indirect striatal output pathways may be viewed as providing counterbalanced or antagonistic regulation of the output of the basal ganglia.

Overlain on the above canonical basal ganglia circuits are a number of additional neuroanatomical features that add to the complexity of the organization of this system. Notable among these is the dopaminergic nigrostriatal system, which provides a massive dopaminergic input to the striatum from the midbrain dopamine neurons in the ventral tegmental area and substantia nigra pars compacta (Fig. 1.1C). In addition, this review describes a number of other features of the basal ganglia organization, including: (1) the organization of the corticostriatal system, which incorporates both a general topographic organization with considerable overlap of corticostriatal inputs from cortical areas that are interconnected; (2) the patch and matrix compartmental organization of the striatum, which is related to the laminar organization of the cerebral cortex and provides differential inputs to the output systems of the basal ganglia and the nigrostriatal outputs in the external segment of the globus pallidus and output nuclei of the basal ganglia.

#### **III. THE CORTICOSTRIATAL SYSTEM**

The striatum is the main input structure of the basal ganglia and the vast majority of its neurons are medium spiny projection neurons, whose activity is determined by excitatory inputs from the cerebral cortex and thalamus. Consequently, the information that striatal projection neurons transmit within the circuits of the basal ganglia is largely determined by the activity of corticostriatal (and thalamostriatal) inputs. Cortical neurons providing striatal inputs are located mostly in layer 5, and in some cases layer 3, of most cortical areas. All corticostriatal neurons are pyramidal neurons and utilize glutamate as a neurotransmitter. The following sections provide an overview of the corticostriatal system. More detailed information on specific aspects of this system is provided in Chapters 12, 18, 19, 20, 24 and 35.

#### A. Subtypes of Corticostriatal Neurons

Subtypes of corticostriatal neurons are distinguished based on their connections within the cortex, their projections to other subcortical areas, and their laminar distribution within the cortex. Two distinct subtypes that have been identified are the corticostriatal pyramidal tract neuron (PT) and the corticostriatal intertelencephalic neuron (IT) (Fig. 1.2) (see Chapter 18). Pyramidal tract corticostriatal neurons are present in the frontal cortex and provide a major projection directly to motor neurons in the brainstem and spinal cord as well as a collateral to the striatum (Donoghue and Kitai, 1981; Landry et al., 1984; Cowan and Wilson, 1994; Lei et al., 2004). The striatal projections of these neurons are



FIGURE 1.2 Tracings of dendrites (black) and cortical (A and B) and striatal axons (A' and B') (grey) of two subtypes of corticostriatal neurons, which had been intracellularly labeled. A. The corticostriatal neuron depicted provides an axonal projection to the pyramidal tract. Axon collaterals within the cortex are distributed in relatively close proximity to the parent neuron. A'. The pyramidal tract axon (arrow) of this neuron gives off collaterals in the striatum, which display a focal terminal arborization. B. The corticostriatal neuron shown, located in the medial agranular cortex (AGm), is a bilaterally projecting cortico-cortical neuron that also extends axon collaterals bilaterally into the striatum. Axon collaterals within the ipsilateral cortex both distribute locally around the parent neuron in AGm, and extend to the adjacent lateral agranular (motor) cortical area (AGl). B'. This neuron has an axon that provides an extensive arborization pattern within the striatum, but does not extend collaterals beyond the striatum. These neuronal tracings are modified from Cowan and Wilson (1994).

significant as they provide a copy of the cortical motor signal that directly regulates movement. Intertelencephalic corticostriatal neurons are very numerous in agranular cortical regions giving rise to bilateral corticocortical and corticostriatal projections. These corticostriatal neurons have extensive connections with other cortical areas. These two types of corticostriatal cells provide distinct patterns of innervation within the striatum, as exemplified by neurons of the rat premotor cortex (Fig. 1.2) (Cowan and Wilson, 1994). The striatal collaterals of PT corticostriatals neurons make one or more relatively focal arborizations, with dimensions of 100–500µm on a side, which suggests a relatively simple topography of the corticostriatal projection formed by these neurons. On the other hand, IT corticostriatal neurons provide arborizations in a much larger striatal volume, with dimensions of 1 mm or greater. Within that volume the axon occupied space in a very sparse fashion, with individual branches running approximately parallel and separated by large uninnervated areas. This pattern is expected from the arborization seen for individual corticostriatal neurons if nearby corticostriatal cells have fine scale similarities in their axonal arborizations. That is, the pattern of labeling seen after extracellular injections of anterograde tracers in the cortex implies that much of the complex topology of corticostriatal axonal arborizations will be shared among neighboring cells in the cortex.

Corticostriatal neurons also display specificity in their targets within the striatum. For example, recent studies indicate that IT and PT corticostriatal neurons differentially target direct and indirect striatal projection neurons, respectively (Lei et al., 2004) (see Chapter 18). Additionally, corticostriatal inputs to the striatal patch and matrix compartments arise from different sublayers within layer 5 of most cortical areas, with neurons projecting to the matrix located in more superficial parts of layer 5 than those projecting to the patch compartment (Gerfen, 1989). Thus, there appears to be a high degree of specificity in the targeting of specific striatal neuron subtypes and compartments by distinct subtypes of corticostriatal neurons.

# **B.** Patterns of Organization of Corticostriatal Afferents

A distinct feature of corticostriatal projections is that the axons of individual neurons are distributed in manner such that they contact a maximum number of neurons but make minimal contacts with each postsynaptic neuron (Zheng and Wilson, 2002). Quantitative data from Wilson and his colleagues provide informative boundaries for the type of information processing that may be taking place within the basal ganglia of the rat. First, there appears to be roughly a 6:1 ratio in terms of the numbers of corticostriatal neurons (17,000,000) and striatal projection neurons (2,800,000; Oorschot, 1996) (see Chapter 3). Second, the volume over which the dendrites of a single medium spiny projection neuron spread (400 µm in diameter) contains approximately 2850 other neurons. Third, approximately 380,000 corticostriatal neurons innervate the volume of the dendritic field of a single medium spiny projection neuron, which contains 2850 neurons. Fourth, a single corticostriatal axon traversing this area has on average 40 synaptic boutons. If,

as is estimated, each axon makes only a single, or a few contacts, with a single medium spiny neuron, then each corticostriatal input makes contact with about 1% of the striatal neurons in the area across which it extends. Taken together these quantitative estimates indicate that the cortical input to a single striatal medium spiny projection neuron is rather unique, that is, no two striatal neurons share common inputs from the cortex. Thus, postsynaptic excitatory activation of individual striatal medium spiny neurons is dependent on convergent input from multiple corticostriatal neurons. Consequently, the pattern of convergence of corticostriatal inputs is critical to understanding the information that is transmitted from the cerebral cortex into the basal ganglia (see also Chapter 19).

Cortical input to the striatum originates from most cortical areas, including primary and higher order sensory areas; motor, premotor and prefrontal regions; as well as from limbic cortical areas (see also Chapters 20 and 24). It is well established that this input is organized in a general topographic manner in that the spatial relationships between cortical areas are maintained in the projections to the striatum (Webster, 1961; Carman et al., 1965; Kemp and Powell, 1970). For example, projections from prefrontal areas are directed mainly to the rostral caudate nucleus, while cortical inputs from motor cortex terminate primarily in the rostral putamen (Kunzle 1975). This pattern of the topographic organization of corticostriatal projections was embodied in the concept of functional regions within the striatum being dependent on the cortical origin of inputs to these regions (Alexander et al., 1986). Thus, dorsal regions of the striatum receiving inputs from premotor and motor cortical areas are characterized as "motor" regions of the striatum, whereas more ventral regions receiving inputs from limbic cortical areas are characterized as "limbic".

More complex is the issue of overlapping projections from functionally related areas. While it is clear that, in general, cortical areas provide input to a much broader area of the striatum than accounted for on the basis of topo graphy alone, the varied and sometimes intricate pattern of this organization have led to a variety of interpretations as to the functional significance (see also Chapter 19). While, the widespread nature of corticostriatal organization is not in doubt, where some have seen patterns of overlap related to patterns of cortical connectivity (Yeterian and Hoesen, 1978), others have seen interdigitation (Selemon and Goldman-Rakic, 1985). Detailed mapping of the organization of corticostriatal inputs has begun to resolve these issues, showing, in some cases, overlap of inputs from interconnected cortical areas that are organized fairly precisely by the somatotopic organization within such areas (Flaherty and Graybiel, 1991; Parthasarathy et al., 1992; Flaherty and Graybiel, 1993).

#### **IV. STRIATUM**

The striatum comprises the caudate nucleus, putamen and nucleus accumbens. The striatum is composed of one principal neuron type, the medium-sized spiny projection neuron (DiFiglia et al., 1976; Wilson and Groves, 1980; Bishop et al., 1982). This medium spiny projection neuron makes up as much as 95% or more of the neuron population (Kemp and Powell, 1971) (see Chapter 3); these neurons are rather homogeneously distributed such that the striatum lacks distinct cytoarchitectural organization, as contrasted with the laminar organization of the cortex, for example. Using retrograde axonal transport methods, Grofova (1975) established that these neurons are the projection neuron of the striatum. Cortical input to the striatum targets primarily spiny projection neurons (Somogyi et al., 1981). Thus the spiny projection neuron is the major input target and the major output neuron of the striatum.

The remaining striatal neurons are interneurons (DiFiglia et al., 1976; Bishop et al., 1982), in that they do not provide projection axons out of the striatum, but rather distribute axons within the striatum, most of which make synaptic contact with spiny projection neurons. Despite being relatively infrequent, striatal interneurons constitute a variety of morphologically and neurochemically defined types (see Chapter 3). Among these are the large aspiny neurons, which utilize acetylcholine as a transmitter (Bolam et al., 1984; Kawaguchi and Kubota, 1993), and medium aspiny neurons (DiFiglia et al., 1976; Bishop et al., 1982), which utilize GABA as a transmitter (Kita, 1993). The latter class of interneurons may be further subdivided on the basis of different peptides and neurochemicals that they contain (Kita, 1993; Kubota and Kawaguchi, 1993; Kubota et al., 1993). These cell types are reviewed in the following sections and are also described in specific chapters in this volume (see below).

#### A. Medium Spiny Projection Neurons

Medium spiny projection neurons (see Chapter 5) take their name from their morphologic appearance (DiFiglia et al., 1976; Wilson and Groves, 1980; Bishop et al., 1982; Chang et al., 1982), with a cell body of approximately 12–20 µm in diameter, from which radiate 7–10 moderately branched dendrites that are densely laden with spines. The dendrites of an individual neuron extend over an area of approximately 200 µm in diameter. These neurons extend a local axon collateral that remains within the striatum, typically distributed over an area roughly equal in size, but not necessarily in the same area, as the dendrites of the parent neuron (Bishop et al., 1982; Kawaguchi et al., 1990), although in some cases it may extend over 1 mm from the parent neuron (Kawaguchi et al., 1990).

As their name implies, medium spiny projection neurons provide an axon collateral which projects out of the striatum to the external segment of the globus pallidus and/ or internal segment of the globus pallidus and substantia nigra (Kawaguchi et al., 1990). Two major subpopulations of medium spiny neurons, of approximately equal numbers, may be defined on the basis of their projection targets (Loopuijt and van der Kooy, 1985; Beckstead and Cruz, 1986; Gerfen and Young, 1988; Kawaguchi et al., 1990). One subset provides an axon projection to the external segment of the globus pallidus. The other subset provides a (minor) axon collateral to the external segment of the globus pallidus, and additional collaterals to the internal segment of the globus pallidus and/or the substantia nigra. These latter neurons constitute the "direct striatal projection pathway" as they provide direct inputs to the output neurons of the basal ganglia, the GABAergic neurons of the internal segment of the globus pallidus and substantia nigra pars reticulata (see Fig. 1.1). The former neurons constitute the "indirect striatal projection pathway", as they are connected indirectly, through connections of the external segment of the globus pallidus and subthalamic nucleus, with the output neurons of the basal ganglia.

Medium spiny projection neurons all contain glutamic acid decarboxylase (GAD), the synthetic enzyme for the neurotransmitter GABA (Kita and Kitai, 1988). In addition, most of the neurons projecting to the external segment of the globus pallidus alone contain the neuropeptide enkephalin, whereas most of those projecting to the internal segment of the globus pallidus and substantia nigra contain the neuropeptides substance P and dynorphin (Haber and Watson, 1983; Beckstead and Kersey, 1985; Gerfen and Young, 1988) (see also below).

#### **B.** Synaptic Inputs to Medium Spiny Neurons

Medium spiny projection neurons receive inputs from the cortex, thalamus and amygdala, which make asymmetric synapses on dendritic spines, and to a lesser degree, dendritic shafts (Fig. 1.3). These are the major excitatory inputs to these neurons. In addition, a number of afferents from outside the striatum and from within the striatum



**FIGURE 1.3** Connections of striatal medium spiny projection neurons. A. Diagram of inputs to medium spiny projection neurons showing the location of cortical glutamatergic synapses on the head of spines, and synapses to the neck and interspine dendritic shafts from nigrostriatal dopamine inputs or GABAergic inputs from other medium spiny neurons. Inputs to the cell body and proximal dendrites are from striatal GABAergic and cholinergic interneurons. B. Diagram of the inputs to the proximal and distal parts of medium spiny neurons. Inputs to distal parts of the dendrites are from the cerebral cortex, nigrostriatal dopamine afferents and thalamus, whereas inputs to the proximal part of the neurons are from GABAergic parvalbumin (PV) and cholinergic (ChAT) interneurons. This organization of inputs appears to be similar for neurons of the direct and indirect pathways, which project, respectively, to the internal segment of the globus pallidus (GPi) (entopeduncular nucleus, EP, in rat)/substantia nigra pars reticulata (SNr) and the external segment of the globus pallidus (GPe), and differentially express D1 and D2 dopamine receptors.

provide inputs that function to modify the responsiveness of spiny neurons to excitatory input. These include dopamine afferents from the substantia nigra, inhibitory GABA (and neuropeptide) inputs from the axon collaterals of other spiny neurons, inhibitory inputs from GABA (and peptide)-containing striatal interneurons, and inputs from cholinergic striatal interneurons (Fig. 1.3).

Corticostriatal afferents make synaptic contact primarily with the expanded head of dendritic spines on spiny neurons (Kemp and Powell, 1970; Hattori et al., 1978; Somogyi et al., 1981; Bouyer et al., 1984). According to a quantitative study in rats (Xu et al., 1989), of all cortical synapses in the striatum, about 90% are formed with dendritic spines, and about 5% with dendritic shafts. The remaining 5% are on somata. Consistent with their excitatory nature, corticostriatal synapses are almost exclusively asymmetric and contain small rounded vesicles. Although cortical innervation of the striatum is relatively dense, as discussed above, input from any individual corticostriatal axon to an individual striatal spiny neuron is very sparse (Cowan and Wilson 1994).

Thalamic afferents from the intralaminar nuclei, including the parafascicular/centromedian complex (see Chapter 22), provide inputs to the striatum that are similar to cortical afferents in the number of synapses formed (Lacey et al., 2005; Raju et al., 2008) and in that they form asymmetric synaptic contacts and have strong excitatory effects on the spiny cells (Dube et al., 1988; Xu et al., 1989). There are two independent thalamostriatal projections of the intralaminar nuclear complex, one originating from the parafascicular/centromedian nuclei and a separate one from rostral parts of the complex including the central lateral and paracentral nuclei. The latter intralaminar projection, unlike the cortical input, makes its asymmetrical synaptic contacts preferentially with the shafts of dendrites rather than the spines, whereas projections arising from the parafascicular/ centromedian nuclei form synapses similar to those formed by corticostriatal fibers (Xu et al., 1989; Lacey et al., 2007).

Inputs from midbrain dopamine neurons make synaptic contact with medium spiny neurons (Fig. 1.3); these have been identified at the ultrastructural level with immunohistochemical localization of either dopamine (Voorn et al., 1986) or the dopamine synthesizing enzyme tyrosine hydroxylase (Arluison et al., 1984; Bouyer et al., 1984; Freund et al., 1984). Most of these afferents make symmetric synapses and contain large round and pleiomorphic vesicles. Of 280 synapses examined by Freund et al. (1984), 59% made synaptic contacts with dendritic spines. Unlike the axospinous synapses formed by cortical or thalamic inputs, these symmetrical synapses were usually not made on the head of the spine but on the neck, and these inputs shared the dendritic spine with another bouton forming an asymmetrical synapse (probably from the cerebral cortex or thalamus). Synapses were made onto dendritic shafts in 35% of the cases, and 6% made synapses with somata (Fig. 1.3). It should be noted, however, that dopaminergic synapses formed on spines is not a targetted phenomenon, as all striatal structures of a similar size have equal probability of being in contact with a dopaminergic axon (Moss and Bolam, 2008).

Medium spiny projection neurons have axon collaterals within the striatum that make symmetric synaptic contact with other spiny neurons (Wilson and Groves, 1980) (see Chapter 5). Ultrastructural analysis of either intracellularly labeled axons (Wilson and Groves, 1980), or axons labeled with immunohistochemical localization of GAD (Bolam et al., 1985) or substance P (Bolam and Izzo, 1988) show similar synaptic relationships. Most spiny projection collaterals contact either the interspine shafts or necks of spines of other spiny projection neurons. These contacts are distributed somewhat closer to the cell body and proximal dendrite parts than are the more distally distributed dopamine contacts (Fig. 1.3).

Striatal interneurons also provide important inputs to medium spiny projection neurons. These interneurons are discussed in more details in the following sections (and in specific chapters of this volume) and are listed here briefly (Fig. 1.3). For example, boutons immunoreactive for choline acetyltransferase (ChAT), indicating input from cholinergic interneurons (see Chapter 7), make synaptic contacts with striatal spiny neurons as well as other striatal cells (Izzo and Bolam, 1988). These cholinergic synapses are symmetric and make contact with the cell somata (20%); dendritic shafts (45%) and with dendritic spines (34%). As with the other symmetrical synapses on dendritic spines, they share the spine with an asymmetrical synapse, usually placed more distally on the spine and resembling afferents from the cerebral cortex and thalamus.

In addition to the GABAergic spiny projection neurons, GABAergic interneurons are present within the striatum (see Chapter 8). GABAergic interneurons were first positively identified by loading with radioactive GABA (Bolam et al., 1983), and were later recognized as a subset of neurons staining more intensely with immunocytochemistry for GAD or GABA (e.g., Bolam et al., 1985). More recently, a subpopulation has been shown to be positive for the calcium-binding protein parvalbumin (Gerfen et al., 1985; Cowan et al., 1990; Kita et al., 1990). These make numerous symmetrical synapses with the somata and dendrites of spiny neurons, as well as other interneurons. More than any other identified source of input, the synapses from the parvalbumin/GABA interneuron preferentially innervate the somata of spiny neurons (Kita et al., 1990).

Another type of aspiny striatal interneurons is identified by its immunocytochemical labeling for somatostatin, neuropeptide Y, and NADPH diaphorase. These cells have also been shown to be distinguishable from parvalbumin/ GABA interneurons on the basis of morphological and physiological criteria (Kawaguchi, 1993). Somatostatinpositive synapses are formed mainly on shafts of dendrites and dendritic spines of spiny neurons (Takagi et al., 1983).

In addition to the dopamine input from the substantia nigra, at least two other downstream parts of the basal ganglia provide feedback axons to the striatum. One of these is the external segment of the globus pallidus, which provides GABAergic input to the striatum (Staines et al. 1981; Beckstead, 1983; Kita and Kitai, 1994; Bevan et al., 1998) (see Chapter 14). About a quarter to a third of globus pallidus neurons project to the striatum and their principal targets are parvalbumin-positive and NOS-positive GABA interneurons (Staines and Fibiger, 1984; Bevan et al., 1998). In addition, the subthalamic nucleus also provides an input to the striatum. This input is relatively sparse as compared to the density of projections of this nucleus to substantia nigra and globus pallidus (Kita and Kitai, 1987). Subthalamic input to the striatum appears to provide asymmetric input to spiny neurons.

While dopamine afferents to the striatum provide the dominant input from the midbrain and brainstem, at least two other forebrain projection systems provide further inputs. These include the serotonergic afferents from the dorsal raphe and the noradrenergic afferents from the locus coeruleus. Added to the list of sources of inputs to the striatum, not covered in depth by this review, but also important for the functional integrity of the basal ganglia, are amygdala and hippocampus. Inputs from these structures are addressed in Chapters 20, 21, 24 and 33.

#### C. Striatal Interneurons

Striatal neurons that extend axons within but not out of the striatum make up 5% or less of the striatal neuron population (Kemp and Powell, 1971; DiFiglia et al., 1976; Bishop et al., 1982; Chang et al., 1982) (see Chapter 3). This class of neurons presents a variety of morphologically and neurochemically distinct subtypes. Two major subtypes are identified (Fig. 1.4). One is the large aspiny neuron, which utilizes acetylcholine as a neurotransmitter (Bolam et al., 1984; Wilson et al., 1990; Kawaguchi, 1992; Kawaguchi, 1993). The other is the medium-sized aspiny GABAergic interneuron, of which there are several varieties (Kita, 1993; Kawaguchi et al., 1995).

#### 1. Large Aspiny Cholinergic Neurons

Striatal cholinergic neurons (see Chapter 7), which utilize acetylcholine as a neurotransmitter, constitute an important type of interneurons (Bolam et al., 1984; Wilson et al., 1990; Kawaguchi, 1993). These neurons are easily identified due to their large size (DiFiglia et al., 1976; Chang et al., 1982; Kawaguchi, 1992), with histochemical staining of acetylcholinesterase (Fibiger, 1982), by immunohistochemical studies



FIGURE 1.4 Striatal interneurons. A. Large aspiny (cholinergic) interneuron. Tracing of dendrites (black) and axon collaterals (grey) of a large aspiny neuron (from Wilson et al., 1990) (left, top), and distribution of cell bodies (black) of striatal neurons immunoreactive for choline acetyltransferase (ChAT) (right) are shown, as well as a photomicrograph of ChATimmunoreactive neurons in the striatum (left, bottom). B. Medium aspiny interneurons. Tracing of dendrites of a medium aspiny neuron (left, top), and distribution of cell bodies of parvalbumin- (black dots) and somatostatin-positive (white dots) medium aspiny interneurons within the striatum (right) are depicted. There is an inverse gradient in the distribution of these two types of striatal interneurons, with parvalbumin neurons more numerous dorsolaterally, and somatostatin neurons more numerous ventrally. Also shown are photomicrographs of parvalbumin-immunoreactive (left, middle) and somatostatin-immunoreactive neurons (left, bottom) in the striatum.

employing antibodies directed against the synthetic enzyme choline acetyltransferase (Bolam et al., 1984; Wilson et al., 1990; Kawaguchi, 1993) (Fig. 1.4), and by intracellular filling studies (Wilson et al., 1990; Kawaguchi, 1992). Striatal cholinergic neurons have a very large cell body, up to  $40 \mu m$ in diameter, from which extend long aspiny dendrites, which may split into secondary and tertiary branches. The dendritic fields may cover an area of over 1 mm with no apparent orientation in any particular axis. Cholinergic neurons extend an axon, which is both extremely fine but extremely extensive in the area that it covers. Intracellular labeling of identified cholinergic neurons has shown axons from individual neurons to extend over an area of as much as 2 mm.

Although it is clear that acetylcholine release is important to striatal function, the neuroanatomical substrates by which this is regulated have been difficult to clearly identify. One possible mechanism involves the reported increase in acetylcholine release mediated through activation of substance P receptors. Such a mechanism is supported by anatomical evidence, not only with the demonstration of synaptic contacts between substance P-containing boutons and cholinergic neurons (Bolam et al., 1986), but also by the localization of substance P (neurokinin-1) receptor mRNA in cholinergic neurons (Elde et al., 1990; Gerfen, 1991).

#### 2. Medium Aspiny GABAergic Interneurons

The second major subtype of striatal interneurons is characterized morphologically as a medium-sized aspiny neuron that utilizes GABA as the main neurotransmitter (Ribak et al., 1979; Bolam et al., 1983; Oertel and Mugnaini, 1984; Smith et al., 1987; Pasik et al., 1988) (see Chapter 8). Interestingly, isoforms of the GABA synthesizing enzyme GAD are differentially expressed by medium spiny projection neurons and medium aspiny interneurons, with GAD67 being expressed at higher levels in interneurons, compared with GAD65, which is expressed in projection neurons. Further classes of GABAergic striatal interneurons are characterized by the patterns of co-expression of neuropeptides, such as somatostatin and neuropeptide Y, and calcium-binding proteins such as parvalbumin and calretinin.

The most abundant type of GABAergic interneurons expresses the calcium-binding protein parvalbumin (Gerfen et al., 1985; Cowan et al., 1990; Kita et al., 1990; Kubota and Kawaguchi, 1993) (Fig. 1.4). Parvalbumin interneurons have very distinct neurophysiological characteristics, marked by a hyperpolarized resting potential, lower input resistance, shorter duration action potential spikes, and abrupt repetitive firing (Kubota and Kawaguchi, 1993). Due to these physiologic features, this type of neuron is often referred to as a fast-spiking interneuron, which is similar to the fast-spiking interneurons in the cerebral cortex. These neurons receive inputs from the cerebral cortex, thalamus and globus pallidus and provide inputs to medium spiny projection neurons. As a result of gap junctions between them, and their high level of activity, these neurons may provide synchronized feed-forward inhibition to restricted regions of the striatum. Although distributed throughout the striatum, parvalbumin neurons are more frequent in the dorsolateral region, and display a dorsolateral to ventral gradient (Fig. 1.4).

A second class of GABAergic interneurons co-expresses somatostatin, neuropeptide Y, or nitric oxide synthetase (Vincent et al., 1983a; Vincent et al., 1983b; Smith and Parent, 1986; Pasik et al., 1988; Chesselet and Robbins, 1989; Dawson et al., 1991) (Fig. 1.4). The distribution of somatostatin neurons also appears to follow a gradient, with higher numbers present in ventral areas than in dorsal areas. Moreover, while somatostatin neurons are located in both patch and matrix compartments, their axons within the striatum are preferentially distributed in the matrix compartment.

#### V. OUTPUT SYSTEMS OF THE STRIATUM

Medium spiny projection neurons make up some 95% of the neuron population of the striatum. These neurons have a common morphology in terms of their size, dendritic organization and local axon collaterals, which extend within the striatum around the parent neuron. Each of these neurons provides an axon that projects out of the striatum. As mentioned above, medium spiny neurons are divided into two subsets of approximately equal numbers and contribute to projection pathways that provide either direct or indirect input to the output neurons of the basal ganglia in the internal segment of the globus pallidus and substantia nigra (Fig. 1.5).

#### A. The Direct and Indirect Pathways

Studies in which individual neurons were intracellularly filled provide the clearest evidence for subsets of medium spiny projection neurons on the basis of the projection axons (Kawaguchi et al., 1990). One type of neuron sends an axon collateral into the external segment of the globus pallidus, which does not arborize extensively, and extends other axon collaterals into the internal segment of the



FIGURE 1.5 The striatal medium spiny projection neuron. A. Photomicrograph of a single medium spiny projection neuron filled with biocytin. A'. High magnification of the intracellularly filled medium spiny neuron in A. B. Tracings of an indirect and a direct striatal projection neuron drawn in place on a sagittal brain diagram. The indirect pathway neuron has a projection axon that extends into the external segment of the globus pallidus (GPe), where it arborizes extensively, but does not extend beyond this nucleus. Direct pathway neurons have projection axons that extend some collaterals into the GPe and project to the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). Higher magnification of the indirect and direct pathway neurons shows their dendrites (black) (red and green) and local axon collaterals within the striatum (grey) (orange and blue). Abbreviations: STN, subthalamic nucleus; VTA, ventral tegmental area; SNc, substantia nigra pars compacta; RR, retrorubral area. C. Functional dissociation of direct and indirect striatal projection neurons. In dopamine-depleted striatum, D1 dopamine receptor stimulation results in phosphorylation of ERK1/2/MAPkinase (green immunoreactive neurons) selectively in direct pathway neurons. Indirect pathway neurons are labeled by localization of mRNA encoding enkephalin (ENK; red neurons). In this field, one neuron is double-labeled for both markers (yellow diagonal arrow). This functional dissociation reflects the differential expression of D1 and D2 receptor subtypes by these neurons (Gerfen et al., 2002; see text). D. Diagram of the direct and indirect pathway neurons. Both neurons are GABAergic and receive glutamatergic corticostriatal inputs. Direct pathway neurons express the D1 receptor subtype, the Gs and Golf stimulatory G-proteins, as well as the neuropeptides substance P (SP) and dynorphin (DYN). These neurons project to the GPe, GPi and SNr. Indirect pathway neurons express the D2 receptor, the A2A adenosine receptor, and the neuropeptide ENK. The D2 receptor is coupled to the inhibitory Gi G-protein, while the A2A receptor is coupled to the stimulatory Golf G-protein. (see Color Plate Section to view the color version of this figure)

globus pallidus and/or the substantia nigra (Fig. 1.5). This type is referred to as a direct pathway neuron in that it provides direct inputs to the output nuclei of the basal ganglia. (In rats, this neuron is often also called "striatonigral" neuron, based on its main projection target.)

A second type (the "striatopallidal" neuron) provides an axon that extends into the external globus pallidus and arborizes there extensively (Fig. 1.5), usually in two separate domains within this nucleus (see below). These neurons do not project beyond the globus pallidus and are thus termed "indirect" striatal projection neurons, in that they connect indirectly to the output of the basal ganglia, through synaptic connections in the globus pallidus and subthalamic nucleus. It is noteworthy that "direct" projection neurons also provide inputs to the globus pallidus, and thus contribute to the "indirect" pathway system. The extent of arborization of this axon collateral is less than that of the indirect projection neuron; however, it exists and appears to make functional synapses with pallidal neurons. Future studies will have to elucidate the functional significance of this collateral.

A major discovery concerning the function of dopamine in the basal ganglia was the demonstration that D1 and D2 dopamine receptors are segregated in the direct and indirect striatal projection neurons (Gerfen et al., 1990) (Fig. 1.5). The mRNA encoding the D1 receptor subtype was shown to be selectively localized in neurons that project to the substantia nigra, and co-localized with substance P and dynorphin, which are selectively expressed by direct projection neurons. Conversely, the mRNA encoding the D2 receptor is selectively localized in neurons that project to the external segment of the globus pallidus and is co-localized with enkephalin, which is selectively expressed in indirect projection neurons. Only a relatively small proportion of neurons express both D1 and D2 receptor mRNAs at comparable levels. While initially somewhat controversial (Surmeier et al., 1992), the general organizational principle of segregated D1 and D2 receptor expression in direct and indirect pathway neurons, respectively, has been confirmed by numerous other studies (e.g., Le Moine et al., 1990; Le Moine et al., 1991; Gerfen et al., 1995; Hersch et al., 1995; Le Moine and Bloch, 1995; Yung et al., 1995; Surmeier et al., 1996; Gong et al., 2003; Gong et al., 2007) and is consistent with receptor binding localization for D1 and D2 receptors in the globus pallidus and substantia nigra, respectively (e.g., Beckstead, 1988; Richfield et al., 1989) (see also Chapters 6 and 28).

The demonstration of a segregation of D1 and D2 receptors in direct and indirect pathway neurons, respectively (Gerfen et al., 1990), provided the basis for understanding of functional changes in movement disorders such as Parkinson's disease (Albin et al., 1989; DeLong, 1990). The central tenet of the theory of movement disorders is that they result from imbalanced activity in the direct and indirect striatal pathways (see also Chapter 39). In Parkinson's disease, which is marked by akinesia, the theory suggested that there is increased activity in the indirect pathway. Neurons of this pathway express the D2 receptor, which is coupled to the inhibitory G protein, Gi. In the normal animal, dopamine binding to the D2 receptors provides an inhibitory function. On the other hand, the D1 receptor expressed on direct pathway neurons is coupled to stimulatory G proteins, Gs and Golf. Consequently, in Parkinson's disease, the loss of dopamine input to the striatum has opposite effects on the direct and indirect pathways, with increased function in the indirect pathway and decreased function in the direct pathway.

#### **B.** Other Nuclei of the Indirect Pathway

Indirect striatal projection neurons extend an axon to the external segment of the globus pallidus, but do not project axon collaterals to either the internal segment of the globus pallidus or substantia nigra. Consequently, indirect striatal projection neurons are connected with the output of the basal ganglia indirectly, through the external globus pallidus and subthalamic nucleus. GABAergic neurons of the external segment of the globus pallidus, the targets of the indirect striatal projection neurons, provide inputs to both the internal segment of the globus pallidus/substantia nigra pars reticulata, as well as to the subthalamic nucleus. The subthalamic nucleus provides a glutamatergic, excitatory input to the internal segment of the globus pallidus and substantia nigra. These two nuclei are reviewed in the following sections and are also addressed in Chapters 13, 14 and 15.

#### 1. External Segment of the Globus Pallidus

There are two major cell types within the external segment of the globus pallidus (Kita and Kitai, 1994) (see Chapter 13). One type has a moderate to large cell soma from which radiate 3-5 dendrites with secondary and tertiary segments (Fig. 1.6); these are aspinous over their entire length and display some varicosities. The dendrites of these neurons are often long, up to 300-400 µm, giving a total maximal dendritic coverage of over 1 mm in some cases. Some aspiny neurons display a discoidal dendritic field in that the dendrites spread mainly in a two-dimensional manner parallel to the border between the external segment of the globus pallidus and striatum. Other aspiny neurons have dendrites that cover a volume with a more 3-dimensional distribution. A second type of pallidal neuron is distinguished by the spines distributed on its dendrites. The cell bodies of these neurons are generally smaller than those of the aspiny neurons. However, the size and extent of the dendritic fields appear to be similar for the two types, except that spiny neurons do not display discoid dendrites. Although all pallidal projection neurons appear to utilize GABA as a transmitter, the differences in morphology are matched by some neurochemical differences. For example, the larger discoidal-type dendrite-bearing neurons contain the calcium-binding protein parvalbumin, whereas the other pallidal projection neurons do not (Kita and Kitai, 1994). Parvalbumin-positive neurons are the more abundant of the two types.

The projections appear to be somewhat different between the two morphologically and neurochemically distinct pallidal neuron populations (Kita and Kitai, 1994a; Kita and Kitai, 1994b). Parvalbumin-positive/discoidal dendrite-bearing neurons provide axon collaterals to the



FIGURE 1.6 Components of the indirect striatal output pathway. A. Tracing of the striatopallidal axons of a single indirect striatal projection neuron in the sagittal plane. Of note are the double arborization zones within the globus pallidus external segment (GPe) from a single striatal neuron. B. Tracings of the dendrites of two GPe neurons in the sagittal plane. Of note is the distribution of the dendrites, which conforms to the same pattern as the striatal afferent axons. The two regions of the GPe, which are defined by the dual terminal patterns of striatal afferents, appear to have distinct populations of pallidal neurons. C. Tracing of a single GPe neuron (dendrites in white in sagittal section) and its axon (black) which provides collaterals to the striatum, subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr). A larger tracing of the dendrites of this neuron is shown on the right. Other GPe neurons also project to the globus pallidus internal segment (GPi) (entopeduncular nucleus in rat). D. Tracing of a single STN neuron (dendrites in white in sagittal section) and its axon (black) which provides collateral inputs to the GPe, GPi and SNr.

subthalamic nucleus, internal segment of the globus pallidus and substantia nigra (Fig. 1.6C), whereas the descending projection of the parvalbumin-negative pallidal neuron is directed primarily to the subthalamic nucleus. Both neuron types appear to also project to the striatum, although not all pallidal neurons provide such a projection (see also Chapter 14).

Most pallidal neurons are GAD-immunopositive and are thus presumed to utilize GABA as a neurotransmitter (Oertel and Mugnaini, 1984; Smith et al., 1987; Pasik et al., 1988). This is consistent with the fact that synaptic contacts of pallidal axon terminals with their target neurons are symmetric (Smith and Bolam, 1989; Smith and Bolam, 1990; Smith and Bolam, 1991). In addition to GADimmunopositive neurons, there are a scattering of cholinergic neurons within the body of the globus pallidus, as well as a large number of cholinergic neurons ventral to the globus pallidus (Fibiger, 1982; Ingham et al., 1985; Grove et al., 1986). In as much as these neurons appear to be the target of some projections from both the dorsal and ventral striatum, these cholinergic neurons might be considered to be part of the basal ganglia (Grove et al., 1986). These cholinergic neurons have been shown to provide projections to the cerebral cortex (Fibiger, 1982; Saper, 1984; Ingham et al., 1985; Grove et al., 1986; Ingham et al., 1988).

Neurons in the external segment of the globus pallidus receive inputs directly from the striatum (Chang et al., 1981; Wilson and Phelan, 1982; Hedreen and DeLong, 1991), which are inhibitory (Park et al., 1982), and inputs from the subthalamic nucleus, which are excitatory (Kita and Kitai, 1987). Inputs from the striatum appear to be the dominant inputs to pallidal neurons and display a distinct synaptic organization (DiFiglia et al., 1982). Individual fibers from the striatum entwine dendrites of pallidal neurons, making numerous synaptic contacts along an extended region of a dendrite. These synapses are symmetric and on the order of 1µm in diameter.

The synaptic organization of the external segment of the globus pallidus, where afferent axons make multiple contacts thus appearing to ensheath pallidal dendrites, has possible consequences for convergence of striatal afferents. The radial orientation of pallidal neuron dendrites, orthogonal to the plane of striatal efferent fibers, had suggested a means of convergence in that individual pallidal neurons would spread dendrites across the paths of outputs of many regions of the striatum. However, an alternative organization is suggested by the fact that individual striatal efferents, rather than remaining "on course" as they traverse the globus pallidus, in fact follow local paths to entwine individual pallidal neuron dendrites. This might suggest that in fact individual striatal efferent neurons make a rather direct transfer to few rather than many pallidal neurons. Such an organization would be decidedly different from that of cortical afferents to the striatum, in which individual axons contact the dendrites of many neurons "en passant".

As mentioned above, descending output of the external segment of the globus pallidus to other components of the basal ganglia is directed principally to the subthalamic nucleus and to the internal segment of the globus pallidus and the substantia nigra (Haber et al., 1985; Kita and Kitai, 1994). Ascending outputs of the globus pallidus provide feedback to the striatum (Staines et al., 1981; Beckstead, 1983; Staines and Fibiger, 1984) (see also Chapters 14 and 24). In addition, there is a projection from the ventral pallidum to the thalamus (Haber et al., 1985; Mogenson et al., 1987; Haber et al., 1993) (see also Chapter 21).

Of particular note is the synaptic organization of pallidal projection terminals, particularly those that provide input to the internal pallidal and substantia nigra neurons. Pallidal afferents onto these neurons are directed to the cell soma and proximal dendrites, whereas the striatal afferent input is directed to the same neurons' more distal dendrites (Smith and Bolam, 1989; Smith and Bolam, 1990; Smith and Bolam, 1991).

#### 2. Subthalamic Nucleus

Based on cellular and dendritic morphology neurons in the subthalamic nucleus appear to be of one main type (Fig. 1.6D), which nonetheless show a variance in the dimensions of the cell soma and dendritic ramifications (Kita et al., 1983a) (see also Chapter 15). In rats the cell soma is ovoid or polygonal with a medium size ranging 10-20µm in diameter. Most subthalamic neurons extend 3-4 primary dendrites which taper and branch into secondary and tertiary dendrites. Dendrites show infrequent spines, which, if present, are located on more distal parts of the dendrites. The dendrites spread in varying patterns within the nucleus. In general, dendrites appear to distribute in an ovoid area in both the frontal and sagittal planes, thus showing a greater extension in the rostro-caudal dimension than in the dorso-ventral dimension. In the horizontal plane, dendrites appear to distribute roughly equally in the medial-lateral dimension as in the rostro-caudal dimension. Subthalamic neurons across species appear to be similar in morphologic type, although the planar distribution patterns of the dendrites vary from species to species. This presumably reflects different geometries of the afferent inputs in different species.

Neurons in the subthalamic nucleus appear to be of one neurochemical type in that most are immunoreactive for glutamate. This is consistent with the fact that the synapses of subthalamic afferents to neurons in both segments of the globus pallidus and substantia nigra are asymmetric (Kita and Kitai, 1987). Moreover, the electrophysiologic response of neurons postsynaptic to subthalamic afferents following stimulation of the subthalamic nucleus confirms the excitatory nature of these inputs (Nakanishi et al., 1987b; Robeldo and Féger, 1990).

Neurons in the subthalamic nucleus receive inputs from the external segment of the globus pallidus, which are inhibitory (Kita et al., 1983b), and inputs from the cortex, which are excitatory (Kita et al., 1983b; Nakanishi et al., 1987a; Nakanishi et al., 1988). Inputs from the cortex are asymmetric and distributed principally to the dendrites of the neurons. Inputs from the external segment of the globus pallidus make large symmetric contact which are directed relatively equally to the cell soma (30%), proximal (39%) and distal (31%) dendrites (Smith et al., 1990). This input is distinguished from pallidal inputs to the substantia nigra in which 90% of the synaptic contact is made with the soma or proximal dendrites (Smith and Bolam, 1990).

Neurons in the subthalamic nucleus send axons that target neurons in both segments of the globus pallidus and substantia nigra (Fig. 1.6D), as well as a sparse projection to the striatum (Kita and Kitai, 1987). These projections provide an excitatory input to each of the target structures (Saper, 1984; Ingham et al., 1985; Nakanishi et al., 1987b; Robeldo and Féger, 1990; Kita and Kitai, 1991).

# C. Dual Projections within Basal Ganglia Circuits

A distinctive feature of striatal output organization is the dual projections from the striatum to subdivisions of the globus pallidus and substantia nigra (Chang et al., 1981; Wilson and Phelan, 1982; Gerfen, 1985) (Fig. 1.7). Initially described in the rat, this organization has also been observed in the primate (Parent and Hazrati, 1994). Thus, striatal projections to the globus pallidus have extensive axon arborizations in a region immediately adjacent to the striatum, and a second arborization zone in the central part of the globus pallidus (Figs 1.6A, 1.7). In the case of the striatopallidal pathway, the dual projections have been demonstrated to arise from individual striatal neurons (Chang et al., 1981). The dual striatonigral projection targets a region in the dorsal part of the substantia nigra pars reticulata, and a second zone that lies immediately above the cerebral peduncle (Fig. 1.7). It has not been demonstrated whether individual striatal neurons contribute projections to both zones of the pars reticulata, although this is likely. At the least they arise from within the striatal matrix



**FIGURE 1.7** Dual projection systems in direct and indirect pathways. A. Illustration of the dual projections from the striatum to the globus pallidus external segment (GPe) and substantia nigra pars reticulata (SNr) are shown. B. The dual projections from the subthalamic nucleus (STN) to the GPe and the SNr are depicted. In each system afferents target the same two regions in the GPe, an area immediately adjacent to the striatum and a second area more central, and the same two regions in the SNr, an area medial and dorsal adjacent to the substantia nigra pars compacta and a second area situated ventrally against the cerebral peduncle. The dual target zones in both the GPe and SNr have neurons whose dendrites appear to conform to the pattern of afferents to these regions. Individual striatal neurons and individual subthalamic neurons provide collaterals to both regions in each nucleus.

and from very closely associated neurons. These dual projection systems are not to be confused with the patchmatrix projections (see Section below).

The dual nature of inputs to the globus pallidus and substantia nigra is not only found in the striatal projections to these nuclei. Kita and Kitai (1987) have also observed a similar organization in the projection of the subthalamic nucleus to these nuclei (Fig. 1.7B). The projection patterns charted in their study bear a remarkable resemblance to those from the striatum. This suggests that this aspect of the organization of basal ganglia circuits is maintained not only in the organization of striatal outputs but also in the organization amongst the nuclei that are the targets of this striatal projection.

In both segments of the globus pallidus and in the substantia nigra the dendritic morphology of neurons conforms to the dual innervation patterns from the striatum (Gerfen, 1985). Thus, in the external segment of the globus pallidus, neurons in the region that is immediately adjacent to the striatum have dendrites that are distributed in a pattern that conforms to a "shell"-like region of the globus pallidus, whereas dendrites of neurons in the central region of the globus pallidus are restricted to the central region and do not appear to extend into the pallidal "shell" region (Kita and Kitai, 1994) (Fig. 1.6B). Note that neurons in different pallidal regions are likely to have different local connections (Sadek et al., 2007). Similarly, in the substantia nigra there are two zones of neurons in the pars reticulata (ignoring the dopamine neurons in the pars reticulata, see Section below). Again, as in the globus pallidus there is one region that forms a "shell"-like structure, in this case forming a region immediately above the cerebral peduncle, and a dorsal zone that is between the ventral "shell" region and the pars compacta. Neurons in these two regions have dendrites that are distributed so as to conform with the shape of the regions (Grofova et al., 1982). This organization was first described by Grofova et al. (1982) based on the morphology of the dendrites of pars reticulata neurons.

The organization of the substantia nigra pars reticulata into subregions appears not only to be related to the inputs from the striatum and subthalamic nucleus, but also to the organization of its outputs. The projections of the substantia nigra pars reticulata to the thalamus and to the superior colliculus (see following Section) appear to maintain a rough topography. This organization has been described by Gerfen et al. (1982) and in considerable details by Deniau and Chevalier (1992). Thus, projections to the ventral medial, mediodorsal, and intralaminar thalamus, as well as those to the superior colliculus, display a topographic organization. This topography involves both the central and peripeduncular "shell" region of the pars reticulata. Neurons projecting to a particular topographically related part of any of these structures arise in one of the two pars reticulata regions. This organization of the nigral output neurons was described by Deniau and Chevalier (1991) to have the appearance of distinct lamellae, much like that of an onion.

The dual nature of these basal ganglia projection systems has repeatedly been remarked upon (e.g., Gerfen et al., 1982; Deniau and Chevalier, 1992; Redgrave et al., 1992). However, its functional significance remains unclear.

#### VI. BASAL GANGLIA OUTPUT NUCLEI: INTERNAL SEGMENT OF GLOBUS PALLIDUS AND SUBSTANTIA NIGRA

Together, the internal segment of the globus pallidus and the substantia nigra are considered the output nuclei of the basal ganglia in that they provide the interface with brain areas outside the basal ganglia, in particular the thalamus and midbrain structures, including the superior colliculus and pedunculopontine nucleus (Fig. 1.8) (see Chapter 23). The neurons that provide these output projections utilize GABA as a transmitter and form a nuclear complex that is continuous from the internal segment of the globus pallidus and substantia nigra pars reticulata. In addition to the GABA neurons in these nuclei, dopamine neurons in the substantia nigra pars compacta provide a feedback pathway to the striatum (see following section).

For the purposes of the present review, details concerning the synaptic connections of the GABA neurons in the substantia nigra pars reticulata are provided. These are comparable to those of the neurons in the internal segment of the globus pallidus, which are addressed in detail in Chapter 13. The distinctions between these nuclei are due to the parts of the body that they are related to. The substantia nigra pars reticulata is involved in movements of the eyes, head and neck, whereas the internal segment of the globus pallidus is involved in limb and axial movements.

#### A. Cell Types

As mentioned above, the substantia nigra is composed of two main neuronal types, those that utilize dopamine (Bjorklund and Lindvall, 1984) and those that utilize GABA as a neurotransmitter (Ribak et al., 1979; Oertel and Mugnaini, 1984; Pasik et al., 1988). Dopamine neurons are located primarily in the pars compacta, which is a



**FIGURE 1.8** Basal ganglia output pathways. Output pathways arise from GABAergic neurons of the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). The GPi output is directed to the ventral lateral (vl) thalamic nucleus, intralaminar/parafascicular (pf) complex and to the lateral habenula (lh). The SNr output is directed to the paralamellar mediodorsal (md), pf and ventromedial (vm) thalamic nuclei, to the intermediate layers of the superior colliculus and to the pedunculopontine tegmental nucleus (PPN).

neuron-dense zone forming the dorsal part of the substantia nigra (Gerfen et al., 1987b) (dopamine neurons are discussed in detail in the following section and in Chapter 16). In addition, dopamine neurons are also located in groupings in the ventral neuron-sparse zone, the pars reticulata. Dopamine neurons in the substantia nigra, as well as those in the adjacent ventral tegmental area and retrorubral area provide inputs to the striatum and other forebrain areas (Beckstead, 1979; Ribak et al., 1979; Oertel and Mugnaini, 1984; Gerfen et al., 1987a,b; Pasik et al., 1988). GABA neurons are localized, for the most part, in the pars reticulata. These neurons provide inputs to the thalamus, superior colliculus and pedunculopontine nucleus (Beckstead, 1979; Ribak et al., 1979; Gerfen et al., 1982; Oertel and Mugnaini, 1984; Pasik et al., 1988).

#### **B.** Inputs

The major sources of input to substantia nigra neurons are inhibitory GABAergic inputs from the striatum and external segment of the globus pallidus, and excitatory inputs from the subthalamic nucleus (see previous sections). That the striatum provides an inhibitory GABAergic input to pars reticulata neurons has been established using electrophysiologic techniques (Deniau et al., 1976; Chevalier et al., 1985; Deniau and Chevalier, 1985). The external segment of the globus pallidus has more recently been established to provide a similar inhibitory input. The synaptic organization of these inputs to the pars reticulata was described in a comprehensive analysis by Smith and Bolam (Smith and Bolam, 1989; Smith and Bolam, 1990; Smith et al., 1990). In these studies, axonally transported tracer labeling of striatal and pallidal input to identified pars reticulata neurons projecting to the superior colliculus were examined at the light and electron microscopic level. Striatal input to pars reticulata neurons form symmetric, relatively small synapses directed principally to distal parts of the dendrites (77% of such input), and only infrequently to the cell soma (3%). In contrast, inputs from the globus pallidus form symmetric, relatively large synapses directed principally to the perikarya (54% of such input), or to proximal dendrites (32%). The differential distribution of inputs from the striatum and globus pallidus to the distal and more proximal dendrites suggests that, if the inputs are comparable in number, the latter afferent system may exert a dominant control over these pars reticulata neurons.

Afferents from the subthalamic nucleus to the pars reticulata provide an excitatory input mediated by the neurotransmitter glutamate (Kita and Kitai, 1987; Nakanishi et al., 1987b). At the synaptic level these inputs form asymmetric contacts principally directed to more distal parts of the dendrites of pars reticulata neurons (Kita and Kitai, 1987). Thus, the distribution pattern of these afferents is similar to that of the striatal inputs.

#### C. Outputs

Output targets of the substantia nigra pars reticulata include the thalamus, superior colliculus and the pedunculopontine nucleus (Beckstead, 1979; Gerfen et al., 1982; Kita and Kitai, 1987; Nakanishi et al., 1987b; Deniau and Chevalier, 1992) (Fig. 1.8). In the thalamus, nigral efferents are directed to two main parts. The first are the set of nuclei, including the intralaminar nuclei, that project back to the striatum (see also Chapter 22). The second thalamic target are nuclei that provide projections to frontal cortical areas. The specific nuclei involved vary from species to species, primarily as a consequence of the organization of cortex. For example, in rodents, the principal target of the substantia nigra is the ventromedial thalamic nucleus, which provides a relatively widespread input to frontal cortical areas, and the paralaminar medial dorsal thalamus, which in turn projects to the cortical areas thought to be equivalent to the frontal eye fields in primates. Conversely, in primates where frontal cortical areas are subdivided into more discrete areas (see also Chapter 24), thalamic inputs to these areas are correspondingly organized. In primates, the principal thalamic targets of the internal segment of the globus pallidus are the ventral lateral, pars oralis and ventral anterior, pars parvocellularis nuclei (Schell and Strick, 1984), and the target of the substantia nigra is the ventral anterior (VAmc) and paralaminar medial dorsal (MDpc) nuclei (Ilinsky et al., 1985). Many individual pars reticulata neurons have collaterals that target two or more of these targets.

# VII. THE NIGROSTRIATAL DOPAMINE SYSTEM

Dopamine neurons in the ventral midbrain, which can be labeled by tyrosine hydroxylase immunolabeling are the origin of the nigrostriatal dopamine system (Fig. 1.9) (see also Chapters 16 and 17). Midbrain areas that contain dopamine neurons include the ventral tegmental area, which is the ventral medial most region of the midbrain, the substantia nigra, including the pars compacta, in which dopamine neurons are densely packed, and the pars reticulata, which is relatively cell sparse compared to the pars compacta, and the retrorubral area, which lies caudal and dorsal to the substantia nigra (Bjorklund and Lindvall, 1984; Gerfen et al., 1987b) (Fig. 1.9). The designation of the subgroupings of dopamine neurons according to regional location – A10 cell group in the ventral tegmental area, A9 cell group in the substantia nigra, and A8 cell group in the retrorubral area – conforms to some extent with their projection targets (Bjorklund and Lindvall, 1984). The A10 dopamine cell group is generally regarded to project to limbic forebrain areas, such as the septal area, prefrontal cortex, olfactory tubercle and nucleus accumbens. The A9 and A8 cell groups are generally regarded as the origin of the projection to the striatum.

#### A. Dorsal Tier Versus Ventral Tier Dopamine Neurons

Dopamine innervation of the striatum (Bjorklund and Lindvall, 1984) is relatively dense and when considered in total appears rather uniform. However, this belies an underlying organization of the nigrostriatal system into patch- and matrix-directed subsystems (Gerfen et al., 1987a,b; Jimenez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989) (Fig. 1.9). The first indication of the compartmental organization of the nigrostriatal dopamine system came from developmental studies which revealed that in the early postnatal striatum dopamine input is distributed in patches (dopamine "islands"), and that during subsequent development innervation of the matrix is completed (Olson et al., 1972; Tennyson et al., 1972). Neuroanatomical tracing studies demonstrated that this developmental sequence is a consequence of the dopamine projections to the patch and matrix compartments arising from distinct sets of dopamine neurons in the substantia nigra (Gerfen et al., 1987a,b).

Dopamine neurons that project to the striatum are distributed in each of these groups, including the A10 cell group. As is also seen, these neurons are distributed in a somewhat continuous manner, such that delineation of subgroupings based on regional location is somewhat arbitrary. A different parcellation of these neurons is suggested based on the morphology of neuronal dendrites, the expression of the calcium-binding protein calbindin, and their projection to either the patch or matrix compartments (Gerfen et al., 1987a,b). Using these determinants the projection of midbrain dopamine neurons to the striatum reveals the following organization. Two sets of dopamine



**FIGURE 1.9** Organization of the nigrostriatal dopamine pathway. The illustration shows the organization of the dopamine (DA) projections from the midbrain to the striatal patch and matrix compartments (sagittal diagram, upper left). The coronal section at a mid-striatal level (A) depicts the innervation of the patch and matrix compartments from different subsets of midbrain DA neurons, shown at three rostrocaudal levels (B,C,D). DA neurons providing inputs to the striatal matrix compartment (light grey in B,C,D) (orange in B,C,D) are located in the ventral tegmental area (B,C,D: VTA; A10 DA cell group), in the dorsal tier of the substantia nigra pars compacta (B,C: SNc; A9) and in the retrorubral area (D: RR; A8). Neurons providing input to the striatal patch compartment (dark grey in B,C,D) (blue in B,C,D) are located in the ventral tier of the SNc (B,C,D; A9) and in the substantia nigra pars reticulata (C,D: SNr; A9). Dorsal tier neurons express the calcium-binding protein calbindin, whereas ventral tier neurons are calbindin-negative. There is a general topography in that medially located cells project to the ventral striatum and laterally located cells project to the dorsal striatum. Neurons at every rostral–caudal level in the midbrain project rather extensively to throughout the rostral–caudal extent of the striatum. (see Color Plate Section to view the color version of this figure)

neurons are distinguished and these are localized in a dorsal and a ventral tier (Fig. 1.9).

The dorsal tier set provides inputs predominantly to the striatal matrix compartment. This set encompasses a continuous group that includes the dopamine neurons projecting to the striatum situated in the ventral tegmental area, the dorsal part of the substantia nigra pars compacta, and the retrorubral area (Fig. 1.9). Several other characteristics apply to this set. First, those neurons in the pars compacta are distinguished from the ventral tier neurons by the extension of their dendrites within the plane of the pars compacta. Second, most of the dorsal tier neurons express, in addition to dopamine, the calcium-binding protein calbindin. Third, there is a rough topography to the organization of the projections to the striatum such that more medially situated neurons project ventrally to the nucleus accumbens and ventral striatum, whereas more lateral and caudal neurons, in the A9 and A8 cell groups, project to the dorsal striatum.

The ventral tier set provides inputs preferentially to the striatal patch compartment. Neurons in this set are situated in the ventral part of the substantia nigra pars compacta and in groups of cells embedded in the pars reticulata (Fig. 1.9). In contrast to dorsal tier neurons, ventral tier pars compacta neurons are distinguished by their extension of dendrites ventrally into the pars reticulata. Ventral tier dopamine neurons do not display calbindin immunoreactivity. These neurons display a topographic organization in their projections to the striatum, with dorsally positioned neurons projecting to the patch compartment in the ventral striatum and nucleus accumbens, and ventrally positioned neurons in the pars reticulata projecting to the dorsal striatal patch compartment.

It is worthwhile to note that the numbers of dopamine neurons located in the ventral substantia nigra pars reticulata increases at more caudal levels. Consequently, the common view of the substantia nigra as being composed of two separate zones, a dorsal pars compacta in which dopamine neurons are located, and a ventral pars reticulata in which GABA neurons are located, applies only to the rostral most levels of this nucleus. This organization appears to be common across species from rat to primates.

#### **B.** Inputs to Dopamine Neurons

Input to pars compacta dopamine neurons appears to be, for the most part, similar to that to the pars reticulata for each of the sources of input described above (see also Chapter 16). Afferents from the striatum, which are identified both directly with anterograde axonal markers, and with GABA or substance P immunoreactivity, appear to provide a major input to pars compacta neurons (Smith and Bolam, 1990). However, in the case of afferents from the globus pallidus the input is somewhat less than that to the pars reticulata neurons (Smith and Bolam, 1990). In addition, there are other known sources of inputs directed to the pars compact that have not been described as being directed to the pars reticulata. One of these is a cholinergic input that provides asymmetric synaptic contacts with pars compacta neurons, which at least in part arises in the pedunculopontine nucleus (see Chapter 23). Another is from the amygdala, which appears to provide inputs to the major components of the dopamine cell groups, but not to the pars reticulata (Gonzales and Chesselet, 1990). In addition, the lateral habenula provides input directed to the pars compacta (Herkenham and Nauta, 1979), which has been identified with electrophysiologic techniques as an inhibitory input (Christoph et al., 1986).

#### VIII. STRIATAL PATCH-MATRIX COMPARTMENTS

#### A. Markers Defining the Patch-Matrix Compartments

As mentioned above, early indication that there are compartments within the striatum came from studies that observed islands or patches of dopamine innervation distributed within the neuropil of the striatum during early postnatal development; this uneven innervation gives way to a homogeneous distribution of dopamine input as development progresses (Tennyson et al., 1972). A number of neurochemical markers were then found to coincide with these patches, including staining for acetylcholinesterase (Graybiel and Ragsdale, 1978) and opiate receptor binding (Herkenham and Pert, 1981). The striatal neurons that are the target of this early dopamine input also develop first, with later born striatal neurons filling in the surrounding matrix regions of the striatum (van der Kooy and Fishell, 1987). These two developmental compartments, the early developing patches or islands and the later developing matrix, give rise to the adult patch (or striosome; Graybiel and Ragsdale, 1978) and matrix compartments of the striatum. As the adult striatum appears homogeneous neurochemical markers can still reveal these compartments; notably, among others, calbindin marks the matrix (Gerfen et al., 1985) and mu opioid receptors mark the patch compartment (Herkenham and Pert, 1981; see Fig. 20.3 in Chapter 20).

#### **B.** Dopamine Inputs to Patches Versus Matrix

As discussed in the previous Section, distinct subsets of dopamine neurons differentially target the striatal patch and matrix compartments (Gerfen et al., 1987a,b). Axonal tracing studies demonstrated that dopamine neuron projections from the ventral tegmental area, dorsal tier of the substantia nigra pars compacta and retrorubral area provide input principally to the striatal matrix compartment, whereas projections from the two groups of ventral tier dopamine neurons of the substantia nigra provide input to the patch compartment (Gerfen et al., 1987a). Moreover, as mentioned above, the

matrix-projecting neurons co-express the calcium-binding protein calbindin, which thus provides a neurochemical marker for these neurons (Gerfen et al., 1985).

To further affirm this differential organization, we took advantage of the differential development of the patch- and matrix-directed dopamine systems. Injecting the neurotoxin 6-hydroxydopamine into the striatum on the day of birth resulted in the selective degeneration of the ventral tier dopamine neurons and the dopamine input to the patch compartment (Gerfen et al., 1987b), as these neurons are already present at birth and thus were destroyed by the neurotoxin. In contrast, the calbindin-expressing dopamine neurons in the ventral tegmental area, dorsal tier of the pars compacta and retrorubral area, which only develop postnatally, survived in adults, as did the dopamine input to the striatal matrix compartment. These findings thus confirmed that distinct sets of mesostriatal dopamine neurons differerentially target the patch and matrix compartments of the striatum.

Such distinct sets of dopamine neurons providing differential input to the striatal patch and matrix compartments have also been demonstrated in the cat and the primate (Gerfen et al., 1985; Jimenez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989). However, this differential innervation is not absolute. A recent study in the rat by Matsuda et al. (2009) used a method that labels the full axonal arborization of single neurons and found that individual dopamine neurons in both the dorsal and ventral tier distribute axons to some degree to both patch and matrix compartments, although each neuron's arborization tended to favor one or the other. The potential significance of this finding is discussed below.

#### C. Cortical and Thalamic Inputs

Dopamine input to striatal medium spiny neurons is directed principally to dendritic shafts and spine necks, and likely functions to modulate excitatory input that is directed to the dendritic spines. There are two main sources of glutamatergic excitatory input, the cerebral cortex and the thalamus, and some aspects of each of these is organized relative to patch and matrix compartments. For the thalamus, parts of the intralaminar thalamic nuclei differentially target the patch-matrix compartments, with projections of the parafascicular and centromedian nuclei directed to the matrix, and projections of the paraventricular nucleus directed to the patch compartment (Herkenham and Pert, 1981; Gerfen et al., 1982; Berendse et al., 1988).

Corticostriatal projections predominantly arise from pyramidal neurons in layer 5. Early studies examining the compartmental targets of corticostriatal projections suggested that different cortical areas projected selectively to one or the other. Limbic cortical areas were shown to provide input to the patch compartment, whereas somatosensory and motor cortical area projections targeted the matrix (Gerfen, 1984; Donoghue and Herkenham, 1986). However, more detailed analysis of corticostriatal projections demonstrated that most cortical areas provide inputs to both compartments, but that neurons in different sublayers of layer 5 differentially project to the patch and matrix compartments (Gerfen, 1989). For each specific cortical area, neurons with patch-directed inputs are located in deep layer 5, whereas those with matrix-directed inputs are located in superficial layer 5 (Fig. 1.10).



**FIGURE 1.10** Connections of the striatal patch–matrix compartments. The organization of the patch-matrix compartments bestows parallel pathways from the cerebral cortex through the striatum that provide differential input to the dopamine and GABA neurons in the substantia nigra. Deep layer 5 corticostriatal neurons project to the patch compartment, whose neurons target dopamine neurons in the substantia nigra pars compacta (SNc). Superficial layer 5 corticostriatal neurons project to the substantia nigra pars reticulata (SNr), which contains the GABA output neurons of the basal ganglia. This organization arises from most neocortical areas, although there is a gradient such that those areas closer to the allocortex provide a greater input to the patch compartment, whereas primary sensorimotor areas provide a greater input to the matrix compartment.

Corticostriatal projections are topographically organized, such that motor cortical areas project to the dorsolateral striatum and prelimbic and infralimbic areas project to the medial and ventral striatum. Importantly, for each cortical area, both patch- and matrix-directed projections target the topographic region within the striatum, such that from a given cortical area, its projections to the matrix surround the patches that it also projects to. While this pattern of organization of corticostriatal projections is apparent in most cortical areas, the relative contribution of inputs to the patch and matrix compartments varies between cortical areas. Neocortical areas, such as motor, supplementary motor and somatosensory cortices, provide greater inputs to the matrix compartment, whereas allocortical and peri-allocortical areas such as the prelimbic and infralimbic cortical areas provide greater inputs to the patch compartment. This transition of a predominance of patch-directed inputs from limbic-related cortical areas to matrix-directed inputs from neocortical areas is likely responsible for the earlier findings, suggesting that different cortical areas provide inputs only to one compartment. The major significance of the organization of corticostriatal projections is that the striatal patch and matrix compartments are related to the laminar organization of the cerebral cortex rather than to tangential or columnar features of its organization (Gerfen, 1989).

#### D. Outputs of Patches Versus Matrix

In addition to inputs, output connections of the striatum are also organized relative to the patch-matrix compartments (Fig. 1.10). The distribution of medium spiny neurons is homogeneous and does not reveal striatal compartments, the dendrites of the neurons in patch and matrix compartments tend to remain confined within their respective compartments (Gerfen, 1985; Bolam et al., 1988). Moreover, axonal tracing studies demonstrated that projections from the striatal patch compartment provide input directed principally to the ventral tier dopamine neurons in the substantia nigra, whereas the striatal matrix neurons project to the external and internal segment of the globus pallidus, and substantia nigra pars reticulata (Gerfen, 1985) (Fig. 1.10). Thus, the striatal output of the patch compartment is directed principally at the same ventral tier dopamine neurons that provide input to this compartment. In this regard, the recent finding that ventral tier neurons provide dopamine input to both patch and matrix compartments (Matsuda et al., 2009) is important. This finding suggests that the striatal patch output is not part of a closed loop with the dopamine neurons that provide patch input, but rather affects dopamine feedback to both compartments.

The target of the output of striatal matrix neurons is directed to components of the basal ganglia that provide the output of this system. These are the internal segment of the globus pallidus and substantia nigra pars reticulata which are composed of GABA neurons that project to the thalamus, superior colliculus and other midbrain systems connected with motor control (see above). Thus, the output of neurons in the striatal patch and matrix, respectively, target dopamine feedback to the striatum and basal ganglia output systems. In summary, the general organization of the patch-matrix compartments provides separate pathways from the cortex, through the striatum, to differentially modulate dopamine and other basal ganglia feedback circuits, or to affect basal ganglia GABAergic output neurons in the internal segment of the globus pallidus and substantia nigra pars reticulata (Fig. 1.10). Thus, the cortical connections through the patch compartment appear to be related to regulation of the dopamine, and possibly serotonin, feedback systems to the striatum, whereas cortical connections through the matrix compartment appear to be related to regulation of the output neurons of the basal ganglia.

#### REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381.
- Arluison M, Dietl M, Thibault J (1984) Ultrastructural morphology of dopaminergic nerve terminals and synapses in the striatum of the rat using tyrosine hydroxylase immunoreactivity: a topographical study. Brain Res Bull 13:269–285.
- Beckstead RM (1979) An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J Comp Neurol 184:43–62.
- Beckstead RM (1983) A pallidostriatal projection in the cat and monkey. Brain Res Bull 11:629–632.
- Beckstead RM (1988) Association of dopamine D1 and D2 receptors with specific cellular elements in the basal ganglia of the cat: the uneven topography of dopamine receptors in the striatum is determined by intrinsic striatal cells, not nigrostriatal axons. Neuroscience 27:851–863.
- Beckstead RM, Cruz CJ (1986) Striatal axons to the globus pallidus, entopeduncular nucleus and substantia nigra come mainly from separate cell populations in cat. Neuroscience 19:147–158.
- Beckstead RM, Kersey KS (1985) Immunohistochemical demonstration of differential substance P-, met-enkephalin-, and glutamic-aciddecarboxylase-containing cell body and axon distributions in the corpus striatum of the cat. J Comp Neurol 232:481–498.
- Berendse HW, Voorn P, te Kortschot A, Groenewegen HJ (1988) Nuclear origin of thalamic afferents of the ventral striatum determines their relation to patch/matrix configurations in enkephalin-immunoreactivity in the rat. J Chem Neuroanat 1:3–10.
- Bevan MD, Booth PAC, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a sub-class of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bishop GA, Chang HT, Kitai ST (1982) Morphological and physiological properties of neostriatal neurons: an intracellular horseradish peroxidase study in the rat.. Neuroscience 7:179–191.
- Bjorklund A, Lindvall O (1984) Dopamine-containing systems in the CNS. *In* Handbook of Chemical Neuroanatomy Vol. 2: Classical Transmitters in the CNS, Part I (Bjorklund A, Hokfelt T, eds), pp. 55–122: Elsevier. Amsterdam.
- Bolam JP, Clarke DJ, Smith AD, Somogyi P (1983) A type of aspiny neuron in the rat neostriatum accumulates [3H]gamma-aminobutyric acid: combination of Golgi-staining, autoradiography, and electron microscopy. J Comp Neurol 213:121–134.
- Bolam JP, Ingham CA, Izzo PN, Levey AI, Rye DB, Smith AD, Wainer BH (1986) Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. Brain Res 397:279–289.
- Bolam JP, Izzo PN (1988) The postsynaptic targets of substance Pimmunoreactive terminals in the rat neostriatum with particular reference to identified spiny striatonigral neurons. Exp Brain Res 70:361–377.
- Bolam JP, Izzo PN, Graybiel AM (1988) Cellular substrate of the histochemically defined striosome/matrix system of the caudate nucleus: a combined Golgi and immunocytochemical study in cat and ferret.. Neuroscience 24:853–875.
- Bolam JP, Powell JF, Wu JY, Smith AD (1985) Glutamate decarboxylaseimmunoreactive structures in the rat neostriatum: a correlated light and electron microscopic study including a combination of Golgi impregnation with immunocytochemistry. J Comp Neurol 237:1–20.
- Bolam JP, Wainer BH, Smith AD (1984) Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. Neuroscience 12:711–718.
- Bouyer JJ, Park DH, Joh TH, Pickel VM (1984) Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylasecontaining terminals in rat neostriatum. Brain Res 302:267–275.
- Carman JB, Cowan WM, Powell TPS (1965) The organization of corticostriate connexions in the rabbit. Brain 86:525–562.
- Chang HT, Wilson CJ, Kitai ST (1981) Single neostriatal efferent axons in the globus pallidus: a light and electron microscopic study. Science 213:915–918.
- Chang HT, Wilson CJ, Kitai ST (1982) A Golgi study of rat neostriatal neurons: light microscopic analysis. J Comp Neurol 208:107–126.
- Chesselet MF, Robbins E (1989) Characterization of striatal neurons expressing high levels of glutamic acid decarboxylase messenger RNA. Brain Res 492:237–244.
- Chevalier G, Vacher S, Deniau JM, Desban M (1985) Disinhibition as a basic process in the expression of striatal functions. I. The striatonigral influence on tecto-spinal/tecto-diencephalic neurons. Brain Res 334:215–226.
- Christoph GR, Leonzio RJ, Wilcox KS (1986) Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. J Neurosci 6:613–619.

- Cowan RL, Wilson CJ, Emson PC, Heizmann CW (1990) Parvalbumincontaining GABAergic interneurons in the rat neostriatum. J Comp Neurol 302:197–205.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- Dawson TM, Bredt DS, Fotuhi M, Hwang PM, Snyder SH (1991) Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc Natl Acad Sci USA 88:7797–7801.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Deniau JM, Chevalier G (1985) Disinhibition as a basic process in the expression of striatal functions. II. The striato-nigral influence on thalamocortical cells of the ventromedial thalamic nucleus. Brain Res 334:227–233.
- Deniau JM, Chevalier G (1992) The lamellar organization of the rat substantia nigra pars reticulata: distribution of projection neurons. Neuroscience 46:361–377.
- Deniau JM, Feger J, LeGuyader C (1976) Striatal evoked inhibition of identified nigro-thalamic neurons. Brain Res 104:152–156.
- DiFiglia M, Pasik P, Pasik T (1976) A Golgi study of neuronal types in the neostriatum of monkeys. Brain Res 114:245–256.
- DiFiglia M, Pasik P, Pasik T (1982) A Golgi and ultrastructural study of the monkey globus pallidus. J Comp Neurol 212:53–75.
- Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397–403.
- Donoghue JP, Kitai ST (1981) A collateral pathway to the neostriatum from corticofugal neurons of the rat sensory-motor cortex: an intracellular HRP study. J Comp Neurol 210:1–13.
- Dube L, Smith AD, Bolam JP (1988) Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum. J Comp Neurol 267:455–471.
- Elde R, Schalling M, Ceddatelli S, Nakanishi S, Hokfelt T (1990) Localization of neuropeptide receptor mRNA in rat brain: initial observations using probes for neurotensin and substance P receptors. Neuroscience Lett 120:134–138.
- Fibiger HC (1982) The organization and some projections of cholinergic neurons of the mammalian forebrain. Brain Res Rev 4:327–338.
- Flaherty AW, Graybiel AM (1991) Corticostriatal transformations in the primate somatosensory system. Projections from physiologically mapped body-part representations. J Neurophysiol 66: 1249–1263.
- Flaherty AW, Graybiel AM (1993) Two input systems for body representations in the primate striatal matrix: experimental evidence in the squirrel monkey. J Neurosci 13:1120–1137.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylaseimmunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 13:1189–1215.
- Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461–464.
- Gerfen CR (1985) The neostriatal mosaic. I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. J Comp Neurol 236:454–476.
- Gerfen CR (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science 246:385–388.

- Gerfen CR (1991) Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain. Brain Res 556:165–170.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 15:133–139.
- Gerfen CR, Baimbridge KG, Miller JJ (1985) The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. Proc Natl Acad Sci U S A 82:8780–8784.
- Gerfen CR, Baimbridge KG, Thibault J (1987a) The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7:3935–3944.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gerfen CR, Herkenham M, Thibault J (1987b) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and nondopaminergic systems. J Neurosci 7:3915–3934.
- Gerfen CR, McGinty JF, Young WS (1991) Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J Neurosci 11:1016–1031.
- Gerfen CR, Keefe KA, Gauda EB (1995) D1 and D2 dopamine receptor function in the striatum: coactivation of D1 and D2 dopamine receptors on separate populations of neurons results in potentiated immediate-early gene response in D1-containing neurons. J Neurosci 15:8167–8176.
- Gerfen CR, Staines WA, Arbuthnott GW, Fibiger HC (1982) Crossed connections of the substantia nigra in the rat. J Comp Neurol 207:283–303.
- Gerfen CR, Young WS (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161–167.
- Gerfen CR, Miyachi S, Paletzki R, Brown P (2002) D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 22:5042–5054.
- Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425:917–925.
- Gong S, Doughty ML, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci 27:9817–9823.
- Gonzales C, Chesselet MF (1990) Amygdalonigral pathway: an anterograde study in the rat with Phaseolus vulgaris leucoagglutinin (PHA-L). J Comp Neurol 297:182–200.
- Graybiel AM, Ragsdale CW Jr (1978) Histochemically distinct compartments in the striatum of human, monkey and cat demonstrated by acetylcholinesterase staining. Proc Natl Acad Sci USA 75:5723–5726.
- Graybiel AM, Chesselet MF (1984) Compartmental distribution of striatal cell bodies expressing [Met]enkephalin-like immunoreactivity. Proc Natl Acad Sci USA 81:7980–7984.
- Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in

striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 87:6912–6916.

- Graybiel AM, Ragsdale CJ, Yoneoka ES, Elde RP (1981) An immunohistochemical study of enkephalins and other neuropeptides in the striatum of the cat with evidence that the opiate peptides are arranged to form mosaic patterns in register with the striosomal compartments visible by acetylcholinesterase staining. Neuroscience 6:377–397.
- Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsalprefrontal topography. Neuroscience 24:379–431.
- Groenewegen HJ, Berendse HW, Haber SN (1993) Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. Neuroscience 57:113–142.
- Groenewegen HJ, Russchen FT (1984) Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. J Comp Neurol 223:347–367.
- Grofova I (1975) The identification of striatal and pallidal neurons projecting to substantia nigra. An experimental study by means of retrograde axonal transport of horseradish peroxidase. Brain Res 91:286–291.
- Grofová I (1979) Types of striatonigral neurons labeled by retrograde transport of horseradish peroxidase. Appl Neurophysiol 42:25–28.
- Grofova I, Deniau JM, Kitai ST (1982) Morphology of the substantia nigra pars reticulata projection neurons intracellularly labeled with HRP. J Comp Neurol 208:352–368.
- Grove EA, Domesick VB, Nauta WJH (1986) Light microscopic evidence of striatal input to intrapallidal neurons of cholinergic cell group Ch4 in the rat: a study employing the anterograde tracer Phaseolus vulgaris leucagglutinin (PHA-L). Brain Res 367:379–384.
- Haber SN, Watson SJ (1983) The comparison between enkephalin-like and dynorphin-like immunoreactivity in both monkey and human globus pallidus and substantia nigra. Life Sci 1:33–36.
- Haber SN, Groenewegen HJ, Grove EA, Nauta WJ (1985) Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. J Comp Neurol 235:322–335.
- Haber SN, Lynd BE, Mitchell SJ (1993) The organization of the descending ventral pallidal projections in the monkey. J Comp Neurol 329:111–128.
- Hanson GR, Alphs L, Pradham S, Lovenberg W (1981) Haloperidolinduced reduction of nigral substance P-like immunoreactivity: a probe for the interactions between dopamine and substance P neuronal systems. J Pharmacol Exp Ther 218:568–574.
- Hanson GR, Merchant KM, Letter AA, Bush L, Gibb JW (1987) Methamphetamine-induced changes in the striato-nigral dynorphin system: role of D-1 and D-2 receptors. Eur J Pharmacol 144:245–246.
- Hattori T, McGeer EG, McGeer PL (1978) Fine structural analysis of cortico-striatal pathway. J Comp Neurol 185:347–354.
- Hedreen JC, DeLong MR (1991) Organization of striatopallidal, striatonigral, and nigrostriatal projections in the macaque. J Comp Neurol 304:569–595.
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, Costa BR, Rice K (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583.
- Herkenham M, Nauta WJH (1979) Efferent connections of the habenular nuclei in the rat. J Comp Neurol 187:19–48.

- Herkenham M, Pert CB (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. Nature 291:415–418.
- Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Ilinsky IA, Jouandet ML, Goldman-Rakic PS (1985) Organization of the nigrothalamocortical system in the rhesus monkey. J Comp Neurol 236:315–330.
- Ingham CA, Bolam JP, Smith AD (1988) GABA-immunoreactive synaptic boutons in the rat basal forebrain: comparison of neurons that project to the neocortex with pallidosubthalamic neurons. J Comp Neurol 273:263–282.
- Ingham CA, Bolam JP, Wainer BH, Smith AD (1985) A correlated light and electron microscopic study of identified cholinergic basal forebrain neurons that project to the cortex in the rat. J Comp Neurol 239:176–192.
- Izzo PN, Bolam JP (1988) Cholinergic synaptic input to different parts of spiny striatonigral neurons in the rat. J Comp Neurol 269:219–234.
- Jimenez-Castellanos J, Graybiel AM (1987) Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. Neuroscience 23:223–242.
- Kawaguchi Y (1992) Large aspiny cells in the matrix of the rat neostriatum in vitro: physiological identification, relation to the compartments and excitatory postsynaptic currents. J Neurophysiol 67:1669–1682.
- Kawaguchi Y (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci 13:4908–4923.
- Kawaguchi Y, Kubota Y (1993) Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindinD28k-immunoreactive neurons in layer V of rat frontal cortex. J Neurophysiol 70:387–396.
- Kawaguchi Y, Wilson CJ, Emson PC (1989) Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. J Neurophysiol 62:1052–1068.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 10:3421–3438.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.
- Kemp JM, Powell TPS (1970) The cortico-striate projection in the monkey. Brain 93:525–546.
- Kemp JM, Powell TPS (1971) The structure of the caudate nucleus of the cat: Light and electron microscopic study. Phil Trans R Soc Lond [Biol] 262:383–401.
- Kincaid AE, Zheng T, Wilson CJ (1998) Connectivity and convergence of single corticostriatal axons. J Neurosci 18:4722–4731.
- Kita H (1993) GABAergic circuits of the striatum. Prog Brain Res 99:51–72.
- Kita H, Kitai ST (1987) Efferent projections of the subthalamic nucleus in the rat: light and electron microscopic analysis with the PHA-L method. J Comp Neurol 260:435–452.
- Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res 447:346–352.

- Kita H, Kitai ST (1991) Intracellular study of rat globus pallidus neurons: membrane properties and responses to neostriatal, subthalamic and nigral stimulation. Brain Res 564:296–305.
- Kita H, Kitai ST (1994a) Parvalbuminin-immunoreactive neurons in rat globus pallidus: a light and electron microscopic study. Brain Res 657:31–41.
- Kita H, Kitai ST (1994b) The morphology of globus pallidus projection neurons in the rat: an intracellular staining study. Brain Res 636:308–319.
- Kita H, Chang HT, Kitai ST (1983a) The morphology of intracellularly labeled rat subthalamic neurons: a light microscopic analysis. J Comp Neurol 215:245–257.
- Kita H, Chang HT, Kitai ST (1983b) Pallidal inputs to subthalamus: intracellular analysis. Brain Res 264:255–265.
- Kita H, Kosaka T, Heizmann CW (1990) Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. Brain Res 536:1–15.
- Kitai ST, Koscis JD, Preston RJ, Sugimori M (1976) Monosynaptic inputs to caudate neurons identified by intracellular injection of horseradish peroxidase. Brain Res 109:601–606.
- Kubota Y, Kawaguchi Y (1993) Spatial distributions of chemically identified intrinsic neurons in relation to patch and matrix compartments of rat neostriatum. J Comp Neurol 332:499–513.
- Kubota Y, Mikawa S, Kawaguchi Y (1993) Neostriatal GABAergic interneurones contain NOS, calretinin or parvalbumin. Neuroreport 5:205–208.
- Kunzle H (1975) Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in Macaca fascicularis. Brain Res 88:195–209.
- Lacey CJ, Boyes J, Gerlach O, Chen L, Magill PJ, Bolam JP (2005) GABA-B receptors at glutamatergic synapses in the rat striatum. Neuroscience 136:1083–1095.
- Lacey CJ, Bolam JP, Magill PJ (2007) Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections. J Neurosci 27:4374–4384.
- Landry P, Wilson CJ, Kitai ST (1984) Morphological and electrophysiological characteristics of pyramidal tract neurons in the rat. Exp Brain Res 57:177–190.
- Langer LF, Graybiel AM (1989) Distinct nigrostriatal projection systems innervate striosomes and matrix in the primate striatum. Brain Res 498:344–350.
- Lei W, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.
- Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426.
- Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc Natl Acad Sci USA 87:230–234.
- Loopuijt LD, van der Kooy D (1985) Organization of the striatum: collateralization of its efferent axons. Brain Res 348:86–99.
- Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, Kaneko T (2009) Single nigrostriatal dopaminergic neurons form

widely spread and highly dense axonal arborizations in the neostriatum. J Neurosci 29:444–453.

Mogenson GJ, Ciriello J, Garland J, Wu M (1987) Ventral pallidum projections to mediodorsal nucleus of the thalamus: an anatomical and electrophysiological investigation in the rat. Brain Res 404:221–230.

- Moss J, Bolam JP (2008) A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. J Neurosci 28:11221–11230.
- Nakanishi H, Kita H, Kitai ST (1987a) Electrical membrane properties of rat subthalamic neurons in an in vitro slice preparation. Brain Res 437:35–44.
- Nakanishi H, Kita H, Kitai ST (1987b) Intracellular study of rat substantia nigra pars reticulata neurons in an in vitro slice preparation: electrical membrane properties and response characteristics to subthalamic stimulation. Brain Res 437:45–55.
- Nakanishi H, Kita H, Kitai ST (1988) An N-methyl-D-aspartate receptor mediated excitatory postsynaptic potential evoked in subthalamic neurons in an in vitro slice preparation of the rat. Neurosci Lett 95:130–136.
- Nauta WJH, Mehler WR (1966) Projections of the lentiform nucleus in the monkey. Brain Res 1:3–42.
- Oertel WH, Mugnaini E (1984) Immunocytochemical studies of GABAergic neurons in rat basal ganglia and their relations to other neuronal systems. Neurosci Lett 47:233–238.
- Olson L, Seiger A, Fuxe K (1972) Heterogeneity of striatal and limbic dopamine innervation: highly fluorescent islands in developing and adult rats. Brain Res 44:283–288.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereological study using the Cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Parent A, Hazrati L-N (1994) Multiple striatal representation in primate substantia nigra. J Comp Neurol 344:305–320.
- Park MR, Falls WM, Kitai ST (1982) An intracellular HRP study of the rat globus pallidus. I. Responses and light microscopic analysis. J Comp Neurol 211:284–294.
- Parthasarathy HB, Schall JD, Graybiel AM (1992) Distributed but convergent ordering of corticostriatal projections: analysis of the frontal eye field and the supplementary eye field in the macaque monkey. J Neurosci 12:4468–4488.
- Pasik P, Pasik T, Holstein GR, Hámori J (1988) GABAergic elements in the neuronal circuits of the monkey neostriatum: a light and electron microscopic immunocytochemical study. J Comp Neurol 270:157–170.
- Penney GR, Wilson CJ, Kitai ST (1988) Relationship of the axonal and dendritic geometry of spiny projection neurons to the compartmental organization of the neostriatum. J Comp Neurol 269:275–289.
- Plenz D, Kitai ST (1999) A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. Nature 400:677–682.
- Raju DV, Ahern TH, Shah DJ, Wright TM, Standaert DG, Hall RA, Smith Y (2008) Differential synaptic plasticity of the corticostriatal and thalamostriatal systems in an MPTP-treated monkey model of parkinsonism. Eur J Neurosci 27:1647–1658.
- Redgrave P, Marrow L, Dean P (1992) Topographical organization of the nigrotectal projection in rat: evidence for segregated channels. Neuroscience 50:571–595.
- Ribak CE, Vaughn JE, Roberts E (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. J Comp Neurol 187:261–284.

- Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 30:767–777.
- Robeldo P, Féger J (1990) Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res 518:47–54.
- Saper CB (1984) Organization of cerebral cortical afferent systems in the rat. II. Magnocellular basal nucleus. J Comp Neurol 222:313–342.
- Selemon LD, Goldman-Rakic PS (1985) Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. J Neurosci 5:776–794.
- Schell GR, Strick PL (1984) The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. J Neurosci 4:539–560.
- Smith Y, Bolam JP (1989) Neurons of the substantia nigra reticulata receive a dense GABA-containing input from the globus pallidus in the rat. Brain Res 493:160–167.
- Smith Y, Bolam JP (1990) The output neurones and the dopaminergic neurones of the substantia nigra receive a GABA-containing input from the globus pallidus in the rat. J Comp Neurol 296:47–64.
- Smith Y, Bolam JP (1991) Convergence of synaptic inputs from the striatum and the globus pallidus onto identified nigrocollicular cells in the rat: a double anterograde labelling study. Neuroscience 44:45–73.
- Smith Y, Bolam JP, vonKrosigk M (1990) Topographical and synaptic organization of the GABA-containing pallidosubthalamic projection in the rat. Eur J Neurosci 2:500–511.
- Smith Y, Parent A (1986) Neuropeptide Y-immunoreactive neurons in the striatum of the cat and monkey: morphological characteristics, intrinsic organization and co-localization with somatostatin. Brain Res 372:241–252.
- Smith Y, Parent A, Seguela P, Descarries L (1987) Distribution of GABAimmunoreactive neurons in the basal ganglia of the squirrel monkey (*Samiri sciureus*). J Comp Neurol 259:50–61.
- Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86:353–387.
- Somogyi P, Bolam JP, Smith AD (1981) Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscopic study using the Golgi-peroxidase transportdegeneration procedure. J Comp Neurol 195:567–584.
- Staines WA, Atmadja S, Fibiger HC (1981) Demonstration of a pallidostriatal pathway by retrograde transport of HRP-labeled lectin. Brain Res 206:446–450.
- Staines WA, Fibiger HC (1984) Collateral projections of neurons of the rat globus pallidus to the striatum and substantia nigra. Exp Brain Res 56:217–220.
- Steiner H, Gerfen CR (1993) Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci 13:5066–5081.
- Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89:10178–10182.
- Surmeier DJ, Reiner A, Levine MS, Ariano MA (1993) Are neostriatal dopamine receptors co-localized?. Trends Neurosci 16:299–305.
- Surmeier DJ, Song W-J, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
- Takagi H, Somogyi P, Somogyi J, Smith AD (1983) Fine structural studies on a type of somatostatin-immunoreactive neuron and its synaptic connections in the rat neostriatum: a correlated light and electron microscopic study. J Comp Neurol 214:1–16.

- Tennyson VM, Barrett RE, Cohen G, Cote L, Heikkila R, Mytilneou C (1972) The developing neostriatum of the rabbit: correlation of fluorescence histochemistry, electron microscopy, endogenous dopamine levels, and [3H]dopamine uptake. Brain Res 46:251–285.
- van der Kooy D, Fishell G (1987) Neuronal birthdate underlies the development of striatal compartments. Brain Res 401:155–161.
- Vincent SR, Johansson O, Hökfelt T, Skirboll L, Elde RP, Terenius L, Kimmel J, Goldstein M (1983a) NADPH-diaphorase: a selective histochemical marker for striatal neurons containing both somatostatin- and avian pancreatic polypeptide (APP-) like immunoreactivity. J Comp Neurol 217:252–263.
- Vincent SR, Staines WA, Fibiger HC (1983b) Histochemical demonstration of separate populations of somatostatin and cholinergic neurons in the rat striatum. Neurosci Lett 35:111–114.
- Voorn P, Jorritsma-Byham B, Dijk CV, Buijs RM (1986) The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopical study with antibodies against dopamine. J Comp Neurol 251:84–99.
- Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95:532–544.
- Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular inject of horseradish peroxidase. J Comp Neurol 194:599–615.

- Wilson CJ, Phelan KD (1982) Dual topographic representation of neostriatum in the globus pallidus of rats. Brain Res 243:354–359.
- Wilson CJ (1986) Postsynaptic potentials evoked in spiny neostriatal projection neurons by stimulation of ipsilateral and contralateral neocortex. Brain Res 367:201–213.
- Wilson CJ (1987) Morphology and synaptic connections of crossed corticostriatal neurons in the rat. J Comp Neurol 263:567–580.
- Wilson CJ, Chang HT, Kitai ST (1990) Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J Neurosci 10:508–519.
- Xu ZC, Wilson CJ, Emson PC (1989) Restoration of the corticostriatal projection in rat neostriatal grafts: electron microscopic analysis. Neuroscience 29:539–550.
- Yeterian EH, Hoesen GWV (1978) Cortico-striate projections in the rhesus monkey: The organization of certain cortico-caudate connections. Brain Res 139:43–63.
- Yung KK, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 65:709–730.
- Zheng T, Wilson CJ (2002) Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 87:1007–1017.

### Chapter 2

# The Conservative Evolution of the Vertebrate Basal Ganglia

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#### I. INTRODUCTION

At the beginning of the 20th century, the traditional view of telencephalic evolution held that the major parts of the telencephalon had evolved in serial order: the globus pallidus in jawed fish, the neostriatum in amphibians, and a primitive cerebral cortex in reptiles (Edinger et al., 1903; Ariëns-Kappers et al., 1936). Mammals were thought to have elaborated cerebral cortex into neocortex, while birds were thought to have expanded the basal ganglia by evolution of a new territory known as the hyperstriatum. By the latter half of the 20th century, more sophisticated techniques for studying cellular neurochemistry, interregional connectivity, and the genetic control of regional brain development have revised the understanding of telencephalic evolution. It is now evident that the evolution of the basal ganglia has been far more conservative than once thought, with both a striatum and pallidum having been part of the basal ganglia since early in vertebrate evolution. In this chapter, I review modern findings on the organization of the basal ganglia in each extant vertebrate group and discuss the implications for basal ganglia evolution. We will begin with an overview of the traits of the mammalian basal ganglia that are pertinent to its identification in nonmammals (see Box 2.1 on homology).

References

## A. Defining Traits of Basal Ganglia in Mammals

In mammals, the region termed the basal ganglia is a rounded territory at the center of the telencephalon surrounded along its dorsolateral margin by the cerebral cortex. The basal ganglia consist of the striatum and the globus pallidus, with the globus pallidus possessing two subdivisions, which in primates are referred to as the external segment (GPe) and the internal segment (GPi) (see Chapter 1). The homologous pallidal subdivisions in nonprimates have been termed the globus pallidus and the entopeduncular nucleus, but we will refer to the pallidal segments in all mammals as the GPe and GPi. Because the striatum and globus pallidus play a role in motor control, the part of the basal ganglia they make up is called the somatic or dorsal

#### Box 2.1 Brain evolution and the term homology

The current review of basal ganglia evolution presents conclusions about the appearance during evolution of the major feature of basal ganglia, and also about differences in basal ganglia features among the different vertebrate groups. In concluding that a given structure characteristic of mammals is present in another vertebrate group - for example the striatum in lamprey - we imply that the mammalian striatum has been inherited via intervening groups from the common ancestor of lamprey and mammals. As commonly defined in biology, the lamprey striatum would, in the present example, be said to be homologous to the mammalian striatum, since structures in two or more species are called homologous if they are thought to derive from the same antecedent structure in their common ancestor (Campbell and Hodos, 1970). Note that identifying homologous brain structures is in actuality problematic because brain does not fossilize in sufficient detail to trace the natural history of given brain structures. The approach that can be taken involves comparing features of the structures in question in extant species, including embryological origin, location within the adult brain, afferent and efferent connections, and neurochemical phenotype. In the simplest case, if candidate lamprey and mammalian homologues (to use the same sample groups) arise from the same developmental primordium and have similar adult features, and if a similar structure is found in intervening groups, then a convincing case can be made that the common jawless fish ancestor of lamprey and mammal had an equivalent structure. Note that the word homologous is a shorthand that is applied to specify a particular evolutionary relationship between structures in two or more species - it is a shorthand for stating that they have been inherited from the same structure in the common ancestor (nearly always now extinct) of the species in question.

basal ganglia. Nucleus accumbens, olfactory tubercle and ventral pallidum, on the other hand, are collectively termed the limbic or ventral basal ganglia (Heimer et al., 1985). This review will focus on somatic basal ganglia, though for some vertebrate groups the data are inadequate to clearly distinguish the limits of the somatic versus the limbic basal ganglia.

Cerebral cortex is part of the telencephalic sector called the pallium and the basal ganglia are part of the telencephalic sector called the subpallium, both of which can be distinguished by the genes they express during development and the major neurotransmitters they employ. Genes controlling the development of subpallium include Dlx1and Dlx2, and the gene Nkx2.1 specifically is critical for development of globus pallidus (Rubenstein et al., 1994). Genes that control pallial development include Emx1, Emx2, and Tbr1. Projection neurons of cerebral cortex characteristically use glutamate as their neurotransmitter, while those of the subpallium are GABAergic (Swanson and Petrovich, 1998). The mammalian striatum is also distinguished from pallium in being rich in medium-sized GABAergic projection neurons with spiny dendrites that contain substance P (SP) or enkephalin (ENK) (Gerfen, 1992) (see Chapters 1 and 5). The striatum also is identifiable because its neuropil is rich in acetylcholinesterase (AChE) and cholinergic terminals, and dopaminergic terminals from midbrain dopaminergic neurons (Parent, 1986; Graybiel, 1990). The striatum possesses two neurochemically and connectionally distinct compartments, the patch (or striosomal) compartment, making up about 15% of the striatum, and the matrix (Chapter 1). Patch neurons are rich in mu-opiate receptors and poor in the calciumbinding protein calbindin, while matrix neurons are the opposite (Graybiel, 1990; Gerfen, 1992; Mansour et al., 1995). The striatum in mammals also contains several distinct types of local circuit neurons, which make up about 3-10% of striatal neurons: (1) large, aspiny cholinergic neurons (see Chapter 7); (2) medium-sized aspiny neurons co-containing somatostatin (SS), neuropeptide Y (NPY) and nitric oxide synthase (NOS); (3) medium-sized aspiny neurons co-containing GABA, the calcium-binding protein parvalbumin (PARV), and the neurotensin-related hexapeptide LANT6; and (4) medium-sized neurons containing the calcium-binding protein calretinin (CALR) (Reiner et al., 1998a) (see Chapter 8). Most of the interneurons of striatum and cerebral cortex are GABAergic, and they migrate in from the Nkx2.1-expressing zone from which globus pallidus forms (Marin and Rubenstein, 2001).

The globus pallidus (see Chapter 13 and Chapter 14) develops from the proliferative zone just inferior to that from which the striatum forms, and in mammals the globus pallidus neurons retain their position ventromedial and below the striatum. Globus pallidus contains large GABAergic projection neurons that also possess the neurotensinrelated neuropeptide LANT6 (Lys<sup>8</sup>-Asn<sup>9</sup>-neurotensin<sup>8-13</sup>) (Reiner, 1987b; Reiner and Carraway, 1987), and it is rich in SP-immunopositive (SP+) and ENK-immunopositive (ENK+) fibers that terminate on the aspiny dendrites of the pallidal GABAergic projection neurons, and relatively poor in dopaminergic fibers and AChE (Haber and Nauta, 1983; Graybiel, 1990; Reiner and Anderson, 1990). The distinctive appearance of the SP+ and ENK+ terminals on pallidal dendrites led Haber and Nauta (1983) to introduce the term "woolly fiber" to describe that appearance. The GPi projects to thalamic cell groups projecting to

motor cortices and to the intralaminar thalamus (Albin et al., 1989; Alexander and Crutcher, 1990; Gerfen, 1992), while the GPe projects mainly to the subthalamic nucleus (STN), and less so to the GPi, thalamic reticular nucleus, and substantia nigra pars reticulata (SNr) (Reiner et al., 1998a). The STN projects heavily, in return, to the GPi (see Chapter 15).

In addition to the midbrain dopaminergic input (see Chapter 17), the striatum receives major inputs from serotonergic brainstem neurons, the cerebral cortex, and the intralaminar thalamus (Albin et al., 1989; Graybiel, 1990). Striatal projection neurons receiving dopaminergic input use the postsynaptic phosphoprotein dopamine receptor second messenger DARPP-32 as part of the dopaminoceptive intracellular signaling cascade (Anderson and Reiner, 1991b, Hemmings et al., 1995), and DARPP-32, and D1 and D2 dopamine receptors are highly abundant in striatum (see Chapter 6). The cortical input to striatum arises from two types of neurons, deep layer 5 neurons whose main axon projects to brainstem and spinal cord via the pyramidal tract (pyramidal tract-type, or PT-type, neurons) and neurons in layer 3 or upper layer 5 that project to basal ganglia and cortex but not outside the telencephalon (intratelencephalically projecting-type, or IT-type, neurons) (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996; Levesque and Parent, 1998; Reiner et al., 2003) (see Chapters 2 and 19). The inputs from cerebral cortex and perhaps thalamus (see Chapter 22) relay critical information on body position and environmental circumstances, while those from substantia nigra and perhaps raphe relay information related to motivation (Schultz et al., 1993) (see Chapter 31). The striatum integrates this input and facilitates appropriate movement via its projections to globus pallidus and SNr, as detailed in the direct-indirect pathway models of basal ganglia function (Albin et al., 1989; DeLong, 1990).

#### **II. BASAL GANGLIA IN ANAMNIOTES**

#### A. Agnathans

Based on anatomical and molecular data, the two living groups of jawless fish, lamprey and hagfish, are thought to be only distantly related, with lamprey being a sister group of jawed vertebrates (Forey and Janvier, 1993). The lamprey telencephalon is partly evaginated and possesses well-developed lateral ventricles, as typically true in jawed vertebrates, consistent with its taxonomic status as a sister group of jawed vertebrates. By contrast, the telencephalic hemispheres in hagfish are largely devoid of lateral ventricles, and possess a highly laminated outer rind that represents the olfactory pallium (Wicht and Northcutt, 1992). While a central region in the hagfish telencephalon possessing some of the neurochemical traits of the basal ganglia has been identified, the resemblance of this region to basal ganglia is not great (Wicht and Northcutt, 1992, 1993, 1994, 1998), and it may be that hagfish are so divergent from the mainstream of vertebrate evolution that they lack a basal ganglia. We will thus focus on lamprey, therefore, which clearly possess a basal ganglia.

The evaginated part of the telencephalon in lamprey is largely pallial (Murakami and Kuratani, 2008) and in receipt of olfactory bulb input (Northcutt and Puzdrowski, 1988), while a more ventromedial region remains unevaginated (Fig. 2.1). This ventromedial zone lies in the same location as the subpallium of most jawed vertebrates (Pombal et al., 1997a,b), and it is rich in SP+ and GABA+ perikarya with spiny dendrites (Fig. 2.1) (Nozaki and Gorbman, 1986; Nozaki et al., 1984; Pombal et al., 1997b; Melendez-Ferro et al., 2002; Auclair et al., 2004; Robertson et al., 2007). This ventromedial region also contains enkephalinergic neurons and many enkephalinergic fibers (Pombal et al., 1997b), as well as some SS/NPY and cholinergic neurons (Hoheisel et al., 1986; Wright, 1986; Yáñez et al., 1992; Chiba, 1999; Pombal et al., 2001). Moreover, this region expresses lamprey Dlx1/2, while the region dorsal to it expresses lamprey Emx1 and Pax6 (Murakami et al., 2001; Neidert et al., 2001; Myojin et al., 2001). This ventromedial telencephalic region also receives a dopaminergic input from the posterior tubercle of the diencephalon and midbrain, the apparent homologue of at least part of the mammalian substantia nigra (Fig. 2.1) (Pierre et al., 1994; Pombal et al., 1997a). Thus, the ventromedial telencephalic region in question appears to be the lamprey striatum. The dopaminergic input to the lamprey striatum is similar in function to that in mammals, since deletion of this input with MPTP yields hypokinesia and diminished movement initiation (Grillner et al., 2000; Thompson et al., 2008). The SP+ neurons in the lamprey striatum project to the dopaminergic neurons in the posterior tubercle of lamprey (Nozaki and Gorbman, 1986, Pombal et al., 1997a,b). Thus, lamprey possess reciprocal projections from the striatum to substantia nigra pars compacta (SNc) and from SNc to striatum. Lamprey striatum also receives serotonergic fibers arising from the raphe region of the isthmic tegmentum (Pombal et al., 1997a,b). The striatum in lamprey also receives telencephalic input from medial pallium, dorsal pallium, lateral



**FIGURE 2.1** Schematics and images illustrating the location of the basal ganglia (A) and substantia nigra in lamprey (B). Image A shows a line drawing reconstruction of a coronal section through the right lamprey telencephalon depicting the location of SP+ perikarya (dots) (redrawn from Fig. 8I of Auclair et al., 2004). Image B is a line drawing of frontal section through the caudal diencephalon of lamprey (redrawn from Fig. 2E of Pierre et al., 1994), illustrating the location of dopaminergic (i.e., tyrosine hydroxylase-containing) neurons (dots) in the posterior tubercle region. Abbreviations: CP – posterior commissure; dmtn – dorsomedial telencephalic neuropil; DPal – dorsal pallium; FR – fasciculus retroflexus; LPal – lateral pallium; MPal – medial pallium; nFLM – nucleus of the medial longitudinal fasciculus; ON – optic nerve; plv – posterior lateral ventricle; PT – posterior tubercle; Str – striatum; TO – optic tract.

(olfactory) pallium, input from dorsal thalamus (possibly comparable to the intralaminar thalamus), and input from the ventral thalamic nucleus (Polenova and Vesselkin, 1993; Northcutt and Wicht, 1997; Pombal et al., 1997a). A globus pallidus, however, is not evident in lamprey (Nieuwenhuys and Nicholson, 1998; Murakami et al., 2001; Murakami and Kuratani, 2008), and lamprey lack an Nkx2.1-expressing subpallial zone (Ogasawara et al., 2001; Osorio et al., 2005; Murakami and Kuratani, 2008). Nonetheless, SP+ woolly fibers in a field of GABAergic neurons ventrolateral to the striatum within what has been called the lateral pallium distinguish a territory that may be an analogue of mammalian pallidum (Nozaki and Gorbman, 1986; Pombal et al., 1997b). Pombal et al. (1997b) have reported that GABAergic pallidal neurons within the ventral lateral pallium project to the ventral thalamus. Ventral thalamus in lamprey, in turn, projects to reticulospinal neurons of the midbrain and hindbrain (Pombal et al., 1997b). Pombal et al. (1997b) have suggested that this may be the major route by which the basal ganglia in lamprey influences movement. This basal ganglia output circuit is without a clear correspondent in mammals, and there is currently no evidence for an SP+ striato-GPi-motor thalamus-motor cortex circuit or an SP+ striato-SNr-tectal circuit in lamprey. Similarly, there is no definitive evidence for an ENK+

striato-GPe-subthalamic nucleus circuit in lamprey. Thus, the motor output circuitry of lamprey basal ganglia, as far as is known, differs from that in mammals. Robertson et al. (2006) have also raised the possibility that the striatum in lamprey influences movement by means of a projection to a pretectal region having input to the tectum.

#### **B.** Chondroicthyans

Skeletal structure in living and extinct jawed fish and molecular data on living fish support a common origin of the two groups of living jawed fish, the cartilaginous fish (chondroichthyes) and bony fish (osteicthyes), from jawed fish with a bony skeleton (Hedges, 2001; Venkatesh et al., 2001). Cartilaginous fish are the most ancient living representatives of the jawed vertebrates, having appeared very early in the paleontological record (Hedges, 2001; Venkatesh et al., 2001). Cartilaginous fish are divided into two sister subclasses, the deep sea-dwelling, primitive holocephalians (ratfish and chimeras), and the elasmobranchs (the sharks, skates and the rays) (Northcutt, 1977). The elasmobranchs are divided into the sharks and the batoids (skates and the rays), with sharks consisting of the more primitive squalomorph sharks and the typically more advanced galeomorph sharks. Cartilaginous fish possess paired evaginated telencephalic hemispheres (Northcutt, 1977, 1978; Smeets, 1990), and pallial development is controlled by some of the same genes as in mammals (Derobert et al., 2002). In holocephalians, the pallium is meager, and possesses a distinct medial (hippocampal) and lateral (olfactory) sector, but no well-defined dorsal sector. In elasmobranchs, medial, dorsal and lateral pallial sectors are all distinct. In sharks, a ventrally directed enlargement of the dorsal pallium, called the central nucleus, is evident at caudal telencephalic levels, where the central nuclei of the two sides of the brain are fused across the midline. This cell group contains distinct subdivisions, is especially large in galeomorph sharks, and receives sensory (including visual) input from the thalamus (Northcutt, 1977, 1978; Ebbesson, 1980; Luiten, 1981b; Smeets, 1990). A central nucleus is also present in batoids, in which the telencephalic expansion is so extreme in some species as to largely obliterate the lateral ventricles.

Neurochemical and hodological evidence suggests that the pallial-subpallial boundary in elasmobranchs is located at the upper lateral edge of a basal cell plate called the area superficialis basalis (ASB), and that the ASB and the region between it and the lateral ventricle (i.e. the ventrolateral





**FIGURE 2.2** Low power images of frontal sections through the right telencephalic hemisphere of the squalomorph shark, the spiny dogfish (*Squalus acanthias*), immunolabeled for SP (A) and ENK (B) (adapted from Fig. 4A,B of Northcutt et al., 1988), and high power images (C,D) of coronal sections through the right caudal diencephalon of *Squalus acanthias* immunolabeled for tyrosine hydroxylase (TH) and SP (adapted from Fig. 4C,D of Northcutt et al., 1988). Note the enrichment of the ASB and area periventricularis ventrolateralis in SP and ENK, which supports their identification as subpallial. Images C and D illustrate the close proximity of the TH+ dopaminergic neurons of the posterior tubercle and the SP+ fibers to this region from the basal ganglia. Abbreviations: APVL – area periventricularis ventrolateralis; ASB – area superficialis basalis; DP – dorsal pallium; LP – lateral pallium; MP – medial pallium; posterior tubercle – PT.

periventricular area, or APVL, of the telencephalon) represent the basal ganglia (Northcutt et al., 1988; Carrera et al., 2008a; Ferreiro-Galve et al., 2008). This also appears to be true of holocephalians, in which an ASB is evident (though less distinct) and the APVL is relatively enlarged. The APVL, while cell-sparse, appears to be the striatal sector, since it contains SP+ and ENK+ perikarya and their processes, spiny GABAergic neurons (likely to be the SP+ and ENK+ neurons), as well as a dopaminergic innervation from the posterior tubercle and midbrain tegmentum (Fig. 2.2) (Meredith and Smeets, 1987; Northcutt et al., 1988; Rodriguez-Moldes et al., 1993; Smeets, 1990; Stuesse et al., 1990, 1991, 1994; Stuesse and Cruce, 1991, 1992; Carrera et al., 2008a; Ferreiro-Galve et al., 2008; Hofmann and Northcutt, 2008). The striatum in cartilaginous fish also contains many serotonergic fibers that are likely to arise from isthmic tegmental serotonergic neurons (Ritchie et al., 1983; Stuesse et al., 1990, 1991; Stuesse and Cruce, 1991, 1992; Yamanaka et al., 1990; Carrera et al., 2008b). A few presumptive interneurons neurons containing SS and/or NPY are evident in APVL (Vallarino et al., 1988; Chiba et al., 1989; Chiba and Honma, 1992). The ASB is enriched in GABAergic neurons and SP+ and ENK+ woolly fibers of presumed APVL origin, and many neurons in its caudolateral part contain the neurotensin-related hexapeptide LANT6 (Fig. 2.2) (Northcutt et al., 1988; Reiner and Carraway, 1985, 1987; Rodriguez-Moldes et al., 1993; Carrera et al., 2008a). For these reasons, at least part of the ASB appears to be comparable to mammalian globus pallidus, and based on the evidence of SP+ and ENK+ striatal projections to the ASB may contain intermingled GPi-type and GPe-type pallidal neurons. In the absence of direct evidence from double-label studies that the SP+ and ENK+ neurons projecting to ASB are separate populations, the conclusion that cartilaginous fish possess both GPi-type and GPe-type pallidal neurons cannot yet, however, be regarded as firm. Note also that the ASB (i.e. presumed globus pallidus) is laterally migrated from its presumptive ventromedial position, as also the case in reptiles and birds (as addressed later). Since parts of the ASB do receive some olfactory bulb input, it is possible that part of ASB is comparable to the mammalian olfactory tubercle (Ebbesson, 1980; Smeets, 1983; Hofmann and Northcutt, 2008). Given that basal ganglia models in mammals involve the notion that striatal neurons must receive an excitatory input that drives them, it seems likely striatal neurons in cartilaginous fish also receive such inputs. The spiny nature of APVL neurons is consistent with this possibility, since the dendritic spines of these neurons in mammals receive excitatory cortical and thalamic input. The prominent development of the pallium in galeomorph sharks and batoids raises the further possibility that these groups possess a motor pallium and a motor thalamus projecting to it. No firm data on thalamic inputs and only limited data showing pallial input to subpallium are, however, available for cartilaginous fish (Smeets, 1990; Hofmann and Northcutt, 2008).

SP+ and ENK+ neurons (much more so SP+ neurons) of the APVL project to the dopaminergic neurons in the posterior tubercle and rostral midbrain tegmentum (Fig. 2.2), and thus striato-SNc and SNc-striatal projections seems to be a feature of basal ganglia organization in cartilaginous fish (Smeets and Boord, 1985; Northcutt et al., 1988; Smeets, 1990; Stuesse et al., 1990, 1991, 1994; Stuesse and Cruce, 1991, 1992; Molist et al., 1993;

Rodriguez-Moldes et al., 1993; Hofmann and Northcutt, 2008). Pathway tracing studies indicate that the posterior tubercle and tegmentum also project to ipsilateral tectum in sharks, skates and rays (Smeets, 1982), suggesting therefore a striato-SNr-tectal circuit in cartilaginous fish. This projection is of interest, because the tectum has descending projections in sharks to premotor and motor cell groups that closely resemble those of the midbrain roof in mammals (Smeets, 1981). Thus, the basal ganglia in cartilaginous fish could influence movement by accessing tectal neurons with descending premotor and motor projections via an SP+ striato-SNr-tectal route. The projection targets of the globus pallidus are unknown for cartilaginous fish, and it is thus not demonstrated whether either a GPi-motor thalamus projection or a GPe-subthalamic projection comparable to that in mammals is present. The available data, however, suggest that the basal telencephalon in cartilaginous fish may project to the subthalamic region (Smeets, 1990). Given what is known about the interaction of the striato-GPe pathway with GPi neurons via the STN in mammals, it seems possible that cartilaginous fish possess an STN.

#### C. Osteicthyes – Ray-Finned Fish

After the divergence of cartilaginous and basal bony fish, bony fish themselves diverged early in their evolution into the ray-finned fish (actinopterygians) and the lobe-finned fish (sarcopterygians) (Hedges, 2001; Venkatesh et al., 2001). The lobe-finned fish possess paired tubular evaginated telencephala, and extinct members of this group (the rhipidistians) were the ancestors of tetrapods. Lobe-finned fish will be discussed in the next section. Ray-finned fish consist of numerous species, which are grouped into superorders termed the polypterids (alternatively called brachiopterygians), the chondrosteans, the holosteans, and the teleosteans (Noack et al., 1996). Among these, teleosts are the most recently evolved and numerous, and they also possess the most complex telencephala among ray-finned fish, with polypterids being the most primitive (Noack et al., 1996). Whereas in all other vertebrate groups the telencephalon develops as a tubular structure by a process of bilateral evagination of the rostral part of the prosencephalon, the pallial part of the telencephalon in ray-finned fish everts during development (Fig. 2.3) (Northcutt and Braford, 1980; Nieuwenhuys and Meek, 1990a). This places the ependymal cells at the dorsal surface of the telencephalon and reverses the normal medial to lateral topography of pallial areas. Due to complex proliferation



**FIGURE 2.3** Schematic depicting the telencephalic transformations that occur during development with inversion and evagination of the rostral end of the neural tube in nonactinopterygians (C to A) and with eversion in actinopterygians (C to B). Brachiopterygians, such as *Polypterus*, show eversion in its simplest form and their telencephalon resembles that shown in B. Note that the topological relations among telencephalic pallial cell groups are the same in A and B, but the topographic locations of the individual cell groups differ. Consequently, the homologue of mammal medial pallium lies laterally in an everted telencephalon and the homologue of mammalian lateral pallium lies medially. Abbreviations: DP – dorsal pallium; LP – lateral (olfactory cortex); MP – medial pallium; Sp – septum; St – striatum.

and migration of telencephalic cell groups and fusion of the pallium and subpallium during telencephalic development in the more advanced ray-finned fish, the homologies of the different parts of the ray-finned fish telencephalon to that in tetrapods has been difficult to decipher (Wullimann and Mueller, 2004; Northcutt, 2006; Yamamoto et al., 2007). In polypterids, the pallium shows a simple eversion, and study of polypterids provided initial clarification of ray-finned fish telencephalic organization. The medial pallial field receives olfactory bulb input and is therefore comparable to lateral cortex in tetrapods (Braford and Northcutt, 1974). The region just below the polypterid medial pallium (called P-1) is, therefore, the dorsalmost subpallial field in polypterids (termed the dorsal part of the ventral area or Vd), and it was found to contain many SP+ and ENK+ neurons, a neuropil relatively rich in SP+ and ENK+ processes, and a dopaminergic input (Fig. 2.4) (Reiner and Northcutt, 1992). These traits indicate Vd to be striatum in polypterids.

It is now clear that the Vd in chondrosteans, holosteans and teleosts also contains many SP+ and ENK+ perikarya, a neuropil rich in SP+ and ENK+ processes (Vecino et al., 1989, 1992, 1995; Batten et al., 1990; Pinuela and Northcutt, 2007), and terminals arising from dopaminergic



FIGURE 2.4 Photographic images of coronal sections through the midtelencephalon of the brachiopterygian ray-finned fish Polypterus senegalus labeled immunohistochemically for SP (A) and ENK (B) (adapted from Fig. 7 of Reiner and Northcutt, 1992), and (C) high contrast image of a Nissl-stained frontal section through the left telencephalon of a goldfish (image kindly provided by R.G. Northcutt) and a juxtaposed line drawing of the right telencephalon showing the main cell groups present, as identified by Northcutt (2006), illustrating the sources of the main pallial inputs to the ray-finned fish striatum (Vd). The major subdivisions of the telencephalon in A and B are indicated and medial is to the left. Note the presence of intense SP+ and ENK+ fiber labeling in the P3, and in Vd and Vv (the dorsal and ventral parts of ventral area). P1 is the term used in Polypterus to refer to the olfactory pallium, while P3 is used to refer to the hippocampal pallium, which is situated laterally in an everted telencephalon. Regarding C, the forebrain territories in ray-finned fish have commonly been dichotomized into two major zones, a dorsal (now recognized as the pallium) and a ventral (now recognized as the subpallium). Within each of these further subdivisions are recognized as shown. The medial parts of the pallium (Dm) have been suggested to be amygdaloid and the lateral parts of the pallium to be hippocampal/olfactory (Wullimann and Mueller, 20004; Northcutt, 2006). Abbreviations: Dc - central part of dorsal area; Dd - dorsal part of dorsal area; Dl - lateral part of dorsal area; Dm medial part of dorsal area; Vd - dorsal part of ventral area; Vl - lateral part of ventral area; Vv - ventral part of ventral area.

neurons of the posterior tubercle and/or midbrain (Parent and Northcutt, 1982; Hornby et al., 1987; Meek et al., 1989; Roberts et al., 1989; Ekström et al., 1990; Sas et al., 1990; Meek, 1994; Rink and Wullimann, 2001, 2002; Huesa et al., 2006; Pinuela and Northcutt, 2007). Moreover, the Vd neurons are GABAergic (Martinoli et al., 1990; Anglade et al., 1999; Mueller and Wullimann, 2008). Consistent with a dopaminergic input, the Vd is enriched in D1 and D2 dopamine receptors (Kapsimali et al., 2000; Vacher et al., 2003), and consistent with a subpallial identity, the Vd of ray-finned fish expresses *Dlx1/2* (Stock et al., 1996; Zerucha et al., 2000; Alunni et al., 2004; Wullimann and Mueller, 2004; Mueller and Wullimann, 2008). By contrast, the region above Vd expresses such pallial genes as *Emx1*, *Emx2*, *Tbr1*, and *Tbr2* (Wullimann and Mueller, 2004; Mueller and Wullimann, 2008).

The location of ray-finned fish globus pallidus is, however, uncertain. Several lines of evidence suggest that pallidal neurons may be intermingled among striatal neurons within Vd, including the presence of SP+ and ENK+ woolly fibers, LANT6+ neurons and cells expressing a homologue of Nkx2.1 in Vd (Reiner and Northcutt, 1992; Rohr et al., 2001; Alunni et al., 2004; Wullimann and Mueller, 2004). The Nkx2.1-expressing neurons in Vd, however, could also be interneurons (Marin and Rubenstein, 2001). There is evidence to suggest an alternative possibility - namely that globus pallidus in ray-finned fish resides ventral to Vd, in the region called the ventral part of the ventral area (or Vv). The Vv also expresses Nkx2.1 and is in the same topographic position as the medial ganglionic eminence of mammals, which gives rise to the globus pallidus (Alunni et al., 2004). Moreover, the GABAergic neurons of Vv express parvalbumin, as characteristic of mammalian globus pallidus (Crespo et al., 1999), and upper Vv is rich in SP+ and to a lesser extent ENK+ woolly fibers (Fig. 2.4) (Reiner and Northcutt, 1992).

The SP+ and ENK+ neurons of Vd are likely to represent GABAergic striatal projection neurons (Martinoli et al., 1990; Anglade et al., 1999; Mueller and Wullimann, 2008). Immunohistochemical and in situ hybridization histochemical studies in diverse ray-finned fish species show that the Vd also sparsely possesses presumptive interneurons containing SS, NPY and/or NOS (Pontet et al., 1989; Sas and Maler, 1991; Pickavance et al., 1992; Reiner and Northcutt, 1992; Arevalo et al., 1995; Brüning et al., 1995; Chiba, 1997, 2005; Chiba and Honma, 1994; Cerda-Revereter et al., 2000a,b; Trabucchi et al., 2002; Canosa et al., 2004; Gaikwad et al., 2004; Adrio et al., 2008). The striatum (Vd) in the five ray-finned fish species studied by immunohistochemistry contains cholinergic fibers and terminals but is devoid of cholinergic neurons (Ekström, 1987; Brantley and Bass, 1988; Adrio et al., 2000; Perez et al., 2000; Mueller et al., 2004). More lateral and ventral subpallial regions in ray-finned fish, however, contain many cholinergic neurons, which may be the source of the cholinergic input to the striatum, as well as to the pallium (Ekström, 1987; Perez et al., 2000). A few LANT6+ (presumptive PARV+) interneurons have been demonstrated in polypterid Vd (Reiner and Northcutt, 1992), as have been GABAergic PARV+ neurons in tench Vd (Crespo et al., 1999). Scattered calretinergic neurons have also been observed in Vd and Vv (Diaz-Regueira and Anadon, 2000; Castro et al., 2003, 2006), which might correspond to the calretinergic interneurons in mammalian striatum.

While ray-finned fish have projections resembling the dopaminergic nigrostriatal system of mammals, dopaminergic neurons appear to be more varied in their abundance and distribution within the midbrain and posterior tubercle (Ekström et al., 1990; Reiner and Northcutt, 1992; Meek, 1994; Rink and Wullimann, 2004). Nonetheless, destruction of dopaminergic neurons in the posterior tubercle in rayfinned fish results in "parkinsonian" symptoms (i.e. slowed movements or bradykinesia), as in mammals (Barbeau et al., 1986; Pollard et al., 1992). Isthmic serotonergic neurons are present in the dorsal raphe in polypterids and teleosts, and appear to be the source of the many serotonergic fibers in the striatum in ray-finned fish (Kah and Chambolle, 1983; Parent et al., 1984; Meek and Joosten, 1989; Johnston et al., 1990; Corio et al., 1991; Reiner and Northcutt, 1992; Rink and Wullimann, 2004; Huesa et al., 2006; Pinuela and Northcutt, 2007).

In some teleost species, such as rainbow trout, zebrafish, dwarf gourami, goldfish and Sabastiscus mormorata, Vd receives input from the medial, central, posterior and/or lateral pallia (Fig. 2.4) (Murakami et al., 1983; Yamamoto and Ito, 2000; Folgueira et al., 2004; Rink and Wullimann, 2004; Northcutt, 2006), regions that have been suggested to resemble amygdaloid and medial cortical territories in mammals (Wullimann and Mueller, 2004). Unresolved is whether similar pallial inputs to Vd exist in chondrosteans, holosteans and polypterids, and whether they were present in basal ray-finned fish, or are newly evolved in teleosts. One study on the chondrostean Acipenser baeri (Huesa et al., 2006) found pallial inputs to the Vd to be meager. This finding, the undeveloped nature of the polypterid pallium, and the absence of a definitive homologue of teleost medial pallium (the putative amygdaloid correspondent) in the polypterid pallium raise the possibility that at least some of the pallial projections to the teleost striatum evolved with the emergence of basal teleosts. Several dorsal thalamic nuclei project to Vd in ray-finned fish (Braford

and Northcutt, 1978; Northcutt, 1981b; Echtheler, 1984; Ito et al., 1986; Nieuwenhuys and Meek, 1990a; Striedter, 1991; Wong, 1997; Folgueira et al., 2004; Rink and Wullimann, 2004; Yamamoto and Ito, 2005; Northcutt, 2006; Huesa et al., 2006). Since these dorsal thalamic nuclei are polysensory and project to pallium as well (Northcutt, 2006), they resemble mammalian intralaminar thalamic nuclei. It seems likely that the pallial and thalamic inputs provide the environmental information and excitatory drive to the striatum needed for its role in motor control.

The SP+ and ENK+ neurons of Vd project to the dopaminergic neurons of the posterior tubercle and rostral midbrain tegmentum (Vecino et al., 1989, 1992, 1995; Batten et al., 1990; Reiner and Northcutt, 1992; Folgueira et al., 2004; Rink and Wullimann, 2004; Pinuela and Northcutt, 2007). Thus, reciprocal striato-SNc and SNcstriatal projections are characteristic of ray-finned fish. A striato-SNr-tectal circuit may also be present in rayfinned fish, since a subdivision of the posterior tubercle and tegmental targets of the basal telencephalic input appears to project to ipsilateral tectum (Luiten, 1981a; Fiebig et al., 1983; Schlussman et al., 1990). Given that the rayfinned fish tectum gives rise to descending motor pathways very similar to those in mammals (Ebbesson and Vanegas, 1976; Northcutt and Butler, 1980), control of descending tectal output via a projection to a tegmental SNr-like region could be a way by which the ray-finned fish basal ganglia influences movement. The existence of both SP+ and ENK+ striatal neurons differing in their distribution and abundance (and thus unlikely to be coextensive) and a possible pallidal region in Vv in ray-finned fish suggests that they may also possess both striato-GPi and striato-GPe circuits. Consistent with a pallidal identity, Vv receives striatal (Vd) input and projects to the thalamus and the posterior tubercle (Rink and Wullimann, 2004; Folgueira et al., 2004; Wong, 1997). Although the latter projection clearly resembles the GPe-nigral pathway of mammals, it is uncertain if the Vv projection to thalamus resembles that of mammalian GPi to either intralaminar or motor thalamus. It is also not clear whether pallido-subthalamic pathways comparable to those in mammals are present in ray-finned fish. The available data suggest that Vv in ray-finned fish projects to the subthalamic region, but the precise projection target is uncertain (Airhart et al., 1988; Wong, 1997; Folgueira et al., 2004; Rink and Wullimann, 2004). Given what is known about the interaction of the striato-GPe pathway with GPi neurons via the STN in mammals, it seems possible that ray-finned fish possess

an STN. Nonetheless, neither an STN, a striato-GPi nor a striato-GPe circuit has been demonstrated experimentally in ray-finned fish, and it may be that the striatal ENK+ neurons mediate their effect on behavior via input to GPe neurons that directly project to GPi or SNr neurons in ray-finned fish.

#### D. Osteicthyes – Lobe-Finned Fish

Lungfish and coelacanths (the crossopterygian *Latimeria*) are the living members of the sarcopterygia (Rosen et al., 1981; Hedges, 2001). The morphology of the basal telencephalon in coelacanths resembles that in lungfish, but the pallium in coelacanths is greatly enlarged (Nieuwenhuys and Meek, 1990b). In the African lungfish, the only lobefinned fish whose basal ganglia has been studied (Reiner and Northcutt, 1987; Vallarino et al., 1995, 1997a,b, 1998; Trabucchi et al., 1999, 2008), the ventrolateral telencephalon contains both a striatum and a globus pallidus, by neurochemical and hodological criteria (Fig. 2.5). The more medial part of this field contains SP+ and ENK+ neurons and receives a dopaminergic innervation (Reiner and Northcutt, 1987; Vallarino et al., 1998), thus identifying it as the striatum. The neuropil of the African lungfish striatum is, in fact, remarkably rich in SP+ fibers and processes (presumably arising from the striatal SP+ neurons) and is thereby sharply demarcated from the overlying pallium (Fig. 2.5). By position and olfactory bulb input, the more ventromedial part of the SP+ and ENK+ striatal field may correspond to olfactory tubercle (Nieuwenhuys and Meek, 1990b). It is likely the SP+ and ENK+ neurons of the striatal sector also contain GABA as well, since the lungfish subpallium is enriched in neurons expressing GAD65 (Trabucchi et al., 2008). The dopaminergic input seems likely to arise from midbrain dopaminergic neurons (Fig. 2.5) (Reiner and Northcutt, 1987; Nieuwenhuys and Meek, 1990b). Additionally, isthmic tegmental serotonergic neurons are present and the striatum contains many serotonergic fibers in lungfish, suggesting an input from the isthmic serotonergic neurons (Reiner and Northcutt, 1987). Presumptive interneurons containing somatostatin and/or NPY are present in African lungfish striatum (Vallarino et al., 1995, 1997; Trabucchi et al., 1999), as are a few LANT6+ neurons (i.e. presumptive PARV+ interneurons) (Reiner and Northcutt, 1987).

The dorsal and lateral sectors of the telencephalon in lungfish are pallial territories receiving olfactory bulb input (Reiner and Northcutt, 1987), and they appear to project



FIGURE 2.5 Images illustrating telencephalic organization in the African lungfish Protopterus annectens (A,B) (adapted from Figs 2D, 4C from Reiner and Northcutt, 1987). Images A and B show coronal views of sections through the right telencephalon of the African lungfish stained for Nissl substance (A) and immunolabeled for SP (B). Note that the subpallium is clearly defined by intense SP immunolabeling. Images C and D show high power views of coronal sections through the right midbrain tegmentum (C,D) (adapted from Fig. 8A,B of Reiner and Northcutt, 1992) of the African lungfish Protopterus annectens immunolabeled for tyrosine hydroxylase (TH) (C) and SP (D). Note that the presence of dopaminergic (TH+) neurons along the ventricle and an SP-rich neuropil juxtaposed to them defines the location of the substantia nigra (SN) of lungfish. The scale bar in A provides the magnification for A and B. The scale bar in C provides the magnification for C and D. Abbreviations: DP dorsal pallium; LP - lateral pallium; MP - medial pallium; Sp - septum; Str - striatum.

to striatum, based on studies of normal fiber staining (Nieuwenhuys and Meek, 1990b). The dorsal thalamus also appears to project to the striatum in lungfish, based again on studies of normal fiber staining (Nieuwenhuys and Meek, 1990b). Given that basal ganglia models in mammals involve the notion that striatal neurons must receive an excitatory input that drives them, it seems likely they do receive at least thalamic input. The striatum sends a return projection from SP+ striatal neurons to the tegmental dopaminergic neurons that appear to project to striatum (Reiner and Northcutt, 1987). Evolutionary inferences from the data for cartilaginous fish and the available data

for sarcopterygians make it likely that sarcopterygians possess an SP+ striato-SNr pathway that mediates its effect on movement via input to the tectum. The more caudolateral part of the lungfish subpallium contains SP+ and ENK+ fibers resembling mammalian pallidal "woolly fibers" and numerous LANT6+ neurons (Reiner and Northcutt, 1987; Vallarino et al., 1998). This part of the telencephalon in lungfish thus appears to be the globus pallidus. In this pallidal field, the SP+ and ENK+ inputs overlap, indicating that sarcopterygians are likely to possess both a striato-GPi and a striato-GPe circuit. Given what is known about the interaction of the striato-GPe pathway with GPi neurons via the STN in mammals, it seems possible that sarcopterygians possess an STN. Nonetheless, one has not been demonstrated, and it may be that the striato-GPe circuit in sarcopterygians mediates its effect on behavior via one of the other demonstrated outputs of the GPe in mammals, namely directly to the GPi, thalamic reticular nucleus or SNr. The role of the apparent SP+ striato-GPi circuit in sarcopterygians is also uncertain, given the meager cortical development in this group. It may be that this circuit has output to intralaminar thalamus and that a striato-SNr circuit is the main motor route.

#### E. Amphibians

Living amphibians are divided into three groups, the limbless amphibaenids, the urodeles (newts and salamanders), and the anurans (frogs and toads) (Northcutt, 1981a; Jenkins and Walsh, 1993). The telencephalon in all three groups is tubular in shape and its neurons largely occupy a periventricular position, especially along the lateral wall (Fig. 2.6A–C). The early amphibians do not appear to have differed greatly from their sarcopterygian predecessors in general body shape, locomotor skills, or their freshwater habitat (Ahlberg and Milner, 1994; Ahlberg, 1995). Little is known about the connections or neurochemistry of the telencephalon in amphibaenids, but it is likely the traits resemble those in urodeles and anurans. The telencephalon of urodeles is less well differentiated than that in frogs, but telencephalic features appear largely similar.

The ventrolateral amphibian telencephalon contains both a striatum and a globus pallidus, by neurochemical, hodological, and molecular developmental criteria (Figs 2.6, 2.7). The striatal sector contains numerous SP+ and ENK+ neurons in anurans and urodeles, and evident cholinergic and dopaminergic innervation (Inagaki et al., 1981a; Taban and Cathieni, 1983; Merchenthaler et al., 1989; González



FIGURE 2.6 Images of frog telencephalon illustrating the cytoarchitectonic organization of the telencephalon as seen in a Nissl-stained section (A) (image provided courtesy of L. Medina), the enrichment of the basal ganglia in GAD-expressing neurons as seen by in situ hybridization histochemistry (B) (image provided courtesy of L. Medina), the enrichment of the basal ganglia in Dlx-expressing neurons as seen by in situ hybridization histochemistry (C) (image provided courtesy of L. Medina), the enrichment of the basal ganglia in SP as seen by immunolabeling (D), the enrichment of the basal ganglia in ENK as seen by immunolabeling (E), and the enrichment of the striatum in dopaminergic fibers and terminals as seen by immunolabeling for tyrosine hydroxylase (TH) (F) (image provided courtesy of A. González). All images are of the right telencephalic hemisphere at a level containing the striatum. Image A shows the thin lateral wall of the telencephalon and the largely periventricular position of its neurons. Images B and C show that the subpallium, including the septum (Sp) and striatum (Str), are defined by their conspicuous enrichment in GAD-expressing and Dlx-expressing neurons. The GADexpressing and Dlx-expressing neurons in the pallial territories represent interneurons that have migrated in from the subpallium. The scale bar in C provides magnification for A-F. Abbreviations: DP - dorsal pallium; LP lateral pallium; MP - medial pallium; Sp - septum; Str - striatum.

and Smeets, 1991, 1994a,b; Marin et al., 1997b, 1998a,b). The identity of the striatum in amphibians is further confirmed by the extensive expression of GAD and amphibian Dlx1/2 (Fig. 2.6) (Papalopulu and Kintner, 1993; Bachy et al., 2002; Brox et al., 2003b). While double-label studies have not firmly established the SP+ and ENK+ striatal neurons to be separate populations, they do differ in their distributions and are thus likely to be in large part separate. The pallidum is recognizable in the caudal ventrolateral telencephalon, based on its enrichment in SP+ and ENK+ fibers woolly fibers (Fig. 2.7) (Marin et al., 1997b,e,



FIGURE 2.7 Images illustrating the cytoarchitectonic organization of the caudal frog telencephalon as seen in a Nissl-stained section (A) (image provided courtesy of L. Medina), the enrichment of the caudal frog striatum and globus pallidus in SP as seen by immunolabeling (B), the enrichment of the caudal frog striatum and globus pallidus in GADexpressing neurons as seen by in situ hybridization histochemistry (C) (image provided courtesy of L. Medina). All images are of the right telencephalic hemisphere at a level containing the striatum. Image A shows the thin lateral wall of the telencephalon and the largely periventricular position of its neurons. Note the conspicuous bed nucleus of the stria terminalis (BNST) and globus pallidus (GP). Image B shows that the BNST possesses an SP-rich neuropil, and the striatum and pallidum are circumscribed by their own SP-rich neuropil. Image C shows that the subpallium, including the septum (Sp), BNST, GP and striatum (Str), are defined by their prominent enrichment in GAD-expressing neurons. The BNST, GP and striatum at this level all consist of separate clusters of GAD+ neurons. Images D and E show transverse sections through the pretectal level of the right side of the frog brain, illustrating a Nissl-stained section showing the cytoarchitecture of the posterior tubercle and juxtacommissural pretectal nucleus (D) (image provided courtesy of L. Medina), and the enrichment of the lateral part of the posterior tubercle and the juxtacommissural pretectal nucleus in GAD-expressing neurons as seen by in situ hybridization histochemistry (E) (image provided courtesy of L. Medina). Images D and E show the location of the GABAergic neurons of the PT (the dopaminergic neurons of PT lying more medially), and the GABAergic nature of the neurons of the juxtacommissural pretectal nucleus (JC). Scale bar in A provides magnification for A-C. The scale bar in E provides the magnification for D and E. Abbreviations: BNST - bed nucleus of the stria terminalis; DP - dorsal pallium; GP - globus pallidus; JC - juxtacommissural pretectal nucleus; LP - lateral pallium; MP medial pallium; PT - posterior tubercle; Sp - septum; Str - striatum.

1998a). The SP+ and ENK+ striatal inputs overlap in the pallidal field, implying that GPi-type and GPe-type neurons are intermingled. Moreover, this region contains large GABAergic neurons, and expresses Nkx2.1 (Fig. 2.7)

(Franzoni and Morino, 1989; Naujoks-Manteuffel et al., 1994; Marin et al., 1998a,b; Brox et al., 2003a,b; González et al., 2002a,b).

Cholinergic neurons are reportedly absent from the striatum in the urodele Pleurodeles waltl and the anuran Xenopus laevis, and scarce in the striatum in the anuran Rana perezi (Marin et al., 1997d). Presumptive interneurons co-containing SS and NOS are abundant, although NPY has not been found in amphibian striatal neurons (Inagaki et al., 1981b; Danger et al., 1985; Perroteau et al., 1988; Lázár et al., 1993; Tuinhof et al., 1994; Munoz et al., 1996; Tostivint et al., 1996; Marin et al., 1998a; González et al., 2003; Huynh and Boyd, 2007; Lopez et al., 2007). A ventral striatal region in amphibians that has been identified as nucleus accumbens is, however, especially rich in SS+ neurons (Inagaki et al., 1981b; Marin et al., 1998a). The cholinergic and SS/NOS interneurons of amphibian striatum express Nkx2.1 and are thus likely to be derived from the same proliferative zone that gives rise to globus pallidus, as true in mammals (González et al., 2002b; Moreno et al., 2008). Scattered CALR+ neurons are present in amphibian striatum, but it is uncertain if they correspond to the CALR interneurons of mammalian striatum (Brox et al., 2003a). Finally, large GABAergic neurons scattered in amphibian striatum may correspond to the PARV+/GABA+ interneurons of mammalian striatum (Brox et al., 2003a,b).

The dopaminergic input to the striatum in amphibians arises from the posterior tubercle and midbrain (Dube et al., 1990; González and Smeets, 1991, 1994a,b; Corio et al., 1992; González et al., 1994; Marin et al., 1997a,b, 1998a,b; Hoke et al., 2007). The dopaminergic neurons of the posterior tubercle and midbrain in amphibians span the tegmental part of so-called prosomeres 1 and 2, plus the midbrain segment, thus showing a great resemblance in location to the A9-A10 dopaminergic neurons of mammals (Puelles and Medina, 1994). While this input is more meager than in mammals, loss of dopaminergic input to striatum in amphibians results in bradykinesia (Barbeau et al., 1986). Isthmic tegmental serotonergic neurons are also present in amphibians, and appear to give rise to a serotonergic input to striatum that is more meager than in mammals (Wilczynski and Northcutt, 1983a; Parent et al., 1984; Dube et al., 1990; Corio et al., 1992; Clairambault et al., 1994; Marin et al., 1997a).

Among pallial regions, only the medial pallium appears to have significant input to amphibian striatum (Marin et al., 1997a; Westhoff and Roth, 2002). The major excitatory input to the striatum instead arises from the thalamus (Kicliter and Northcutt, 1975; Kicliter, 1979; Wilczynski and Northcutt, 1983a; Wicht and Himstedt, 1986; Dube et al., 1990; Marin et al., 1997a, 1998b; Westhoff and Roth, 2002; Laberge and Roth, 2007; Roth et al., 2007). In amphibians, the dorsal thalamic nuclei projecting to the striatum receive visual and auditory input via the midbrain roof, thus making them resemble specific sensory dorsal thalamic nuclei in mammals (Wilczynski and Northcutt, 1983a; Butler, 1994a). Unlike such nuclei in mammals, these nuclei in amphibians have only a slight projection to the pallium at best but a major projection to the striatum, although an additional small cell group in the anterior thalamus does have a restricted projection to the medial part of the rostral dorsal pallium (Kicliter and Northcutt, 1975; Wilczynski and Northcutt, 1983a; Wicht and Himstedt, 1986; Sassoe et al., 1991; Laberge and Roth, 2007; Roth et al., 2007; Laberge et al., 2008; Mangiamele and Burmeister, 2007). The fact that thalamic sensory nuclei have only a limited projection to the dorsal or lateral pallium in frogs may explain why the pallium does not project to the amphibian striatum. Since the pallium does not receive extensive thalamic sensory input, it would not be a useful source of such information for the striatum. Instead, the thalamus appears to directly provide this information in amphibians. It is uncertain, however, if the dorsal thalamic nuclei projecting to striatum in amphibians are comparable to mammalian intralaminar nuclei (which also receive sensory input) or to the specific sensory nuclei of mammalian dorsal thalamus. Dorsal thalamic specific sensory nuclei possess some collateral projections to the striatum in mammals, which could be a remnant of the relatively larger projection these thalamic nuclei putatively had to striatum in ancestral amphibians (Butler, 1994a).

In amphibians, SP+ neurons and ENK+ neurons of the striatum project to the posterior tubercle of the diencephalo-mesencephalic junction, as well as to the midbrain tegmentum (Fig. 2.7D,E) (Wilczynski and Northcutt, 1983b; Marin et al., 1997c,f; Marin et al., 1998a; Roth et al., 2004; Laberge and Roth, 2005). Thus, amphibians have a projection resembling the striato-SNc circuit of mammals, as well as one resembling the dopaminergic SNc-striatal pathway of mammals. A striato-SNr projection also seems to be present in amphibians (Wilczynski and Northcutt, 1977, 1983b; Marin et al., 1997a,b,f, 1998a; Roth et al., 2004). The tegmental part of the striatal target area, in particular, appears comparable to mammalian SNr, because it receives a prominent input from the striatum (Kokoros and Northcutt, 1977; Vesselkin et al., 1980; Wilczynski and Northcutt, 1983b; Marin et al., 1997c,f, 1998a; Roth et al., 2004), projects to the tectum (Wilczynski and Northcutt, 1977; Finkenstädt et al., 1983; Rettig, 1988; Zittlau et al., 1988; Masino and Grobstein, 1990; Marin et al., 1997f), does not contain dopaminergic neurons (Marin et al., 1997b, 1998a), and does contain GABAergic neurons (Franzoni and Morino, 1989; Naujoks-Manteuffel et al., 1994). The tectum in amphibians has descending projections to premotor cell groups of the brainstem similar to those in mammals (Rubinson, 1968; Ten Donkelaar et al, 1981b; Lázár et al., 1983; Wilczynski and Northcutt, 1983b; Masino and Grobstein, 1989a,b; Smeets, 1991), and the evidence for an involvement of the descending tectal projections in visuomotor behavior in amphibians is strong, particularly in frogs (Ingle, 1983; Ewert, 1984; Masino and Grobstein, 1989a,b, 1990). Thus, the basal ganglia is likely to influence motor functions via the tectum in amphibians (Marin et al., 1997f). The projections of globus pallidus in amphibians include the dorsal thalamus (Wilczynski and Northcutt, 1983b; Marin et al., 1997c; Endepols et al., 2004). The functional role of this presumptive SP+ striato-GPi circuit in amphibians is uncertain, given the meager cortical development in this group. It may be that this circuit has output to intralaminar thalamus and that the striato-SNr circuit is the main motor route in amphibians. In amphibians, the pallidal region also has a projection to a subthalamic region that projects back to the basal ganglia (Wilczynski and Northcutt, 1983b; Marin et al., 1997a,c). This may correspond to the mammalian GPe-subthalamic nucleus-GPi pathway. Given what is known about the interaction of the striato-GPe pathway with GPi neurons via the STN in mammals, and about basal telencephalic projections to the ventral thalamus in amphibians, it seems possible that amphibians possess an STN. Nonetheless, one has not been demonstrated unequivocally, and it may be that in amphibians the striato-GPe circuit mediates its effect on behavior via one of the other demonstrated outputs of the GPe in mammals, namely the GPi, thalamic reticular nucleus or SNr.

A pathway from the basal ganglia to the tectum via a pretectal cell group containing ENK+ and GABA+ neurons has been demonstrated in amphibians (Wilczynski and Northcutt, 1977, 1983b; Finkenstädt et al., 1983; Naujoks-Manteuffel and Manteuffel, 1986; Rettig, 1988; Franzoni and Morino, 1989; Merchenthaler et al., 1989; Naujoks-Manteuffel et al., 1994; Schmidt et al., 1989; Lázár et al., 1990; Masino and Grobstein, 1990; Marin et al., 1997b,f; Brox et al., 2003b). This cell group has been termed the juxtacommissural pretectal nucleus (Fig. 2.7) (Brox et al., 2003b). The projection to the juxtacommissural pretectal nucleus appears to arise from both striatal and pallidal neurons (Marin et al., 1997b). Since the pretectal target neurons of this basal ganglia projection are GABAergic, this circuit functionally resembles the striato-SNr-tectal pathway. The apparent absence of basal ganglia output pathways to the pallium via the dorsal thalamus in amphibians indicates that the basal ganglia of amphibians may exert its major influence on movement via its outputs to the midbrain tectum via the tegmentum (the SNr) and the pretectum (the juxtacommissural nucleus). As will be noted in the review of the basal ganglia in amniotes, reptiles and birds but not mammals possess a clear homologue of the juxtacommissural pretectal cell group.

#### F. Summary and Overview of Basal Ganglia Evolution in Anamniotes

Despite limitations in the available data, a number of conclusions can be reached about the evolutionary history of the anamniote basal ganglia from the preceding overview. First, at least the striatal part of the basal ganglia was present in the vertebrate telencephalon as of the appearance of jawless fish (lamprey), and both a striatum and pallidum were present in the early jawed fish. In the earliest jawed vertebrates, the striatum must have been characterized by SP+ and ENK+ spiny projections neurons, and somatostatinergic and large GABAergic (parvalbuminergic) interneurons. The inconsistent presence of cholinergic neurons in striatum among anamniotes suggests that these were not added as a core constituent of the striatum during amniote evolution. As early as jawless fish, the striatum was characterized by a dopaminergic input from midbrain and a predominantly SP+ GABAergic projection back to the midbrain dopaminergic neurons, though the dopaminergic input appears to have been relatively modest and the abundance of tegmental dopaminergic neurons relatively low. By the appearance of jawed vertebrates, SP+ striatal projections to midbrain GABAergic neurons projecting to tectum had emerged as a major motor output pathway of the basal ganglia. Additionally, striato-GPe and striato-GPi pathways of yet undetermined functional significance must also have been present in early jawed vertebrates, although double-label studies are desirable to firmly establish the separateness of SP+ and ENK+ striatal neurons in the diverse anamniote groups. The main excitatory input to striatum in anamniotes with a simple tubular telencephalon appears likely to have been the thalamus, although pallial inputs to the striatum may have evolved independently in cartilaginous and ray-finned fish groups with expanded telencephalic pallia. By amphibians (if not before), the basal ganglia had evolved a projection to a pretectal cell group as one of its major motor output pathways. The basal ganglia of lobe-finned fish is unlikely to have been notably different in its organization or neurochemistry from that of the earliest tetrapods (amphibians) that evolved from lobe-finned fish, although it is unknown if lobe-finned fish possess a basal ganglia output to the pretectum comparable to that seen in amphibians. One peculiarity of the basal ganglia (and the entire telencephalon in some cases) in nontetrapods that has not been noted until here is that it contains scattered dopaminergic neurons that may contribute to the dopaminergic innervation of the striatum (e.g. Northcutt et al., 1988; Steusse et al., 1994; Rink and Wullimann, 2002). It is uncertain if these dopaminergic neurons are homologous to the infrequent dopaminergic neurons of the striatum in mammals (Betarbet et al., 1997). Finally, further studies are needed to define the limits of the limbic and somatic basal ganglia, especially in nontetrapods.

#### **III. BASAL GANGLIA IN AMNIOTES**

#### A. Reptiles

Mammals share with reptiles a common origin from early stem amniote descendants of amphibians (Gauthier et al., 1988; Lee, 1993, 1997), but mammals and reptiles evolved divergently beginning very early in amniote evolution. Paleontological data indicate that living reptiles are a paraphyletic group whose members represent separate branchings from the diapsid reptilian stock that itself diverged from the earliest reptiles (the other divergent branch being the anapsids). The diapsid lineages include turtles (which arose early in reptile evolution and were once considered anapsids), lepidosaurians (lizards, snakes, and Sphenodon) and archosaurians (which include crocodilians and dinosaurs) (Gauthier et al., 1988; Lee, 1993, 1997; deBraga and Rieppel, 1997; Li et al., 2008). Sphenodon is an early and relatively unchanged offshoot of the lepidosaurian lineage (with lizards and snakes arising later). Thus, both turtles and Sphenodon diverged early during reptile evolution, and retain primitive reptilian features, including their telencephalon. Despite, the somewhat differing telencephalic morphologies of the various groups of living reptiles, all evidence suggests that the main features of basal ganglia organization are the same.

During development, the telencephalic hemispheres of reptiles possess the same tubular evaginated structure as seen in adult amphibians. As embryonic development progresses, however, the pallial and subpallial sectors of the telencephalon come to be large and cell rich (Källén, 1951). The basal ganglia of reptiles thus reflects its inheritance from amphibians, but shows differences that stem from the expansion of the thalamus, basal ganglia and pallium characteristic of reptiles (traits that were presumably inherited from stem amniotes). For example, as in amphibians, the ventrolateral telencephalon of reptiles contains both a striatum and a globus pallidus. As in amphibians, the striatum is characterized by SP+ and ENK+ GABAergic neurons with spiny dendrites (Fig. 2.8) (Reiner, 1987a; Russchen et al., 1987; Bennis et al., 1991, 1994; Reiner et al., 1998a), and expression of Dlx1/2 (Smith-Fernandez et al., 1998). The neurons of the striatum occupy much of the ventrolateral telencephalic wall (i.e. are not mainly periventricular as in amphibians), and the ventrolateral telencephalic wall is much thicker than in amphibians. Enrichment in cholinesterase, dopaminergic terminals, DARPP32, and dopamine receptors also characterize the striatum of reptiles, more so than in amphibians (Parent, 1986; Richfield et al., 1987; Bissoli and Contestabile, 1988; Smeets, 1994; Smeets et al., 2003). The globus pallidus possesses large GABAergic neurons with aspiny dendrites that also contain LANT6, and in many cases parvalbumin, which are enveloped in a dense mat of overlapping SP+ and ENK+ woolly fibers (Fig. 2.8) (implying that GPi-type and GPe-type neurons are intermingled) (Reiner, 1987a; Reiner and Carraway, 1987; Bennis et al., 1991, 1994; Reiner et al., 1998a; Guirado et al., 1999b). A limbic striatum and limbic pallidum also are present in reptiles (Russchen et al., 1987; Smeets et al., 1986, 1987; Smeets, 1988; Smeets and Medina, 1995; Guirado et al., 1999a; Martinez-Marcos et al., 2005).

Three types of striatal interneurons are present in reptiles. These make up approximately 5–10% of striatal neurons and they include: (1) sparse, large, aspiny cholinergic neurons (Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993); (2) medium-sized aspiny neurons co-containing somatostatin (SS) and neuropeptide Y (NPY), and perhaps also NOS (Bear and Ebner, 1983; Reiner and Oliver, 1987; Medina et al., 1992; Brüning et al., 1994; Bennis et al., 2001); and (3) mediumsized aspiny neurons co-containing GABA, parvalbumin, and LANT6 (Reiner and Carraway, 1987; Guirado et al., 1999b).

The reptilian striatum receives a more substantial dopaminergic input from the midbrain than is the case in any



FIGURE 2.8 Images of turtle telencephalon illustrating the enrichment of the basal ganglia in SP (A), and of the basal ganglia in ENK (B). Images A and B show frontal sections through the basal ganglia of the right telencephalic hemisphere that had been immunostained for SP (A) or enkephalin (ENK) (B). Note the intense SP+ and ENK+ immunoreactivity in the ventrolateral telencephalon that defines the region of the striatum and globus pallidus, and distinguishes the subpallium from the dorsal ventricular ridge (DVR) of the overlying pallium. Images C-F present views of turtle midbrain brain showing the dopaminergic neurons of substantia nigra in turtle (C) as visualized by tyrosine hydroxylase (TH) immunolabeling, the striatal input to the substantia nigra in turtle (D) immunolabeled for SP, the neurons of the substantia nigra pars reticulata of turtle (E) immunolabeled for LANT6 (adapted from Fig. 11D of Reiner and Carraway, 1987), and neurons of the turtle dorsal nucleus of the posterior commissure (F) immunolabeled for ENK (adapted from Fig. 12C of Reiner, 1987a). Scale bar in B provides the magnification for A. The scale bar in D shows the magnification for C-E. Abbreviations: DVR dorsal ventricular ridge; LP - lateral (olfactory) pallium; Sp - septum.

anamniote (Parent, 1986; Smeets, 1994). The major source of dopaminergic input to the dorsal striatum is the SNc, which in more primitive reptiles such as turtles closely resembles its apparent mammalian homologue in its shape and tegmental location (Fig. 2.8). Within the striatum in reptiles, the dopaminergic input typically ends on the spine necks of spiny striatal projection neurons and these neurons contain DARPP32 (Henselmans and Wouterlood, 1994; Smeets et al., 2001, 2003). As in mammals, dopaminergic effects on striatum are mediated by D1 and D2 dopamine receptors, with dopamine agonists inducing hyperkinesia and dopamine antagonists yielding hypokinesia (Andersen et al., 1975; Richfield et al., 1987; Greenberg et al., 1989; Clark et al., 2000). The striatum in reptiles also receives a prominent input from serotonergic neurons in the isthmic brainstem (Parent, 1986; Brauth and Kitt, 1980; Ueda et al., 1983; Wolters et al., 1985; Smeets and Steinbusch, 1988; Bennis et al., 1990).

The pallium in reptiles consists of two major sectors, a laminated cortex overlying the lateral ventricle and a large subcortical sector protruding into the lateral ventricle called the dorsal ventricular ridge (Reiner, 1993). The cortex possesses medial (hippocampal), dorsal (neocortex-like) and lateral (olfactory) sectors, and the anterior DVR is neocortex-like in its connectivity and function, but in most reptiles predominantly nuclear in its cytoarchitecture. Excitatory input to the striatum in reptiles that appears to be glutamatergic arises from visual, somatosensory and auditory parts of the cortex and DVR (Voneida and Sligar, 1979; Bruce and Butler, 1984a,b; González et al., 1990; Ulinski, 1990; Butler, 1994a,b; Fowler et al., 1999). Moreover, the cell-type specific glutamate receptors employed by striatal projection neurons and interneurons to respond to this excitatory input are very similar to that in mammals (Fowler et al., 1999). The simplest interpretation of the commonality in corticostriatal organization between reptiles and mammals is that a major elaboration of corticostriatal circuitry occurred with the evolutionary appearance of stem amniotes. In reptiles, several dorsomedial thalamic nuclei also project to the striatum (Ten Donkelaar and De Boer-van-Huizan, 1981a; González et al., 1990; Butler, 1994a). These nuclei too are glutamatergic, and additionally have widespread projections to the dorsal cortex and DVR (Hall and Ebner, 1970; Balaban and Ulinski, 1981; Bruce and Butler, 1984a,b; González et al., 1990; Fowler et al., 1999; Zhu et al., 2005). The connectivity and neurochemistry of these dorsomedial thalamic nuclei supports the interpretation that they correspond to the mammalian intralaminar thalamic nuclei (Hoogland, 1981; Künzle and Woodson, 1982; Reiner, 1987a; Medina et al., 1993; Butler, 1994a; Reiner et al., 1998a).

The striatum in reptiles has a prominent projection to the tegmentum, and this projection mainly arises from SP+ GABAergic neurons of medial striatum (Fig. 2.8) (Hoogland, 1977; Reiner et al., 1980; Ten Donkelaar and De Boer-van-Huizan, 1981a; Brauth et al., 1983; Wolters et al., 1986; Russchen and Jonker, 1988; Anderson and Reiner, 1990a). The striato-tegmental projection ends on tegmental dopaminergic neurons and a region of lateral tegmentum that appears homologous to mammalian SNr, since it is rich in GABAergic neurons co-containing PARV and/or LANT6 (Fig. 2.8) (Wolters et al., 1986; Reiner and Carraway, 1987; Bennis et al., 1991). A lesser ENK+ projection from the striatum, which is somewhat more prominent in snakes and some lizards, also ends on nigral dopaminergic neurons (Brauth, 1984; Reiner, 1987a; Smeets, 1991; Smeets and Medina, 1995). In reptiles, the neurons of SNr are located lateral or dorsolateral to the majority of the dopaminergic neurons of SNc (Fig. 2.8). Nonetheless, SNr neurons in reptiles do receive SP+ striatal input, as well as ENK+ striatal input, the latter of which varies in its extent among reptiles (Brauth, 1984; Reiner, 1987a; Medina and Smeets, 1991). The reptilian SNr, like that in mammals, projects to the midbrain tectum, which itself projects to premotor and motor centers in the brainstem and spinal cord (Reiner et al., 1980; Reiner, 1994; Medina and Smeets, 1991). In mammals, SNr neurons have high firing rates and are metabolically active, as seems likely to be true of reptilian SNr neurons as well (Baker-Cohen, 1968). By their SP-rich striatal input, their high firing rates, their neurotransmitter content, their PARV content and their output to tectal premotor neurons, the SNr neurons in reptiles and mammals resemble GPi neurons. Thus, a basal ganglia route to the midbrain tectum via the SNr may be involved in promoting movement in both reptiles and mammals.

The globus pallidus in reptiles receives prominent SP+ and ENK+ GABAergic input from lateral striatum (Fig. 2.8) (Hoogland, 1977; Anderson and Reiner, 1990a; Russchen and Jonker, 1988), with SP+ input presumably ending on GPi-type neurons and ENK+ input ending on GPe-type neurons. There is currently suggestive evidence for a GPi-intralaminar thalamus projection and a GPimotor thalamus projection in reptiles (Hoogland, 1977; Voneida and Sligar, 1979; Brauth and Kitt, 1980; Reiner et al., 1980; Brauth, 1988; Russchen and Jonker, 1988). The dorsomedial and dorsolateral nuclei of reptiles, the GPi intralaminar target, resemble the mammalian intralaminar nuclei by topography, histochemistry, and outputs to striatum and pallium, and a pallidal projection to them has been demonstrated in lizards (Russchen and Jonker, 1988). In reptiles, the globus pallidus also projects to a specific nucleus located in the subthalamus, called the anterior entopeduncular nucleus (ENa) (Brauth and Kitt,

1980; Brauth, 1988; Russchen and Jonker, 1988). The ENa contains glutamatergic neurons (Fowler et al., 1999) and projects back to the pallidum, as well as to the SNr (Brauth and Kitt, 1980), thus making it resemble the mammalian STN. Additionally, pallidal neurons in reptiles and mammals possess glutamate receptors, consistent with a glutamatergic input from the subthalamic nucleus and its reptilian homologue, respectively (Götz et al., 1997; Fowler et al., 1999). The topographic location of ENa within the diencephalon also supports its identification as the subthalamic nucleus. It is possible that the putative ENK+ striato-GPe-subthalamic pathway is involved in suppression of unwanted movements in reptiles, as it is in mammals (Albin et al., 1989; DeLong, 1990). As in mammals, inhibition of ENK+ striato-GPe neurons with D2-acting dopamine receptor agonists is functionally akin to ablation of the STN, leading to disinhibition of unwanted movements (Andersen et al., 1975). Thus, the major parts of the direct-indirect circuit plan seen in mammals appear to be present in reptiles, implying their existence in the stem amniote common ancestors of reptiles and mammals.

In turtles, crocodiles and lacertid lizards, the basal ganglia has a major projection to a pretectal cell group whose neurons co-contain ENK, GABA and LANT6 (Fig. 2.8) (Brauth, 1984; Reiner, 1987a; Reiner and Carraway, 1987; Medina and Smeets, 1991). This cell group has been called the dorsal nucleus of the posterior commissure (nDCP) in reptiles (Reiner et al., 1980; Brauth, 1988; Medina and Smeets, 1991), and it projects to the deeper layers of the midbrain tectum, including those that project to premotor cell groups of the hindbrain (Reiner, 1987a, 1994; Medina and Smeets, 1991). This circuit provides an additional route by which the basal ganglia may promote movement in reptiles (Reiner et al., 1980; Medina and Smeets, 1991). These pretectal neurons are functionally akin to pallidal neurons since they are GABAergic, metabolically active and thus likely to have a high firing rate (Baker-Cohen, 1968; Reiner et al., 1984a; Bennis et al., 1991). This pretectal cell group appears homologous to the juxtacommissural pretectal nucleus of amphibians. Nonetheless, a well-defined basal ganglia-pretecto-tectal pathway seems to be absent in some lizard groups and in snakes (Russchen and Jonker, 1988; Medina and Smeets, 1991). Both the striatum and pallidum are the likely source of the basal ganglia projection to the reptilian pretectum (Smeets et al., 2003). Thus, the basal ganglia in reptiles has its major output to motor areas via a striatal projection to pallidal-type neurons of the pretectum (nDCP) and the tegmentum (SNr), which are likely to affect movements by input to tectal neurons with descending projections to brainstem premotor cell groups (Reiner et al., 1980; Medina and Smeets, 1991). Activation of the striatal input is likely to produce inhibition of their target pretectal and SNr GABAergic neurons, resulting in disinhibition of tectal neurons that have descending projections to hindbrain motor-related centers, as occurs in mammalian basal ganglia circuitry (Chevalier et al., 1985).

#### **B.** Birds

Birds evolved from archosaurian reptiles, of which crocodilians are the only other living representative (Chiappe, 1995). Unsurprisingly, therefore, the basal ganglia in birds highly resembles that in reptiles, with the main differences stemming from the yet further telencephalic enlargement in birds. Other than the evolution of a distinct basal ganglia cell group in songbirds devoted to vocal control (as discussed later in this section), basal ganglia anatomy is relatively similar among the diverse living avian groups. The ventrolateral telencephalon in birds contains both a striatum and a globus pallidus, identifiable by neurochemical, hodological, and developmental molecular criteria. The striatum is distinguished by its enrichment in SP+ and ENK+ neurons and their processes (Fig. 2.9) (Reiner et al., 1983, 1984b; Molnar et al., 1994; Aste et al., 1995; Dubbeldam et al., 1999; den Boer-Visser and Dubbeldam, 2002), with the identity of the striatum further confirmed by its high expression of GAD (Veenman and Reiner, 1994; Sun et al., 2005), and Dlx1/2 (Smith-Fernandez et al., 1998; Puelles et al., 2000). The SP+ and ENK+ neurons possess spiny dendrites and together make up the vast majority of striatal neurons (Tömböl et al., 1988; Karle et al., 1992, 1994). The avian striatum also is identifiable by its richness in dopaminergic terminals arising from midbrain (Fig. 2.9), and in cholinergic terminals arising from intrinsic cholinergic interneurons (Medina and Reiner, 1994; Reiner et al., 1994; Karle et al., 1996; Dubbeldam et al., 1999). Concomitant with the abundance of these types of terminals, the striatum is enriched in cholinesterase, muscarinic receptors, D1 and D2 type dopamine receptors, and DARPP32 (Karten and Dubbeldam, 1973; Parent, 1986; Richfield et al., 1987; Dietl and Palacios, 1988; Dietl et al., 1988; Wächtler and Ebinger, 1989; Moons et al., 1994; Ball et al., 1995; Kohler et al., 1995; Wynne and Güntürkün, 1995; Reiner et al., 1998b; Dubbeldam et al., 1999; Durstewitz et al., 1998, 1999; Sun and Reiner 2000; Absil et al., 2001). Although striatal neurons with the characteristics of patch neurons (mu-opiate receptor-rich and



**FIGURE 2.9** Images of avian telencephalon illustrating the enrichment of the basal ganglia in SP (A), ENK (B), and in dopaminergic fibers and terminals (C). Images A and B show frontal sections through the basal ganglia of the right telencephalic hemisphere of pigeon that had been immunostained for SP (A) or enkephalin (ENK) (B) (adapted from Figs 2C,D of Reiner et al., 1998b). Note the intense SP+ and ENK+ immunoreactivity in the ventrolateral telencephalon that defines the region of the striatum and globus pallidus, and distinguishes the subpallium from the dorsal ventricular ridge (DVR) of the overlying pallium. Image C shows a frontal section through the basal ganglia of the right telencephalic hemisphere of pigeon that had been immunostained for dopamine (adapted from Fig. 2B of Reiner et al., 1998b). Note the conspicuous enrichment of the striatum in dopaminergic fibers and terminals. Images D–F of pigeon brain illustrate tyrosine hydroxylase-containing neurons (D) in substantia nigra pars compacta (adapted from Fig. 4F of Reiner et al., 2000b), SP+ fibers (E) in the substantia nigra, and ENK+ immunolabeling (D) of the neurons of the lateral spiriform nucleus of the pretectum. Note that the neurons of SpL are rich in ENK. The scale bar in A provides the magnification for A–C. Scale bar in D provides magnification for D–F. Abbreviations: DVR – dorsal ventricular ridge; Meso – mesopallium; Nido – nidopallium; SNc – substantia nigra pars compacta; SNr – substantia nigra pars reticulata; Sp – septum; SpL – lateral spiriform nucleus; TeO – optic tectum.

calbindin-poor) may be present in avian striatum, they are not organized into recognizable islands resembling the striatal patches in mammals (Karten and Dubbeldam, 1973; Brauth, 1988; Reiner et al., 1989; Csillag et al., 1990; Braun et al., 1991; Dubbeldam et al., 1999). The globus pallidus is identifiable by its large GABAergic neurons that also contain LANT6 and PARV, by its enrichment in SP+ and ENK+ woolly fibers (Fig. 2.9), and by its expression of Nkx2.1 (Karten and Dubbeldam, 1973; Hall et al., 1984; Reiner and Carraway, 1987; Reiner and Anderson, 1993; Veenman and Reiner, 1994; Puelles et al., 2000; Laverghetta et al., 2006). Limbic striatal cell groups such as the nucleus accumbens and olfactory tubercle, and the limbic pallidal cell group the ventral pallidum have also been identified in birds (Reiner et al., 1983, 1984b; Aste et al., 1998; Reiner et al., 2004b; Yamamoto and Reiner, 2005; Balint and Csillag, 2007).

In birds, about 10% of striatal neurons are interneurons and three major populations have been identified: (1) large, aspiny cholinergic neurons (Medina and Reiner, 1994; Cookson et al., 1996); (2) medium-sized aspiny neurons co-containing SS, NPY, and/or NOS (Anderson and Reiner, 1990b; Brüning, 1993; Cozzi et al., 1997; Atoji et al., 2001); and (3) medium-sized aspiny neurons cocontaining GABA, parvalbumin, and LANT6 (Reiner and Carraway, 1987; Reiner and Anderson, 1993; Reiner and Anderson, 1993; Laverghetta et al., 2006). The striatum in birds also contains neurons that localize CALR, but these neurons also contain PARV and thus no not correspond to the unique CALR interneurons in mammals (Laverghetta et al., 2006).

The major source of dopaminergic input to the dorsal striatum is the SNc, and this input accounts for the dense dopaminergic innervation observed in the striatum of birds (Fig. 2.9) (Parent et al., 1984; Bons and Oliver, 1986; Kitt and Brauth, 1986a,b; Bailhache and Balthazart, 1993; Moons et al., 1994; Reiner et al., 1994; Wynne and Güntürkün, 1995; Metzger et al., 1996; Durstewitz et al., 1998, 1999; Absil et al., 2001; Roberts et al., 2001). The dopaminergic input typically ends on the spine necks of striatal projection neurons in birds, and it targets both SP+ and ENK+ striatal neurons (Karle et al., 1992, 1994). As in mammals, dopaminergic effects in striatum are mediated by D1 and D2 class dopamine receptors, with dopamine agonists inducing hyperkinesia and dopamine antagonists yielding hypokinesia (Gargiulo et al., 1981; Barrett, 1983; Nistico et al., 1983; Akbas et al., 1984; Richfield et al., 1987; Dietl and Palacios, 1988; Ball et al., 1995; Demchyshyn et al., 1995; Durstewitz et al., 1999; Sun et al., 2000; Ding and Perkel, 2002). The striatum in birds also receives a prominent input from serotonergic neurons of the isthmic brainstem (Bons and Oliver, 1986; Brauth et al., 1978; Dubé and Parent, 1981; Yamada et al., 1984; Yamada and Sano, 1985; Cozzi et al., 1991; Kitt and Brauth, 1986a).

The pallium in birds contains the same subdivisions as in reptiles but is much expanded, with the dorsal part of the pallium enlarged into a medially situated sensorimotor region called the Wulst (comparable to reptile dorsal cortex), and the DVR (consisting of the mesopallium and nidopallium) juxtaposed to a basocaudal motor output area called the arcopallium. The Wulst, DVR and arcopallium give rise to a massive excitatory input to the avian striatum (Zeier and Karten, 1971; Karten and Dubbeldam, 1973; Karten et al., 1973; Nottebohm et al., 1976; Brauth et al., 1978; Dubbeldam and Visser, 1987; Wild, 1987b, 1989; Wild et al., 1993; Veenman et al., 1995b; Veenman and Reiner, 1996; Davies et al., 1997; Dubbeldam et al., 1997). As in mammals, the avian "corticostriatal" projection utilizes the excitatory neurotransmitter glutamate (Veenman and Reiner, 1996; Csillag et al., 1997; Laverghetta et al., 2006; Adam and Csillag, 2006). The communication between pallium and striatum in birds is mediated by the same two "corticostriatal" cell types as in mammals, the pyramidal tract type (located in the hyperpallium apicale subdivision of the Wulst and the arcopallium) and the intratelencephalically projecting type (located mainly in the nidopallium) (Cowan and Wilson, 1994; Veenman et al., 1995b; Reiner et al., 2001, 2003), and by the same cell-type specific postsynaptic glutamate receptors (Reiner, 2002; Wada et al., 2004; Laverghetta et al., 2006).

In birds, several dorsomedial thalamic nuclei project to the striatum (Kitt and Brauth, 1982; Miceli and Repérant, 1985; Bons and Oliver, 1986; Wild, 1987a; Veenman et al., 1995a, 1997; Montagnese et al., 2003). This input too is excitatory and ends on the spine heads of striatal projection neurons (Veenman et al., 1995a). These striatal afferent nuclei include the dorsomedial and dorsolateral thalamic nuclei, as well as the dorsointermediate posterior nucleus, which also have widespread projections to pallium. The neurochemistry and connectivity of these nuclei supports the interpretation that they are homologues of the mammalian intralaminar thalamic nuclei (Zeier and Karten, 1971; Kitt and Brauth, 1986a,b; Miceli et al., 1987; Wild, 1987b, 1989; Korzeniewska and Güntürkün, 1990; Arends and Zeigler, 1991; Medina and Reiner, 1994, 1997; Veenman et al., 1997; Bruce et al., 2002). As in mammals, part of the intralaminar thalamus, namely the dorsointermediate posterior nucleus,

receives pallidal input (Medina and Reiner, 1997; Veenman et al., 1997).

A major, topographically ordered projection from medial striatum to tegmental dopaminergic neurons arises from GABAergic spiny neurons that contain SP (Fig. 2.9), and a lesser one from GABAergic spiny neurons that contain ENK (Kitt and Brauth, 1981; Reiner et al., 1983; Hall et al., 1984; Anderson and Reiner, 1990a, 1991a; Veenman and Reiner, 1994; Mezey and Csillag, 2002, 2003). The terminals of both SP+ and ENK+ neurons make synaptic contact with tegmental dopaminergic neurons in birds (Anderson et al., 1991; Medina et al., 1995). An SNr whose neurons are rich in GABA, PARV and LANT6, and in receipt of synaptic input from SP+ terminals of striatal origin, is also present in birds (Reiner and Carraway, 1987; Anderson and Reiner, 1991a; Anderson et al., 1991; Reiner and Anderson, 1993; Veenman and Reiner, 1994). The SNr of birds also receives input from pallidal neurons receiving input from ENK/GABA striatal neurons, as it does in mammals (Reiner et al., 1998a). The avian SNr projects to the midbrain tectum (Reiner et al., 1983; Hunt and Brecha, 1984; Veenman and Reiner, 1994), and also to the avian motor thalamus, as it does in mammals (Medina et al., 1997). By their SP-rich striatal input, high firing rates, high metabolic activity (Baker-Cohen, 1968; Streit et al., 1980), neurotransmitter content, PARV content and output to thalamic ventral tier and tectal premotor neurons, the SNr neurons in birds resemble GPi neurons. Thus, the basal ganglia route to the avian tectum via the SNr may promote movement. As in reptiles, the neurons of SNr in birds are located dorsolateral to the dopaminergic neurons of SNc (Fig. 2.9) (Reiner and Carraway, 1987; Reiner and Anderson, 1993; Veenman and Reiner, 1994). Moreover, the avian SNr does not coincide with the most dense part of the tegmental field of SP+ fibers, which mostly overlaps the dopaminergic tegmental neurons in pigeons (Fig. 2.9).

SP+ and ENK+ neurons of lateral striatum in birds project to separate populations of large aspiny GABAergic neurons in globus pallidus (Fig. 2.9) (Anderson and Reiner, 1990a; Jiao et al., 2000; Person et al., 2008). The pallidal neurons receiving SP+ input correspond to GPitype neurons and those receiving ENK+ input correspond to GPe-type neurons. GPi-type neurons in birds give rise to a projection resembling that of GPi to motor thalamus in mammals to a small thalamic region termed the ventrointermediate area (VIA) in birds (Fig. 2.10) (Karten and Dubbeldam, 1973; Kitt and Brauth, 1982; Medina et al., 1997; Person et al., 2008). The VIA projects to the rostral





FIGURE 2.10 Circuit diagram showing the functional organization of the avian basal ganglia. The pluses and minuses indicate whether projections are excitatory (+) or inhibitory (-). Striatal projection neuron types, which all use GABA as their primary neurotransmitter, are distinguished by their characteristic neuropeptide. The terminology used for basal ganglia subdivisions in birds is now similar to that in mammals, per the recent revision in avian brain nomenclature (Reiner et al., 2004b). As in mammals, the striatal and pallidal output circuitry in birds is organized into direct SP+ striatal outputs to pallidal neurons promoting movement and ENK+ striatal outputs to pallidal neurons inhibiting unwanted movement. The pallidal neurons of the indirect pathway project directly to the targets of the SP+ striatal neurons (i.e. GPi, SNr and SpL), as well indirectly to these same targets via the subthalamic nucleus. In mammals, SP+ neurons target two sets of pallidal-type neurons (GPi and SNr), while in birds three are targeted (GPi, SNr and SpL). It is not yet certain, however, if GPe-like neurons in the avian globus pallidus (where they are intermingled with GPi-type neurons) have a projection to GPi-type neurons of globus pallidus. Such a projection is present in mammals. Abbreviations: ENK - enkephalin; GLUT - glutamate; GPe - external segment of globus pallidus; GPi - internal segment of globus pallidus; SNr - substantia nigra pars reticulata; SP - substance P; SpL - nucleus spiriformis lateralis; STN - subthalamic nucleus.

part of the Wulst, which is comparable by location and connectivity to mammalian primary somatosensory/somatomotor cortex (Wild, 1987b, 1989, 1992; Korzeniewska and Güntürkün, 1990; Medina and Reiner, 2000). It is unknown if this circuit is better developed in birds with a larger Wulst than in pigeons, such as owls. In addition to the input from the globus pallidus, the VIA also receives

input from the SNr and the lateral and internal cerebellar nuclei (Medina et al., 1997). The dorsointermediate posterior (DIP) nucleus of the avian intralaminar thalamus also receives a projection from the pallidum, presumably from GPi type neurons (Karten and Dubbeldam, 1973; Medina and Reiner, 1997). The DIP projects to striatum and to the pallium, and it seems specifically comparable to the mammalian parafascicular nucleus in its connectivity, neurochemistry and location (Kitt and Brauth, 1981, 1982; Wild, 1987a; Butler, 1994a; Veenman et al., 1995a,b; Veenman et al., 1997; Bruce et al., 2002). GPe-type pallidal neurons in birds project to the subthalamic nucleus, which contains glutamatergic neurons and projects back to globus pallidus and SNr (Fig. 2.10) (Karten and Dubbeldam, 1973; Medina and Reiner, 1997; Jiao et al., 2000; Person et al., 2008). Consistent with a glutamatergic input, pallidal neurons in birds possess glutamate receptors (Jiao et al., 2000; Wada et al., 2004; Laverghetta et al., 2006). As in mammals, STN destruction causes hyperkinesia (Jiao et al., 2000). Thus, as in mammals, an ENK+ striato-GPe-STN circuit is present that is involved in suppressing unwanted movements.

In birds, lateral striatal SP+ neurons and GPe-type neurons of the globus pallidus have a projection to a pretectal cell group known as the lateral spiriform nucleus (SpL), whose neurons co-contain ENK, GABA, parvalbumin and LANT6 (Figs. 2.9, 2.10) (Reiner et al., 1982a,b; Reiner and Carraway, 1987; Domenici et al., 1988; Reiner and Anderson, 1993; Veenman and Reiner, 1994). The avian SpL is homologous to the juxtacommissural pretectal nucleus of amphibians and the dorsal nucleus of the posterior commissure of reptiles. Since SpL projects to deep tectal layers that project to premotor cell groups of the hindbrain (Reiner and Karten, 1982; Reiner et al., 1982a,b; Hellmann et al., 2004), it provides a route by which the basal ganglia may influence movement. The neurons of SpL are functionally akin to pallidal neurons in that they are highly metabolically active, have a high firing rate and contain parvalbumin (Streit et al., 1980; Reiner et al., 1984a; Reiner and Anderson, 1993). Since they also resemble pallidal neurons in that they use GABA as their primary neurotransmitter (Veenman and Reiner, 1994; Sun et al., 2005), it seems likely that they exert a tonic inhibitory influence on their target tectal neurons, which are released from this inhibition by activation of the SP+ neurons projecting to SpL. The GPe-type pallidal input to SpL seems akin to GPe input to GABAergic neurons of SNr. Thus, the avian basal ganglia has its major output to motor areas via a projection to the pretectal nucleus SpL and the tegmental nucleus SNr, which affect movements by input to tectal neurons with descending motor projections (Fig. 2.10).

The striatal output pathways to pretectum, tegmentum and thalamus are all "direct pathway" type outputs that are likely to facilitate behavior (Fig. 2.10). As noted above, birds also possess a subthalamic nucleus and "indirect pathway" circuit, as well (Jiao et al., 2000). The organization of the avian basal ganglia into direct and indirect striatal output circuits closely resembling those in mammals, and the presence of both SP+ and ENK+ striatal outputs in reptiles as well, suggests that the direct-indirect pathway plan of basal ganglia functional organization was present already in stem amniotes. The striato-GPi-thalamic circuit to motor cortex is, however, less well developed in birds than in mammals. Instead, basal ganglia influences on motor function appear to be mediated more so by outputs to the tectum in birds. While birds and mammals do share one of these circuits to the tectum (the striato-SNr-tectal circuit), the other (the striato-SpL-tectal circuit) is not readily discernible in mammals. Additionally, the STN of the striato-GPe-STN-GPi circuit is better developed in mammals than in birds. The well-developed striato-GPe pathway in birds may mainly play its role via a direct projection of GPe neurons to GPi neurons. This may explain why GPe and GPi neurons remain intermingled in birds and in nonmammals in general. Although a GPe to GPi projection is present in mammals, the expansion of the STN-GPi circuit in mammals may explain the segregation of GPe and GPi neurons in mammals, with GPi neurons moving closer to STN.

## Basal Ganglia Specializations in Vocalizing Birds

Two telencephalic circuits underlie song learning and production in oscine songbirds (Doupe and Kuhl, 1999). These circuits (Fig. 2.11) are present in males in those species in which only males sing and in both males and females in those species in which both sing (Langmore, 1998). One of these circuits, called the anterior forebrain pathway, is routed through a specialized part of the basal ganglia called area X (Fig. 2.11). This circuit is an uncrossed multisynaptic pathway connecting a higher order pallial song control area called HVC (or the "higher vocal center") to the robust nucleus of the arcopallium (RA), whose full components are: HVC – Area X of the basal ganglia – the dorsolateral medial nucleus of the thalamus (DLM) – the lateral magnocellular nucleus of the anterior nidopallium (LMAN) – RA (Nottebohm et al., 1976; Bottjer, 1997; Nordeen and



**FIGURE 2.11** Images illustrating (A) the two song control circuits in male songbirds (adapted from Fig. 1 of Reiner et al., 2004a), and (B) immunolabeling for SP in area X of male zebra finch (adapted from Fig. 4A of Reiner et al., 2004a). Image B shows SP+ immunolabeling of Area X at a low magnification, as seen in a transverse section through the right telencephalon of a male zebra finch. Note that Area X stands out in SP-immunolabeled material. Abbreviations: NIDO – nidopallium.

Nordeen, 1997). The other song control circuit involves a direct projection from HVC to ipsilateral RA and is necessary for song production. By contrast, the X–DLM–LMAN– RA pathway is necessary for initial song learning (Bottjer et al., 1984; Scharff and Nottebohm, 1991), and rehearsal-based adjustments in song during adulthood (Doupe and Solis, 1997; Jarvis et al., 1998; Mello and Ribeiro, 1998; Brainard and Doupe, 2000). Area X is located in the medial striatum, and, consistent with its location, predominantly appears to contain small GABAergic spiny neurons with the anatomical, neurochemical (e.g. many contain SP), and physiological characteristics of spiny striatal projection neurons (Fig. 2.11) (Grisham and Arnold, 1994; Farries and Perkel, 2002). Area X also contains a less numerous

population of larger GABAergic neurons that are the neurons of area X that project to the thalamic nucleus DLM (Luo and Perkel, 1999a,b). These neurons possess pallidal neurochemistry, physiology and dendritic morphology (Fig. 2.11) (Luo and Perkel, 1999a; Farries and Perkel, 2002; Reiner et al., 2004a). The HVC input to Area X seems likely to terminate extensively on spiny striatal-type neurons, with these spiny neurons then having their major efferent projection to pallidal-type neurons that project to DLM.

Parrots and hummingbirds also use learned vocalizations to communicate with conspecifics (Farabaugh et al., 1994; Durand et al., 1997; Pepperberg, 1999; Hile et al., 2000; Jarvis and Mello, 2000; Jarvis et al., 2000). While the vocalizing abilities of parrots, hummingbirds and song birds are likely to have evolved independently, a similar pair of neural circuits subserves vocalization in all three (Ball, 1994; Striedter, 1994; Durand et al., 1997; Jarvis et al., 2000). The circuits in hummingbirds and parrots resembling the anterior forebrain pathway of songbirds include a portion of the medial striatum called the magnocellular nucleus of medial striatum (MStm) in parrots and the vocal nucleus of the anterior paleostriatum (VAP) in hummingbirds (Ball, 1994; Striedter, 1994; Durand et al., 1997; Jarvis and Mello, 2000; Jarvis et al., 2000). While some details of the neurochemistry and circuitry of parrot MStm are known (Ball, 1994; Roberts et al., 2002), it is unknown if parrot MStm and hummingbird VAP possess the unique mix of striatal and pallidal neuron types found in area X.

## C. Overview of Basal Ganglia Evolution in Amniotes

The anamniote to stem amniote transition must have been accompanied by several changes in basal ganglia organization. First, cell abundance in the telencephalon must have undergone a great increase, as reflected in an expansion of both the pallium and basal ganglia. As part of this expansion, both thalamo-pallial sensory pathways and palliostriatal pathways must have become prominent. Moreover, the tegmental dopaminergic field and its input to striatum also must have become more substantial. Two evolutionary scenarios seem possible to account for this. In one scenario, the amphibians giving rise to stem amniotes are presumed to have only possessed intralaminar thalamic nuclei, and specific sensory nuclei are presumed to have evolved during the amphibian – reptile transition. In the second scenario, the amphibians giving rise to stem amniotes are presumed to have possessed specific sensory nuclei in the dorsal thalamus, but during the amphibian - reptile transition the major projection of these neurons shifted from the striatum to the cortex. As in amphibians, the major output to motor areas in stem amniotes must have been via a projection to the pretectum and SNr, with both affecting movements by an input to tectal neurons with descending projections. In stem amniotes, basal ganglia circuitry must have been organized into direct and indirect pathways. In mammals, however, the direct pathway to the pretectum became de-emphasized and the direct pathway to GPi emphasized instead. Thus, in mammals, the basal ganglia effects its role in motor control via pallial motor areas more so than in reptiles and birds. Additionally, mammalian striatum is compartmentalized into interlaced zones called striosomes and a much larger sector in which the striosomes are embedded called the matrix. These two striatal sectors, which differ in their connectivity with cortex and midbrain, consist of neuronal populations that may be more uniformly interspersed in striatum in birds and reptiles. Finally, the globus pallidus is laterally migrated in reptiles and birds. Since it is medially situated in lobe-finned fish and amphibians, it is likely that the medial position of globus pallidus as seen in mammals is the stem amniote condition, and that the lateral migration of pallidal neurons evolved in the reptile-bird lineage.

Several major changes in telencephalic morphology appear to have occurred in the evolution of the mammalian basal ganglia from the stem amniote condition inferred from commonalities among extant reptiles, birds and mammals. First, from a simple, poorly laminated state, the pallium evolved into multilayered neocortex (Karten, 1969; Reiner et al., 2005). By a process of extensive lateroventral migration from the pallium where it meets the subpallium, the neocortex comes to surround the basal ganglia in mammals (Alvarez-Bolado and Swanson, 1996). As a consequence, the basal ganglia occupies a more central position in the telencephalon than it does in nonmammals. The embryonic development of the basal ganglia versus the neocortex, however, reflects its ancestral basal position. Unlike in nonmammals in which GPi and GPe neurons are intermingled, in mammals these pallidal neuronal populations occupy separate sectors, in either of two arrangements. In primates, the GPe and GPi are segregated, but contiguous and distinguishable by their differential neurochemistry, namely an enrichment in terminals arising from ENK+ striatal neurons in GPe and an enrichment in terminals arising from SP+ striatal neurons in GPi. All other mammalian groups show a pallidal arrangement that must therefore be the primitive pattern for mammals. In this primitive pallidal pattern, the GPi and GPe are noncontiguous, with the GPi enveloped by the internal capsule and seemingly dragged medially to a thalamic proximity. The gap between the two pallidal segments is so large, that they have customarily been identified by different names than in primates, the globus pallidus instead of the GPe and the entopeduncular nucleus instead of the GPi.

#### IV. BASAL GANGLIA EVOLUTION – OUTDATED CONCEPTS AND TERMINOLOGY

The preceding overview of basal ganglia evolution in vertebrates reveals that both the striatum and pallidum are ancient structures, both likely present in the jawed fish ancestral to modern jawed vertebrates. Thus, the notion that the pallidum (i.e., the so-called paleostriatum) evolved first and is older than the striatum (i.e., the so-called neostriatum) is incorrect. Because the terms paleostriatum and neostriatum perpetuate outdated ideas about basal ganglia evolution, their abandonment is recommended. The actual evolutionary history of the basal ganglia seems characterized by an increase in neuron number as the telencephalon expanded during the anamniote-amniote transition, with the elaboration of prominent cortical glutamatergic inputs and midbrain dopaminergic inputs, and an increased role for telencephalic circuitry in motor control occurring in stem amniotes. In mammals, especially the primate lineage, this trend has been accelerated. Nonetheless, the basic direct-indirect pathway circuit plan by which the basal ganglia regulate movement may have already been in place in early anamniotes.

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#### REFERENCES

- Absil P, Foidart A, Hemmings HC Jr, Steinbusch HWM, Ball GF, Balthazart J (2001) Distribution of DARPP-32 immunoreactive structures in the quail brain: anatomical relationship with dopamine and aromatase. J Chem Neur 21:23–39.
- Adam AS, Csillag A (2006) Differential distribution of L-aspartate and L-glutamate-immunoreactive structures in the arcopallium and medial striatum of the domestic chick (*Gallus domesticus*). J Comp Neurol 498:266–276.
- Adrio F, Anadon R, Rodriguez-Moldes I (2000) Distribution of choline acetyltransferase (ChAT) immunoreactivity in the central nervous system of a Chondrostean, the Siberian Sturgeon (*Acipenser baeri*). J Comp Neurol 426:602–621.
- Adrio F, Anadon R, Rodriguez-Moldes I (2008) Distribution of somatostatin immunoreactive neurons and fibres in the central nervous system of a chondrostean, the Siberian sturgeon (*Acipenser baeri*). Brain Res 1209:92–104.
- Ahlberg PE (1995) *Elginerpeton pancheni* and the earliest tetrapod clade. Nature 373:420–425.
- Ahlberg PE, Milner AR (1994) The origin and early diversification of tetrapods. Nature 368:507–514.
- Airhart MJ, Shirk JO, Kriebel RM (1988) Telencephalic projections to the goldfish hypothalamus: An anterograde degeneration study. Brain Res Bull 20:503–514.
- Akbas O, Verimer T, Onur R, Kayaalp SO (1984) The effects of yohimbine and neuroleptics on apomorphine-induced pecking behavior in the pigeon. Neuropharmacol 23:1261–1264.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: Neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Alunni A, Blin M, Deschet K, Bourra F, Vernier P, Retaux S (2004) Cloning and developmental expression patterns of *Dlx2*, *Lhx7*, and *Lhx9* in the medaka fish (*Oryzias latipes*). Mechan Develop 121:977–983.
- Alvarez-Bolado G, Swanson LW (1996) Developmental Brain Maps: Structure of the Embryonic Rat Brain. Amsterdam: Elsevier.
- Andersen H, Baestrup C, Randrup A (1975) Apomorphine-induced stereotyped biting in the Tortoise in relation to dopaminergic mechanisms. Brain Behav Evol 11:365–373.
- Anderson KD, Reiner A (1990a) Extensive co-occurrence of substance P and dynorphin in striatal projection neurons: an evolutionarily conserved feature of basal ganglia organization. J Comp Neurol 295:339–369.
- Anderson KD, Reiner A (1990b) Distribution and relative abundance of neurons in the pigeon forebrain containing somatostatin, neuropeptide Y, or both. J Comp Neurol 299:261–282.
- Anderson KD, Reiner A (1991a) Striatonigral projection neurons: A retrograde labeling study of the percentages that contain substance P or enkephalin in pigeons. J Comp Neurol 303:658–673.
- Anderson KD, Reiner A (1991b) Immunohistochemical localization of DARPP-32 in striatal projection neurons and striatal interneurons: Implications for the localization of D1 dopamine receptors on different types of striatal neurons. Brain Res 568:235–243.
- Anderson KD, Karle EJ, Reiner A (1991) Ultrastructural single- and double-label immunohistochemical studies of substance P-containing terminals and dopaminergic neurons in the substantia nigra in pigeons. J Comp Neurol 309:341–362.

- Anglade I, Mazurais D, Douard V, Le Jossic-Corcos C, Mananos EL, Michel D, Kah O (1999) Distribution of glutamic acid decarboxylase mRNA in the forebrain of the rainbow trout as studied by in situ hybridization. J Comp Neurol 410:277–289.
- Arends JJA, Zeigler HP (1991) Organization of the cerebellum in the pigeon (*Columba livia*): II. Projections of the cerebellar nuclei. J Comp Neurol 306:245–272.
- Arevalo R, Alonso JR, Garcia-Ojeda E, Brinson JG, Crespo C, Aijon J (1995) NADPH-diaphorase in the central nervous system of the tench (*Tinca tinca* L., 1758). J Comp Neurol 352:398–420.
- Ariëns-Kappers CU, Huber GC, Crosby E (1936) The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. New York: Hafner Press.
- Aste N, Viglietti-Panzica C, Fasolo A, Panzica GC (1995) Mapping of neurochemical markers in quail central nervous system: VIP- and SP-like immunoreactivity. J Chem Neur 8:87–102.
- Aste N, Balthazart J, Absil P, Grossmann R, Mulhbauer E, Viglietti-Panzica C, Panzica GC (1998) Anatomical and neurochemical definition of the nucleus of the stria terminalis in Japanese quail (*Coturnix japonica*). J Comp Neurol 396:141–157.
- Atoji Y, Yamamoto Y, Suzuki Y (2001) Distribution of NADPH diaphorase-containing neurons in the pigeon central nervous system. J Chem Neur 2:1–22.
- Auclair F, Lund JP, Dubuc R (2004) Immunohistochemical distribution of tachykinins in the CNS of the lamprey *Petromyzon marinus*. J Comp Neurol 479:328–346.
- Bachy I, Berthon J, Retaux S (2002) Defining pallial and subpallial divisions in the developing Xenopus forebrain. Mechanisms of Development 117:163–172.
- Bailhache T, Balthazart J (1993) The catecholaminergic system of the quail brain: Immunocytochemical studies of dopamine β-hydroxylase and tyrosine hydroxylase. J Comp Neurol 329:230–256.
- Baker-Cohen F (1968) Comparative enzyme histochemical observations on submammalian brains. Ergebnisse Anatomische Entwicklungs Geschichte 40:1–70.
- Balaban CD, Ulinski PS (1981) Organization of thalamic afferents to the anterior dorsal ventricular ridge in turtles: I. Projections of thalamic nuclei. J Comp Neurol 200:95–129.
- Balint E, Csillag A (2007) Nucleus accumbens subregions: hodological and immunohistochemical study in the domestic chick (*Gallus domesticus*). Cell Tissue Res 327:221–230.
- Ball GF (1994) Neurochemical specializations associated with vocal learning and production in songbirds and budgerigars. Brain Behav Evol 44:234–246.
- Ball GF, Casto JM, Balthazart J (1995) Autoradiographic localization of D1-like dopamine receptors in the forebrain of male and female Japanese quail and their relationship with immunoreactive tyrosine hydroxylase. J Chem Neur 9:121–133.
- Barbeau A, Dallaire L, Buu NT, Veilleux F, Boyer H, Donaldson J, Irwin I, Langston EB, Langston JW (1986) MPTP effects in frogs. In: Recent Developments in Parkinson's Disease (Fahn S, Marsden CD, Jenner P, Teychenne P, eds), pp. 155–163. New York: Raven Press.
- Barrett JE (1983) Comparison of the effects of antipsychotic drugs on the schedule-controlled behavior of squirrel monkeys and pigeons. Neuropharmacol 22:519–524.
- Batten TFC, Cambre ML, Moons L, Vandesande F (1990) Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, *Poecilia latipinna*. J Comp Neurol 302:893–919.

- Bear MF, Ebner FF (1983) Somatostatin-like immunoreactivity in the forebrain of *Pseudemys* turtles. Neuroscience 9:297–307.
- Bennis M, Gamrani H, Geffard M, Calas A, Kah O (1990) The distribution of 5HT immunoreactive systems in the brain of a saurian, the chameleon. J Hirnforschung 31:563–574.
- Bennis M, Calas A, Geffard M, Gamrani H (1991) Distribution of GABA immunoreactive systems in the forebrain and midbrain of the chameleon. Brain Res Bull 26:891–898.
- Bennis M, Araneda S, Calas A (1994) Distribution of substance P-like immunoreactivity in the chameleon brain. Brain Res Bull 34: 349–357.
- Bennis M, Ba m'hamed S, Rio JP, Le Cren D, Reperant J, Ward R (2001) The distribution of NPY-like immunoreactivity in the chameleon brain. Anat Embryol 203:121–128.
- Betarbet R, Turner R, Chockkan V, DeLong MR, Allers KA, Walters J, Levey AI, Greenamyre JT (1997) Dopaminergic neurons intrinsic to the primate striatum. J Neurosci 17:6761–6768.
- Bissoli R, Contestabile A (1988) Evolution of neurotransmitter-related markers in the vertebrate telencephalon. Comparative microchemical study in discrete brain regions of a frog and a turtle. Comp Biochem Physiol 89C:241–248.
- Bons N, Oliver J (1986) Origin of the afferent connections to the parolfactory lobe in the quail shown by retrograde labeling with a fluorescent neuron tracer. Exp Brain Res 63:125–134.
- Bottjer SW, Miesner EA, Arnold AP (1984) Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science 224:901–903.
- Bottjer SW (1997) Building a bird brain: Sculpting neural circuits for a learned behavior. BioEssays 19:1109–1116.
- Braford MR, Northcutt RN (1974) Olfactory bulb projections in the bichir, *Polypterus*. J Comp Neurol 156:165–178.
- Braford MR, Northcutt RG (1978) Correlation of telencephalic afferents and SDH distribution in the bony fish *Polypterus*. Brain Res 152:157–160.
- Brainard MS, Doupe AJ (2000) Interruption of a basal ganglia forebrain circuit prevents plasticity of learned vocalizations. Nature 404: 762–766.
- Brantley RK, Bass AH (1988) Cholinergic neurons in the brain of a teleost fish (*Porichthys notatus*) located with a monoclonal antibody to choline acetyltransferase. J Comp Neurol 275:87–105.
- Braun K, Scheich H, Braun S, Rogers JH, Heizmann CW (1991) Parvalbumin-, calretinin- and calbindin-D28k-immunoreactivity and GABA in a forebrain region involved in auditory filial imprinting. Brain Res 539:31–44.
- Brauth SE (1984) Enkephalin-like immunoreactivity within the telencephalon of the reptile *Caiman crocodilus*. Neuroscience 11:345–358.
- Brauth SE (1988) The organization and projections of the paleostriatal complex in *Caiman crocodilus*. In: The Forebrain of Reptiles. Current Concepts of Structure and Function (Schwerdtfeger WK, Smeets WJAJ, eds), pp. 60–76. Basel: Karger.
- Brauth SE, Kitt CA (1980) The paleostriatal system of *Caiman crocodilus*. J Comp Neurol 189:437–465.
- Brauth SE, Ferguson JL, Kitt CA (1978) Prosencephalic pathways related to the paleostriatum of the pigeon (*Columba livia*). Brain Res 147:205–221.
- Brauth SE, Reiner A, Kitt CA, Karten HJ (1983) The substance P-containing striatotegmental path in reptiles: An immunohistochemical study. J Comp Neurol 219:305–327.

- Brauth SE, Kitt CA, Price DL, Wainer BH (1985) Cholinergic neurons in the telencephalon of the reptile, *Caiman crocodilus*. Neurosci Lett 58:235–240.
- Brox A, Ferreiro B, Puelles L, Medina L (2003a) The telencephalon of the frog *Xenopus* based on calretinin immunostaining and gene expression patterns. Brain Res Bull 57:381–384.
- Brox A, Puelles L, Ferreiro B, Medina L (2003b) Expression of the genes GAD67 and Distal-less-4 in the forebrain of Xenopus laevis confirms a common pattern in tetrapods. J Comp Neurol 461:370–393.
- Bruce LL, Butler AB (1984a) Telencephalic connections in lizards. I. Projections to cortex. J Comp Neurol 229:585–601.
- Bruce LL, Butler AB (1984b) Telencephalic connections in lizards. II. Projections to anterior dorsal ventricular ridge. J Comp Neurol 229:602–615.
- Bruce LL, Kornblum HI, Seroogy KB (2002) Comparison of thalamic populations in mammals and birds: Expression of ErbB4 mRNA. Brain Res Bull 57:455–461.
- Brüning G (1993) Localization of NADPH-diaphorase in the brain of the chicken. J Comp Neurol 334:192–208.
- Brüning G, Wiese S, Mayer B (1994) Nitric oxide synthase in the brain of the turtle *Pseudemys scripta elegans*. J Comp Neurol 358:353–382.
- Brüning G, Katzbach R, Mayer B (1995) Histochemical and immunocytochemical localization of nitric oxide synthase in the central nervous system of the goldfish *Carassius auratus*. J Comp Neurol 348:183–206.
- Butler AB (1994a) The evolution of the dorsal thalamus of jawed vertebrates, including mammals: cladistic analysis and a new hypothesis. Brain Res Rev 19:29–65.
- Butler AB (1994b) The evolution of the dorsal pallium in the telencephalon of amniotes: cladistic analysis and a new hypothesis. Brain Res Rev 19:66–101.
- Campbell CB, Hodos W (1970) The concept of homology and the evolution of the nervous system. Brain Behav Evol 3:353–367.
- Canosa LF, Cerda-Reverter JM, Peter RE (2004) Brain mapping of three somatostatin encoding genes in the goldfish. J Comp Neurol 474:43–57.
- Carrera I, Ferreiro-Galve S, Sueiro C, Anadon R, Rodriguez-Moldez I (2008a) Tangentially migrating GABAergic cells of subpallial origin invade massively the pallium in developing sharks. Brain Res Bull 75:405–409.
- Carrera I, Molist P, Anadon R, Rodriguez-Moldez I (2008b) Development of the serotonergic system in the central nervous system of a shark, the lesser Spotted Dogfish *Scyliorhinus canicula*. J Comp Neurol 511:804–831.
- Castro A, Becerra M, Manso MJ, Anadon R (2003) Distribution and development of calretinin-like immunoreactivity in the telencephalon of the brown trout, *Salmo trutta fario*. J Comp Neurol 467:254–269.
- Castro A, Becerra M, Manso MJ, Anadon R (2006) Calretinin immunoreactivity in the brain of the Zebrafish, *Danio rerio*: Distribution and comparison with some neuropeptides and neurotransmittersynthesizing enzymes. I. Olfactory organ and forebrain. J Comp Neurol 494:435–459.
- Cerda-Reverter JM, Anglade I, Martinez-Rodriguez G, et al. (2000a) Characterization of neuropeptide Y expression in the brain of a perciform fish, the sea bass (*Dicentrarchus labrax*). J Chem Neur 19:197–210.
- Cerda-Reverter JM, Martinez-Rodriguez G, Anglade I, Kah O, Zanuy S (2000b) Peptide YY (PYY) and fish pancreatic peptide Y (PY) expression in the brain of the sea bass (*Dicentrarchus labrax*) as revealed by in situ hybridization. J Comp Neurol 426:197–208.

- Chevalier G, Vacher S, Deniau JM, Desban M (1985) Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tectospinal/tectodiencephalic neurons. Brain Res 334:215–226.
- Chiappe LM (1995). The first 85 million years of avian evolution Nature 378:349–355.
- Chiba A, Honma Y (1992) Distribution of neuropeptide Y-like immunoreactivity in the brain and hypophysis of the cloudy dogfish, *Scyliorhinus torazazame*. Cell Tissue Res 268:453–461.
- Chiba A, Honma Y (1994) Neuropeptide Y-like immunoreactive structures in the telencephalon and diencephalon of the white sturgeon, *Acipenser transmontanus*, with special regard to the hypothalamohypophyseal system. Arch Histol Cytol 57:77–86.
- Chiba A, Honma Y, Ito S, Honma S (1989) Somatostatin-immunoreactivity in the brain of the gummy shark, *Mustelus manazo* Bleeker, with special regard to the hypothalamo-hypophyseal system. Biomed Res 10, Supplement 3:1–12.
- Chiba A (1997) Distribution of neuropeptide Y-like immunoreactivity in the brain of the bichir, *Polypterus senegalus*, with special regard to the terminal nerve. Cell Tissue Res 289:275–284.
- Chiba A (1999) Immunohistochemical distribution of neuropeptide Y-related substance in the brain and hypophysis of the Arctic lamprey, *Lethenteron japonica*. Brain Behav Evol 53:102–109.
- Chiba A (2005) Neuropeptide Y-immunoreactive (NPY-ir) structures in the brain of the gar *Lepisosteus oculatus* (Lepisosteiformes, Osteichthyes) with special regard to their anatomical relations to gonadotropinreleasing hormone (GnRH)-ir structures in the hypothalamus and the terminal nerve. Gen Comp Endocrinol 142:336–346.
- Clairambault P, Christophe N, Pairault C, Ward R, Reperant J (1994) Organization of the serotonergic system in the brain of two amphibian species, *Ambystoma mexicanum* (Urodela) and *Typhlonectes com*pressicauda (Gymnophiona). Anat Embryol 190:87–99.
- Clark EC, Baxter LR Jr, Dure LS, Ackermann RF, Kemp GF, Bachus SE (2000) Mammal-like striatal functions in *Anolis*. II. Distribution of dopamine D1 and D2 receptors, and a laminar pattern of basal ganglia sub-systems. Brain Behav Evol 56:249–258.
- Cookson KK, Hall WS, Heaton JT, Brauth SE (1996) Distribution of choline acetyltransferase and acetylcholinestrase in vocal control nuclei of the budgerigar (*Melopsittacus undulatus*). J Comp Neurol 369:220–235.
- Corio M, Peute J, Steinbusch HWM (1991) Distribution of serotonin- and dopamine-immunoreactivity in the brain of the teleost *Claria gariepinus*. J Chem Neur 4:79–95.
- Corio M, Thibault J, Peute J (1992) Distribution of catecholaminergic and serotonergic systems in forebrain and midbrain of the newt, *Triturus* alpestris (Urodela). Cell Tissue Res 268:377–387.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- Cozzi B, Viglietti-Panzica C, Aste N, Panzica GC (1991) The serotonergic system in the brain of the Japanese quail. An immunohistochemical study. Cell Tissue Res 263:271–284.
- Cozzi B, Massa R, Panzica GC (1997) The NADPH-diaphorasecontaining system in the brain of the budgerigar (*Melopsitticus undulatus*). Cell Tissue Res 287:101–112.
- Crespo C, Porteros A, Arevalo R, Brinon JG, Aijon J, Alonso JR (1999) Distribution of parvalbumin immunoreactivity in the brain of the tench (*Tinca* β L 1758). J Comp Neurol 413:549–571.
- Csillag A, Bourne RC, Stewart M (1990) Distribution of mu, delta and kappa opioid receptor binding sites in the brain of the one-day-old domestic

chick (*Gallus domesticus*): An in vitro quantitative autoradiographic study. J Comp Neurol 302:541–543.

- Csillag A, Szekely AD, Stewart MG (1997) Synaptic terminals immunolabeled against glutamate in the lobus parolfactorius of domestic chicks in relation to afferents from archistriatum. Brain Res 750:171–179.
- Danger JM, Guy J, Benyamina M, Jegou S, Leboulenger F, Cote J, Tonon MC, Pelletier G, Vaudry H (1985) Localization and identification of neuropeptide Y (NPY)-like immunoreactivity in the frog brain. Peptides 6:1225–1236.
- Davies DC, Csillag A, Szekeley AD, Kabai P (1997) Efferent connections of the domestic chick archistriatum: A *Phaseolus* lectin anterograde tracing study. J Comp Neurol 389:679–693.
- deBraga M, Rieppel O (1997) Reptile phylogeny and the interrelationships of turtles. Zool J Linnean Soc 120:281–354.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Demchyshyn LL, Sugamori KS, Lee FJS, Hamadanizadeh SA, Niznik HB (1995) The dopamine D1D receptor. Cloning and characterization of three pharmacologically distinct D1-like receptors from *Gallus domesticus*. J Biol Chem 270:4005–4012.
- den Boer-Visser AM, Dubbeldam JL (2002) The distribution of dopamine, substance P, vasoactive intestinal polypeptide and neuropeptide Y immunoreactivity in the brain of the collared dove, *Streptopelia decaocto.* J Chem Neur 23:1–27.
- Derobert Y, Plouhinec JL, Sauka-Spengler T, Le Mentec C, Baratte B, Jaillard D, Mazan S (2002) Structure and expression of three *Emx* genes in the dogfish *Scyliorhynus canicula*: Functional and evolutionary implications. Devel Biol 247:390–404.
- Diaz-Regueira S, Anadon R (2000) Calretinin expression in specific neuronal systems in the brain of an advanced teleost, the grey mullet (*Chelon labrosus*). J Comp Neurol 426:81–105.
- Dietl MM, Palacios JM (1988) Neurotransmitter receptors in the avian brain. I. Dopamine receptors. Brain Res 439:354–359.
- Dietl MM, Cortes R, Palacios JM (1988) Neurotransmitter receptors in the avian brain. I. Muscarinic cholinergic receptors. Brain Res 439:360–365.
- Ding L, Perkel DJ (2002) Dopamine modulates the excitability of spiny neurons in the avian basal ganglia. J Neurosci 22:5210–5218.
- Domenici L, Waldvogel HJ, Matute C, Streit P (1988) Distribution of GABA-like immunoreactivity in the pigeon brain. Neuroscience 25:931–950.
- Doupe AJ, Kuhl PK (1999) Birdsong and human speech: common themes and mechanisms. Ann Rev Neurosci 22:567–631.
- Doupe AJ, Solis MM (1997) Song- and order-selective neurons develop in the songbird anterior forebrain during vocal learning. J Neurobiol 33:694–709.
- Dubbeldam JL, Visser AM (1987) The organization of the nucleus basalis-neostriatum complex of the mallard (*Anas platyrhynchos* L) and its connections with the archistriatum and the paleostriatum complex. Neuroscience 21:487–517.
- Dubbeldam JL, Ommen MH, den Boer-Visser AM (1999) Immunohistochemical characterization of forebrain areas in the collared dove (*Streptopelia decaocto*). Eur J Morph 37:134–138.
- Dubbeldam JL, den Boer-Visser AM, Bout RG (1997) Organization and efferent connections of the archistriatum of the mallard, *Anas platyrhynchos* L: An anterograde and retrograde tracing studies. J Comp Neurol 388:632–657.
- Dubé L, Parent A (1981) The monoamine-containing neurons in avian brain: 1. A study of the brain stem of the chicken (*Gallus domesticus*)

by means of fluorescence and acetylcholinesterase histochemistry. J Comp Neurol 196:695–708.

- Dubé L, Clairambault P, Malacarne G (1990) Striatal afferents in the newt *Triturus cristatus*. Brain Behav Evol 35:212–226.
- Durand SE, Heaton JT, Amateau SK, Brauth SE (1997) Vocal control pathways through the anterior forebrain of a parrot (*Melopsittacus* undulatus). J Comp Neurol 377:179–206.
- Durstewitz D, Kroner S, Hemmings HC Jr, Gunturkun O (1998) The dopaminergic innervation of the pigeon telencephalon: Distribution of DARPP-32 and co-occurrence with glutamate decarboxylase and tyrosine hydroxylase. Neuroscience 83:763–779.
- Durstewitz D, Kroner S, Gunturkun O (1999) The dopaminergic innervation of the avian telencephalon. Prog Neurobiol 59:161–195.
- Ebbesson SOE (1980) On the organization of the telencephalon in elasmobranchs. In: Comparative Neurology of the Telencephalon (Ebbesson SOE ed), pp. 1–16. New York: Plenum Press.
- Ebbesson SOE, Vanegas H (1976) Projections of the optic tectum in two teleost species. J Comp Neurol 165:161–180.
- Echteler SM (1984) Connections of the auditory midbrain in a teleost fish, *Cyprinus carpio*. J Comp Neurol 230:536–551.
- Edinger L, Wallenberg A, Holmes GM (1903) Untersuchungen über die vergleichende Anatomie des Gehirns. 3. Das Vorderhirn der Vögel. Abhand 1.d. Senekenberate Gesellschaft. Frankfurt am Main 20:343–426.
- Ekström P (1987) Distribution of choline acetyltransferaseimmunoreactive neurons in the brain of a Cyprinid teleost (*Phoxinus phoxinus* L.). J Comp Neurol 256:494–515.
- Ekström P, Honkanen T, Steinbusch HWM (1990) Distribution of dopamine-immunoreactive neuronal perikarya and fibres in the brain of a teleost, *Gasterosteus aculeatus* L. Comparison with tyrosine hydroxylase- and dopamine-beta-hydroxylase-immunoreactive neurons. J Chem Neur 3:233–260.
- Endepols H, Roden K, Luksch H, Gicke U, Walkowiak W (2004) Dorsal striatopallidal system in anurans. J Comp Neurol 468:299–310.
- Ewert JP (1984) Tectal mechanisms that underlie prey catching and avoidance in toads. In: Comparative Neurology of the Optic Tectum (Vanegas H ed), pp. 247–416. New York: Plenum Press.
- Farabaugh SM, Linzenbold A, Dooling RJ (1994) Vocal plasticity in budgerigars (*Lelopsittacus undulatus*): evidence for social factors in the learning of contact calls. J Comp Psychol 108:81–92.
- Farries MA, Perkel DJ (2002) A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus. J Neurosci 22:3776–3787.
- Ferreiro-Galve S, Carrera I, Candal E, Villar-Cheda B, Anadon R, Mazan S, Rodriguez-Moldes I (2008) The segmental organization of the developing shark brain based on neurochemical markers, with special attention to the prosencephalon. Brain Res Bull 75:236–240.
- Fiebig E, Ebbesson SOE, Meyer DL (1983) Afferent connections of the optic tectum in the piranha (*Serrasalmus nattereri*). Cell Tissue Res 231:55–72.
- Finkenstädt T, Ebbesson SOE, Ewert JP (1983) Projections to the midbrain tectum in Salamandra salamandra L. Cell Tissue Res 234:39–55.
- Folgueria M, Anadon R, Yanez J (2004) An experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: Olfactory bulb and ventral area. J Comp Neurol 480:180–203.
- Forey P, Janvier P (1993) Agnathans and the origin of jawed vertebrates. Nature 361:129–133.
- Fowler M, Medina L, Reiner A (1999) Immunohistochemical localization of NMDA and AMPA type glutamate receptor subunits in the basal ganglia of red-eared turtles. Brain Behav Evol 54:276–289.

- Franzoni MF, Morino P (1989) The distribution of GABA-likeimmunoreactive neurons in the brain of the newt, *Triturus cristatus carnifex*, and the green frog, *Rana esculenta*. Cell Tissue Res 255:155–166.
- Gaikwad A, Biju KC, Saha SG, Subhedar N (2004) Neuropeptide Y in the olfactory system, forebrain and pituitary of the teleost, *Clarias batrachus*. J Chem Neur 27:55–70.
- Gauthier J, Kluge AJ, Rowe T (1988) Amniote phylogeny and the importance of fossils. Cladistics 4:105–209.
- Gargiulo G, Nistico G, Rotiroti D, Silvestri R, Stephenson JD (1981) Stereotyped behavior in fowls elicited by apomorphine given into the optic ventricle and into the nucleus spiriformis lateralis. Br J Pharmacol 72:124P.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Ann Rev Neurosci 15:285–320.
- González A, Smeets WJAJ (1991) Comparative analysis of dopamine and tyrosine hydroxylase immunoreactivities in the brain of two amphibians, the anuran *Rana ridibunda* and the urodele *Pleurodeles waltlii*. J Comp Neurol 303:457–477.
- González A, Smeets WJ (1994a) Distribution of tyrosine hydroxylase immunoreactivity in the brain of *Typhlonectes compressicauda* (Amphibian, Gymnophiona): further assessment of primitive and derived traits of amphibian catecholamine systems. J Chem Neur 8:19–32.
- González A, Smeets WJAJ (1994b) Catecholamine systems in the CNS of amphibians. In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 77–102. Cambridge: Cambridge University Press.
- González A, Russchen FT, Lohman AHM (1990) Afferent connections of the striatum and the nucleus accumbens in the lizard *Gekko gecko*. Brain Behav Evol 36:39–58.
- González A, Muñoz M, Muñoz A, Marin O, Smeets WJAJ (1994) On the basal ganglia of amphibians: dopaminergic mesostriatal projections. Eur J Morph 32:2–4.
- González A, Lopez JM, Marin O (2002a) Expression of the homeobox protein *Nkx2.1* in the developing *Xenopus* forebrain. Gene Expression Patterns 1:181–185.
- González A, Lopez JM, Sanchez-Camacho C, Marin O (2002b) Regional expression of the homeobox gene Nkx2.1 defines pallidal and interneuronal populations in the basal ganglia of amphibians. Neuroscience 114:567–575.
- González A, Moreno N, Morona R, Lopez JM (2003) Somatostatin immunoreactivity in the brain of the urodele amphibian *Pleurodele waltl*: Colocalization with catecholamines and nitric oxide. Brain Res 965:246–258.
- Götz T, Kraushaar U, Geiger J, Lübke J, Berger T, Jones P (1997) Functional properties of AMPA and NMDA receptors expressed in identified types of basal ganglia neurons. J Neurosci 17:204–215.
- Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–253.
- Greenberg N, Burghardt GM, Crews D, Font E, Jones RE, Vaughan G (1989) Reptile models for biomedical research. In: Nonmammalian Animal Models for Biomedical Research (Woodhead AD, ed.), pp. 289–308. Boca Raton, Florida: CRC Press.
- Grillner S, Cangiano L, Hu GY, Thompson R, Hill R, Wallen P (2000) The intrinsic function of a motor system – from ion channels to networks and behaviour. Brain Res 886:224–236.
- Grisham W, Arnold AP (1994) Distribution of GABA-like immunoreactivity in the song system of the zebra finch. Brain Res 651:115–122.
- Guirado S, Davila JC, Real MA, Medina L (1999a) Nucleus accumbens in the lizard *Psammodromus algirus*: Chemoarchitecture and cortical afferent connections. J Comp Neurol 405:15–31.

- Guirado S, Martinez-Garcia F, Andreu MJ, Davila JC (1999b) Calciumbinding proteins in the dorsal ventricular ridge of the lizard *Psammodromus algirus*. J Comp Neurol 405:32–44.
- Haber SN, Nauta WJH (1983) Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry. Neuroscience 9:245–260.
- Hall WC, Ebner FF (1970) Thalamotelencephalic projections in the turtle (*Pseudemys scripta*). J Comp Neurol 140:101–122.
- Hall K, Brauth SE, Kitt CA (1984) Retrograde transport of [3H]GABA in the striatotegmental system of the pigeon. Brain Res 310:157–163.
- Hedges SB (2001) Molecular evidence for the early history of living vertebrates. In: Major Events in Early Vertebrate Evolution: Palaeontology, Phylogeny, Genetics and Development (Ahlberg PE ed), pp. 119–134. London: Taylor & Francis.
- Heimer L, Alheid GF, Zaborszky L (1985) Basal ganglia. In: The Rat Nervous System (Paxinos G ed), pp. 37–86. Orlando, Florida: Academic Press.
- Hellmann B, Güntürkün O, Manns M (2004) Tectal mosaic: Organization of the descending tectal projections in comparisons to the ascending tectofugal pathway in the pigeon. J Comp Neurol 472:395–410.
- Hemmings HC Jr, Nairn AC, Bibb JA, Greengard P (1995) Signal transduction in the striatum: DARPP32, a molecular integrator of multiple signaling pathways. In: Molecular and Cellular Mechanisms of Neostriatal Function (Ariano MA, Surmeier DJ, eds), pp. 283–297. Berlin: Springer-Verlag.
- Henselmans JML, Wouterlood FG (1994) Light and electron microscopic characterization of cholinergic and dopaminergic structures in the striatal complex and the dorsal ventricular ridge of the lizard *Gekko* gecko. J Comp Neurol 345:69–83.
- Hile AG, Plummer TK, Striedter GF (2000) Male vocal imitation produces call convergence during pair bonding in budgerigars (*Melopsittacus* undulatus). Anim Behavior 59:1209–1218.
- Hofmann MH, Northcutt RG (2008) Organization of major telencephalic pathways in an elasmobranch, the Thornback Ray *Platyrhinoidis triseriata*. Brain Behav Evol 72:307–325.
- Hoheisel VG, Petter H, Sterba G (1986) Somatostatin im Gehim des Bachneunauges (*Lampetra planeri* Bloch). Acta Histochem Supplement-Band 33S:259–264.
- Hoke KL, Ryan MJ, Wilzynski W (2007) Functional coupling between substantia nigra and basal ganglia homologues in amphibians. Behav Neurosci 121:1393–1399.
- Hoogland PV (1977) Efferent connections of the striatum in *Tupinambis* nigropunctatus. J Morphol 152:229–246.
- Hoogland PV (1981) Spinothalamic projections in a lizard, Varanus exanthematicus: An HRP study. J Comp Neurol 198:7–12.
- Hoogland PV, Vermeulen-VanderZee E (1990) Distribution of choline acetyltransferase immunoreactivity in the telencephalon of the lizard *Gekko gecko*. Brain Behav Evol 36:378–390.
- Hornby PJ, Piekut DT, Demski LS (1987) Localization of immunoreactive tyrosine hydroxylase in the goldfish brain. J Comp Neurol 261:1–14.
- Huesa G, Anadon R, Yanez J (2006) Topography and connections of the telencephalon in a chondrostean, *Acipenser baeri*: An experimental study. J Comp Neurol 497:519–541.
- Hunt SP, Brecha N (1984) The avian optic tectum: A synthesis of morphology and biochemistry. In: Comparative Neurology of the Optic Tectum (Vanegas H, ed.), pp. 619–648. New York: Plenum Press.
- Huynh P, Boyd SK (2007) Nitric oxide synthase and NADPH diaphorase distribution in the bullfrog (*Rana catesbeiana*) CNS: Pathways and fuctional implications. Brain Behav Evol 70:145–163.

- Inagaki S, Senba E, Shiosaka S, Takagi H, Kawai Y, Takatsuki K, Sakanaka M, Matsuzaki T, Tohyama M (1981a) Regional distribution of substance P-like immunoreactivity in the frog brain and spinal cord: immunohistochemical analysis. J Comp Neurol 201:243–254.
- Inagaki S, Shiosaka S, Takatsuki K, Sakanaka M, Takagi H, Kawai Y, Senba E, Matsuzaki T, Tohyama M (1981b) Distribution of somatostatin in the frog brain, *Rana catesbiana*, in relation to location of catecholamine-containing neuron system. J Comp Neurol 202:89–101.
- Ingle DJ (1983) Brain mechanisms of visual localization in frogs and toads. In: Advances in Vertebrate Neuroethology (Ewert JP, Capranica RR, Ingle DJ, eds), pp. 177–226. New York: Plenum Press, NY.
- Ito H, Murakami T, Fukuoka T, Kishida R (1986) Thalamic fiber connections in a teleost (*Sebastiscus marmoratus*): visual, somatosensory, olfactory, octavolateral, cerebellar relay region to the telencephalon. J Comp Neurol 250:215–227.
- Jarvis ED, Scharff C, Grossman MR, Ramos JA, Nottebohm F (1998) For whom the bird sings: Context-dependent gene expression. Neuron 2:775–788.
- Jarvis ED, Mello CV (2000) Molecular mapping of brain areas involved in parrot vocal communication. J Comp Neurol 419:1–31.
- Jarvis ED, Ribeiro S, da Silva ML, Ventura D, Vielliard J, Mello CV (2000) Behaviourally driven gene expression reveals song nuclei in hummingbird brain. Nature 406:628–632.
- Jenkins FA Jr, Walsh DM (1993) An early Jurassic caecilian with limbs. Nature 365:246–250.
- Jiao Y, Medina L, Veenman CL, Toledo C, Puelles L, Reiner A (2000) Identification of the anterior nucleus of the ansa lenticularis in birds as the homolog of the mammalian subthalamic nucleus. J Neurosci 20:6998–7010.
- Johnston SA, Maler L, Tinner B (1990) The distribution of serotonin in the brain of *Apteronotis leptorhynchus*: an immunohistochemical study. J Chem Neur 3:429–465.
- Kah O, Chambolle P (1983) Serotonin in the brain of the goldfish, Carassius auratus. Cell Tiss Res 234:319–333.
- Källén B (1951) On the ontogeny of the reptilian forebrain. Nuclear structures and ventricular sulci. J Comp Neurol 95:397–447.
- Kapsimali M, Vidal B, González A, Dufour S, Vernier P (2000) Distribution of the mRNA encoding the four dopamine D1 receptor subtypes in the brain of the European eel (*Anguilla anguilla*): Comparative approach to the function of D1 receptors in vertebrates. J Comp Neurol 419:320–343.
- Karle EJ, Anderson KD, Reiner A (1992) Ultrastructural double-labeling directly reveals synaptic contact between dopaminergic terminals and substance P-containing striatal neurons in pigeons. Brain Res 572:303–309.
- Karle EJ, Anderson KD, Reiner A (1994) Dopaminergic terminals form synaptic contacts with enkephalinergic striatal neurons in pigeons: An electron microscopic study. Brain Res 646:149–156.
- Karle EJ, Anderson KD, Medina L, Reiner A (1996) Light and electron microscopic immunohistochemical study of dopaminergic terminals in pigeon striatum using antisera against tyrosine hydroxylase and dopamine. J Comp Neurol 369:109–124.
- Karten HJ (1969). The organization of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. *In* Comparative and Evolutionary Aspects of the Vertebrate Central Nervous System (Petras J, ed.)167, pp. 146–179: New York:Annals of the New York Academy of Sciences.
- Karten HJ, Dubbeldam JL (1973) The organization and projections of the paleostriatal complex in the pigeon (*Columba livia*). J Comp Neurol 148:61–90.

- Karten HJ, Hodos W, Nauta WJH, Rezvin AM (1973) Neural connections of the "visual Wulst" of the avian telencephalon. Experimental studies in the pigeon (*Columba livia*) and owl (*Speotyto cunicularia*). J Comp Neurol 150:253–278.
- Kicliter E (1979) Some telencephalic connections in the frog, *Rana pipiens*. J Comp Neurol 185:75–86.
- Kicliter E, Northcutt RG (1975) Ascending afferents to the telencephalon of ranid frogs: An ascending degeneration study. J Comp Neurol 161:239–254.
- Kitt CA, Brauth SE (1981) Projections of the paleostriatum upon the midbrain tegmentum in the pigeon. Neuroscience 6:1551–1566.
- Kitt CA, Brauth SE (1982) A paleostriatal-thalamic-telencephalic path in pigeons. Neuroscience 7:2735–2751.
- Kitt CA, Brauth SE (1986a) Telencephalic projections from midbrain and isthmal cell groups in the pigeon. I. Locus coeruleus and subcoeruleus. J Comp Neurol 247:69–91.
- Kitt CA, Brauth SE (1986b) Telencephalic projections from midbrain and isthmal cell groups in the pigeon. II. The nigral complex. J Comp Neurol 247:92–110.
- Kohler EC, Messer WS Jr, Bingman VP (1995) Evidence for muscarinic acetylcholine receptor subtypes in the pigeon telencephalon. J Comp Neurol 362:271–282.
- Kokoros JJ, Northcutt RG (1977) Telencephalic efferents of the tiger salamander, Ambystoma tigrinum (Green). J Comp Neurol 173:613–628.
- Korzeniewska E, Güntürkün O (1990) Sensory properties and afferents of the n dorsolateralis posterior thalami of the pigeon. J Comp Neurol 292:457–479.
- Künzle H, Woodson W (1982) Mesodiencephalic and other target regions of ascending spinal projections in the turtle, *Pseudemys scripta elegans*. J Comp Neurol 212:349–364.
- Laberge F, Roth G (2005) Connectivity and cytoarchitecture of the ventral telencephalon in the salamander *Plethodon shermani*. J Comp Neurol 482:176–200.
- Laberge F, Roth G (2007) Organization of the sensory input to the telencephalon in the fire-bellied toad, *Bombina orientalis*. J Comp Neurol 502:55–74.
- Laberge F, Mühlenbrock-Lentner S, Dicke U, Roth G (2008) Thalamotelencephaloic pathways in the fire-bellied toad *Bombina orientalis*. J Comp Neurol 508:806–823.
- Langmore NE (1998) Functions of duet and solo songs of female birds. Trends Ecol Evol 13:136–140.
- Laverghetta AV, Toledo C, Veenman CL, Reiner A (2006) Cellular localization of AMPA-type glutamate receptor subunits in the basal ganglia of pigeons (*Columba livia*). Brain Behav Evol 67:10–38.
- Lázár G, Toth P, Csank G, Kicliter E (1983) Morphology and location of tectal projection neurons in frogs: A study with HRP and cobaltfilling. J Comp Neurol 215:108–120.
- Lázár G, Maderdrut JL, Merchenthaler I (1990) Some enkephalinergic pathways in the brain of *Rana esculenta* : an experimental analysis. Brain Res 521:238–246.
- Lázár G, Maderdrut JL, Trasti SL, Liposits Z, Tóth P, Kozicz P, Merchenthaler I (1993) Distribution of proneuropeptide Y-derived peptides in the brain of *Rana esculenta* and *Xenopus laevis*. J Comp Neurol 327:551–571.
- Lee MSY (1993) The origin of the turtle body plan: Bridging a famous morphological gap. Science 261:1716–1720.
- Lee MSY (1997) Reptile relationships turn turtle. Nature 389:245-246.
- Levesque M, Parent A (1998) Axonal arborization of corticostriatal and corticothalamic fibers arising from prelimbic cortex in the rat. Cerebral Cortex 8:602–613.

- Levesque M, Charara A, Gagnon S, Parent A, Descenes M (1996) Corticostriatal projections from layer V cells in rat are collaterals of long-range corticofugal axons. Brain Res 709:311–315.
- Li C, Wu XC, Rieppel O, Wang LT, Zhao LJ (2008) An ancestral turtle from the late Triassic of southwestern China. Nature 456:497–501.
- Lopez JM, Moreno N, Morona R, Munoz M, Dominguez L, Gonzalez A (2007) Distribution of somatostatin-like immunoreactivity in the brain of the Caecilian *Dermophis mexicanus* (Amphibia: Gymnophiona): Comparative aspects in amphibians. J Comp Neurol 501:413–430.
- Luiten PGM (1981a) Afferent and efferent connections of the optic tectum in the carp (*Cyprinus carpio* L). Brain Res 220:51–65.
- Luiten PGM (1981b) Two visual pathways to the telencephalon in nurse shark (*Ginglymostoma cirratum*). II. Ascending thalamotelencephalic connections. J Comp Neurol 196:539–548.
- Luo M, Perkel DJ (1999a) Long range GABAergic projection in a circuit essential for vocal learning. J Comp Neurol 403:68–84.
- Luo M, Perkel DJ (1999b) A GABAergic, strongly inhibitory projection to a thalamic nucleus in the zebra finch song system. J Neurosci 19:6700–6711.
- Mangiamele LA, Burmeister SS (2007) Acoustically evoked immediate early gene expression in the pallium of male Tungara frogs. Brain Behav Evol 72:239–250.
- Mansour A, Fox CA, Akil H, Watson SJ (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 18:22–29.
- Marin O, González A, Smeets WJAJ (1997a) Basal ganglia organization in amphibians: Afferent connections to the striatum and the nucleus accumbens. J Comp Neurol 378:16–49.
- Marin O, Smeets WJAJ, González A (1997b) Basal ganglia organization in amphibians: Catecholaminergic innervation of the striatum and the nucleus accumbens. J Comp Neurol 378:50–69.
- Marin O, González A, Smeets WJAJ (1997c) Basal ganglia organization in amphibians: Efferent connections of the striatum and the nucleus accumbens. J Comp Neurol 380:23–50.
- Marin O, Smeets WJAJ, González A (1997d) Distribution of choline acetyltransferase immunoreactivity in the brain of Anuran (*Rana perezi*, *Xenopus laevis*) and Urodele (*Pleurodeles waltl*) amphibians. J Comp Neurol 382:499–534.
- Marin O, Smeets WJAJ, González A (1997e) Basal ganglia organization in amphibians: Development of the striatal and nucleus accumbens connections with emphasis on the catecholinergic inputs. J Comp Neurol 378:16–49.
- Marin O, Smeets WJAJ, González A (1997f) Anatomical substrate of amphibian basal ganglia involvement in visuomotor behavior. Eur J Neurosci 9:2100–2109.
- Marin O, Smeets WJAJ, González A (1998a) Basal ganglia organization in amphibians: chemoarchitecture. J Comp Neurol 392:285–312.
- Marin O, Smeets WJ, González A (1998b) Evolution of the basal ganglia in tetrapods: a new perspective based on recent studies in amphibians. Trends Neurosci 21:487–494.
- Marin O, Rubenstein JL (2001) A long, remarkable journey: tangential migration in the telencephalon. Nature Rev Neurosci 2:780–790.
- Martinez-Marcos A, Ubeda-Banon I, Lanuza E, Halpern M (2005) Chemoarchitecture and afferent connections of the "olfactostriatum": a specialized vomeronasal structure within the basal ganglia of snakes. J Chem Neur 29:49–69.
- Martinoli MG, Dubourg P, Gefard M, Calas A, Kah O (1990) Distribution of GABA-immunoreactive neurons in the forebrain of the goldfish, *Carassius auratus*. Cell Tissue Res 260:77–84.

- Masino T, Grobstein P (1989a) The organization of descending tectofugal pathways underlying orienting in the frog, *Rana pipiens*.
  I. Lateralization, parcellation and an intermediate spatial representation. Exp Brain Res 75:227–244.
- Masino T, Grobstein P (1989b) The organization of descending tectofugal pathways underlying orienting in the frog, Evidence for the involvement of a tecto-tegmento-spinal pathway. Exp Brain Res 75:245–264.
- Masino T, Grobstein P (1990) Tectal connectivity in the frog *Rana pipiens*: tectotegmental projections and a general analysis of topographic organization. J Comp Neurol 291:103–127.
- Medina L, Reiner A (1994) Distribution of choline acetyltransferase immunoreactivity in the pigeon brain. J Comp Neurol 342:497–537.
- Medina L, Reiner A (1997) The efferent projections of the dorsal and ventral pallidal parts of the pigeon basal ganglia, studied with biotinylated dextran amine. Neuroscience 81:773–802.
- Medina L, Reiner A (2000) Do birds possess homologues of mammalian primary visual, somatosensory and motor cortices? Trends Neurosci 23:1–12.
- Medina L, Smeets WJAJ (1991) Comparative aspects of the basal gangliatectal pathways in reptiles. J Comp Neurol 308:614–629.
- Medina L, Smeets WJAJ (1992) Cholinergic, monoaminergic and peptidergic innervation of the primary visual centers in the brain of the lizards *Gekko gecko* and *Gallotia galloti*. Brain Behav Evol 40:157–181.
- Medina L, Martí E, Artero C, Fasolo A, Puelles L (1992) Distribution of neuropeptide Y-like immunoreactivity in the brain of the lizard *Gallotia galloti*. J Comp Neurol 319:387–405.
- Medina L, Smeets WJAJ, Hoogland PV, Puelles L (1993) Distribution of choline acetyltransferase immunoreactivity in the brain of the lizard *Gallotia galloti*. J Comp Neurol 33:261–285.
- Medina LM, Anderson KD, Karle EJ, Reiner A (1995) An ultrastructural double-label immunohistochemical study of the enkephalinergic input to dopaminergic neurons of the substantia nigra in pigeons. J Comp Neurol 357:408–432.
- Medina L, Veenman CL, Reiner A (1997) New evidence for an avian dorsal thalamic center comparable to the mammalian VA/VL nuclei. J Comp Neurol 384:86–108.
- Meek J (1994) Catecholamines in the brains of Osteichthyes (bony fishes). In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 49–76. Cambridge: Cambridge University Press.
- Meek J, Joosten HWJ (1989) Distribution of serotonin in the brain of the mormyrid teleost Gnathonemus petersii. J Comp Neurol 281:206–224.
- Meek J, Joosten HWJ, Steinbusch HWM (1989) Distribution of dopamine immunoreactivity in the brain of the mormyrid teleost *Gnathonemus petersii*. J Comp Neurol 28:362–383.
- Melendez-Ferro M, Perez-Costas E, Villar-Cheda B, Abalo XM, Rodriguez-Munoz R, Rodicio MC, Anadon R (2002) Ontogeny of gamma-aminobutyric acid-immunoreactive neuronal populations in the forebrain and midbrain of the sea lamprey. J Comp Neurol 446:360–376.
- Mello VV, Ribeiro S (1998) ZENK protein regulation by song in the brain of songbirds. J Comp Neurol 393:426–438.
- Merchenthaler I, Lázár G, Maderdrut JL (1989) Distribution of proenkephalin-derived peptides in the brain of *Rana esculenta*. J Comp Neurol 281:23–39.
- Meredith G, Smeets WJAJ (1987) Immunocytochemical analysis of the dopamine system in the forebrain and midbrain of *Raja radiata*: Evidence for a substantia nigra and a ventral tegmental area in cartilaginous fish. J Comp Neurol 265:530–548.

- Metzger M, Jiang S, Wang J, Braun K (1996) Organization of the dopaminergic innervation of forebrain areas relevant to learning: A combined immunohistochemical/retrograde tracing study in the domestic chick. J Comp Neurol 376:1–27.
- Mezey S, Csillag A (2002) Selective striatal connections of midbrain dopaminergic nuclei in the chick (*Gallus domesticus*). Cell Tissue Res 308:35–46.
- Mezey S, Csillag A (2003) The light and electron microscopic characterization of identified striato-ventrotegmental projection neurons in the domestic chick (*Gallus domesticus*). Neurosci Res 47: 299–308.
- Miceli D, Repérant J (1985) Telencephalic afferent projections from the diencephalon and brainstem in the pigeon. A retrograde multiplelabel fluorescent study. Exp Biol 44:71–99.
- Miceli D, Repérant J, Villalobos J, Dionne L (1987) Extratelencephalic projections of the avian visual wulst. A quantitative autoradiographic study in the pigeon *Columba livia*. J Hirnforschung 28:45–57.
- Molist P, Rodríguez-Moldes I, Anadón R (1993) Organization of catecholaminergic systems in the hypothalamus of two elasmobranch species, *Raja undulata* and *Scyliorhinus canicula*. A histofluorescence and immunohistochemical study. Brain Behav Evol 41:290–302.
- Molnar M, Casini G, Davis BM, Bagnoli P, Brecha N (1994) Distribution of proenkephalin mRNA in the chicken and pigeon telencephalon. J Comp Neurol 348:419–432.
- Montagnese CM, Mezey SE, Csillag A (2003) Efferent connections of the dorsomedial thalamic nuclei of the domestic chick (*Gallus domesticus*). J Comp Neurol 459:301–326.
- Moons L, van Gils J, Ghijsels E, Vandesande F (1994) Immunocytochemical localization of L-DOPA and dopamine in the brain of the chicken (*Gallus domesticus*). J Comp Neurol 346:97–118.
- Moreno N, Gonzalez A, Retaux S (2008) Evidences for tangential migration in *Xenopus* telencephalon: developmental patterns and cell tracking experiments. Develop Neurobiol 68:504–520.
- Mueller T, Vernier P, Wullimann MF (2004) The adult central nervous cholinergic system of a neurogenic model animal, the zebrafish *Danio rerio*. Brain Res 1011:156–169.
- Mueller T, Wullimann MF (2008) Early teleostean basal ganglia development visualized by zebrafish *Dlx2a*, *Lhx6*, *Lhx7*, *Tbr2* (eomesa), and *GAD67* gene expression. J Comp Neurol 507:1245–1257.
- Munoz M, Munoz A, Marin O, Alonso JR, Arevalo R, Porteros A, González A (1996) Topographical distribution of NADPH-diaphorase activity in the central nervous system of the frog, *Rana perezi*. J Comp Neurol 367:54–69.
- Murakami T, Morita Y, Ito H (1983) Cytoarchitecture and topographic projections of the gustatory centers in a teleost, *Sebastiscus marmoratus*. J Comp Neurol 216:115–131.
- Murakami Y, Ogasawara M, Sugahara F, Hirano S, Satoh N, Kuratani S (2001) Identification and expression of the lamprey *Pax6* gene: Evolutionary origin of the segmented brain of vertebrates. Development 128:3521–3531.
- Murakami Y, Kuratani S (2008) Brain segmentation and trigeminal projections in the lamprey; with reference to vertebrate brain evolution. Brain Res Bull 75:218–224.
- Myojin M, Ueki T, Sugahara F, Murakami Y, Shigetani Y, Aizawa S, Hirano S, Kuratani S (2001) Isolation of DIx and Emx gene cognates in an agnathan species, *Lampetra japonica*, and their expression patterns during embryonic and larval development: conserved and diversified regulatory patterns of homeobox genes in vertebrate head evolution. J Exp Zool 291:68–84.

- Naujoks-Manteuffel C, Manteuffel G (1986) Internuclear connections between the pretectum and the accessory optic system in *Salamandra salamandra*. Cell Tissue Res 243:595–602.
- Naujoks-Manteuffel C, Himstedt W, Gläsener-Cipollone G (1994) Distribution of GABA-immunoreactive neurons in the brain of adult and developing salamanders (*Pleurodeles waltli, Triturus alpestris*). Cell Tissue Res 276:485–501.
- Neidert AH, Virupannavar V, Hooker GW, Langeland JA (2001) Lamprey *Dlx* genes and early vertebrate evolution. Proc Natl Acad Sci USA 98:1665–1670.
- Nieuwenhuys R, Meek J (1990a) The telencephalon of actinopterygian fishes. In: Cerebral Cortex. Volume 8A. 1. Nonmammalian Vertebrates (Jones EG, Peters A, eds), pp. 31–73. New York: Plenum Press.
- Nieuwenhuys R, Meek J (1990b) The telencephalon of sarcopterygian fishes. In: Cerebral Cortex. Volume 8A. 1. Nonmammalian Vertebrates (Jones EG, Peters A, eds), pp. 75–106. New York: Plenum Press.
- Nieuwenhuys R, Nicholson C (1998) Lampreys, Petromyzontoidea. In: The Central Nervous System of Vertebrates (Nieuwenhuys R, ten Donkelaar HJ, Nicholson C, eds), pp. 397–495. Berlin: Springer Verlag.
- Nistico G, Rotiroti D, Stephenson JD (1983) Neurotransmitters and stereotyped behavior in birds. In: Progress in Nonmammalian Brain Research Volume II (Nistico G, Bolis L, eds), pp. 89–105. New York: CRC Press.
- Noack K, Zardoya R, Meyer A (1996) The complete mitochondrial DNA sequence of the bichir (*Polypterus ornatipinnis*), a basal ray-finned fish: ancient establishment of the consensus vertebrate gene order. Genetics 144:1165–1180.
- Nordeen KW, Nordeen EJ (1997) Anatomical and synaptic substrates for avian song learning. J Neurobiol 33:532–548.
- Northcutt RG (1977) Elasmobranch central nervous system organization and its possible evolutionary significance. Am Zool 17:411–429.
- Northcutt RG (1978) Brain organization in the cartilaginous fishes. In: Sensory Biology of Sharks, Skates, and Rays, Chapter II. Vision (Hodgson ES, Mathewson RF, eds), pp. 117–193. Washington DC: Office of Naval Research, Department of the Navy.
- Northcutt RG (1981a) Evolution of the telencephalon in nonmammals. Ann Rev Neurosci 4:301–350.
- Northcutt RG (1981b) Localization of neurons afferent to the telencephalon in a primitive bony fish *Polypterus palmas*. Neurosci Lett 22:219–222.
- Northcutt RG (2006) Connections of the lateral and medial divisions of the goldfish telencephalic pallium. J Comp Neurol 494:903–943.
- Northcutt RG, Braford MR (1980) New observations on the organization and evolution of the telencephalon in actinopterygian fishes. In: Comparative Neurology of the Telencephalon (Ebbesson SOE, ed.), pp. 41–98. New York: Plenum Press.
- Northcutt RG, Butler AB (1980) Projections of the optic tectum in the Longnose Gar, *Lepisosteus osseus*. Brain Res 190:333–346.
- Northcutt RG, Puzdrowski RL (1988) Projections of the olfactory bulb and nervus terminalis in the silver lamprey. Brain Behav Evol 32:96–107.
- Northcutt RG, Wicht H (1997) Afferent and efferent connections of the lateral and medial pallia of the silver lamprey. Brain Behav Evol 49:1–19.
- Northcutt RG, Reiner A, Karten HJ (1988) Immunohistochemical study of the telencephalon of the spiny dogfish, *Squalus acanthias*. J Comp Neurol 277:250–267.
- Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary. J Comp Neurol 165:457–486.

- Nozaki M, Gorbman A (1986) Occurrence and distribution of substance P-related immunoreactivity in the brain of adult lampreys, *Petromyzon marinus* and *Entosphenus tridentus*. Gen Comp Endocrinol 62:217–229.
- Nozaki M, Tsukahara T, Kobayashi H (1984) An immunohistochemical study on the distribution of neuropeptides in the brain of certain species of fish. Biomed Res 4:135–145.
- Ogasawara M, Shigetani Y, Suzuki S, Kuratani S, Satah N (2001) Expression of thyroid transcription factor-1 (TTF-1) gene in the ventral forebrain and endostyle of the agnathan vertebrate, *Lampetra japonica*. Genesis 30:51–58.
- Osorio J, Mazan S, Retaux S (2005) Organization of the lamprey (*Lampetra fluviatilis*) embryonic brain: Insights from *LIM*-homeodomain, *Pax* and *hedgehog* genes. Devel Biol 288:100–112.
- Papalopulu N, Kintner C (1993) *Xenopus* Distal-less related homeobox genes are expressed in the developing forebrain and are induced by planar signals. Development 117:961–975.
- Parent A (1986) Comparative Neurobiology of the Basal Ganglia. New York: John Wiley.
- Parent A, Northcutt RG (1982) The monoamine-containing neurons in the brain of the garfish, *Lepisosteus osseus*. Brain Res Bull 9:189–204.
- Parent A, Poitras D, Dubé L (1984) Comparative anatomy of central monoaminergic systems. In: Handbook of Chemical Neuroanatomy Vol. 2: Classical Transmitters in the CNS. Part I (Bjorklund A, Hokfelt T, eds), pp. 409–439. Amsterdam, Netherlands: Elsevier.
- Pepperberg IM (1999) The Alex Studies. Harvard University Press. Cambridge, MA:
- Perez SE, Yanez J, Marın O, Anadon R, Gonzalez A, Rodriguez-Moldes I (2000) Distribution of ChAT-ir in the brain of the adult trout and tract-tracing observations on the connections of the nuclei of the isthmus. J Comp Neurol 428:450–474.
- Perroteau I, Danger JM, Biffo S, Pelletier G, Vaudry H, Fasolo A (1988) Distribution and characterization of neuropeptide Y-like immunoreactivity in the brain of the crested newt. J Comp Neurol 275:309–325.
- Person AL, Gale SD, Farries MA, Perkel DJ (2008) Organization of the songbird basal ganglia, including area X. J Comp Neurol 508:840–866.
- Pickavance LC, Staines WA, Fryer JN (1992) Distributions and colocalization of neuropeptide Y and somatostatin in the goldfish brain. J Chem Neur 5:221–233.
- Pierre J, Rio JP, Mahouche M, Repérant J (1994) Catecholamine systems in the brain of cyclostomes, the lamprey, *Lampreta fluviatilis*. In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 7–19. Cambridge: Cambridge University Press.
- Pinuela C, Northcutt RG (2007) Immunohistochemical organization of the forebrain in the white sturgeon, *Acipenser transmontanus*. Brain Behav Evol 69:229–253.
- Polenova OA, Vesselkin NP (1993) Olfactory and nonolfactory projections in the river lamprey (*Lampetra fluviatilis*) telencephalon. J Hirnforschung 34:261–279.
- Pollard HB, Dhariwal K, Adeyemo OM, Markey CJ, Caohuy H, Levine M, Markey S, Youdim MBH (1992) A parkinsonian syndrome induced in the goldfish by the neurotoxin MPTP. FASEB J 6:3108–3116.
- Pombal MA, El Manira A, Grillner S (1997a) Afferents of the lamprey striatum with special reference to the dopaminergic system: A combined tracing and immunohistochemical study. J Comp Neurol 386:71–91.
- Pombal MA, El Manira A, Grillner S (1997b) Organization of the lamprey striatum – transmitters and projections. Brain Res 766:249–254.

- Pombal MA, Marin O, González A (2001) Distribution of choline acetyltransferase-immunoreactive structures in the lamprey brain. J Comp Neurol 431:105–126.
- Pontet A, Danger JM, Dubourg P, Peletier G, Vaudry H, Calas A, Kah O (1989) Distribution and characterization of neuropeptide Y-like immunoreactivity in the brain and pituitary of the goldfish. Cell Tissue Res 255:529–538.
- Powers AS, Reiner A (1993) The distribution of cholinergic neurons in the central nervous system of turtles. Brain Behav Evol 4:326–345.
- Puelles L, Medina L (1994) Development of neurons expressing tyrosine hydroxylase and dopamine in the chicken brain: a comparative segmental analysis. In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 381–404. Cambridge: Cambridge University Press.
- Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J, Smiga S, Rubenstein JL (2000) Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes *Dlx-2*, *Emx-1*, *Nkx-2.1*, *Pax-6*, *Tbr-1*. J Comp Neurol 424:409–438.
- Reiner A (1987a) The distribution of proenkephalin-derived peptides in the central nervous system of turtles. J Comp Neurol 259:65–91.
- Reiner A (1987b) A LANT6-like substance that is distinct from neuromedin N is present in pallidal and striatal neurons in monkeys. Brain Res 422:186–191.
- Reiner A (1993) Neurotransmitter organization and connections of turtle cortex: Implications for the evolution of mammalian isocortex. Comp Biochem Physiol 104A:735–748.
- Reiner A (1994) Laminar distribution of the cells of origin of the ascending and descending tectofugal pathways in turtles: Implications for the evolution of tectal lamination. Brain Behav Evol 43:254–292.
- Reiner A (2002) Functional circuitry of the avian basal ganglia: Implications for basal ganglia organization in stem amniotes. Brain Res Bull 57:513–528.
- Reiner A, Anderson KD (1990) The patterns of neurotransmitter and neuropeptide co-occurrence among striatal projection neurons: conclusions based on recent findings. Brain Res Rev 15:251–265.
- Reiner A, Anderson KD (1993) Co-occurrence of gamma-aminobutyric acid, parvalbumin and the neurotensin-related neuropeptide LANT6 in pallidal, nigral and striatal neurons in pigeons and monkeys. Brain Res 624:317–325.
- Reiner A, Carraway RE (1985) The presence and phylogenetic conservation of a neurotensin-related hexapeptide in neurons of globus pallidus. Brain Res 341:365–371.
- Reiner A, Carraway RE (1987) Immunohistochemical and biochemical studies on Lys<sup>8</sup>-Asn<sup>9</sup>-neurotensin<sup>8–13</sup> (LANT6)-related peptides in the basal ganglia of pigeons, turtles, and hamsters. J Comp Neurol 257:453–476.
- Reiner A, Karten HJ (1982) Laminar distribution of the cells of origin of the descending tectofugal pathways in the pigeon (*Columba livia*). J Comp Neurol 204:165–187.
- Reiner A, Northcutt RG (1987) An immunohistochemical study of the telencephalon of the African lungfish, *Protopterus annectens*. J Comp Neurol 256:463–481.
- Reiner A, Northcutt RG (1992) An immunohistochemical study of the telencephalon of the Senegal bichir (*Polypterus senegalus*). J Comp Neurol 319:359–386.
- Reiner A, Oliver JR (1987) Somatostatin and neuropeptide Y are almost exclusively found in the same neurons in the telencephalon of turtles. Brain Res 426:149–156.

- Reiner A, Brauth SE, Kitt CA, Karten HJ (1980) Basal ganglionic pathways to the tectum: Studies in reptiles. J Comp Neurol 193:565–589.
- Reiner A, Brecha NC, Karten HJ (1982a) Basal ganglia pathways to the tectum: The afferent and efferent connections of the lateral spiriform nucleus of pigeon. J Comp Neurol 208:16–36.
- Reiner A, Karten HJ, Brecha NC (1982b) Enkephalin-mediated basal ganglia influences over the optic tectum: Immunohistochemistry of the tectum and the lateral spiriform nucleus in pigeon. J Comp Neurol 208:37–53.
- Reiner A, Karten HJ, Solina AR (1983) Substance P: Localization within paleostriatal-tegmental pathways in the pigeon. Neuroscience 9:61–85.
- Reiner A, Brauth SE, Karten HJ (1984a) Evolution of the amniote basal ganglia. Trends Neurosci 7:320–325.
- Reiner A, Davis BM, Brecha NC, Karten HJ (1984b) The distribution of enkephalin-like immunoreactivity in the telencephalon of the adult and developing domestic chicken. J Comp Neurol 228:245–262.
- Reiner A, Brauth SE, Kitt CA, Quirion R (1989) The distribution of mu, delta and kappa opiate receptors in the pigeon forebrain and midbrain. J Comp Neurol 280:359–382.
- Reiner A, Karle EJ, Anderson KD, Medina L (1994) Catecholaminergic perikarya and fibers in the avian nervous system. In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 135–181. Cambridge: Cambridge University Press.
- Reiner A, Medina L, Veenman CL (1998a) Structural and functional evolution of the basal ganglia in vertebrates. Brain Res Reviews 28:235–285.
- Reiner A, Perera M, Paullus R, Medina L (1998b) Immunohistochemical localization of DARPP-32 in striatal projection neurons and striatal interneurons in pigeons. J Chem Neur 16:17–33.
- Reiner A, Stern EA, Wilson CJ (2001) Physiology and morphology of intratelencephalically projecting corticostriatal-type neurons in pigeons as revealed by intracellular recording and cell filling. Brain Behav Evol 58:101–114.
- Reiner A, Jiao Y, Del Mar N, Laverghetta AV, Lei WL (2003) Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. J Comp Neurol 457:420–440.
- Reiner A, Laverghetta AV, Meade CA, Cuthbertson SL, Bottjer SW (2004a) An immunohistochemical and pathway tracing study on the striatopallidal organization of Area X in the male zebra finch. J Comp Neurol 469:239–261.
- Reiner A, Perkel DJ, Bruce LL, et al. (2004b) Revised nomenclature for avian telencephalon and some related brainstem nuclei. J Comp Neurol 473:377–414.
- Reiner A, Yamamoto K, Karten HJ (2005) Organization and evolution of the avian forebrain. Anat Record 287A:1080–1102.
- Rettig G (1988) Connections of the tectum opticum in two urodeles, *Salamandra salamandra* and *Bolitoglossa subpalmata*, with special reference to the nucleus isthmi. J Hirnforschung 29:5–16.
- Richfield EK, Young AB, Penney JB (1987) Comparative distribution of D-1 and D-2 receptors in the basal ganglia of turtles, pigeons, rats, cats, and monkeys. J Comp Neurol 262:446–463.
- Rink E, Wullimann RF (2001) The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tubercle). Brain Res 889:316–330.
- Rink E, Wullimann RF (2002) Connections of the ventral telencephalon and tyrosine hydroxylase distribution in the zebrafish brain (*Danio rerio*) lead to identification of an ascending dopaminergic system in a teleost. Brain Res Bull 57:385–387.

- Rink E, Wullimann RF (2004) Connections of the ventral telencephalon (subpallium) in the zebrafish (*Danio rerio*). Brain Res 1011:206–220.
- Ritchie TC, Livingston CA, Hughes CA, McAdoo MG, Leonard RB (1983) The distribution of serotonin in the CNS of an elasmobranch fish: Immunocytochemical and biochemical studies in the Atlantic stingray, *Dasyatis sabina*. J Comp Neurol 22:429–443.
- Roberts BL, Meredith GE, Maslam S (1989) Immunocytochemical analysis of the dopamine system in the brain and spinal cord of the European eel, *Anguilla anguilla*. Anat Embryol 180:401–412.
- Roberts TF, Cookson KK, Heaton KJ, Hall WS, Brauth SE (2001) Distribution of tyrosine hydroxylase-containing neurons and fibers in the brain of the budgerigar (*Melopsittacus undulatus*): General patterns and labeling in vocal areas. J Comp Neurol 429:436–454.
- Roberts TF, Hall WS, Brauth SE (2002) Organization of the avian basal forebrain: chemical anatomy in the parrot (*Melopsittacus undulatus*). J Comp Neurol 454:383–408.
- Robertson B, Saitoh K, Menard A, Grillner S (2006) Afferents of the lamprey optic tectum with special reference to the GABA input: Combined tracing and immunohistochemical study. J Comp Neurol 499:106–119.
- Robertson B, Auclair F, Menard A, Grillner S, Dubuc R (2007) GABA distribution in lamprey is phylogenetically conserved. J Comp Neurol 503:47–63.
- Rodriguez-Moldes I, Manso MJ, Becerra M, Molist P, Anadon R (1993) Distribution of substance P-like immunoreactivity in the brain of the elasmobranch *Scyliorhinus canicula*. J Comp Neurol 335:228–244.
- Rohr KB, Barth KA, Varga ZM, Wilson SW (2001) The Nodal pathway acts upstream of Hedgehog signaling to specify ventral telencephalic identity. Neuron 29:341–351.
- Rosen DE, Forey PL, Gardiner BG, Patterson C (1981) Lungfishes, tetrapods, paleontology and plesiomorphy. Bull Am Museum Nat History 167:163–275.
- Roth G, Muhlenbrock-Lentner S, Grunwald W, Laberge F (2004) Morphology and axonal projection patterns in the telencephalon of the fire-bellied toad *Bombina orientalis*: An anterograde, retrograde, intracellular biocytin labeling study. J Comp Neurol 478:35–61.
- Roth G, Laberge F, Muhlenbrock-Lentner S, Grunwald W (2007) Organization of the pallium in the fire-bellied toad *Bombina orientalis* I: Morphology and axonal projection pattern of neurons revealed by biocytin labeling. J Comp Neurol 501:443–464.
- Rubenstein JLR, Martinez S, Shimamura K, Puelles L (1994) The embryonic vertebrate forebrain: The prosomeric model. Science 266:578–580.
- Rubinson K (1968) Projections of the tectum opticum of the frog. Brain Behav Evol 1:529–561.
- Russchen FT, Jonker AJ (1988) Efferent connections of the striatum and the nucleus accumbens in the lizard *Gekko gecko*. J Comp Neurol 276:61–80.
- Russchen FT, Smeets WJAJ, Hoogland PV (1987) Histochemical identification of pallidal and striatal structures in the lizard *Gekko gecko*: Evidence for compartmentalization. J Comp Neurol 256:329–341.
- Sas E, Maler L (1991) Somatostatin-like immunoreactivity in the brain of an electric fish (*Asperonotus leptorhynchus*) identified with monoclonal antibodies. J Chem Neur 4:155–186.
- Sas E, Maler L, Tinner B (1990) Catecholaminergic systems in the brain of a Gymnotid teleost fish: An immunohistochemical study. J Comp Neurol 292:127–162.
- Sassoe M, Pognetto C, Pairault C, Clairambault P, Fasolo A (1991) The connections of the anterior pallium in *Pleurodeles waltl* and *Triturus carnifex*: An HRP study. J Hirnforschung 32:397–407.
- Scharff C, Nottebohm F (1991) A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. J Neurosci 11:2896–2913.
- Schlussman SD, Koobylack MA, Dunn-Meynell AA, Sharma SC (1990) Afferent connections of the optic tectum in channel catfish *Ictalurus punctatus*. Cell Tissue Res 262:531–541.
- Schmidt A, Roth G, Ernst M (1989) Distribution of substance P-like, leucine-enkephalin-like, and bombesin-like immunoreactivity and acetylcholinesterase activity in the visual system of salamanders. J Comp Neurol 288:123–135.
- Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci 13:900–913.
- Smeets WJAJ (1981) Efferent tectal pathways in two chondroichthyans, the shark Scyliorhinus canicula and the ray Raja clavata. J Comp Neurol 195:13–23.
- Smeets WJAJ (1982) The afferent connections of the tectum mesencephalic in two chondroichthyans, the shark *Scyliorhinus canicula* and the ray *Raja clavata*. J Comp Neurol 205:139–152.
- Smeets WJAJ (1983) The secondary olfactory connections in two chondroichthyans, the shark *Scyliorhinus canicula* and the ray *Raja clavata*. J Comp Neurol 218:334–344.
- Smeets WJAJ (1988) Distribution of dopamine immunoreactivity in the forebrain and midbrain of the snake *Python regius*: A study with antibodies against dopamine. J Comp Neurol 271:115–129.
- Smeets WJAJ (1990) The telencephalon of cartilaginous fishes. In: Cerebral Cortex. Volume 8A. 1. Nonmammalian Vertebrates (Jones EG, Peters A, eds), pp. 3–30. New York: Plenum Press.
- Smeets WJAJ (1991) Comparative aspects of the distribution of substance P and dopamine immunoreactivity in the substantia nigra of amniotes. Brain Behav Evol 37:179–188.
- Smeets WJAJ (1994) Catecholamine systems in the CNS of reptiles: structure and functional correlations. In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 103–133. Cambridge: Cambridge University Press.
- Smeets WJAJ, Boord RL (1985) Connections of the lobus inferior hypothalami of the clearnose skate *Raja eglanteria* (Chondroichthyes). J Comp Neurol 234:380–392.
- Smeets WJAJ, Medina L (1995) The efferent connections of the nucleus accumbens in the lizard Gekko gecko. A combined tract-tracing/ transmitter-immunohistochemical study. Anat Embryol 191:73–81.
- Smeets WJAJ, Steinbusch HWM (1988) Distribution of serotonin immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko*. J Comp Neurol 27:419–434.
- Smeets WJAJ, Hoogland PV, Voorn P (1986) The distribution of dopamine immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko*: An immunohistochemical study with antibodies against dopamine. J Comp Neurol 253:46–60.
- Smeets WJAJ, Jonker AJ, Hoogland PV (1987) Distribution of dopamine in the forebrain and midbrain of the red-eared turtle, *Pseudemys scripta elegans*, reinvestigated using antibodies against dopamine. Brain Behav Evol 30:121–142.
- Smeets WJAJ, Lopez JM, González A (2001) Immunohistochemical localization of DARPP-32 in the brain of the lizard, *Gekko Gecko*: Co-occurrence with tyrosine hydroxylase. J Comp Neurol 435:194–210.
- Smeets WJAJ, Lopez JM, González A (2003) Immunohistochemical localization of DARPP-32 in the brain of the turtle, *Pseudemys*

*scripta elegans*: further assessment of its relationship with dopaminergic systems in reptiles. J Chem Neur 25:83–95.

- Smith-Fernandez A, Pieau C, Reperant J, Boncinelli E, Wassef M (1998) Expression of the Emx-1 and Dlx-1 homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes. Development 125:2099–2111.
- Stock DW, Ellies DL, Zhao Z, Ekker M, Ruddle FH, Weiss KM (1996) The evolution of the vertebrate *Dlx* gene family. Proc Nat Acad Sci USA 93:10858–10863.
- Striedter GF (1991) Auditory, electrosensory and mechanosensory lateral line pathways through the forebrain in channel catfishes. J Comp Neurol 312:311–331.
- Striedter GF (1994) The vocal control pathways in budgerigars differ from those in songbirds. J Comp Neurol 343:35–56.
- Streit P, Burkhalter A, Stella M, Cuenod M (1980). Patterns of activity in pigeon brain visual relays as revealed by the [14C]2-deoxyglucose method Neuroscience 5:1053–1066.
- Stuesse SL, Cruce WLR (1991) Immunohistochemical localization of serotoninergic, enkephalinergic, and catecholaminergic cells in the brainstem and diencephalon of a cartilaginous fish, *Hydrolagus colliei*. J Comp Neurol 309:535–548.
- Stuesse SL, Cruce WLR (1992) Distribution of tyrosine hydroxylase, serotonin, and leu-enkephalin immunoreactive cells in the brainstem of a shark, *Squalus acanthias*. Brain Behav Evol 39:77–92.
- Stuesse SL, Cruce WLR, Northcutt RG (1990) Distribution of tyrosine hydroxylase- and serotonin-immunoreactive cells in the central nervous system of the thornback guitarfish, *Platyrhinoidis triseariata*. J Chem Neur 3:45–58.
- Stuesse SL, Cruce WLR, Northcutt RG (1991) Localization of serotonin, tyrosine hydroxylase, and leu-enkephalin immunoreactive cells in the brainstem of the horn shark, *Heterodontus francisci*. J Comp Neurol 308:277–292.
- Stuesse SL, Cruce WLR, Northcutt RG (1994) Localization of catecholamines in the brains of Chondrichthyes (cartilaginous fishes). In: Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates (Smeets WJAJ, Reiner A, eds), pp. 21–47. Cambridge: Cambridge University Press.
- Sun Z, Reiner A (2000) Localization of dopamine D1A and D1B receptor mRNAs in the forebrain and midbrain of the domestic chick. J Chem Neur 19:211–224.
- Sun Z, Wang HB, Laverghetta AV, Yamamoto K, Reiner A (2005) The distribution and cellular localization of glutamic acid decarboxylase-65 (GAD65) mRNA in the forebrain and midbrain of domestic chick. J Chem Neur 29:265–281.
- Swanson LW, Petrovich GD (1998) What is the amygdala? Trends Neurosci 21:323–331.
- Taban CH, Cathieni M (1983) Distribution of substance P-like immunoreactivity in the brain of the newt (*Triturus cristatus*). J Comp Neurol 216:453–470.
- Ten Donkelaar HJ, de Boer-van Huizen R (1981a) Basal ganglia projections to the brain stem in the lizard Varanus exanthematicus as demonstrated by retrograde transport of horseradish peroxidase. Neuroscience 6:1567–1590.
- Ten Donkelaar HJ, de Boer-van Huizen R, Schouten FTM, Eggen SJ (1981b) Cells of origin of descending pathways to the spinal cord in the clawed toad (*Xenopus laevis*). Neuroscience 6:2297–2312.

- Thompson RH, Menard A, Pombal M, Grillner S (2008) Forebrain dopamine depletion impairs motor behavior in lamprey. Eur J Neurosci 27:1452–1460.
- Tömböl T, Csillag A, Stewart MG (1988) Cell types of the paleostriatal complex of the domestic chicken (*Gallus domesticus*): A Golgi study. J Hirnforschung 29:493–507.
- Tostivint H, Lihrmann I, Bucharales C, Vieau D, Coulouarn Y, Fournier A, Conlon JM, Vaudry H (1996) Occurrence of two somatostatin variants in the frog brain: Characterization of the cDNAs, distribution of the mRNAs, and receptor-binding affinities of the peptides. Proc Nat Acad Sci USA 93:12605–12610.
- Trabucchi M, Tostivint H, Lihrmann I, Jegou S, Vallarino M, Vaudry H (1999) Molecular cloning of the cDNAs and distribution of the mRNAs encoding two somatostatin precursors in the African lungfish *Protopterus annectens*. J Comp Neurol 410:643–652.
- Trabucchi M, Tostivint H, Lihrmann I, Sollars C, Vallarino M, Dores RM, Vaudry H (2002) Polygenic expression of somatostatin in the sturgeon *Acipenser transmontanus*: Molecular cloning and distribution of the mRNAs encoding two somatostatin precursors. J Comp Neurol 443:332–345.
- Trabucchi M, Trudeau VL, Drouin G, Tostivint H, Lihrmann I, Vallarino M, Vaudry H (2008) Molecular characterization and comparative localization of the mRNAs encoding two glutamic acid decarboxylases (GAD65 and GAD67) in the brain of the African lungfish, *Protopterus annectens.* J Comp Neurol 506:979–988.
- Tuinhof R, González A, Smeets WJAJ, Roubos EW (1994) Neuropeptide Y in the developing and adult brain of the South American clawed toad *Xenopus laevis*. J Chem Neur 7:271–283.
- Ueda S, Takeuchi Y, Sano Y (1983) Immunohistochemical demonstration of serotonin neurons in the central nervous system of the turtle (*Clemmys japonica*). Anat Embryol 168:1–19.
- Ulinski PS (1990). Neuronal organization of the striatum in the alligator, *Alligator mississippiensis*. In: The Forebrain in Nonmammals: New Aspects of Structure and Development. (Schwerdtfeger WK, Germoth P, eds), Exp Brain Res Series 19, pp. 119–133: Berlin: Springer-Verlag.
- Vacher C, Pellegrini E, Anglade I, Ferriere F, Saligaut C, Kah O (2003) Distribution of dopamine D2 receptor mRNAs in the brain and the pituitary of female rainbow trout: An *in situ* hybridization study. J Comp Neurol 458:32–45.
- Vallarino M, Danger JM, Fasolo A, Peletier G, Saint-Pierre S, Vaudry H (1988) Distribution and characterization of neuropeptide Y in the brain of an elasmobranch fish. Brain Res 448:67–76.
- Vallarino M, Tranchand-Bunel D, Thoumas JL, Masini MA, Conlon JM, Fournier A, Pelletier G, Vaudry H (1995) Neuropeptide tyrosine in the brain of the African lungfish, *Protopterus annectens*: Immunohistochemical localization and biochemical characterization. J Comp Neurol 356:537–551.
- Vallarino M, Transchand-Bunel D, Thoumas JL, Masini MA, Conlon JM, Fournier A, Pelletier G, Vaudry H (1997a) Neuropeptide tyrosine in the brain of the African Lungfish, *Protopterus annectens*: Immunohistochemical localization and biochemical characterization. J Comp Neurol 356:537–551.
- Vallarino M, Trabucchi M, Masini MA, Chartrel N, Vaudry H (1997b) Immunocytochemical localization of somatostatin and autoradiographic distribution of somatostatin binding sites in the brain of the African lungfish, *Protopterus annectens*. J Comp Neurol 388:337–353.
- Vallarino M, Thoumas JL, Masini MA, Trabucchi M, Chartrel N, Vaudry H (1998) Immunohistochemical localization of enephalins in the brain of the African lungfish, *Protopterus annectens*, provides evidence

for differential distribution of met-enkephalin and leu-enkephalin. J Comp Neurol 396:275–287.

- Vecino E, Covenas R, Alonso JR, Lara J, Aijon J (1989) Immunocytochemical study of substance P-like cell bodies and fibers in the brain of the rainbow trout, *Salmo gairdneri*. J Anat 165:191–200.
- Vecino E, Pinuela C, Arevalo R, Lara J, Alonso JR, Aijon J (1992) Distribution of enkephalin-like immunoreactivity in the central nervous system of the rainbow trout: an immunocytochemical study. J Anat 180:435–453.
- Vecino E, Perez MTR, Ekstrom P (1995) Localization of enkephalinergic neurons in the central nervous system of the salmon (*Salmo salar* L) by in situ hybridization and immunocytochemistry. J Chem Neur 9:81–97.
- Veenman CL, Reiner A (1994) The distribution of GABA-containing perikarya, fibers, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. J Comp Neurol 339:209–250.
- Veenman CL, Reiner A (1996) Ultrastructural study of the targets of cortical afferents in the avian striatum. Brain Res 707:1–12.
- Veenman CL, Karle EJ, Anderson KD, Reiner A (1995a) Thalamostriatal projections neurons in birds utilize LANT6 and neurotensin: A light and electron microscopic double-labeling study. J Chem Neur 9:1–16.
- Veenman CL, Wild JM, Reiner A (1995b) Organization of the avian "Corticostriatal" projection system: A retrograde and anterograde pathway tracing study in pigeons. J Comp Neurol 354:87–126.
- Veenman CL, Medina L, Reiner A (1997) The avian homologues of the mammalian intralaminar, mediodorsal and midline thalamic nuclei: Immunohistochemical and hodological evidence. Brain Behav Evol 49:78–98.
- Venkatesh B, Erdmann MV, Sydney Brenner S (2001) Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. Proc Nat Acad Sci USA 98:11382–11387.
- Vesselkin NP, Ermakova TV, Kenigfest NB, Goikovic M (1980) The striatal connections in frog *Rana temporaria*: An HRP study. J Hirnforschung 21:381–392.
- Voneida TJ, Sligar CM (1979) Efferent projections of the dorsal ventricular ridge and the striatum in the Tegu lizard, *Tupinambis nigropunctatus*. J Comp Neurol 186:43–64.
- Wächtler K, Ebinger P (1989) The pattern of muscarinic acetylcholine receptor binding in the avian forebrain. J Hirnforschung 30:409–414.
- Wada K, Sakaguchi H, Jarvis ED, Hagiwara M (2004) Differential expression of glutamate receptors in avian neuronal pathways for learned vocalizations. J Comp Neurol 476:44–64.
- Westhoff G, Roth G (2002) Morphology and projection pattern of medial and dorsal pallial neurons in the frog *Disoglossus pictus* and the salamander *Plethodon jordani*. J Comp Neurol 445:97–121.
- Wicht H, Himstedt W (1986) Two thalamo-telencephalic pathways in a urodele, *Triturus alpestris*. Neurosci Lett 68:90–94.
- Wicht H, Northcutt RG (1992) The forebrain of hagfish: A cladistic reconstruction of the ancestral craniate forebrain. Brain Behav Evol 40:25–64.
- Wicht H, Northcutt RG (1993) Secondary olfactory projections and pallial topography in the Pacific hagfish, *Eptatretus stouti*. J Comp Neurol 337:529–542.
- Wicht H, Northcutt RG (1994) An immunohistochemical study of the telencephalon and the diencephalon in a myxinoid jawless fish, *Eptatretus stouti*. Brain Behav Evol 43:140–161.
- Wicht H, Northcutt RG (1998) Telencephalic connections in the Pacific hagfish (*Eptatretus stouti*), with special reference to the thalamopallial system. J Comp Neurol 395:245–260.

- Wilczynski W, Northcutt RG (1977) Afferents to the optic tectum of the leopard frog: An HRP study. J Comp Neurol 173:219–230.
- Wilczynski W, Northcutt RG (1983a) Connections of the bullfrog striatum: afferent organization. J Comp Neurol 214:321–332.
- Wilczynski W, Northcutt RG (1983b) Connections of the bullfrog striatum: efferent projections. J Comp Neurol 214:333–343.
- Wild JM (1987a) Thalamic projections of the paleostriatum and neostriatum in the pigeon (*Columba livia*). Neuroscience 20:305–327.
- Wild JM (1987b) The avian somatosensory system: connections of regions of body representation in the forebrain of the pigeon. Brain Res 412:205–223.
- Wild JM (1989) Avian somatosensory system: II. Ascending projections of the dorsal column and external cuneate nuclei in the pigeon. J Comp Neurol 287:1–18.
- Wild JM (1992) Direct and indirect "cortico"-rubral and rubro-cerebellar cortical projections in the pigeon. J Comp Neurol 326:623–636.
- Wild JM, Karten HJ, Frost BJ (1993) Connections of the auditory forebrain in the pigeon (*Columba livia*). J Comp Neurol 337:32–62.
- Wilson CJ (1987) Morphology and synaptic connections of crossed corticostriatal neurons in the rat. J Comp Neurol 263:567–580.
- Wolters JG, Ten Donkelaar HJ, Steinbusch HWM, Verhofstad AAJ (1985) Distribution of serotonin in the brainstem and spinal cord of the lizard Varanus exanthematicus: An immunohistochemical study. Neuroscience 14:169–193.
- Wolters JG, Ten Donkelaar HJ, Verhofstad AAJ (1986) Distribution of some peptides (substance P, [Leu]enkephalin, [Met]enkephalin) in the brainstem and spinal cord of a lizard *Varanus exanthematicus*. Neuroscience 18:917–946.
- Wong CJH (1997) Connections of the basal forebrain of the weakly electric fish, *Eigenmannia virescens*. J Comp Neurol 389:49–64.
- Wright GM (1986) Immunocytochemical demonstration of growth hormone, prolactin and somatostatin-like immunoreactivities in the brain of larval, young adult and upstream migrant adult sea lamprey, *Petromyzon marinus*. Cell Tissue Res 246:23–31.
- Wullimann MF, Mueller T (2004) Teleostean and mammalian forebrains contrasted: Evidence from genes to behavior. J Comp Neurol 475:143–162.
- Wynne B, Güntürkün O (1995) Dopaminergic innervation of the telencephalon of the pigeon (*Columba livia*): A study with antibodies against tyrosine hydroxylase and dopamine. J Comp Neurol 357:446–464.

- Yamada H, Sano Y (1985) Immunohistochemical studies on the serotonin neuron system in the brain of the chicken (*Gallus domesticus*). I. The distribution of the nerve fibers. Biogenic Amines 2:21–36.
- Yamada H, Takeuchi Y, Sano Y (1984) Immunohistochemical studies on the serotonin neuron system in the brain of the chicken (*Gallus domesticus*). I. The distribution of the neuronal somata. Biogenic Amines 1:83–94.
- Yamamoto K, Reiner A (2005) Distribution of the limbic systemassociated membrane protein (LAMP) in pigeon forebrain and midbrain. J Comp Neurol 486:221–242.
- Yamamoto N, Ito H (2000) Afferent sources to the ganglion of the terminal nerve in teleosts. J Comp Neurol 428:355–375.
- Yamamoto N, Ito H (2005) Fiber connections of the anterior preglomerular nucleus in cyprinids with notes on telencephalic connections of the preglomerular complex. J Comp Neurol 491:212–233.
- Yamanaka S, Honma Y, Ueda S, Sano Y (1990) Immunohistochemical demonstration of serotonin neuron system in the central nervous system of the Japanese dogfish *Scyliorhinus torazame* (Chondroichthyes). J Hirnforschung 3:385–397.
- Yamamato N, Ishikawa Y, Yoshimoto M, Xue HG, Bahaxar N, Sawai N, Yang CY, Ozawa H, Ito H (2007) A new interpretation on the homology of the teleostean telencephalon based on hodology and a new eversion model. Brain Behav Evol 69:96–104.
- Yáñez J, Rodriguez-Moldes I, Anadon R (1992) Distribution of somatostatin-immunoreactivity of the larval lamprey (*Petromyzon marinus*). J Chem Neur 5:511–520.
- Zeier H, Karten HJ (1971) The archistriatum of the pigeon: Organization of afferent and efferent connections. Brain Res 31:313–326.
- Zerucha T, Stühmer T, Hatch G, Park BK, Long QM, Yu GY, Gambarotta A, Schultz JR, Rubenstein JLR, Ekker M (2000) A highly conserved enhancer in the *Dlx5/Dlx6* intergenic region is the site of crossregulatory interactions between Dlx genes in the embryonic forebrain. J Neurosci 20:709–721.
- Zhu D, Lustig KH, Bifulco K, Kiefer J (2005) Thalamocortical connections in the pond turtle *Pseudemyscripta elegans*. Brain Behav Evol 65:278–292.
- Zittlau KE, Claas B, Munz H (1988) Horseradish peroxidase study of tectal afferents in *Xenopus laevis* with special emphasis on their relationship to the lateral line system. Brain Behav Evol 32:208–219.

# Cell Types in the Different Nuclei of the Basal Ganglia

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# I. INTRODUCTION

# A. Overview of the Basal Ganglia Nuclei in Rodents and Higher Vertebrates

The basal ganglia can be subdivided into "dorsal" and "ventral" aspects. The dorsal aspect of the basal ganglia in rodents and higher vertebrates consists of four major nuclei (Tepper et al., 2007). These nuclei are the striatum, the globus pallidus, the subthalamic nucleus (STN) and the substantia nigra (SN) (see Fig. 1.1 in Chapter 1). The striatum in rodents is a single nucleus, also called the caudate-putamen. In higher vertebrates the striatum is comprised of the caudate nucleus and the putamen which are partitioned by the internal capsule. The globus pallidus consists of external (GPe) and internal (GPi) segments. In rodents, the external segment is also known as the "globus pallidus" and the internal segment is equivalent to the entopeduncular nucleus. In this chapter, GPe will be used when referring to the rodent globus pallidus and GPi will be used when referring to the rodent entopeduncular nucleus. Each pallidal segment has different afferents and efferents and is functionally unique (see further details below). The SN is also divided into two parts that are functionally distinct. The pars reticulata (SNr) and the pars compacta (SNc) share mostly similar afferents but have different outputs. The main output of the SNr is to the thalamus, while the SNc primarily consists of dopaminergic neurons that principally innervate the striatum. The SNr and GPi are the major output nuclei of the basal ganglia (see Fig. 1.1 in Chapter 1).

The ventral aspect of the basal ganglia consists of the nucleus accumbens, the ventral pallidum, and the medial parts of the STN and SN. The dorsal aspect of the basal ganglia is primarily involved with motor and associative functions, while the ventral aspect is primarily involved with limbic or emotional functions (Tepper et al., 2007).



**FIGURE 3.1** (A) Schematic diagram illustrating the local axonal collaterals of striatal spiny projection neurons in the rat brain. Adapted with permission from Heimer et al. (1995). (B) A horseradish peroxidase-filled rat neostriatal spiny projection neuron with densely spiny dendrites (black) and a projecting axon (grey, upper figure) and local axonal collaterals (grey, lower figure). Reprinted from Wilson and Groves (1980) with permission from John Wiley & Sons, Inc.

In rodents, the dorsal basal ganglia receive a massive input from the cerebral cortex to the largest input structure, the striatum (Webster, 1961; McGeorge and Faull, 1989; Hontanilla et al., 1994) (see Chapter 1). From the striatum several pathways reach other basal ganglia nuclei (Grofova, 1975; Bunney and Aghajanian, 1976; Cuello and Paxinos, 1978; van der Kooy et al., 1981; Haber and Nauta, 1983; Heimer et al., 1985; Loopuijt and van der Kooy, 1985; Kawaguchi et al., 1990; Bevan et al., 1994), namely, the GPe, the GPi, the STN, and the SNr and SNc (see Fig. 1.1 in Chapter 1). These internal loops are thought to regulate the activity of the GPi and SNr, which then convey the output of the basal ganglia to the thalamus and onto the supplementary motor area and premotor area of the frontal cortex (Graybiel, 1990). The circuitry in higher vertebrates is very similar to that of rodents (DeLong, 2000). Since both the input and the output of the dorsal basal ganglia are intimately related to motor (and other) areas of the cerebral cortex, the dorsal basal ganglia are thought to control the direction and the velocity of movement that is initiated by the cerebral cortex (Alexander et al., 1986; Wickens, 1993). The dorsal aspect of the basal ganglia also contributes to associative functions (Tepper et al., 2007).

Within the striatum of rodents and higher vertebrates, there are two types of projection neurons. Since these projection neurons have numerous spines on their dendrites (Fig. 3.1b), and have a medium-sized soma (Table 3.1), they are generally referred to as either spiny projection neurons or medium-spiny neurons. The first type of projection neuron projects to the SNr and GPi to form the direct pathway to the output nuclei of the basal ganglia (see Fig. 1.1 in Chapter 1). These projection neurons of the direct

pathway contain GABA, substance P and dynorphin. The second type of projection neuron projects to the GPe and contains GABA and enkephalin. Since the GPe neurons project in turn to the SNr and GPi, the striatal GABA/ enkephalin neurons form the indirect pathway to the output nuclei of the basal ganglia (Fig. 1.1 in Chapter 1).

In the ventral aspect of the basal ganglia, the nucleus accumbens has a number of striatal characteristics (Heimer et al., 1995). For example, it is innervated by neurons in the cerebral cortex and by midbrain dopaminergic neurons. It also consists principally of spiny projection neurons that project to pallidal neurons. Yet, it is also important to acknowledge that some of the detailed connections do differ [i.e., the pallidal connection is primarily to the ventral pallidum (Heimer et al., 1995)] and there are relatively sparse nigral afferents to the nucleus accumbens (Gerfen et al., 1987a). The shell region of the nucleus accumbens is also unique, in that it also projects to the lateral hypothalamus (Heimer et al., 1995). The ventral pallidum is located inferior to the anterior commissure and receives input from the nucleus accumbens which is mainly substance P-positive (Heimer et al., 1995). The ventral pallidum projects in turn to the SN, as well as other brain regions (Heimer et al., 1995). Additional structural and functional aspects of the ventral part of the basal ganglia are discussed in greater detail in Chapter 21.

# B. Overview of Recent Findings on the Circuitry and Nuclei of the Basal Ganglia

Several discoveries on the circuitry of the basal ganglia and related structures were made over the last decade, and some of these are highlighted in this Section. The principal neurons

Nucleus	Cell type (neurotransmitter)	Size	References
Striatum	Spiny projection neuron (GABA)	Medium, 20–10 µm	Ribak et al., 1979
	Interneuron (acetylcholine)	Large, 35–20µm	Bolam et al., 1984
	Interneuron (somatostatin/nitric oxide synthase/neuropeptide Y/possibly GABA)	Medium, 25–15 µm	Aoki and Pickel, 1988
	Interneuron (GABA/parvalbumin)	Medium, 20–14 $ imes$ 12–11 $\mu$ m	Gerfen et al., 1985; Cowan et al., 1990
	Interneuron (GABA/parvalbumin) (2% subpopulation of GABA/parvalbumin interneurons)	Large, >∼20µm	Cowan et al., 1990
	Interneuron (GABA/calretinin)	Medium, 17–9µm	Bennett and Bolam, 1993
GPe	Projection neuron (GABA)	Large, 28–13 µm	Oertel et al., 1984; Moriizumi and Hattori, 1992
	Projection neuron (acetylcholine)	Large, 26–14µm	Johnston et al., 1979; Ingham et al., 1985
	Interneuron (calretinin/GABA?)	Small, 11–9µm	Cooper and Stanford, 2002
GPi	Projection neuron (GABA)	Medium, $\sim 15-7\mu m$	Oertel et al., 1984; Rajakumar et al., 1994
STN	Projection neuron (glutamate)	Medium, 25–10µm	Chang et al., 1983; Bevan et al., 1994
	Interneuron (GABA)	Size unknown	Mugnaini and Oertel, 1985
SNr	Projection neuron (GABA)	Medium, 22–13 µm	Gonzalez–Hernandez and Rodriguez, 2000
	Projection neuron (dopamine)	Medium, 20–11 µm	German and Manaye, 1993
SNc	Projection neuron (dopamine)	Medium, 22–11 µm	German and Manaye, 1993
	Interneuron (GABA)	Medium, ~20–10μm	Hebb and Robertson, 2000

of the striatum, the GABAergic spiny projection neurons, are connected by local axonal collateral synapses (Wilson and Groves, 1980; Somogyi et al., 1981; Kitai and Wilson, 1982; see Fig. 3.1). Electrophysiological evidence of the existence of local inhibitory synapses between striatal spiny projection neurons, however, remained elusive for 20 years. Only in 2002, two publications provided the first evidence for these synaptic connections (Czubayko and Plenz, 2002; Tunstall et al., 2002) (see Chapter 5). This evidence has since been confirmed by a number of studies (e.g., Gustafson et al., 2006). The existence of local inhibitory connections between striatal spiny projection neurons is critical to increasing knowledge on the structure, computations and functions that are performed by the striatum and the basal ganglia. From a theoretical perspective, local inhibitory synaptic connections are common features of neural network models (Wickens, 1993; Plenz,

2003; Tepper et al., 2004; Wickens et al., 2007), endowing them with computational and learning capacities (Taverna et al., 2008). Aspects of the structural, computational and functional consequences of these local inhibitory connections are discussed further in Section IV.A (see also Chapter 5).

Other recent studies indicate that the lateral habenula (LHb) may play a role in regulating midbrain dopaminergic neurons. In the monkey, a smaller pathway exists from the GPi to the LHb (Nauta, 1974; Herkenham and Nauta, 1977; Nagy et al., 1978), and recent electrophysiological evidence indicates that this connection is involved in reward evaluation rather than motor execution (Hong and Hikosaka, 2008). The LHb may provide a key source of input to the dopaminergic neurons in the SN and the ventral tegmental area (VTA) of the rat (Aghajanian and Wang, 1977; Wang and Aghajanian, 1977; Ji and Shepard, 2007) and the monkey (Matsumoto

and Hikosaka, 2007). This GPi-LHb-SN/VTA pathway may signal non-rewarding or disappointing outcomes (Hong and Hikosaka, 2008; Wickens, 2008). The detailed inhibitory or excitatory pathways that subserve this function require further anatomical and behavioural research (see also Section IV.B).

In the rat, other more extensively characterized inputs to the dopaminergic neurons of the SNc and VTA are derived from the pedunculopontine nucleus (Jackson and Crossman, 1983), the superior colliculus (Comoli et al., 2003), the projection neurons within the patch compartment of the striatum (Gerfen, 1985), and GABA and substance P neurons in general including some derivation from the striatum (Bolam and Smith 1990). The projection neurons within the patch compartment of the striatum are discussed further in Chapter 1. The other inputs are discussed further in Chapters 16 and 23).

The dopaminergic input from the SNc to the striatum (Anden et al., 1964) is one of the best known pathways in the brain. The nigral dopaminergic neurons are known to synapse with the striatal spiny projection neurons (Freund et al., 1984) and some of the striatal interneurons (see Section IV.C below). Progress over the past 10 years indicates that this dopaminergic pathway to the projection neurons is critically involved in reward-related learning (e.g., Reynolds et al., 2001). Dopamine acts principally as a neuromodulator in the striatum via modulation of voltage-gated sodium, potassium and calcium channels in spiny projection neurons. This modulation leads directly to complex and state-dependent changes in striatal neuronal excitability (Surmeier, 2006) (see also Chapters 6 and 12).

In recent years, a second major pathway from the cerebral cortex to the basal ganglia has gained increasing prominence. This is the pathway from the cerebral cortex to the STN (Kita, 1994), which is also know as the corticosubthalamic or hyperdirect pathway. Since the STN regulates the activity of the SNr and GPi neurons, this pathway is considered the fastest route by which cortical information can influence the output nuclei (Tepper et al., 2007). This pathway may also be critically involved in Parkinson's disease, since a lesion of the STN can reduce Parkinsonian symptoms and major L-DOPA-associated motor complications (Steiner et al., 2008). This topic is discussed in detail in Chapters 36 and 39.

# II. PROJECTION NEURONS WITHIN THE DIFFERENT NUCLEI OF THE BASAL GANGLIA

In the adult, the projection neurons of the different nuclei of the dorsal aspect of the basal ganglia are mostly GABAergic inhibitory neurons (Tepper et al., 2007). Glutamatergic excitatory neurons constitute the cortical input to the basal ganglia, as well as the projection neurons of the STN. The functional consequences of this mix of inhibitory and excitatory neurons is discussed below.

Within the striatum of rodents and higher vertebrates there are two main types of projection neurons, the GABA/ substance P/dynorphin neurons of the direct pathway and the GABA/enkephalin neurons of the indirect pathway (see Section I.A). A third putative type of striatal projection neuron may be the large  $(25 \times 16-12 \,\mu\text{m})$  neurons that are located in the ventral region of the rat striatum (Bolam et al., 1981, 1984). Alternatively, these large neurons may be displaced pallidal neurons since they are structurally like GPe neurons and their synaptic input is similar (Bolam, personal communication). Further characterization of these large neurons is needed.

The GPe of higher vertebrates and rodents contains projection neurons which have a large soma and are mainly GABAergic (Table 3.1). There are also scattered clusters of large cholinergic neurons in the ventromedial portion of the rat GPe (Table 3.1). In the monkey the equivalent neurons contribute to the "cholinergic group 4" (Ch4) group of the substantia innominata (Mesulam and Van Hoesen, 1976). There is a similar population of cholinergic neurons in humans (Saper and Chelimsky, 1984). In rats, monkeys and humans these cholinergic neurons project to the cerebral cortex (Mesulam and Van Hoesen, 1976; Saper and Chelimsky, 1984; Ingham et al., 1985) and may integrate limbic and basal ganglia functions (Ingham et al., 1985).

In the rat GPe, recent evidence indicates that the GABAergic projection neurons co-express either calbindin or parvalbumin or neither of these calcium-binding proteins (Cooper and Stanford, 2002). Since calcium-binding proteins are known to have unique buffering kinetics, the differential expression of these proteins may underlie the electrophysiological heterogeneity observed in the rat GPe (Cooper and Stanford, 2002). In living cells, calbindin is considered to be a fast calcium buffer, whereas parvalbumin is a slow-onset calcium buffer (Schwaller et al., 2002). The basis for this difference is that the metal binding sites in calbindin are calcium-specific, whereas those in parvalbumin are also capable of interacting with magnesium (Schwaller et al., 2002). Under resting conditions within a cell parvalbumin is mainly bound to magnesium, but the magnesium ions are displaced by calcium when the intracellular calcium concentration is elevated (Schwaller et al., 2002). Since the dissociation of magnesium from parvalbumin is relatively slow, parvalbumin is therefore considered to be a slow-onset calcium buffer (Schwaller et al., 2002).

The GPi and SNr, the output nuclei of the basal ganglia, are also comprised mainly of medium-sized GABAergic projection neurons (Table 3.1). The GPi consists of two subpopulations of projection neurons. Fluorescent tracer studies in the rat have shown that its rostral neurons project to the LHb in the epithalamus, while the caudal neurons project to the thalamus (Rajakumar et al., 1993). These projections come from the rostral third or caudal two-thirds of the GPi neurons, respectively (Rajakumar et al., 1993). Progress on possible functions of this circuitry are discussed further in Section IV.B.

The SNr consists principally of GABAergic projection neurons and a small subpopulation of dopaminergic projection neurons (Table 3.1). A recent study in the rat has shown that the GABAergic projection neurons express calcium binding proteins, specifically parvalbumin or calretinin or both (Lee and Tepper, 2007). This in contrast to the striatum where these calcium binding proteins are expressed by interneurons (see Section III). The significance of this difference is unclear. Although the GABAergic projection neurons of the SNr are heterogeneous in terms of their calcium binding proteins, the neurons are physiologically and morphologically homogeneous (Lee and Tepper, 2007). This is different from the rat GPe and requires further investigation.

In the rat, the dopaminergic neurons within the SNr and the ventral tier of the SNc innervate the striatal patches and the dopaminergic neurons within the dorsal tier of the SNc innervate the striatal matrix (Gerfen et al., 1987a,b) (for further discussion of the striatal matrix and patch/striosome compartments, see Chapter 1).

In contrast to the GPi and SNr, the STN consists of glutamatergic projection neurons (Table 3.1) which innervate the GABAergic projection neurons of the GPi and SNr (Bevan et al., 1994; Levesque and Parent, 2005). In the primate STN, there may be five different types of projection neurons (Rafols and Fox, 1976). Recent studies have revealed that the five distinctive types are: (i) neurons projecting to the SNr, GPi and GPe; (ii) neurons targeting the SNr and GPe; (iii) neurons projecting to the GPi and GPe; (iv) neurons targeting the GPe only; and (v) neurons sending axons toward the striatum but whose terminal arborization could not be visualized (Parent and Parent, 2007).

The connections among the projection neurons of the dorsal basal ganglia are usually considered functionally in terms of the direct and indirect pathways and the role of the STN. Since the STN neurons are tonically active they are thought to excite the GPi and SNr neurons of the output ganglia, which, in turn, inhibit their thalamic target neurons. This is considered to be the normal "steady-state". The role of the direct pathway neurons (Fig. 1.1 in Chapter 1) is to inhibit the GPi and SNr neurons, which in turn disinhibits the thalamus and allows excitation of the cerebral cortical neurons. Thus the direct pathway is thought to facilitate the generation of a desired movement. The role of the indirect pathway is to remove any inhibition by the GPe neurons on the STN, GPi and SNr (Fig. 1.1 in Chapter 1), which in turn facilitates the inhibition of the thalamus by the GPi and SNr neurons. The precise role of the indirect pathway in movement is debated. It may have a role in inhibiting the undesired or antagonist movement (Alexander and Crutcher, 1990) or it may have a role in terminating the movement associated with the preceding activation of the direct pathway (Alexander and Crutcher, 1990; Smith et al., 1998). The experimental evidence appears to favor the latter view (see review by Smith et al., 1998).

### III. INTERNEURONS WITHIN THE NUCLEI OF THE BASAL GANGLIA

The number of interneurons in the nuclei of the dorsal aspect of the basal ganglia is generally small, or seemingly nonexistent for the GPi. In spite of the relatively small number of interneurons, their role(s) are likely to be functionally profound (see further details in Section IV.C for the striatum).

Four major types of interneurons have been identified within the striatum (Kawaguchi et al., 1995). These are the large cholinergic interneurons, and the medium-sized GABA/parvalbumin interneurons, GABA/calretinin interneurons, and somatostatin/neuropeptide Y/nitric oxide synthase/possibly GABA interneurons (Table 3.1). Within the striatum, it is well known that the cholinergic, GABA/parvalbumin, and somatostatin/neuropeptide Y interneurons innervate the spiny projection neurons (see review by Kawaguchi et al., 1995; Oorschot, 2000). Whether the GABA/calretinin neurons innervate the spiny projection neurons remains to be investigated. All striatal interneurons are aspiny in comparison to the striatal spiny projection neurons.

Within the output nuclei of the basal ganglia, there may be a low number of putative interneurons in the SNr (Deniau et al., 2007) and appear to be no interneurons in the GPi. In the GPe, small calretinin-positive interneurons have recently been identified (Cooper and Stanford, 2002; Table 3.1). Within the STN, GABA interneurons have been observed in the primate (Rafols and Fox, 1976) and rat (Oertel and



**FIGURE 3.2** Schematic diagram illustrating the unilateral total number of neurons within each subdivision of the rat basal ganglia in a parasagittal section. Note that the entopeduncular nucleus is the equivalent of the GPi. The respective location and size of each basal ganglia subdivision is also shown. Note also that the hippocampus has been omitted for clarity. Reprinted from Oorschot (1996) with permission from John Wiley & Sons, Inc.

Mugnaini, 1984; Mugnaini and Oertel, 1985; Table 3.1), and more recently in the human (Levesque and Parent, 2005). A small subpopulation of GABAergic neurons, which are possibly interneurons, have also been identified in the SNc (Hebb and Robertson, 2000; Table 3.1). Specific roles of each type of interneuron in these basal ganglia remain to be elucidated.

# IV. ABSOLUTE NUMBERS OF NEURONS IN THE BASAL GANGLIA: FUNCTIONAL IMPLICATIONS

Anatomical and electrophysiological evidence of the last 10–15 years has increased our understanding of the circuitry of the basal ganglia. Two examples of this increased knowledge is new data on the absolute number of neuronal cell types within each nucleus (Oorschot, 1996; Hardman et al., 2002) and electrophysiological evidence of inhibitory interactions between nearby striatal spiny projection neurons (Czubayko and Plenz, 2002; Tunstall et al., 2002; Gustafson et al., 2006).

# A. Absolute Number of Projection Neurons in the Striatum and its Targets

There is a noticeable trend in rodents and higher vertebrates that the striatum contains far more output neurons



**FIGURE 3.3** A schematic illustration of the original domain hypothesis. Reprinted from Wickens et al. (1995) with permission from John Wiley & Sons, Inc.

(i.e., medium-sized, spiny projection neurons) than the total number of neurons in all its targets. For example, in the rat there are 29 times more striatal output neurons compared with all the pallidal and nigral target neurons (Oorschot, 1996; Fig. 3.2). The resulting anatomical convergence, in both the rat and the human, has been interpreted as a loss of information through the basal ganglia. However, the domain hypothesis (Wickens, 1993; Wickens et al., 1995) challenged this interpretation.

The domain hypothesis in its original form (Wickens, 1993) stated that each domain is a population of spiny projection neurons that have mutually inhibitory connections. As part of this hypothesis, it was also proposed that a spiny projection neuron may inhibit all nearby spiny projection neurons via its local axonal collaterals (Figs 3.1, 3.2, 3.3;

Wickens et al., 1995). In the human, there are thought to be, bilaterally, 100 million medium-sized spiny striatal projection neurons, with a (shrinkage-corrected) N<sub>v</sub> of 11,000 per mm<sup>3</sup> (Lange et al., 1976). Assuming a radius of contact inhibition for each striatal projection neuron of 250 µm, Wickens (1993) calculates the number of neurons in a human striatal inhibitory domain to be 720 (i.e., number in a domain = N<sub>v</sub>.4 $\pi$ /3.r<sup>3</sup>), and the total number of domains to be 139,000. This latter number is remarkably similar to estimates of the number of neurons in the human GPi (157,000) and SNr (160,000; Percheron et al., 1987) and is given the interpretation that information is preserved in this pathway (Wickens, 1993).

For the rat, the radius of contact inhibition for each striatal projection neuron is likely to be 100 µm (Penny et al., 1988; Kawaguchi et al., 1989). Coupled with the total number of medium-sized neurons in the neostriatum of 5.58 million (bilaterally; Fig. 3.2), and their (shrinkage-corrected)  $N_{y}$ of 84,900 per mm<sup>3</sup>, the number of neurons in a rat domain would be 356 and the total number of domains would be 15,674 (Oorschot, 1996). This number of domains is only 2.4 times the total number of entopeduncular neurons (6400; bilaterally; Fig. 3.2) and 0.3 of the total number of SNr neurons (52,600; bilaterally; Fig. 3.2), thereby implicating that information may also be preserved in the rat basal ganglia pathway (Oorschot, 1996). Thus, a circuit which is somewhat perplexing in terms of information theory at the macroscopic level, becomes less perplexing in the context of the domain hypothesis (Wickens, 1993; Wickens et al., 1995).

Electrophysiological evidence gained in the last 10 years indicates, however, that the local circuit diagram for striatal spiny projection neurons may be more complex. For example, only 1 in 5 pairs of neighboring rat spiny projection neurons may be synaptically connected and the amplitude of an inhibitory postsynaptic potential can be small (Tunstall et al., 2002). By contrast, the amplitude of an inhibitory postsynaptic potential between a rat GABA/ parvalbumin interneuron and a spiny projection neuron is larger (Koos and Tepper, 1999). Nonetheless, some inhibitory postsynaptic potentials between spiny projection neurons can be just as large as that recorded between a GABA/parvalbumin neuron and a spiny projection neuron (Koos and Tepper, 1999; Gustafson et al., 2006). In addition, smaller inhibitory postsynaptic potentials from a spiny projection neuron can effectively inhibit nearby spiny projection neurons in realistic computer models (Oorschot et al., 2002; Wickens et al., 2007). Taken together, the local inhibition of spiny projection neurons by nearby spiny projection neurons and by GABA/parvalbumin neurons may still yield domains of inhibition that allow information to be preserved in the basal ganglia pathway. Specifically, the spiny projection neurons may modulate, via inhibition, the timing of action potentials generated by nearby spiny projection neurons during their up-state (Oorschot et al., 2002). The aspiny neostriatal GABA/parvalbumin interneurons may delay the onset of cortically driven action potential firing at the start of the up-state (Oorschot et al., 2002).

An alternative interpretation for the role of striatal spiny projection neurons is that they act principally on postsynaptic dendrites to modulate local dendritic excitability as well as the overall level of activity of spiny projection neurons (Tepper et al., 2008). Clarification of this will require electron microscopic studies on the postsynaptic location of synapses between identified spiny projection neurons.

# B. Absolute Number of GPe, GPi, SNr and STN Neurons

In the rat, there are, on average and unilaterally, 46,000 GPe projection neurons, 3,200 GPi projection neurons, 26,300 SNr neurons and 13,600 STN neurons (Oorschot, 1996; see Fig. 3.2). This data has been confirmed in the rat and quantified in other species (Hardman et al., 2002).

As discussed above, the GPi consists of two subpopulations of projection neurons. Fluorescent tracer studies in the rat have shown that its rostral neurons project to the LHb in the epithalamus while the caudal neurons project to the thalamus (Rajakumar et al., 1993). This study also showed that these projections come from the rostral third or caudal two-thirds of the GPi neurons, respectively (Rajakumar et al., 1993). Recent total number data for the rat LHb (Zhang and Oorschot, 2006) has provided greater insight into information processing in the GPi/LHb/SNc pathway. Based on the unilateral total number of GPi neurons in the rat of 3200 to 6300 neurons (Oorschot, 1996; Hardman et al., 2002; Fig. 3.2), approximately 1000 to 2000 GPi neurons may project to the unilateral LHb, which is primarily to the lateral LHb (L-LHb; Nauta, 1974; Herkenham and Nauta, 1977; Nagy et al., 1978). In the rat, 13,000 neurons are within a unilateral LHb (Zhang and Oorschot, 2006). Half of these neurons, namely 6500, might be located within the L-LHb. This indicates that about 6500 L-LHb neurons can receive or be innervated by about 1000 to 2000 GPi neurons. This suggests that all the information conveyed by one third of the neurons in the GPi can be preserved in this pathway (Zhang and Oorschot, 2006).

Furthermore, the medial LHb (M-LHb) and the L-LHb neurons both project to the SNc (Wang and Aghajanian, 1977; Aghajanian and Wang, 1977). Quantitative studies have shown that the total number of neurons in one rat SNc is 7,200 (Oorschot, 1996; Fig. 3.2) and that there are 13,700 neurons in both SNc of the normal adult mice (Chadi et al., 1993). Thus, the total information from a unilateral GPi and L-LHb can be received by a unilateral SNc even if there exists point-to-point connection between them. Therefore, it is suggested that the information from the GPi and LHb might be preserved in this pathway (Zhang and Oorschot, 2006) and that there is unlikely to be loss of information from the basal ganglia to their targets as was originally thought between the striatum and its nigral and pallidal targets (see Oorschot, 1996, and Section IV.A).

It needs to be recognized, however, that this important total neuronal number data (Zhang and Oorschot, 2006) is a first step in understanding information processing. Other data are now needed before firm conclusions can be made. The data now needed includes the total number of synaptic connections made by an afferent neuron, the spatial extent and local configuration of an axonal arborization, physiological considerations about how information is encoded in spike trains, and the number of converging afferents that are required to generate a decisive effect on a postsynaptic neuron (Zhang and Oorschot, 2006).

For the GPe, approximately one-quarter to one-third of the GPe neurons also innervate the striatal GABAergic/parvalbumin interneurons (Bevan et al., 1998). The GPe is thus in a position to provide some sort of spatiotemporal selection of these neurons and, indirectly, of their target striatal spiny projection neurons (Tepper et al., 2007). This in turn may modify the flow of cortical information through the striatum and the basal ganglia (Bolam et al., 2000). This is in addition to the role of the GPe in regulating the STN and the output nuclei of the basal ganglia, the SNr and GPi (see Section I).

#### C. Absolute Number of Interneurons

Over the past 10–15 years, the absolute number of interneuronal subtypes within the rat striatum has been quantified using modern stereological methods. These studies have revealed that there are, unilaterally, 21,300 somatostatin/ neuropeptide Y/possibly GABA interneurons (West et al., 1996), 12,200 cholinergic interneurons (Oorschot et al., 1999), 16,900 GABA/parvalbumin interneurons (Luk and Sadikot, 2001) and 13,200 GABA/calretinin interneurons (Rymar et al., 2004). When compared to the total unilateral

number of medium-sized striatal projection neurons of 2,791,000 (Oorschot, 1996; Fig. 3.2), these data suggest that less than 3% of rat striatal neurons are interneurons (Oorschot et al., 2002; Rymar et al., 2004).

Using the known anatomy, biochemistry, pharmacology and physiology of the striatal projection neurons and interneurons (e.g. Kawaguchi et al., 1995), it has been proposed (Oorschot, 2000) that the major role of the interneurons and the nigral dopaminergic input within the striatum may be as follows: (i) the GABAergic interneurons may assist in the inhibition of the vast majority of spiny projection neurons which are silent at any one point in time as proposed by the domain hypothesis; (ii) the somatostatin/neuropeptide Y/nitric oxide synthase interneurons may excite the "winning" spiny projection neurons across a number of domains and inhibit the non-firing neurons and regulate local blood flow at any one point in time; (iii) the nigral dopaminergic input may drive the "winning" spiny projection neurons by increasing competition within a neostriatal domain at any one point in time; and (iv) the role of the cholinergic interneurons may occur after a set of spiny projection neurons, each in a different domain, have fired. In this context, the role of the cholinergic interneurons may be to "reset the domains" in readiness for the next movement sequence. In other words, their role may be to "restore a level playing field" by decreasing the competition that has been generated by the previous firing sequence (Oorschot, 2000). This proposal suggests an underlying functional coherence to the specific neuronal cell types within the striatum. It is likely, however, to be an oversimplistic account of biological reality. Nonetheless it provides a framework for further research on the specific details of the anatomical microcircuitry and the biophysical consequences of each part of the circuitry.

The striatal interneurons are discussed in further detail in Chapters 7 and 8.

### V. GLIAL CELL TYPES WITHIN THE DIFFERENT NUCLEI

The three major types of glial cells within the central nervous system, namely astrocytes, oligodendrocytes, and resting microglia, reside within all the nuclei of the dorsal and ventral aspects of the basal ganglia. Stereological studies over the past 10–15 years have revealed the total number of astrocytes within some nuclei of the dorsal aspect of the basal ganglia. The relationship between the total number of astrocytes and the characteristics of the resident neurons within each of these ganglia has proven insightful.

# A. Absolute Number of Glial Cells: Neuronto-Astrocyte Ratios in some of the Basal Ganglia Nuclei of the Rat

Astrocytes are thought to have a pivotal role in equilibrating the extracellular ionic environment after neuronal activity (Walz, 1989; White et al., 1992). The number of astrocytes per neuron possibly reflects a number of neuronal characteristics, including neuronal size, neuronal activity rate and active neuron location. These three neuronal characteristics vary, sometimes markedly, between the rat basal ganglia. For example, in the rat striatum only a minority of the predominant medium-sized spiny neurons (Table 3.1) are thought to be active at any one time, with active neurons widely separated (Wilson and Groves, 1981; Wickens, 1993). By contrast, the SN consists of medium-size projection neurons that are larger (Table 3.1) and exhibit tonic or pacemaker-like activity (Nakanishi et al., 1987; Lacey, 1993). The pacemaker-like activity for the SN is evident for both the dopamine neurons of the SNc (Lacey, 1993) and the GABA neurons of the SNr (Nakanishi et al., 1987). It is therefore pertinent to ask: Do neuron-to-astrocyte ratios reflect characteristics of the resident neurons?

Neuron-to-astrocyte ratios can be derived from recent studies on the absolute number of neurons and astrocytes within two adult rat basal ganglia, the striatum and the SN. In the normal, rat striatum, there are unilaterally 2,791,000 medium-spiny neurons (Oorschot, 1996; Fig. 3.2) and unilaterally 35,000 astrocytes that stain positively with glial fibrillary acidic protein (GFAP; Gomide et al., 2005). This yields a neuron-to-astrocyte ratio of approximately 80 to one. By contrast, there are 33,500 neurons in total in the unilateral SN (Oorschot, 1996; Fig. 3.2) and 25,500 GFAP-positive astrocytes unilaterally (Janson and Møller, 1993). This yields a neuron-to-astrocyte ratio of approximately 1.3 to one. It is thus evident that the striatum has an abundance of neurons in comparison to astrocytes, while the SN consists of approximately the same number of astrocytes and neurons. These findings suggest that neuron-to-astrocyte ratios do reflect characteristics of the resident neurons within the rat striatum and SN.

In particular, neuron-to-astrocyte ratios within these ganglia seem to reflect neuronal size, neuronal activity rate and active neuron location. The supporting evidence for this interpretation is as follows: In terms of neuronal size, the SN (Juraska et al., 1977) consists of far more larger (greater than  $20 \mu m$ ) neurons relative to the striatum, where almost all the 2.85 million neurons in one hemisphere

are medium-sized,  $10-20\,\mu$ m, neurons (Oorschot, 1996; Oorschot et al., 2002; Table 3.1). It seems reasonable that the presence of relatively more larger neurons requires a greater number of astrocytes per neuron since this would ensure that ionic conditions along the entire surface area of each large neuron are adequately controlled.

With respect to neuronal activity rate, the dominant population of GABA neurons within the SNr are able to fire spontaneously at high frequency and are tonically activated by excitatory glutamatergic neurons of the STN (Nakanishi et al., 1987; Bevan et al., 1994). Within the SNc, its dominant population of dopamine neurons seem to sustain pacemaker-like firing that is intrinsically generated, as well as bursting activity when stimulated (Lacey, 1993). By contrast, only a minority of the predominant population of GABA medium-spiny neurons within the striatum are thought to be activated at any one point in time (Wickens, 1993). It seems that each striatal medium-spiny neuron requires the relatively rare occurrence of temporally coincident activity in a large number of its glutamatergic afferent axons in order to be depolarized sufficiently to generate an action potential (Wilson and Groves, 1981). The striatal medium-spiny neurons also have a low intrinsic spontaneous activity (Wilson and Groves, 1981). It seems reasonable that these marked differences in activity rate result in far fewer astrocytes for the relatively inactive striatal neurons and a much greater number of astrocytes per neuron for the substantially more active nigral neurons. With respect to active neuron location, the neurons that are active within the striatum at any one time are thought to be widely separated from each other (Wickens, 1993).

It seems therefore that the SN may have a much higher astrocyte number per neuron because its neurons are relatively larger and tonically active. The striatum seems to have a much lower number of astrocytes per neuron, because the medium-spiny neurons within it that are active at any one time are not large, clustered nor constantly active.

Further research is now needed on whether neuron-toastrocyte ratios reflect characteristics of the resident neurons in the rat GPe, STN and GPi. This data is also needed for other species.

# VI. CONCLUSIONS: THE PAST AND THE NEXT 10–15 YEARS

Considerable knowledge on the different types of neurons and interneurons in the basal ganglia has been gained in the past 10-15 years. There has also been an advance in

knowledge of the absolute number of neurons, interneurons and astrocytes within the dorsal aspect of the basal ganglia, as well as the absolute number of neurons in associated structures such as the LHb, using modern stereological methods. The electrophysiological detection of local inhibitory connections between neighboring neostriatal spiny projection neurons has been another major advance in 2002. In combination, this anatomical and electrophysiological knowledge has advanced understanding of information processing within the basal ganglia and associated structures. Major investigations are now needed to unravel the detailed synaptic circuitry between the projection neurons of the basal ganglia nuclei, and between the interneurons and projection neurons within each nuclei. Progress may be facilitated by recent technical advances in serial sectioning and image collection for electron microscopy, and in two photon microscopy.

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#### REFERENCES

- Aghajanian GK, Wang RY (1977) Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique. Brain Res 122:229–242.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–270.
- Alexander GE, De Long MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Ann Rev Neurosci 9:357–381.
- Anden NE, Carlsson A, Dahlstrom A, Fuxe K, Hillarp NA, Larsson K (1964) Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sciences 3:523–530.
- Aoki C, Pickel VM (1988) Neuropeptide Y-containing neurons in the rat striatum: Ultrastructure and cellular relations with tyrosine hydroxylase-containing terminals and with astrocytes. Brain Res 459:205–225.
- Bennett BD, Bolam JP (1993) Characterization of calretininimmunoreactive structures in the striatum of the rat. Brain Res 609:137–148.
- Bevan MD, Bolam JP, Crossman AR (1994) Convergent synaptic input from the neostriatum and the subthalamus onto identified nigrothalamic neurons in the rat. Eur J Neurosci 6:320–334.
- Bevan MD, Booth PAC, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bolam JP, Somogyi P, Totterdell S, Smith AD (1981) A second type of striatonigral neuron: A comparison between retrogradely labelled and Golgi-stained neurons at the light and electron microscopic levels. Neurosci 6:2141–2157.

- Bolam JP, Wainer BH, Smith AD (1984) Characterisation of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. Neurosci 12:711–718.
- Bolam JP, Smith Y (1990) The GABA and substance P input to dopaminergic neurons in the substantia nigra of the rat. Brain Res 529:57–78.
- Bolam JP, Hanley JJ, Booth PAC, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196:527–542.
- Bunney BS, Aghajanian GK (1976) The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res 117:423–435.
- Chadi G, Møller A, Rosén L, Janson A, Agnati L, Goldstein M, Ögren S-O, Pettersson R, Fuxe K (1993) Protective actions of human recombinant basic fibroblast growth factor on MPTP-lesioned nigrostriatal dopamine neurons after intraventricular infusion. Exp Brain Res 97:145–158.
- Chang HT, Kita H, Kitai ST (1983) The fine structure of the rat subthalamic nucleus: An electron microscopic study. J Comp Neurol 221:113–123.
- Comoli E, Coizet V, Boyes J, Bolam JP, Canteras NS, Quirk RH, Overton PG, Redgrave P (2003) A direct projection from superior colliculus to substantia nigra for detecting salient visual events. Nat Neurosci 6:974–980.
- Cooper AJ, Stanford IM (2002) Calbindin D-28k positive projection neurons and calretinin positive interneurons of the rat globus pallidus. Brain Res 929:243–251.
- Cowan RL, Wilson CJ, Emson PC, Heizmann CW (1990) Parvalbumincontaining GABAergic interneurons in the rat neostriatum. J Comp Neurol 302:197–205.
- Cuello AC, Paxinos G (1978) Evidence for a long leu-enkephalin striatopallidal pathway in the rat brain. Nature 271:178–180.
- Czubayko U, Plenz D (2002) Fast synaptic transmission between striatal spiny projection neurons. Proc Natl Acad Sci USA 99:15764–15769.
- DeLong MR (2000) The basal ganglia. In: Principles in Neural Science, 4th edn (Kandel ER, Schwartz JH, Jessell TM, eds), pp. 853–867. New York: McGraw-Hill.
- Deniau JM, Mailly P, Maurice N, Charpier S (2007) The pars reticulata of the substantia nigra: a window to basal ganglia output. Prog Brain Res 160:151–172.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylaseimmunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neurosci 13:1189–1215.
- Gerfen CR (1985) The neostriatal mosaic. 1. Compartmental organization of projections from the striatum to the substantia nigra in the rat. J Comp Neurol 236:454–476.
- Gerfen CR, Baimbridge KG, Miller JJ (1985) The neostriatal mosaic: Compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. Proc Natl Acad Sci USA 82:8780–8784.
- Gerfen R, Herkenham M, Thibault J (1987a) The neostriatal mosaic. II. Patch- and matrix-directed mesostriatal dopaminergic and nondopaminergic systems. J Neurosci 7:3915–3934.
- Gerfen R, Baimbridge KG, Thibault J (1987b) The neostriatal mosaic. III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7:3935–3944.
- German DC, Manaye KF (1993) Midbrain dopaminergic neurons (nuclei A8, A9, and A10): Three-dimensional reconstruction in the rat. J Comp Neurol 331:297–309.

- Gomide V, Bibancos T, Chadi G (2005) Dopamine cell morphology and glial cell hypertrophy and process branching in the nigrostriatal system after striatal 6-OHDA analyzed by specific sterological tools. Int J Neurosci 115:557–582.
- Gonzalez-Hernandez T, Rodriguez M (2000) Compartmental organization and chemical profile of dopaminergic and GABAergic neurons in the substantia nigra of the rat. J Comp Neurol 421:107–135.
- Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254.
- Grofova I (1975) The identification of striatal and pallidal neurons projecting to the substantia nigra: An experimental study by means of retrograde axonal transport of horseradish peroxidase. Brain Res 91:286–291.
- Gustafson N, Gireesh-Dharmaraj E, Czubayko U, Blackwell KT, Plenz D (2006) A comparative voltage and current-clamp analysis of feedback and feedforward synaptic transmission in the striatal microcircuit in vitro. J Neurophysiol 95:737–752.
- Haber S, Nauta WJH (1983) Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry. Neurosci 9:245–260.
- Hardman CD, Henderson JM, Finkelstein DI, Horne MK, Paxinos G, Halliday GM (2002) Comparison of the basal ganglia in rats, marmosets, macaques, baboons, and humans: Volume and neuronal number for the output, internal relay, and striatal modulating nuclei. J Comp Neurol 445:238–255.
- Hebb MO, Robertson HA (2000) Identification of a subpopulation of substantia nigra pars compacta gamma-aminobutyric acid neurons that is regulated by basal ganglia activity. J Comp Neurol 416:30–44.
- Heimer L, Alheid GF, Zaborszky L (1985) Basal ganglia. In: The Rat Nervous System (Paxinos G ed), pp. 37–86. North Ryde: Academic Press.
- Heimer L, Zahm DS, Alheid GF (1995) Basal ganglia. In: The Rat Nervous System (Paxinos G ed), pp. 579–628. San Diego: Academic Press.
- Herkenham M, Nauta WJH (1977) Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study with a note of the fiber-of-passage problem. J Comp Neurol 173:123–146.
- Hong S, Hikosaka O (2008) The globus pallidus sends reward-related signals to the lateral habenula. Neuron 60:720–729.
- Hontanilla B, de las Heras S, Giménez-Amaya JM (1994) Organization of the striatal projections from the rostral caudate nucleus to the globus pallidus, the entopeduncular nucleus, and the pars reticulata of the substantia nigra in the cat. Anat Rec 238:114–124.
- Ingham CA, Bolam JP, Wainer BH, Smith AD (1985) A correlated light and electron microscopic study of identified cholinergic basal forebrain neurons that project to the cortex in the rat. J Comp Neurol 239:176–192.
- Jackson A, Crossman AR (1983) Nucleus tegmenti pedunculopontinus: Efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase. Neurosci 10:725–765.
- Janson AM, Møller A (1993) Chronic nicotine treatment counteracts nigral cell loss induced by a partial mesodiencephalic hemitran section: An analysis of the total number and mean volume of neurons and glia in the substantia nigra of the male rat. Neurosci 57: 931–941.
- Ji H, Shepard PD (2007) Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA<sub>A</sub> receptor-mediated mechanism. J Neurosci 27:6923–6930.
- Johnston MV, McKinney M, Coyle JT (1979) Evidence for a cholinergic projection to neocortex from neurons in basal forebrain. Proc Natl Acad Sci USA 76:5392–5396.

- Juraska JM, Wilson CJ, Groves PM (1977) The substantia nigra of the rat: A Golgi study. J Comp Neurol 172:585–600.
- Kawaguchi Y, Wilson CJ, Emson PC (1989) Intracellular recording of identified neostriatal patch and matrix spiny neurons in a slice preparation preserving cortical inputs. J Neurophysiol 62:1052–1068.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 10:3421–3438.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurons: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.
- Kita H (1994) Physiology of two disynaptic pathways from the sensorimotor cortex to the basal ganglia output nuclei. In: The Basal Ganglia IV (Percheron G ed), pp. 263–276. New York: Plenum Press.
- Kitai ST, Wilson CJ (1982) Intracellular labeling of neurons in the mammalian brain. In: Cytochemical Methods in Neuroanatomy (Chan-Palay V, Palay SL, eds), pp. 533–549. New York: Alan R. Liss.
- Koos T, Tepper JM (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nature Neurosci 2:467–472.
- Lacey MG (1993) Neurotransmitter receptors and ionic conductances regulating the activity of neurons in substantia nigra pars compacta and ventral tegmental area. Prog Brain Res 99:251–276.
- Lange H, Thörner G, Hopf A, Schröder KF (1976) Morphometric studies of the neuropathological changes in choreatic disease. J Neurol Sci 28:401–425.
- Lee CR, Tepper JM (2007) Morphological and physiological properties of parvalbumin- and calretinin-containing gamma-aminobutyric acidergic neurons in the substantia nigra. J Comp Neurol 500:958–972.
- Levesque JC, Parent A (2005) GABAergic interneurons in human subthalamic nucleus. Mov Disord 20:574–584.
- Loopuijt LD, van der Kooy D (1985) Organization of the striatum: collateralization of its efferent axons. Brain Res 348:86–99.
- Luk KC, Sadikot AF (2001) GABA promotes survival but not proliferation of parvalbumin-immunoreactive neurons in rodent neostriatum: An *in vivo* study with stereology. Neurosci 104:93–103.
- Matsumoto M, Hikosaka O (2007) Lateral habenula as a source of negative reward signals on dopamine neurons. Nature 447:1111–1115.
- McGeorge AJ, Faull RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat.. Neurosci 29:503–537.
- Mesulam MM, Van Hoesen GW (1976) Acetylcholinesterase-rich projections from the basal forebrain of the rhesus monkey to neocortex. Brain Res 109:152–157.
- Moriizumi T, Hattori T (1992) Separate neuronal populations of the rat globus pallidus projecting to the subthalamic nucleus, auditory cortex and pedunculopontine tegmental area. Neurosci 46:701–710.
- Mugnaini E, Oertel WH (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry part 1. *In* Handbook of Chemical Neuroanatomy: GABA and Neuropeptides in the CNS, vol. 4 (Björklund A, Hökfelt T, eds), pp. 436–608: Elsevier Science Publishers. Amsterdam.
- Nagy JI, Carter DA, Lehmenn J, Fibiger HC (1978) Evidence for a GABA-containing projection from the entopeduncular nucleus to the lateral habenular in the rat. Brain Res 145:360–364.
- Nakanishi H, Kita H, Kitai ST (1987) Intracellular study of rat substantia nigra pars reticulata neurons in an *in vitro* slice preparation: Electrical membrane properties and response characteristics to subthalamic stimulation. Brain Res 437:45–55.
- Nauta WJH (1974) Evidence of a pallidohabenular pathway in the cat. J Comp Neurol 156:19–28.

- Oertel WH, Mugnaini E (1984) Immunocytochemical studies of GABAergic neurons in rat basal ganglia and their relations to other neuronal systems. Neurosci Lett 47:233–238.
- Oertel WH, Nitsch C, Mugnaini E (1984). Immunocytochemical demonstration of the GABA-ergic neurons in rat globus pallidus and nucleus entopeduncularis and their GABA-ergic innervation. *In* Advances in Neurology: Parkinson-Specific Motor and Mental Disorders (Hassler RG, Christ JF, eds)vol. 40, pp. 91–98: Raven Press. New York.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereolo gical study using the Cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Oorschot DE, Zhang R, Wickens JR (1999) Absolute number and threedimensional spatial distribution of rat neostriatal large interneurons: A first and second order stereological study. Proc Xth Int Congr Stereol:87.
- Oorschot DE (2000) The domain hypothesis: A central organising principle for understanding neostriatal circuitry?. In: Conceptual Advances in Brain Research, Brain Dynamics and the Striatal Complex (Miller R, Wickens JR, eds), pp. 151–163. Reading: Gordon and Breach.
- Oorschot DE, Tunstall MJ, Wickens JR (2002) Local connectivity between striatal spiny projection neurons: A re-evaluation. In: The Basal Ganglia VII (Nicholson L, Faull RLM, eds), pp. 421–434. New York: Plenum Press.
- Parent M, Parent A (2007) The microcircuitry of primate subthalamic nucleus.. Parkinsonism Relat D 13:S292–S295.
- Penny GR, Wilson CJ, Kitai ST (1988) Relationship of the axonal and dendritic geometry of spiny projection neurons to the compartmental organization of the neostriatum. J Comp Neurol 269:275–289.
- Percheron G, Francois C, Yelnik J (1987) Spatial organization and information processing in the core of the basal ganglia. In: The Basal Ganglia II (Carpenter MB, Jayaraman A, eds), pp. 205–226. New York: Plenum Press.
- Plenz D (2003) When inhibition goes incognito: feedback interaction between spiny projection neurons in striatal function. Trends Neurosci 26:436–443.
- Rafols JA, Fox CA (1976) The neurons in the primate subthalamic nucleus: A Golgi and electron microscopic study. J Comp Neurol 168:75–111.
- Rajakumar N, Elisevich K, Flumerfelt BA (1993) Compartmental origin of the striato-entopeduncular projection in the rat. J Comp Neurol 331:286–296.
- Rajakumar N, Elisevich K, Flumerfelt BA (1994) Parvalbumin-containing GABAergic neurons in the basal ganglia output system of the rat. J Comp Neurol 350:324–336.
- Reynolds JNJ, Hyland BIH, Wickens JR (2001) A cellular mechanism of reward-related learning. Nature 413:67–70.
- Ribak CE, Vaughn JE, Roberts E (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. J Comp Neurol 187:261–283.
- Rymar VV, Sasseville R, Luk KC, Sadikot AF (2004) Neurogenesis and stereological morphometry of calretinin-immunoreactive GABAergic interneurons of the neostriatum. J Comp Neurol 469:325–339.
- Saper CB, Chelimsky TC (1984) A cytoarchitectonic and histochemical study of nucleus basalis and associated cell groups in the normal human brain. Neurosci 13:1023–1037.
- Schwaller B, Meyer M, Schiffmann S (2002) "New" functions for "old" proteins: The role of the calcium-binding proteins calbindin D-28K, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. Cerebellum 1:241–258.
- Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neurosci 86:353–387.

- Somogyi P, Bolam JP, Smith AD (1981) Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscopic study using the Golgi-peroxidase transportdegeneration procedure. J Comp Neurol 195:567–584.
- Steiner B, Kupsch A, Siebert E, Hosmann K, Klempin F, Morgenstern R, Winter C (2008) Unilateral lesion of the subthalamic nucleus transiently provokes bilateral subacute glial cell proliferation in the adult rat substantia nigra. Neurosci Lett 430:103–108.
- Surmeier DJ (2006) Microcircuits in the striatum: Cell types, intrinsic properties and neuromodulation. In: Microcircuits – The Interface Between Neurons and Global Brain Function (Grillner S, Graybiel AM, eds), pp. 105–112. Cambridge: MIT Press.
- Taverna S, Ilijic E, Surmeier DJ (2008) Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's Disease. J Neurosci 28:5504–5512.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tepper JM, Abercrombie ED, Bolam JP (2007) Basal ganglia macrocircuits. Prog Brain Res 160:3–7.
- Tepper JM, Wilson CJ, Koos T (2008) Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons. Brain Res Rev 58:272–281.
- Tunstall MJ, Oorschot DE, Kean A, Wickens JR (2002) Inhibitory interactions between spiny projection neurons in the rat striatum. J Neurophysiol 88:1263–1269.
- van der Kooy D, Hattori T, Shannak K, Hornykiewicz O (1981) The pallido-subthalamic projection in the rat: Anatomical and biochemical studies. Brain Res 204:253–268.
- Wang RY, Aghajanian GK (1977) Physiological evidence for habenular as major link between forebrain and midbrain raphe. Science 197:89–91.
- Walz W (1989) Role of glial cells in the regulation of the brain ion microenvironment. Prog Neurobiol 33:309–333.
- Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95:532–544.
- West MJ, Østergaard K, Andreassen OA, Finsen B (1996) Estimation of the number of somatostatin neurons in the striatum: an in situ hybridization study using the optical fractionator method. J Comp Neurol 370:11–22.
- White HS, Chow SY, Yen-Chow YC, Woodbury DM (1992) Effect of elevated potassium on the ion content of mouse astrocytes and neurons. Can J Physiol Pharmacol 70:S263–S268.
- Wickens JR (1993) A Theory of the Striatum pp 32. Oxford: Pergamon.
- Wickens JR, Kotter R, Alexander ME (1995) Effects of local connectivity on striatal function: Simulation and analysis of a model. Synapse 20:281–298.
- Wickens JR, Arbuthnott GW, Shindou T (2007). Simulation of GABA function in the basal ganglia: computational models of GABAergic mechanisms in basal ganglia function. *In* GABA and the Basal Ganglia (Tepper JM, Abercrombie ED, Bolam JP, eds)Vol. 160, pp. 313–329: Elsevier. Edinburgh.
- Wickens J (2008) Toward an anatomy of disappointment: Reward-related signals from the globus pallidus. Neuron 60:530–531.
- Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: A study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615.
- Wilson CJ, Groves PM (1981) Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. Brain Res 220:67–80.
- Zhang R, Oorschot DE (2006) Total number of neurons in the habenular nuclei of the rat epithalamus: A stereological study. J Anat 208:577–585.

# Neurotransmitter Receptors in the Basal Ganglia

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### I. INTRODUCTION

The concept that neurotransmitters produce their physiological effects by acting on "receptive substances" or receptors on cells was developed by Langley (1905; see review by Maehle, 2004). This receptor concept was further developed and refined by distinguished pharmacologists such as Hill, Clark and Gaddum (for overview, see Rang, 2006) This concept has been amply confirmed and, over the past years, a number of receptor proteins have been cloned, identified and are being subject to detailed study. Neurotransmitter receptors fall into two classes, ionotropic, including acetylcholine, serotonin, and GABA receptors, and metabotropic or serpentine receptors, which include dopamine, GABA, opioid, tachykinin, adenosine and glutamate receptors and if orphan receptors are included make up a family of up to 1000 receptors. The principal distinction is that ionotropic receptors when activated open to let Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> or Cl<sup>-</sup> ions pass through, either depolarizing or hyperpolarizing the cell, whilst the serpentine seven transmembrane metabotropic receptors exert their slower effects after ligand binding through activation of heterotrimeric G-proteins that

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. influence downstream signaling pathways. Other important features of transmitter receptors include saturability, specificity, supersensitivity and reversibility. It is the tools of molecular biology, particularly the technique of expression cloning where cDNAs were expressed in Xenopus oocytes, which allowed the identification of the majority of transmitter receptors (for detailed examples, see Houamed et al., 1991; Masu et al., 1991; Hollmann and Heinemann, 1994; Kaupmann et al., 1997). More recently transgenic techniques using homologous recombination in embryonic stem cells which allow selective, regional or conditional knockouts and knock-ins of specific receptors have allowed the localization and roles of individual receptors to be studied in detail. This overview will focus on the main features of the most abundant basal ganglia ionotropic and metabotropic receptors, whilst more specific aspects of signaling are covered in other chapters. For detailed localization in the CNS for many of the receptors briefly discussed here the excellent Gensat and Allen Brain atlases, Gensat http://www.gensat.org/index.html and Allen http://www. brain-map.org, provide gene expression data in the mouse brain. Table 4.1 provides a limited overview of the basal

**TABLE 4.1** Localization of neurotransmitter receptors in the basal ganglia nuclei. Neurotransmitter receptors associated pre- or postsynaptically with afferents to the different neuronal types in the various nuclei of the basal ganglia.

Nucleus	Neuron type	Afferent neurotransmitter	Receptor
Striatum	Projection neuron, striatonigral (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 2, 5
		GABA	GABA-A α2α3β2,3γ2 subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M1, M4
		Dopamine	D1, D3
		Serotonin	5-HT1, 5-HT3
		Histamine	H2, H3
		Opioid	Mu, delta and kappa
		Cannabinoid	CB1
		Glycine	Glycine
		Somatostatin	Sst2
		Adenosine	A1
		Cholecystokinin	CCK2
		Noradrenaline	Alpha 2
Striatum	Projection neuron, striatopallidal (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 4, 5
		GABA	GABA-A α2α3β2,3γ2 subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M1
		Dopamine	D2, D3
		Serotonin	5-HT1, 5-HT3
		Histamine	H2, H3
		Opioid	Mu, delta, kappa
		Cannabinoid	CB1
		Glycine	Glycine
		Somatostatin	Sst2
		Adenosine	A2a
		Cholecystokinin	CCK2
		Noradrenaline	Alpha 2
			(Continued)

Nucleus	Neuron type	Afferent neurotransmitter	Receptor
Striatum	Interneuron (acetylcholine)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A a3, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M2
		Dopamine	D5
		Serotonin	5-HT3
		Histamine	H2, H3
		Opioid	Delta
		Cannabinoid	CB1
		Glycine	Glycine
		Tachykinin	NK1
		Somatostatin	Sst2
		Noradrenaline	Beta adrenoreceptors
Striatum	Interneuron, Somatostatin/ NOS (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A
		Acetylcholine	Nicotinic, Muscarinic M2
		Dopamine	D5
		Serotonin	5-HT3
		Opioid	Opiate
		Cannabinoid	CB1
		Glycine	Glycine
		Tachykinin	NK1
		Somatostatin	Sst2
		Noradrenalin	Beta adrenoreceptors
Striatum	Interneuron, Parvalbumin (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A $\alpha 1\beta 2,3\gamma 2$ subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M2

Nucleus	Neuron type	Afferent neurotransmitter	Receptor
		Dopamine	D5
		Opioid	Opiate
		Cannabinoid	CB1
		Glycine	Glycine
		Somatostatin	Sst2
		Noradrenalin	Beta adrenoreceptors
itriatum	Interneuron, Calretinin (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A $\alpha 1\beta 2, 3\gamma 2$ subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M2
		Dopamine	D5
		Serotonin	5-HT3
		Glycine	Glycine
		Opioid	Opiate
		Tachykinin	NK1
		Somtatostatin	Sst2
		Noradrenalin	Beta adrenoreceptors
Globus pallidus internal and external)	Projection neuron (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A $\alpha$ 1 $\beta$ 2,3 $\gamma$ 2 subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M2
		Dopamine	D2
		Serotonin	5-HT1A, 5-HT1B
		Glycine	Glycine
		Tachykinin	NK1
		Neurotensin	NTR1
		Somatostatin	Sst1, 2 and 4
			(Continued

Nucleus	Neuron type	Afferent neurotransmitter	Receptor
	Interneuron	Not known	
Subthalamic nucleus	Projection neuron (glutamate)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
		GABA	GABA-A, GABA-B
		Acetylcholine	Nicotinic
		Dopamine	D1/D5, D2, D3
		Serotonin	5-HT2A/C
	Interneuron (GABA)	Not known	
Substantia nigra pars compacta	Projection neuron (dopamine)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR5
		GABA	GABA-A $lpha 3\gamma 2$ subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M5
		Dopamine	D2
		Serotonin	5-HT1, 5-HT2, 5-HT4
		Histamine	H3
		Opioid	Mu, delta, kappa
		Cannabinoid	CB1
		Glycine	Glycine
		Tachykinin	NK1, NK3
		Neurotensin	NTR1
	Interneuron (GABA)	Not known	
Substantia nigra	Projection neuron (GABA)	Glutamate	NR1, NR2, AMPA, Kainate, mGluR1, 5
pars reticulata to read reticulata		GABA	GABA-A α1β2,3γ2 subunits, GABA-B
		Acetylcholine	Nicotinic, Muscarinic M2
		Serotonin	5-HT1A, 5-HT1B
		Opioid	Delta, kappa
		Tachykinin	NK1, NK3
		Interneuron	Not known

ganglia cell body/nuclear localization of some receptors discussed in the text (see text for relevant references) (see Chapter 1, for an overview of basal ganglia anatomy).

#### **II. IONOTROPIC RECEPTORS**

#### A. Glutamate Receptor Ion Channels

These glutamate receptors are homo- or heteromeric complexes of integral membrane protein subunits (Hollmann and Heinemann, 1994). Each subunit has a typical structure (Fig. 4.1) consisting of three transmembrane (TM) domains  $(\alpha$ -helices) and a pore-forming membrane-residing domain which does not cross the membrane. The N-terminal domain is extracellular and the C-terminal domain intracellular. The distal N-terminal domain has similarities to the leucine/isoleucine/valine binding protein (LIVBP), a bacterial periplasmic binding protein (Mayer and Armstrong, 2004; Mayer, 2005, 2006), whilst the agonist binding domain of ionotropic glutamate receptors (S1/S2) is made up of the C-terminal part of the N-terminus and a homologous part of the second transmembrane domain. This S1/S2 ligand binding domain has similarities to bacterial glutamine binding proteins and can be expressed separately from the rest of the subunits allowing crystallographic studies to be done (Madden, 2002; Mayer and Armstrong, 2004; Mayer, 2005, 2006). Ionotropic glutamate receptors can be divided into three types based on their affinities for synthetic agonists: (1) N-methyl-D-aspartate (NMDA); (2)  $\alpha$ -amino-3hydroxy-5-methyl-4-isoxazole propionate (AMPA); and (3) kainate receptors. These receptors may have developed from a prokaryotic glutamate receptor (Chen et al., 1999). The NMDA receptors are permeable to monovalent cations and calcium, whilst AMPA and kainate receptors are permeable primarily to monovalent cations but not calcium. There has been considerable interest in developing antagonists for these receptors as treatments for stroke and brain injury (for recent review, see Kew and Kemp, 2005).

#### 1. NMDA Receptors (Fig. 4.1B)

The NMDA receptor is a heteromeric ligand-gated ion channel. There are three subunits of the NMDA receptor: the ubiquitously expressed NR1 subunit, which is critical for the formation of functional channels, four NR2 (A–D) subunits, and two NR3 (A–B) subunits, although the NR3 subunits have not been reported in the basal ganglia. The NMDA receptor subunits share a high degree of amino acid sequence similarity with some 20% identity between NR1 and NR2 subunits, and around 50–60% identity between NR2A/NR2B and NR2C or NR2D subunits (Matsuda et al.,



**FIGURE 4.1** A. Schematic representation of an ionotropic glutamate receptor. Note the extracellular N-terminal domain which has similarities to the bilobed agonist binding domains of mGluR and GABAb receptors. There are three transmembrane domains and a pore forming domain which resides in the membrane. The agonist binding domain is S1/S2 B. Structure of an NMDA receptor made up of NR1 and NR2 subunits. Glycine binds to NR1 and glutamate binds to NR2. Magnesium ions block the channel until the cell is depolarized. C. General structure (simplified) of an AMPA/kainate receptor. The AMPA receptors in the basal ganglia are predominantly GluR1 and GluR3 whilst the kainate receptors are assemblies of GLuK1-3 and GluK4-5 subunits (see text for details).

2002). There are also a number of isoforms particularly of the NR1 subunit (Monyer et al., 1992; Monyer et al., 1994; Ciabarra et al., 1995). In situ hybridization studies indicate that the basal ganglia contain mRNAs for the ubiquitous NR1, NR2A and at lower levels NR2B, with NR2C present in glial-like cells in the caudate-putamen (Monyer et al., 1992; Monyer et al., 1994; Standaert et al., 1996; Standaert et al., 1999) and NR2D in low levels in the globus pallidus (Standaert et al., 1996). NMDA receptors are heterotetrameric cation channels with subunits in an (NR1)<sub>2</sub> and (NR2)<sub>2</sub> organization (Laube et al., 1998). There is evidence for both binary and ternary complexes of subunits, that is NR1/NR2A or NR1/NR2A/NR2B (Sheng et al., 1994; Chazot and Stephenson, 1997). In the caudate-putamen, Dunah and Standaert (2003) have provided immunoprecipitation evidence for the presence of ternary receptors in synaptosomal membrane fractions. This is important because the subunit composition will alter the pharmacology and physiology of the resulting channel. The NR1/ NR2A binary complex has been shown in in vitro studies to deactivate faster than the NR1/NR2B complex, whilst the ternary complex is slowly deactivating.

The extracellular S1/S2 domain of the NR2 subunit is the site of binding for glutamate, whilst the extracellular domain NR1 subunit binds glycine (Kuryatov et al., 1994; Wafford et al., 1995; Hirai et al., 1996; Anson et al., 1998; Laube et al., 1998); this feature of requiring co-agonists, glutamate and glycine, is unique amongst ligand-gated ion channels.  $Zn^{2+}$  ions are known to be concentrated and released at some glutamatergic synapses and may inhibit NMDA receptors through voltage-dependent and voltage-independent mechanisms (reviewed by Dingledine et al., 1999; Paoletti and Neyton, 2007). The NR2A subunit is allosterically inhibited by nanomolar concentrations of  $Zn^{2+}$  such that sensitivity is approximately 100-fold higher for NR2A over the other NR2 subunits (Kew and Kemp, 2005; Paoletti and Neyton, 2007). The other cation critically involved with the NMDA receptor is magnesium, which binds to the channel in a voltagedependent fashion so that activity of other receptors such as AMPA receptors is required to unblock the receptor channel. Antagonists of the NMDA receptor have been a subject of much research for the treatment of neurological illness especially stroke (for review, see Kew and Kemp, 2005).

There is a considerable literature on interactions between NMDA receptors and other proteins through the cytoplasmic C-terminal which are responsible for dendritic transport and clustering at the synapse. The trafficking and anchoring of all the receptors discussed here is a rapidly developing field and most progress has so far been made with ionotropic receptors. Many of these proteins for the NMDA receptor are membrane-associated guanylate kinases which anchor the NMDA receptor at the synapse. A typical member of this family is postsynaptic density protein(PSD) 95, that contains three PDZ domains, an SH3 domain, and a guanylate kinase (GK) domain (Wenthold et al., 2003). The PDZ domain binds directly to the NMDA receptor subunit C-termini and is a common structural domain found in PSD signaling MAGUK proteins, including PSD-95, the synapse-associated protein of 102 kDa (SAP-102), SAP-97, and PSD-93 (Husi and Grant, 2001b; Sheng and Sala, 2001). The PSD is a specialized structure localized at the postsynaptic membrane and acts as a scaffold for the proteins that are involved with signal transduction (Husi and Grant, 2001a; Wenthold et al., 2003). Specific interactions between NR2 subunits and the PSD proteins are critical for the synaptic localization of receptors. For example, NR2B subunit delivery to the plasma membrane involves an interaction between SAP-102 and an exocyst protein, Sec8 (Sans et al., 2003). The exocyst is a complex of eight proteins that are involved in targeting secretory vesicles to the cell (Wenthold et al., 2003). NMDA receptors are also critically involved in long-term depression (LTD) and long-term potentiation (LTP) (Calabresi et al., 2007).

#### 2. AMPA Receptors (Fig. 4.1C)

Fast synaptic transmission in the basal ganglia is primarily mediated by AMPA receptors which are composed of subunits GluR1-4. In the basal ganglia, GluR1-3 are the main forms of GluR, depending on species (Stefani et al., 1998; Beneyto and Meador-Woodruff, 2003; Deng et al., 2007). The ligand-binding domain S1/S2 is present as expected in all GluRs. The C-terminal has PDZ- and NSF-binding domains which will influence the binding of intracellular proteins involved in trafficking and synaptic targeting. As with NMDA receptors many of these proteins are membraneassociated guanylate kinases (MAGUKs). MAGI-2 is a candidate for linking the four-pass transmembrane AMPA receptor-regulating proteins (TARPS) (including stargazin) which are responsible for synaptic trafficking of AMPA receptors (Tomita et al., 2004; Milstein and Nicoll, 2008). For example, in the stargazin mutant mouse the surface expression of AMPA receptors is substantially reduced in the cerebellar granule cells, resulting in ataxia.

All AMPA receptor subunits exist as two splice variants, *flip* and *flop*, in the extracellular S2 domain. Flip and flop variants have effects on the rate of desensitization of heteromers and sensitivity to allosteric modulators. Another feature of the GluR2 subunit is post-transcriptional editing of the mRNA, changing a single amino-acid from glutamine (Q) to arginine (R). GluR2(R) is the major form in the CNS and this form is calcium-impermeable, whilst the GluR2(Q) is calcium-permeable. The calcium impermeability and its intracellular interactions through PDZ and NSF domains make GluR2 the most important AMPA receptor subunit.

#### 3. Kainate Receptors (Fig. 4.1C)

Kainate receptors (KAR) constitute a separate group from the NMDA and AMPA receptors. Although they share many structural characteristics with the other ionotropic glutamate receptors, they do not cross-assemble. The nomenclature of the KARs has changed recently; the subtypes once termed GluR5-7 are now termed GLuK1-3, and the KA1-2 subtypes are now GLuK4-5, corresponding to the gene nomenclature GIRK1-5 as decided by IUPHAR (Collingridge et al., 2008). They are built from multimeric assemblies of GLuK1-3 and GLuK4-5 subunits; the GLuK4-5 subtypes can not assemble into functional channels and require a partner from the GLuK1-3 group, whilst members of the GLuK1-3 can form homomers. Like the other ionotropic glutamate receptors, they possess an extracellular N-terminus that, together with a loop between TMIII and TMIV, forms the ligand-binding domain (S1/S2), and a re-entrant loop (TMII) that forms the lining of the pore region of the ion channel. There is around 70-80% homology between GLuK1-3, 60-70% between GLuK4 and GLuK5, and some 45% between the two groups.

In the basal ganglia, GLuK2 and GLuK5 are the main forms detected by in situ hybridization (Wullner et al., 1997). As with the other ionotropic glutamate receptors, the functional kainate receptor is probably a tetramer with ligand-binding domains similar to NR1 or GluR2. Recent structural studies have suggested a basis for the differences in agonist binding between GLuK1 and 2 (Mayer and Armstrong, 2004; Mayer, 2005; Nanao et al., 2005; Naur et al., 2005; Mayer, 2006). There is editing or alternative splicing of kainate receptors and, as with the AMPA receptor Q/R site, editing determines permeability to calcium ions. C-terminal domain variants determine the intracellular trafficking and interactions with proteins at the synapse (Pinheiro and Mulle, 2006). In recent years selective agonists for the GLuK1 subunit have been developed (Jane et al., 2008) and progress is being made to develop selective antagonists for individual subunits (Pinheiro and Mulle,

2006; Jane et al., 2008). Knockout mice have allowed concentrations of agonists to be defined to selectively activate specific kainate receptors.

## B. Ligand-Gated Ion Channels (Fig. 4.2)

Members of the ligand-gated ion channel family, which are also sometimes termed cys-loop ligand-gated ion channels (LIGC) because of the cystine loop in the extracellular N-terminal domain (Sine and Engel, 2006; Fig. 4.2A), are abundant in the mammalian CNS. In the basal ganglia, they include nicotinic acetylcholine receptors (nAChR), GABA-A receptors, serotonin (5-HT3) receptors and glycine receptors. Each subunit has four transmembrane domains, and the subunits are arranged in a pentameric structure around the ion channel (Fig. 4.2B,C) based on the model of Unwin (Unwin, 2005). There is only limited sequence homology of the extracellular domains between the families. However, models based on the structure of the soluble snail acetylcholine binding protein (AChBP) allow correct prediction of ligand-binding domains, indicating that the secondary structure (Zhou et al., 2002) is to some extent conserved between the families (Brejc et al., 2001; Connolly and Wafford, 2004). The GABA-A and glycine receptors are anion/chloride channels, whilst the nicotinic and 5-HT3 channels are cation channels with permeability to  $Ca2^+$  as well as  $Na^+$  and  $K^+$ .

#### 1. Nicotinic Receptors

As members of the cys-loop ligand-gated receptor family neuronal nicotinic receptors are pentamers of  $\alpha$  and  $\beta$ subunits in a ratio of  $3\alpha:2\beta$  (Jones et al., 1999). Neuronal subunits include  $\alpha 2-\alpha 6$  and  $\beta 2-\beta 4$  with  $\alpha 7$  and  $\alpha 9$  being



**FIGURE 4.2** A. Schematic of a typical ligand-gated channel subunit with four membrane-spanning domains. B. Schematic of a pentameric arrangement of a ligand gated channel. C. Schematic of the heteromeric arrangement of a GABA<sub>A</sub> receptor made up of two  $\alpha$ 1 subunits, two  $\beta$ 2 subunits and one  $\gamma$ 2 subunit. The agonist GABA binds between  $\alpha$ 1 and  $\beta$ 2 subunits, with benzodiazepines binding between the  $\alpha$ 1 and  $\gamma$ 2 subunits.

capable of forming homomers and  $\alpha 9$  possibly forming heteromers with  $\alpha 10$ . The most common combination in the basal ganglia are the  $\alpha 2$  and  $\beta 4$  and in the midbrain  $\alpha 3-\alpha 7$  and  $\beta 2-\beta 4$  (for reviews, see (Wonnacott, 1997; Zhou et al., 2002). The most described effect of nicotinic receptors is presynaptic, namely, modulation of transmitter release through regulation of the calcium response; this is best established for dopamine release and to a lesser extent glutamate (Wonnacott, 1997; Zhou et al., 2002). Dopamine neurons express nAChR \beta2 subunits, and knockout of \beta2 dramatically reduces the effects of nicotine on dopamine release in the basal ganglia (Picciotto and Wickman, 1998; Picciotto et al., 1998). It is likely that many of the major effects of acetylcholine released by the cholinergic interneurons in the caudate-putamen are mediated through presynaptic regulation of transmitter release. Studies of striatal synaptosomes show that nicotinic receptors mobilize  $Ca^{2+}$  as do 5-HT3 receptors (for a recent review, see (Dani and Bertrand, 2007).

#### 2. 5-HT<sub>3</sub> Receptors

The serotonin 5-Hydroxytryptamine type-3 receptor (5-HT<sub>3</sub>), in contrast to the other 5-HT receptors in the basal ganglia which are metabotropic G-protein coupled receptors, belongs to the ligand gated channel (LIGC) family of receptors and like all the members of this family is made of a pentamer of subunits around the central ion channel. The first subunit isolated was the 5HT3A subunit which was isolated by expression cloning. Expression of this subunit did not however mimic the precise physiological properties of the 5-HT<sub>3</sub> receptor in neurons and additional subunit genes were subsequently identified. Examination of the human genome sequence identified 5-HT3B-E which require the presence of the 5-HT3A to form 5-HT binding sites and functional receptors. So far, the physiological properties of the subunits forming heteromers with 5-HT3A have not been tested in detail (Barnes et al., 2008). In the striatum, binding studies using tissue from patients with a diagnosis of Parkinson's disease (PD) and Huntington's disease (HD) indicate that, for the human brain at least, the 5-HT<sub>3</sub> receptor is localized on GABA output cells that die in HD, and not on the dopamine terminals which degenerate in PD (Steward et al., 1993b; Steward et al., 1993a).

#### 3. Glycine Receptors

Like its cousins the nicotinic, 5-HT3 and  $GABA_A$  receptors, the glycine receptor (GlyR) is a member of the cys-loop

ligand gated channels (LIGC). Compared to the GABA<sub>A</sub> receptor there has been relatively little work on basal ganglia glycine receptors. Expression studies indicate that glycine receptors are expressed by medium sized spiny neurons and giant cholinergic neurons in the rat and mouse striatum (Sergeeva, 1998; Darstein et al., 2000; Sergeeva and Haas, 2001). Immunocytochemical studies on human brain found glycine receptor immunoreactivity to be highly expressed in substantia nigra neurons and on neurons of the IML and MML (Waldvogel et al., 2007). In the human striatum, GlyRs were detected in interneuron populations including cholinergic and parvalbumin positive neurons (Waldvogel et al., 2007). Similar studies have not been done in rodent species. Glycine receptors are strychnine sensitive and like other LIGCs form pentamers of  $\alpha$ 1–4 and  $\beta$  subunits (Cascio, 2004; Lynch, 2004; Grudzinska et al., 2005). The protein gephyrin is responsible for clustering of glycine and GABA<sub>A</sub> receptors at the synapse. Gephyrin is a multimeric PSD component which couples directly to GlyRs and indirectly to GABA receptors. (For recent reviews see (Kneussel and Loebrich, 2007; Fritschy et al., 2008).

#### 4. GABA<sub>A</sub> Receptors (Fig. 4.2C)

GABA is the major inhibitory neurotransmitter in the brain and the main output transmitter of the basal ganglia nuclei. As with other cys-loop LIGC, GABA<sub>A</sub> receptors are composed of five subunits with a large extracellular domain. A number of subunits including  $\alpha 1$ –6,  $\beta 1$ –3,  $\gamma 1$ –3,  $\delta$ ,  $\varepsilon$ ,  $\pi$ ,  $\theta$ , and  $\rho 1-3$  have been cloned and sequenced, which is the most for any mammalian ion channel receptor (Sieghart, 2006; Olsen and Sieghart, 2008). This complexity allows an extensive range of pharmacological and electrophysiological properties; however, the most common subunits are combinations of  $\alpha$ ,  $\beta$  and  $\gamma$ , usually in a combination of alternating  $\alpha$  and  $\beta$  subunits with one  $\gamma$  subunit (Pritchett et al., 1989; McKernan and Whiting, 1996). The  $\gamma 2$  subunit is necessary to form a benzodiazepine sensitive receptor with the benzodiazepine binding site localized between an  $\alpha$  and a  $\gamma 2$  subunit (Sieghart, 2006). Indeed, reduced expression of the  $\gamma 2$  subunit created an "anxious" mouse. Activation of a functional GABA<sub>A</sub> receptor lets chloride ions into the neuron causing a strong inhibitory hyperpolarization. In a detailed overview of the distribution of GABAA receptors on neurons in the basal ganglia, Wisden and colleagues (Goetz et al., 2007) note that a majority of GABA<sub>A</sub> receptors are of the standard benzodiazepine type  $\alpha 1\beta 2\gamma 3$  and that the subthalamic nucleus, substantia nigra and globus pallidus are regions of high benzodiazepine binding. Based on abundance Wisden and colleagues predict that receptors expressing  $\alpha 2$  and  $\beta 3$  subunits are on the GABA-ergic medium sized output neurons of the caudate-putamen. This is also generally true in the human basal ganglia, where Faull and colleagues have studied changes in Huntington's disease (Faull et al., 1993). In the human the most common receptor type is also  $\alpha 1\beta 2\gamma 3$  found on parvalbumin positive interneurons in the striatum as well as on the pallidal neurons and those in the SNr. The  $\alpha$ 3 subunit is mainly associated with cholinergic interneurons in the striatum and the pars compacta neurons of the substantia nigra (Waldvogel et al., 1999; Waldvogel et al., 2004; Waldvogel et al., 2008). GABA<sub>A</sub> receptors are cycled rapidly in the neuron and respond to changes in GABA levels (Fenelon and Herbison, 1996). As noted above synaptic anchoring of GABA<sub>A</sub> receptors involves the protein gephryin in association with other intermediate proteins such as GABA receptor associated protein (GABARAP), dystrophin and others (Luscher and Keller, 2004) with synaptic receptors being recruited from an extrasynaptic pool (Kneussel and Loebrich, 2007; Connolly, 2008). Clustering of GABA<sub>A</sub> receptors extrasynaptically may involve gephyrin or the protein radixin which is a member of the ERM family (ezrin/radixin/moesin) (Kneussel and Loebrich, 2007).

### **III. METABOTROPIC RECEPTORS**

Compared to the ionotropic families of receptors, the serpentine G-protein coupled receptors (GPCRs) are a very large family of at least a 1000 members. They mediate most of the effects of peptides and hormones in the body. They can be divided into three families. Family 1 is the largest and contains the majority of GPCRs, including opioid, muscarinic, adenosine, cannabinoid, dopamine, somatostatin and tachykinin receptors, amongst others. Family 2 includes a number of peptide hormones and is not significantly represented in the basal ganglia. Family 3 is a small family but includes the metabotropic glutamate and GABA receptors which are of considerable importance in the basal ganglia. Family 1 receptors are members of the rhodopsin family, and the structures of rhodopsin,  $\beta$  and  $\beta_2$ -adrenergic and most recently the  $_{A2a}$ adenosine receptors have been determined at high resolution (Palczewski et al., 2000; Rasmussen et al., 2007; Jaakola et al., 2008). Figure 4.3 shows a two-dimensional view of the somatostatin-2 receptor based on the rhodopsin structure (Palczewski et al., 2000). Using this receptor as a general example of GPCR structure, there is an N-terminal extracellular domain containing sites for glycosylation which may alter the receptor properties, influencing ligand binding or interactions with other receptors. There are three extracellular loops between the seven transmembrane domains. On the intracellular side are three cytoplasmic loops and a C-terminal sequence which contains sites for phosphorylation and palmitoylation; these are important for internalization and desensitization (Fig. 4.3). Phosphorylation for example may influence the receptor's G-protein coupling. This has been shown for the  $\beta_2$  adrenergic receptor that has reduced ability to interact with G<sub>s</sub> and acquires an ability to interact with G<sub>i</sub> after phosphorylation (Daaka et al., 1997). The cytoplasmic structure of GPCRs is relatively conserved, probably reflecting the need to interact with and bind G-proteins of which there are relatively few compared to the 100s of GPCRs. Family 3 receptors are characterized by a much larger N-terminus than family 1 receptors; it is this N-terminal that is responsible for ligand binding and it contains domains such as the leucine/ isoleucine/valine binding domain (LIVP) with homology to bacterial periplasmic proteins which also bind amino acids. Studies of the GABA<sub>B</sub> receptor (see below) showed that this receptor exists as a dimer of two subunits. This discovery prompted many studies of potential dimerization/oligomerization between GPCRs which may have important physiological and pharmacological consequences (see (Chabre and le Maire, 2005; Eilers et al., 2005; Fuxe et al., 2007).

The trimeric G protein (G $\alpha\beta\gamma$ ) bound to the GPCR consists of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. There is considerable diversity in G-proteins and there are at least twenty  $G\alpha$ , six  $G\beta$ and thirteen  $G\gamma$  subunits interacting with different signaling pathways. Ligand binding to the GPCR triggers the release of GDP from  $G\alpha$ , which can bind GTP and dissociate from  $G\beta\gamma$ . Both  $G\alpha$  and  $G\beta\gamma$  can activate signaling pathways. Hydrolysis of GTP in  $G\alpha$  increases its affinity for  $G\beta\gamma$  and for its receptor, resulting in the trimeric  $G\alpha\beta\gamma$ binding to its receptor. There are four families of  $G\alpha$  subunits (for reviews, see (Gilman, 1987; Simon et al., 1991; Neves et al., 2002) of which only three, G<sub>i</sub>, G<sub>s</sub> and G<sub>a</sub>, are directly involved in basal ganglia receptor signaling. The G<sub>i</sub> family is usually termed inhibitory as these G-proteins inhibit adenylyl cyclases and are sensitive to pertussis toxin ribosylation which prevents them from being activated by GPCRs. This family includes a number of  $G\alpha_i$  forms including  $G\alpha_{i 1-3}$ ,  $G\alpha_z$ , as well as  $G\alpha_o$ ,  $G\alpha_o$  provides a good example of the variety of signaling pathways G-proteins may activate. It is neuronal specific and, as expected, inhibits adenylyl cyclase, but also inhibits Ca<sup>2+</sup> channels, activates mitogen activated protein kinases (MAPKs) and can



FIGURE 4.3 Schematic structure of the somatostatin-2 receptor as an example of a typical family 1 GPCR. Note sites for glycosylation in the N-terminal domain, the seven transmembrane spanning domains and the C-terminal domain, which also contains a site for palmitoylation. Palmitoylation at the amino acid cystine effectively creates a fourth intracellular domain. Reproduced from Emson PC (2009) In: Encyclopedia of Neuroscience (Squire LR, ed), pp 121–127. Oxford: Academic Press.

interact with other proteins including the growth associated protein GAP43 and the Alzheimer's gene product presenilin-1. This complexity of "signaling mechanisms/targets" needs to be borne in mind in the space-limited descriptions of some of the basal ganglia GPCRs that follows. Members of the G<sub>s</sub> family, which includes G<sub>olf</sub>, all stimulate adenylyl cyclase, but can also activate tyrosine kinases (G<sub>s</sub>) or stimulate phosphoinositide turnover (G<sub>olf</sub>). There are some nine members of the adenylyl cyclase family as potential targets for G<sub>s</sub> or G<sub>i</sub>. Members of the G<sub>q</sub> family stimulate the various phospholipase C $\beta$  forms.

#### A. Family 1

#### 1. Dopamine Receptors

Since the discovery of the major tranquillizers and the localization studies of dopamine and other amines in the basal ganglia there has been ongoing interest in the basal ganglia dopamine receptors as targets for drugs for the treatment of schizophrenia and basal ganglia disorders (Bjorklund and Dunnett, 2007; Iversen and Iversen, 2007). Dopamine receptors can be divided into two subfamilies,  $D_1$ -like ( $D_1$ and D<sub>5</sub>), which stimulate adenylate cyclase, and D<sub>2</sub>-like  $(D_2, D_3, and D_4)$  which inhibit adenylate cyclase (Hartman and Civelli, 1997). Of these the D<sub>1</sub> and D<sub>2</sub> receptors are enriched in the striatum and its projection areas. In general, the D<sub>1</sub> receptor can be used to define medium spiny neurons (MSNs) projecting directly to the substantia nigra in the so-called direct pathway, whilst MSNs of the indirect pathway are characterized by the D<sub>2</sub> receptor (see Chapters 1 and 6 for details). It was more difficult to show that the  $D_3$  receptor inhibits adenylate cyclase. In the basal ganglia this receptor is found in lower amounts in the ventral striatum, both in direct and indirect pathway neurons. The D<sub>4</sub> receptor is particularly localized in the cerebral cortex, as is D<sub>5</sub>, although the D<sub>5</sub> receptor is also enriched in striatal interneurons (Rivera et al., 2002). Gene expression studies of the subthalamus indicate neurons in this area express a spectrum of dopamine receptors including D1, D2, D3 and D5 (Flores et al., 1999; Augood et al., 2000; Baufreton et al., 2003) For recent reviews of dopamine signaling, see (Strange, 2001; Girault and Greengard, 2004). There have been a number of studies involving over-expression of receptors in cell lines suggesting dopamine receptors may form oligomers with other GPCRs including somatostatin and adenosine receptors. Whether this occurs in *in vivo* neurons, however, remains to be established (Chabre and le Maire, 2005; Eilers et al., 2005).

#### 2. Opioid Receptors

After dopamine historically the opioid peptides and tachykinins were of considerable interest in providing chemical markers for the striatonigral and striatopallidal systems. In the case of the direct pathway, the opioid peptide dynorphin A was found in direct pathway MSNs, whilst enkephalin was found in the indirect pathway (Lee et al., 1997) (see also Chapter 29). The concentration of enkephalin in the external segment of the globus pallidus is equivalent to that found in spinal nociceptive pathways. Opioid receptors can be divided into three subfamilies ( $\mu$ ,  $\delta$ ,  $\kappa$ ) plus an opioid-related receptor, N/OFQ, that has a pharmacology distinct from the three "classical" opioid receptors. Of the three opioid receptors both the  $\mu$  receptor and the  $\delta$  receptor are enriched in the striatum, with the  $\kappa$  receptor being localized more in the ventral striatum/nucleus accumbens. The  $\mu$ opioid receptor is particularly concentrated in the patches or striosomes (Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981; Mansour et al., 1995; Wang et al., 1996). The N/OFQ receptor is present in the cerebral cortex but is virtually absent from the striatum (Bunzow et al., 1994). All opioid receptors couple through G<sub>i</sub> or G<sub>o</sub> to inhibit adenylate cyclase so that, for example, locally released enkephalin will presynaptically inhibit GABAergic inputs.

In situ localization studies indicate the  $\mu$ .  $\delta$  and  $\kappa$  receptors are found in both striatopallidal and striatonigral neurons, with the  $\delta$  receptor in cholinergic interneurons (Le Moine et al., 1994; Mansour et al., 1995; Wang and Pickel, 2001)

#### 3. Tachykinin Receptors

Of the three tachykinins (substance P, neurokinin A and neurokinin B), substance P is the most abundant and is localized in the direct pathway, although all the tachykinins are enriched in the striatum. Tachykinin receptors, especially that for substance P, termed SP-R or NK1, are markers for cholinergic and somatostatin/NOS containing striatal interneurons (Shigemoto et al., 1993), whereas the NK1 and NK3 receptor are found on the target cells of the direct pathway in the substantia nigra. Tachykinin receptors couple to Gq and G11 and exert their effects through the phosphoinositide system generating inositol1,4,5 triphosphate (IP3) and diacylglycerol (DAG) (Guard et al., 1988; Guard et al., 1991) and will tend to activate GABAergic MSNs.

#### 4. Cannabinoid Receptors

The behavioral effects of  $\Delta$ 9-tetrahydrocannabinol the major ingredient of the marijuana plant (Cannabis sativa) are mediated through cannabinoid receptors in the CNS (see also Chapter 9). There are two GPCRs, CB1 and CB2, but it is primarily the CB1 type that is found in the CNS to mediate the effects of cannabis. The CB1 receptor is amongst the most abundant GPCRs in the brain. CB1 is coupled through G<sub>0</sub> or G<sub>i</sub> to inhibit adenylate cyclase. In the striatum CB1 mRNA is found in most MSNs both in the direct and indirect pathways and in patch and matrix (Herkenham et al., 1991; Hohmann and Herkenham, 2000), and in some interneurons, especially those containing parvalbumin (Fusco et al., 2004). Immunoreactivity and autoradiographic labeling studies for CB1 is concentrated in the pallidum and substantia nigra consistent with most of the receptor protein being transported in the axons of striatonigral and striatopallidal MSNs to their terminals (Glass et al., 1997; Julian et al., 2003). This presynaptic localization allows cannabinoids to modulate transmitter release and influence dopamine cell activity, possibly underlying the psychoactive actions of cannabis. Consistent with these observations, human patients with Huntington's disease had dramatically depleted CB1 binding (Glass et al., 1993; Glass et al., 2000) and immunostaining in the GP and SN (Allen et al., 2009).

#### 5. Muscarinic Receptors

There are five subclasses of muscarinic receptors, M<sub>1</sub>-M<sub>5</sub> (Caulfield and Birdsall, 1998). M<sub>2</sub> and M<sub>4</sub> couple through Gi/o to inhibit adenylate cyclase, whilst M1, M3 and M5 couple through G<sub>q/11</sub> to mobilize phosphoinositides to generate IP<sub>3</sub> and DAG (Eglen, 2005, 2006; Wess et al., 2007). In the basal ganglia, as with many other receptors the localization of muscarinic receptors has been facilitated by the use of subclass specific knockout mice (Zhou et al., 2001; Zhang et al., 2002; Wess, 2003; Yamada et al., 2003; Oki et al., 2005; Wess et al., 2007). M<sub>1</sub> and M<sub>4</sub> receptors (Hersch et al., 1994) are particularly concentrated in the striatum, with M<sub>1</sub> being found in all MSNs both in the indirect and direct pathway, and in cholinergic and NOS interneurons. M<sub>2</sub> is found in cholinergic interneurons and M<sub>4</sub> in substance P-containing MSNs. M<sub>3</sub> staining is diffusely distributed, but was not found in identified neuronal

types (Hersch et al., 1994).  $M_5$  is expressed by the dopamine cells of the substantia nigra and ventral tegmental area and by endothelial cells of the vascular beds (Yamada et al., 2003). For more detailed discussions of muscarinic pharmacology the reader is referred to recent reviews (Eglen, 2005, 2006; Ishii and Kurachi, 2006).

#### 6. Somatostatin Receptors

All of the somatostatin receptors are typical G-protein coupled receptors (Fig. 4.3) with the classical seven transmembrane domains and sites for glycosylation and/or palmitoylation on the N-termini. They can be grouped into two subfamilies on the basis of pharmacology, with receptors sst1 and sst4 in one group, and sst2A, sst2B, sst3 and sst5 forming another group. The peptide analogues seglitide and octreotide can be used to distinguish the subfamilies; radioligand binding studies show that sst1 and sst4 possess only a low affinity for both analogues, whilst sst<sub>2</sub>, sst<sub>3</sub> and sst<sub>5</sub> display high affinity. The homology between the subfamilies is less than 50%, but within the subfamilies, sequence similarities exceed 80% in transmembrane regions. Somatostatin receptors couple to signal cascades through both pertussis toxin (PTX)-sensitive and PTXinsensitive G-proteins G<sub>i</sub>/G<sub>o</sub> and G<sub>q</sub>. The precise coupling depends on the neuronal type and the different isoforms of G-proteins available in the target cell. All the receptors have been shown to inhibit adenylyl cyclase activity in a PTX-sensitive fashion and to couple to phospholipase C (Selmer et al., 2000). In the striatum, sst2 is localized to MSNs in the matrix consistent with the ability of this receptor to respond to somatostatin released by the NOS/ somatostatin interneurons (Allen et al., 2003). Sst2 is also present in cholinergic interneurons in the striatum and in the substantia nigra on GABAergic cells of the pars reticulata (Allen et al., 2003).

#### 7. Adenosine Receptors

In the CNS, adenosine may be generated by dephosphorylation of ATP in relation to activity or by the action of ecto-nucleotidase on ATP released together with classical neurotransmitter, and adenosine is regarded as a neuromodulator (see also Chapter 11). There are four adenosine receptors, A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub> and A<sub>3</sub>. The A<sub>2</sub> subtypes are  $G_s/G_{olf}/G_q$ coupled, stimulating adenylyl cyclase, whilst A<sub>1</sub> and A<sub>3</sub> couple to  $G_i/G_o$  and inhibit adenylyl cyclase. In the basal ganglia, the receptors of interest are A<sub>1</sub> and A<sub>2a</sub>. A<sub>2a</sub> receptors are found on the enkephalin/striatopallidal cells, whilst A<sub>1</sub> receptors are found on the substance P/direct pathway neurons (Glass et al., 2000). This allows inhibitory  $A_1$ receptors to modulate the action of excitatory D<sub>1</sub> receptors, and A<sub>2a</sub> receptors can antagonize the action of D<sub>2</sub> receptors (Fink et al., 1992; Schiffmann and Vanderhaeghen, 1993; Svenningsson et al., 1998; Schiffmann et al., 2007). The localization of the A<sub>2a</sub> receptor with enkephalin cells has led to the suggestion that A<sub>2a</sub> antagonists may be useful in the treatment of Parkinson's disease, by reducing the imbalance between direct and indirect pathways (Schwarzschild et al., 2006; and Chapter 11), although the role of extrastriatal  $A_{2a}$  receptors may complicate this picture (Shen et al., 2008). The crystal structure of the  $A_{2a}$  receptor bound to an antagonist has recently been determined at 2.6 angstroms (Jaakola et al., 2008) and it is likely that knowledge of this structure this will lead to development of more selective adenosine receptor compounds. It is noteworthy for example that the binding pocket for this receptor differs significantly in structure from those found in the adrenergic and rhodopsin receptors whose structures have been determined (Jaakola et al., 2008) As with other GPCRs the formation of homo and heterodimers such as  $A_{2a}/D_2$  may have important physiological roles in the striatum.

#### 8. Serotonin Receptors

After dopamine serotonin, 5-hydroxytryptamine (5-HT), is the most abundant amine in the basal ganglia, with 5-HT projections arising from the raphe nuclei and other brainstem groups. Serotonin receptors, with the exception of the ligand gated 5-HT<sub>3</sub> receptor (see earlier), are all GPCRs and comprise a number of subtypes that can be divided into three main families, the 5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>6</sub> families (for review, see (Barnes and Sharp, 1999; Hoyer et al., 2002; Bockaert et al., 2006)). In the basal ganglia, in situ hybridization or ligand binding experiments demonstrated that representatives of each family are present. These include 5-HT<sub>1b</sub>, 5-HT<sub>1d</sub>, 5-HT<sub>5A</sub>, and 5-HT<sub>7</sub> for the 5-HT<sub>1</sub> family, 5-HT<sub>2a</sub> and 5-HT<sub>2c</sub> for the 5-HT<sub>2</sub> family and 5-HT<sub>4</sub> and 5-HT<sub>6</sub> for the 5-HT<sub>3</sub> family. The members of the 5-HT<sub>1</sub> family negatively couple to adenylyl cyclase, 5-HT<sub>2</sub> receptors couple positively to phospholipase C to generate IP<sub>3</sub> and DAG, whilst 5-HT<sub>3</sub> members couple positively to adenylyl cyclase.

Studies show that 5-HT<sub>1b</sub> receptors are present throughout the basal ganglia including striatum, pallidum and substantia nigra, although mRNA is found only in the striatum, indicating that this receptor is present on axons to the pallidum and substantia nigra (Bruinvels et al., 1994). 5-HT<sub>1d-f</sub> mRNAs are also expressed in the striatum (Bach et al., 1993; Bruinvels et al., 1994) as are 5-HT<sub>5</sub> receptors (Grailhe et al., 1999). Of the 5-HT<sub>2</sub> family, autoradiography studies have localized 5-HT<sub>2a</sub> and 5-HT<sub>2c</sub> receptors in the striatum and substantia nigra (Lopez-Gimenez et al., 1997). 5-HT<sub>4</sub> is localized to the striatum, globus pallidus and SNr are but have not been conclusively found on dopaminergic neurons of the SNc (Eglen et al., 1995) and 5-HT<sub>6</sub> is found in the striatum (Kohen et al., 1996). The widespread distribution of 5-HT receptors in the basal ganglia and their effects on dopamine release has led to interest in these receptors as targets for the treatment of Parkinson's disease (especially 5-HT<sub>1b</sub> and 5-HT<sub>4</sub>) and schizophrenia (5-HT<sub>1a</sub> and 5-HT<sub>2a</sub>). Indeed, antipsychotic drugs often interact with 5-HT receptors, in addition to dopamine receptors.

#### 9. Cholecystokinin Receptors

Interest in the role of CCK in the basal ganglia was stimulated by the discovery by Hokfelt and colleagues (1980) of CCK-like immunoreactivity in the dopamine cells of the substantia nigra of the rat. This led to a number of studies of the effects of CCK on dopamine release, for example (Marshall et al., 1991). Two CCK receptors have been cloned, CCK<sub>1</sub> (CCK<sub>A</sub>) and CCK<sub>2</sub> (CCK<sub>B</sub>). CCK<sub>1</sub> is found in the periphery and in selected CNS areas including the hypothalamus and interpeduncular nucleus. As its original name implies (CCK<sub>B</sub>), CCK<sub>2</sub> is the major form in the brain, being particularly expressed in the cerebral cortex and in lower amounts in the striatum and nucleus accumbens (Wank, 1995; Noble and Roques, 1999), although cellular localization is not yet established. CCK<sub>1</sub> receptors couple through G<sub>s</sub> to adenylyl cyclase and through G<sub>g</sub> to phospholipase C. CCK<sub>2</sub> receptors couple through G<sub>i</sub> /G<sub>o</sub> or G<sub>q</sub>.

#### 10. Neurotensin Receptors

The interest in the peptide neurotensin (NT) and its receptors is rather similar to CCK in that neurotensin immunoreactivity is found in dopamine cells of the ventral tegmental area, in MSNs of the ventral striatum and, after neuroleptic treatment, also in neurons in the dorsal striatum (for recent reviews, see Vincent et al., 1999; Binder et al., 2001; Dobner, 2005)). Neurotensin mRNA coexists with enkephalin mRNA in striatal MSNs, indicating that neurotensin is found in D<sub>2</sub>/enkephalin-expressing neurons of the indirect pathway (Augood et al., 1997). Interestingly neurotensin binding was found in an annular region surrounding striosomes of the striatum (Faull et al., 1989).

There are two established neurotensin GPCRs. NTR-1 is a high affinity receptor for neurotensin, whilst NTR-2 is closely related to NTR-1 but has a lower affinity for NT and is sensitive to the antihistamine drug levocabastine. NTR-1 couples through  $G_q/G_i$  and phospholipase C, mobilizing IP3 and DAG. NTR-2 is widely expressed in the CNS, including the cerebral cortex and different hypothalamic nuclei. However, its role as a functional NT receptor has been questioned as NT antagonists activate signaling pathways in cells transfected with NTR-2, which NT itself antagonizes (Dobner, 2005). In contrast, adding to the interest in NT/dopamine interactions, NTR-1 receptor mRNA is specifically expressed by the nigral dopamine cells, and NTR-1 immunoreactivity is found in the substantia nigra and striatum consistent with expression of the receptor on cell bodies and axons of the dopamine cells (Vincent et al., 1999; Fassio et al., 2000; Binder et al., 2001; Dobner, 2005).

#### 11. Histamine Receptors

Although histamine is not a major amine in the basal ganglia by comparison with dopamine or serotonin, a histaminergic system originating from neurons in the posterior hypothalamus and innervating the forebrain, including striatum and substantia nigra, has been described (Watanabe et al., 1983; Panula et al., 1984; Steinbusch et al., 1986). There are currently four known histamine receptors, H1-H4. H1 couples through Gq/11 H2 couples through G<sub>s</sub>, and H<sub>3</sub>-H<sub>4</sub> couple through Gi/o. In the striatum, H<sub>2</sub> and H<sub>3</sub> receptor mRNAs are strongly expressed in the majority of MSNs. In the substantia nigra only low levels of H<sub>2</sub> mRNA are present, although strong H<sub>2</sub> binding is found in this region, suggesting the binding is present on afferents from the striatum, which is supported by lesion studies and studies in HD cases (Vizuete et al., 1997). In contrast to H<sub>2</sub>, H<sub>3</sub> mRNA is expressed by the dopamine cells of the pars compacta (Pillot et al., 2002).

#### 12. Adrenergic Receptors

Adrenoreceptors can be grouped into three classes,  $\alpha_1$ -adrenoreceptors which couple through  $G_{q/11}$ ,  $\alpha_2$ -adrenoreceptors which couple through  $G_{i/o}$  and  $\beta$ -adrenoreceptors which couple through  $G_s$ . In the striatum, where the main amine is dopamine, it is thought that the  $\alpha_1$  and  $\beta$ -adrenoreceptors respond to dopamine rather than noradrenaline. In terms of localization, the  $\beta$ -adrenoreceptors are localized on striatal cholinergic neurons (Pisani et al., 2003) and the  $\alpha_{2c}$ -adrenoreceptors on striatal MSNs (Holmberg et al., 1999).

#### 13. Neuropeptide Y Receptors

Receptors for the neuropeptide NPY include five family members,  $Y_1-Y_5$ , all of which couple through  $G_{i/o}$ . Most interest has focused on the roles of these receptors in obesity, although the peptide NPY is abundant throughout the CNS (Parker et al., 2002). The  $Y_2$  receptor is particularly localized in the basal forebrain (Stanic et al., 2006).

#### 14. Other Family 1 GPCRs

In providing a brief overview of basal ganglia receptors, the focus has been on receptors with reported roles in the basal ganglia, particularly in the striatum; other identified receptors for neuropeptides such as neuromedin U, TRH or galanin, for example, have not been discussed. Similarly, there are other "orphan" receptors in the basal ganglia where the ligand remains to be identified. These include, for example, somatostatin- or opioid-like receptors (Lee et al., 2001),the glucocorticoid induced/NPY like receptor GPR 83 (De Moerlooze et al., 2000) and the biogenic amine receptor GPR88 (Mizushima et al., 2000)

#### B. Family 3

1. GABA<sub>B</sub> Receptors (Fig. 4.4)

In the basal ganglia and throughout the mammalian CNS, the actions of GABA are mediated by two classes of GABA receptors, the GABA<sub>A</sub> (discussed earlier) and GABA<sub>B</sub> receptors. The first class discovered were the GABA<sub>A</sub> receptors which could be detected by binding assays using <sup>3</sup>H-GABA. Such binding is blocked by the antagonist bicuculline (Curtis et al., 1974) and dependent on chloride (Zukin et al., 1974). In contrast, in 1979 Bowery and colleagues, studying the release of <sup>3</sup>H-noradrenaline from the rat atria, reported that GABA would reduce this release, but this process was not chloride-dependent or blockable by bicuculline, suggesting the presence of a separate, pharmacologically distinct GABA receptor (Bowery et al., 1979; Bowery et al., 1980; Bowery et al., 1981b; Bowery et al., 1981a). While this was disputed initially (Bowery, 1993), further studies identified  $\beta$ -p-chlorophenyl-GABA (baclofen) as an agonist at this putative GABA receptor, and the use of the <sup>3</sup>H-labeled form of baclofen allowed Bowery and colleagues to reveal a  $Ca^{2+}$ dependent binding of <sup>3</sup>H-baclofen and <sup>3</sup>H-GABA at the site they had earlier termed the GABA<sub>B</sub> site (Hill and Bowery, 1981). These studies established  $GABA_B$  as a CNS as well



**FIGURE 4.4** Schematic structure of the heterodimeric GABA<sub>B</sub> receptor. The functional receptor is made of two separate family 3 GPCRs, GABA<sub>B1</sub> and GABA<sub>B2</sub>, which have the typical serpentine seven transmembrane domains and large N-terminal sequences. GABA binds to the N-terminal bilobed domain of GABA<sub>B1</sub> whilst GABA<sub>B2</sub> is thought to bind G-proteins. Also in the N-terminal domain of GABA<sub>B1</sub> are two complement control proteins (CCP) or sushi domains. There are a number of proteins that have been reported to bind to the coiled–coil domains of the C-terminal sequences of GABA<sub>B1</sub> and GABA<sub>B2</sub> which will influence receptor function and localization for example, 14-3-3 and Mupp-1, see figure for further examples (Emson, 2007). G<sub>i</sub>/G<sub>o</sub> binds to adenylyl cyclase (AC), and the  $\beta/\gamma$  subunit can influence ion channels (Reproduced from Emson PC (2007) In: GABA and the basal ganglia pp 43–57 Progress in Brain Research Vol. 160, Edited by Tepper JM, Abercrombie ED and Bolam JP. Elsevier, Amsterdam).

as a peripheral receptor (Curtis and Johnston, 1974; Bowery et al., 1983). Baclofen was already known to decrease transmitter release and depress neuronal activity probably through an action on calcium channels. Subsequent work established a physiological role for a postsynaptic GABA<sub>B</sub> receptor whose activation produced an increase in membrane potassium conductance and neuronal hyperpolarization (Dutar and Nicoll, 1988; Nicoll, 2004). Thus activation of presynaptic GABA<sub>B</sub> receptors will inhibit the release of other neurotransmitters (e.g. noradrenaline) through a decrease in membrane Ca2+ conductance, whereas postsynaptic receptors induce an increase in membrane potassium conductance through G-protein coupling to inwardly rectifying GIRK or Kir3 potassium channels (Nicoll, 2004); see schematic Fig. 4.4). The GABA<sub>B</sub> receptor, in contrast to the GABA<sub>A</sub> channel, is coupled to G-proteins and is a family 3 metabotropic receptor (Hill, 1985; Karbon and Enna, 1985). Despite the relatively early recognition (1981) of the GABA<sub>B</sub> receptor and the identification of <sup>3</sup>H-baclofen as a ligand, it was not until 1997 that the first GABA<sub>B</sub> receptor was cloned (now termed GABA<sub>B(1)</sub>) by Kaupmann and colleagues (Kaupmann et al., 1997). Subsequently a number of groups established that the functional GABA<sub>B</sub> receptor exists as a heterodimer of two components, subunits GABA<sub>B(1)</sub> and GABA<sub>B(2)</sub> (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Ng et al., 1999). The cloning of these two GABA<sub>B</sub> receptor subunits and the realization that GPCRs can exist as heterodimers led to a large number of ongoing studies on oligomerization between GPCRs and the realization that the properties of oligomeric GPCRs may differ substantially from their monomers (Bulenger et al., 2005). Localization studies indicate that  $GABA_{B(1)}$  mRNA is localized throughout the forebrain, with lower amounts of GABA<sub>B(2)</sub> in the basal ganglia. However, immunostaining and receptor binding in the human indicate expression of the functional receptor throughout the basal ganglia (Waldvogel et al., 2004).

#### 2. Metabotropic Glutamate Receptors (mGluRs)

In contrast to the ionotropic glutamate receptors, the members of the mGluR family are serpentine G-protein-coupled receptors. They were originally identified as glutamate receptors linked to inositol phospholipid metabolism (Sugiyama et al., 1987). Subsequently, eight members of this family have been identified by molecular cloning (for recent reviews, see Kew and Kemp, 2005; Ferraguti and Shigemoto, 2006). Like GABAB receptors and the classical 7-transmembrane domain receptors, mGluRs have a large bi-lobed N-terminal domain that contains the glutamate binding site. The family can be divided into three groups on the basis of pharmacology, sequence and second messenger signaling, group 1 (mGluR1 and 5) which usually couple through  $G_q/G_{11}$  to phospholipase C, group 2 (mGluR2 and 3) and group 3 (mGluR4, 6, 7 and 8). Groups 2 and 3 usually couple through  $G_i/G_0$  to inhibit adenylate cyclase. In the basal ganglia, group 1 mGluRs, particularly mGluR5, are found in most regions, as are group 2 mGluRs. mGluR2 is found in the neuropil of the striatum as well as on the striatal terminals in the globus pallidus and substantia nigra pars reticulata, but is not found on compacta neurons (Phillips et al., 2000). From group 3, mGluR7 is the main form in the basal ganglia. Localization studies suggest that mGluR5 is found on indirect MSNs, cholinergic interneurons and globus pallidus neurons, whilst mGluR1 is on direct MSNs, neurons of the globus pallidus and dopamine neurons of the substantia nigra. mGluR4 is found on indirect MSN and dopamine cells, and mGluR4 immunoreactivity is concentrated in patches in the striatum (for review, see Feeley Kearney and Albin, 2003; Conn et al., 2005). mGluRs differ considerably in their C-terminal regions which determines their ability to interact with intracellular proteins. mGluR7a is found in the striatum, whilst the 7b variant is found in the globus pallidus in rodents. The intracellular binding proteins include calmodulin, homer and PICK1 (Dev et al., 2001; Pin et al., 2003). Competitive ligands are believed to interact with the bi-lobed N-terminal domain, whilst non-competitive ligands bind within the transmembrane domains (for review, see Kew and Kemp, 2005).

#### **IV. CONCLUSIONS**

The basal ganglia contains a complex variety of both ionotropic and metabotropic neurotransmitter receptors which reflects the major neurotransmitters of the system. The basal ganglia system functions primarily by GABAergic inhibition/disinhibition mechanisms and does not contain excitatory output neurons with the exception of the subthalamus which has glutamatergic projection neurons. The major input to the striatum is via excitatory glutamatergic afferents from the cerebral cortex and thalamus, and dopaminergic afferents from the substantia nigra. Local striatal interneurons use GABA and acetylcholine as well as a range of neuropeptides (e.g., NPY, somatostatin, nitric oxide). Fast transmission occurs mainly through ionotropic ion channel receptors (especially AMPA, NMDA and GABA<sub>A</sub>) while metabotropic receptors modulate the size and nature of these responses and provide slower signaling through G-protein modulation of basal ganglia signaling.

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#### REFERENCES

- Allen JP, Hathway GJ, Clarke NJ, Jowett MI, Topps S, Kendrick KM, Humphrey PP, Wilkinson LS, Emson PC (2003) Somatostatin receptor 2 knockout/lacZ knockin mice show impaired motor coordination and reveal sites of somatostatin action within the striatum. Eur J Neurosci 17:1881–1895.
- Allen KL, Waldvogel HJ, Glass M, Faull RL (2009) Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. J Chem Neuroanat 37:266–281.
- Anson LC, Chen PE, Wyllie DJ, Colquhoun D, Schoepfer R (1998) Identification of amino acid residues of the NR2A subunit that control glutamate potency in recombinant NR1/NR2A NMDA receptors. J Neurosci 18:581–589.
- Augood SJ, Westmore K, Emson PC (1997) Phenotypic characterization of neurotensin messenger RNA-expressing cells in the neuroleptic-treated rat striatum: a detailed cellular co-expression study.. Neuroscience 76:763–774.
- Augood SJ, Hollingsworth ZR, Standaert DG, Emson PC, Penney JB Jr. (2000) Localization of dopaminergic markers in the human subthalamic nucleus. J Comp Neurol 421:247–255.
- Bach AW, Unger L, Sprengel R, Mengod G, Palacios J, Seeburg PH, Voigt MM (1993) Structure functional expression and spatial distribution of a cloned cDNA encoding a rat 5-HT1D-like receptor. J Recept Res 13:479–502.
- Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function.. Neuropharmacology 38:1083–1152.
- Barnes NM, Hales TG, Lummis SC, Peters JA (2009) The 5-HT(3) receptor the relationship between structure and function. Neuropharmacology 56:273–284.
- Baufreton J, Garret M, Rivera A, de la Calle A, Gonon F, Dufy B, Bioulac B, Taupignon A (2003) D5 (not D1) dopamine receptors potentiate burst-firing in neurons of the subthalamic nucleus by modulating an L-type calcium conductance. J Neurosci 23:816–825.
- Beneyto M, Meador-Woodruff JH (2003) AMPA- and NMDA-associated postsynaptic protein expression in the human dorsolateral prefrontal cortex. Ann N Y Acad Sci 1003:352–355.
- Binder EB, Kinkead B, Owens MJ, Nemeroff CB (2001) Neurotensin and dopamine interactions. Pharmacol Rev 53:453–486.
- Bjorklund A, Dunnett SB (2007) Fifty years of dopamine research. Trends Neurosci 30:185–187.
- Bockaert J, Claeysen S, Becamel C, Dumuis A, Marin P (2006) Neuronal 5-HT metabotropic receptors: fine-tuning of their structure, signaling, and roles in synaptic modulation. Cell Tissue Res 326:553–572.
- Bowery NG (1993) GABAB receptor pharmacology. Annu Rev Pharmacol Toxicol 33:109–147.

- Bowery NG, Hill DR, Hudson AL (1983) Characteristics of GABAB receptor binding sites on rat whole brain synaptic membranes. Br J Pharmacol 78:191–206.
- Bowery NG, Doble A, Hill DR, Hudson AL, Shaw JS, Turnbull MJ (1979) Baclofen: a selective agonist for a novel type of GABA receptor [proceedings]. Br J Pharmacol 67:444P–445P.
- Bowery NG, Doble A, Hill DR, Hudson AL, Turnbull MJ, Warrington R (1981a) Structure/activity studies at a baclofen-sensitive, bicuculline-insensitive GABA receptor. Adv Biochem Psychopharmacol 29:333–341.
- Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M (1980) (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94.
- Bowery NG, Doble A, Hill DR, Hudson AL, Shaw JS, Turnbull MJ, Warrington R (1981b) Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. Eur J Pharmacol 71:53–70.
- Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411:269–276.
- Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, Hoyer D, Palacios JM (1994) Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. Neuropharmacology 33:367–386.
- Bulenger S, Marullo S, Bouvier M (2005) Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends Pharmacol Sci 26:131–137.
- Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, Grandy DK (1994) Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. FEBS Lett 347:284–288.
- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopaminemediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Cascio M (2004) Structure and function of the glycine receptor and related nicotinicoid receptors. J Biol Chem 279:19383–19386.
- Caulfield MP, Birdsall NJ (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 50:279–290.
- Chabre M, le Maire M (2005) Monomeric G-protein-coupled receptor as a functional unit. Biochemistry 44:9395–9403.
- Chazot PL, Stephenson FA (1997) Molecular dissection of native mammalian forebrain NMDA receptors containing the NR1 C2 exon: direct demonstration of NMDA receptors comprising NR1, NR2A, and NR2B subunits within the same complex. J Neurochem 69: 2138–2144.
- Chen GQ, Cui C, Mayer ML, Gouaux E (1999) Functional characterization of a potassium-selective prokaryotic glutamate receptor. Nature 402:817–821.
- Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA (1995) Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. J Neurosci 15:6498–6508.
- Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels.. Neuropharmacology 56:2–5.
- Conn PJ, Battaglia G, Marino MJ, Nicoletti F (2005) Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat Rev Neurosci 6:787–798.

- Connolly CN (2008) Trafficking of 5-HT(3) and GABA(A) receptors (Review). Mol Membr Biol 25:293–301.
- Connolly CN, Wafford KA (2004) The Cys-loop superfamily of ligand-gated ion channels: the impact of receptor structure on function. Biochem Soc Trans 32:529–534.
- Curtis DR, Johnston GA (1974) Amino acid transmitters in the mammalian central nervous system. Ergeb Physiol 69:97–188.
- Curtis DR, Johnston GA, Game CJ, McCulloch RM (1974) Central action of bicuculline. J Neurochem 23:605–606.
- Daaka Y, Luttrell LM, Lefkowitz RJ (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390:88–91.
- Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:699–729.
- Darstein M, Landwehrmeyer GB, Kling C, Becker CM, Feuerstein TJ (2000) Strychnine-sensitive glycine receptors in rat caudatoputamen are expressed by cholinergic interneurons.. Neuroscience 96:33–39.
- De Moerlooze L, Williamson J, Liners F, Perret J, Parmentier M (2000) Cloning and chromosomal mapping of the mouse and human genes encoding the orphan glucocorticoid-induced receptor (GPR83). Cytogenet Cell Genet 90:146–150.
- Deng YP, Xie JP, Wang HB, Lei WL, Chen Q, Reiner A (2007) Differential localization of the GluR1 and GluR2 subunits of the AMPA-type glutamate receptor among striatal neuron types in rats. J Chem Neuroanat 33:167–192.
- Dev KK, Nakanishi S, Henley JM (2001) Regulation of mglu(7) receptors by proteins that interact with the intracellular C-terminus. Trends Pharmacol Sci 22:355–361.
- Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7–61.
- Dobner PR (2005) Multitasking with neurotensin in the central nervous system. Cell Mol Life Sci 62:1946–1963.
- Dunah AW, Standaert DG (2003) Subcellular segregation of distinct heteromeric NMDA glutamate receptors in the striatum. J Neurochem 85:935–943.
- Dutar P, Nicoll RA (1988) A physiological role for GABAB receptors in the central nervous system. Nature 332:156–158.
- Eglen RM (2005) Muscarinic receptor subtype pharmacology and physiology. Prog Med Chem 43:105–136.
- Eglen RM (2006) Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. Auton Autacoid Pharmacol 26:219–233.
- Eglen RM, Wong EH, Dumuis A, Bockaert J (1995) Central 5-HT4 receptors. Trends Pharmacol Sci 16:391–398.
- Eilers M, Hornak V, Smith SO, Konopka JB (2005) Comparison of class A and D G protein-coupled receptors: common features in structure and activation. Biochemistry 44:8959–8975.
- Emson PC (2007) GABA(B) receptors: structure and function. Prog Brain Res 160:43–57.

Emson PC (2009) Somatostatin. Oxford: Academic Press.

- Fassio A, Evans G, Grisshammer R, Bolam JP, Mimmack M, Emson PC (2000) Distribution of the neurotensin receptor NTS1 in the rat CNS studied using an amino-terminal directed antibody. Neuropharmacology 39:1430–1442.
- Faull RL, Dragunow M, Villiger JW (1989) The distribution of neurotensin receptors and acetylcholinesterase in the human caudate nucleus: evidence for the existence of a third neurochemical compartment. Brain Res 488:381–386.

- Faull RL, Waldvogel HJ, Nicholson LF, Synek BJ (1993) The distribution of GABAA-benzodiazepine receptors in the basal ganglia in Huntington's disease and in the quinolinic acid-lesioned rat. Prog Brain Res 99:105–123.
- Feeley Kearney JA, Albin RL (2003) mGluRs: a target for pharmacotherapy in Parkinson disease. Exp Neurol 184(Suppl 1):S30–S36.
- Fenelon VS, Herbison AE (1996) In vivo regulation of specific GABAA receptor subunit messenger RNAs by increased GABA concentrations in rat brain. Neuroscience 71:661–670.
- Ferraguti F, Shigemoto R (2006) Metabotropic glutamate receptors. Cell Tissue Res 326:483–504.
- Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM, Reppert SM (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Brain Res Mol Brain Res 14:186–195.
- Flores G, Liang JJ, Sierra A, Martinez-Fong D, Quirion R, Aceves J, Srivastava LK (1999) Expression of dopamine receptors in the subthalamic nucleus of the rat: characterization using reverse transcriptase-polymerase chain reaction and autoradiography. Neuroscience 91:549–556.
- Fritschy JM, Harvey RJ, Schwarz G (2008) Gephyrin: where do we stand, where do we go? Trends Neurosci 31:257–264.
- Fusco FR, Martorana A, Giampa C, De March Z, Farini D, D'Angelo V, Sancesario G, Bernardi G (2004) Immunolocalization of CB1 receptor in rat striatal neurons: a confocal microscopy study. Synapse 53:159–167.
- Fuxe K, Ferre S, Genedani S, Franco R, Agnati LF (2007) Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 92:210–217.
- Gilman AG (1987) G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56:615–649.
- Girault JA, Greengard P (2004) The neurobiology of dopamine signaling. Arch Neurol 61:641–644.
- Glass M, Faull RL, Dragunow M (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease.. Neuroscience 56:523–527.
- Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77:299–318.
- Glass M, Dragunow M, Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97:505–519.
- Goetz T, Arslan A, Wisden W, Wulff P (2007) GABA(A) receptors: structure and function in the basal ganglia. Prog Brain Res 160:21–41.
- Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Geyer MA, Hen R (1999) Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 22:581–591.
- Graybiel AM, Ragsdale CW Jr. (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci USA 75:5723–5726.
- Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H, Laube B (2005) The beta subunit determines the ligand binding properties of synaptic glycine receptors. Neuron 45:727–739.

- Guard S, Watling KJ, Watson SP (1988) Neurokinin3-receptors are linked to inositol phospholipid hydrolysis in the guinea-pig ileum longitudinal muscle-myenteric plexus preparation. Br J Pharmacol 94:148–154.
- Guard S, McKnight AT, Watling KJ, Watson SP (1991) Evidence for two types of tachykinin receptors on cholinergic neurons of the guinea pig ileum myenteric plexus. Ann NY Acad Sci 632:400–403.
- Hartman DS, Civelli O (1997) Dopamine receptor diversity: molecular and pharmacological perspectives. Prog Drug Res 48:173–194.
- Herkenham M, Pert CB (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. Nature 291:415–418.
- Herkenham M, Lynn AB, de Costa BR, Richfield EK (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. Brain Res 547:267–274.
- Hersch SM, Gutekunst CA, Rees HD, Heilman CJ, Levey AI (1994) Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. J Neurosci 14:3351–3363.
- Hill DR (1985) GABAB receptor modulation of adenylate cyclase activity in rat brain slices. Br J Pharmacol 84:249–257.
- Hill DR, Bowery NG (1981) 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. Nature 290:149–152.
- Hirai H, Kirsch J, Laube B, Betz H, Kuhse J (1996) The glycine binding site of the N-methyl-D-aspartate receptor subunit NR1: identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. Proc Natl Acad Sci USA 93:6031–6036.
- Hohmann AG, Herkenham M (2000) Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a doublelabel in situ hybridization study. Synapse 37:71–80.
- Hokfelt T, Skirboll L, Rehfeld JF, Goldstein M, Markey K, Dann O (1980) A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. Neuroscience 5:2093–2124.
- Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annu Rev Neurosci 17:31–108.
- Holmberg M, Scheinin M, Kurose H, Miettinen R (1999) Adrenergic alpha2C-receptors reside in rat striatal GABAergic projection neurons: comparison of radioligand binding and immunohistochemistry. Neuroscience 93:1323–1333.
- Houamed KM, Kuijper JL, Gilbert TL, Haldeman BA, O'Hara PJ, Mulvihill ER, Almers W, Hagen FS (1991) Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. Science 252:1318–1321.
- Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533–554.
- Husi H, Grant SG (2001a) Proteomics of the nervous system. Trends Neurosci 24:259–266.
- Husi H, Grant SG (2001b) Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. J Neurochem 77:281–291.
- Ishii M, Kurachi Y (2006) Muscarinic acetylcholine receptors. Curr Pharm Des 12:3573–3581.
- Iversen SD, Iversen LL (2007) Dopamine: 50 years in perspective. Trends Neurosci 30:188–193.
- Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC (2008) The 2.6 angstrom crystal structure

of a human A2A adenosine receptor bound to an antagonist. Science 322:1211–1217.

- Jane DE, Lodge D, Collingridge GL (2009) Kainate receptors: Pharmacology, function and therapeutic potential. Neuropharmacology 56:90–113.
- Jones KA, Borowsky B, Tamm JA, et al. (1998) GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. Nature 396:674–679.
- Jones S, Sudweeks S, Yakel JL (1999) Nicotinic receptors in the brain: correlating physiology with function. Trends Neurosci 22:555–561.
- Julian MD, Martin AB, Cuellar B, Rodriguez De Fonseca F, Navarro M, Moratalla R, Garcia-Segura LM (2003) Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. Neuroscience 119:309–318.
- Karbon EW, Enna SJ (1985) Characterization of the relationship between gamma-aminobutyric acid B agonists and transmitter-coupled cyclic nucleotide-generating systems in rat brain. Mol Pharmacol 27:53–59.
- Kaupmann K, Huggel K, Heid J, et al. (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. Nature 386:239–246.
- Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B (1998) GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature 396:683–687.
- Kew JN, Kemp JA (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) 179:4–29.
- Kneussel M, Loebrich S (2007) Trafficking and synaptic anchoring of ionotropic inhibitory neurotransmitter receptors. Biol Cell 99:297–309.
- Kohen R, Metcalf MA, Khan N, et al. (1996) Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. J Neurochem 66:47–56.
- Kuner R, Kohr G, Grunewald S, Eisenhardt G, Bach A, Kornau HC (1999) Role of heteromer formation in GABAB receptor function. Science 283:74–77.
- Kuryatov A, Laube B, Betz H, Kuhse J (1994) Mutational analysis of the glycine-binding site of the NMDA receptor: structural similarity with bacterial amino acid-binding proteins. Neuron 12:1291–1300.
- Langley JN (1905) On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. J Physiol 33:374–413.
- Laube B, Kuhse J, Betz H (1998) Evidence for a tetrameric structure of recombinant NMDA receptors. J Neurosci 18:2954–2961.
- Le Moine C, Kieffer B, Gaveriaux-Ruff C, Befort K, Bloch B (1994) Deltaopioid receptor gene expression in the mouse forebrain: localization in cholinergic neurons of the striatum. Neuroscience 62:635–640.
- Lee DK, George SR, Evans JF, Lynch KR, O'Dowd BF (2001) Orphan G protein-coupled receptors in the CNS. Curr Opin Pharmacol 1:31–39.
- Lee T, Kaneko T, Taki K, Mizuno N (1997) Preprodynorphin-, preproenkephalin-, and preprotachykinin-expressing neurons in the rat neostriatum: an analysis by immunocytochemistry and retrograde tracing. J Comp Neurol 386:229–244.
- Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT (1997) Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL 100,907. Naunyn Schmiedebergs Arch Pharmacol 356:446–454.
- Luscher B, Keller CA (2004) Regulation of GABAA receptor trafficking, channel activity, and functional plasticity of inhibitory synapses. Pharmacol Ther 102:195–221.

- Lynch JW (2004) Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 84:1051–1095.
- Madden DR (2002) The inner workings of the AMPA receptors. Curr Opin Drug Discov Devel 5:741–748.
- Maehle AH (2004) "Receptive substances": John Newport Langley (1852–1925) and his path to a receptor theory of drug action. Med Hist 48:153–174.
- Mansour A, Fox CA, Akil H, Watson SJ (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 18:22–29.
- Marshall FH, Barnes S, Hughes J, Woodruff GN, Hunter JC (1991) Cholecystokinin modulates the release of dopamine from the anterior and posterior nucleus accumbens by two different mechanisms. J Neurochem 56:917–922.
- Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S (1991) Sequence and expression of a metabotropic glutamate receptor. Nature 349:760–765.
- Matsuda K, Kamiya Y, Matsuda S, Yuzaki M (2002) Cloning and characterization of a novel NMDA receptor subunit NR3B: a dominant subunit that reduces calcium permeability. Brain Res Mol Brain Res 100:43–52.
- Mayer ML (2005) Glutamate receptor ion channels. Curr Opin Neurobiol 15:282–288.
- Mayer ML (2006) Glutamate receptors at atomic resolution. Nature 440:456–462.
- Mayer ML, Armstrong N (2004) Structure and function of glutamate receptor ion channels. Annu Rev Physiol 66:161–181.
- McKernan RM, Whiting PJ (1996) Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci 19:139–143.
- Milstein AD, Nicoll RA (2008) Regulation of AMPA receptor gating and pharmacology by TARP auxiliary subunits. Trends Pharmacol Sci 29:333–339.
- Mizushima K, Miyamoto Y, Tsukahara F, Hirai M, Sakaki Y, Ito T (2000) A novel G-protein-coupled receptor gene expressed in striatum.. Genomics 69:314–321.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529–540.
- Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256:1217–1221.
- Nanao MH, Green T, Stern-Bach Y, Heinemann SF, Choe S (2005) Structure of the kainate receptor subunit GluR6 agonist-binding domain complexed with domoic acid. Proc Natl Acad Sci USA 102:1708–1713.
- Naur P, Vestergaard B, Skov LK, Egebjerg J, Gajhede M, Kastrup JS (2005) Crystal structure of the kainate receptor GluR5 ligand-binding core in complex with (S)-glutamate. FEBS Lett 579:1154–1160.
- Neves SR, Ram PT, Iyengar R (2002) G protein pathways. Science 296:1636–1639.
- Ng GY, Clark J, Coulombe N, et al. (1999) Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. J Biol Chem 274:7607–7610.
- Nicoll RA (2004) My close encounter with GABA(B) receptors. Biochem Pharmacol 68:1667–1674.
- Noble F, Roques BP (1999) CCK-B receptor: chemistry, molecular biology, biochemistry and pharmacology. Prog Neurobiol 58:349–379.
- Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, Yamada S (2005) Quantitative analysis of binding parameters of

[3H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. Brain Res Mol Brain Res 133:6–11.

- Olsen RW, Sieghart W (2009) GABA(A) receptors: Subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141–148.
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M (2000) Crystal structure of rhodopsin: A G proteincoupled receptor. Science 289:739–745.
- Panula P, Yang HY, Costa E (1984) Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA 81:2572–2576.
- Paoletti P, Neyton J (2007) NMDA receptor subunits: function and pharmacology. Curr Opin Pharmacol 7:39–47.
- Parker E, Van Heek M, Stamford A (2002) Neuropeptide Y receptors as targets for anti-obesity drug development: perspective and current status. Eur J Pharmacol 440:173–187.
- Phillips T, Rees S, Augood S, Waldvogel H, Faull R, Svendsen C, Emson P (2000) Localization of metabotropic glutamate receptor type 2 in the human brain. Neuroscience 95:1139–1156.
- Picciotto MR, Wickman K (1998) Using knockout and transgenic mice to study neurophysiology and behavior. Physiol Rev 78:1131–1163.
- Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391:173–177.
- Pillot C, Heron A, Cochois V, Tardivel-Lacombe J, Ligneau X, Schwartz JC, Arrang JM (2002) A detailed mapping of the histamine H(3) receptor and its gene transcripts in rat brain. Neuroscience 114:173–193.
- Pin JP, Galvez T, Prezeau L (2003) Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther 98:325–354.
- Pinheiro P, Mulle C (2006) Kainate receptors. Cell Tissue Res 326:457–482.
- Pisani A, Bonsi P, Centonze D, Martorana A, Fusco F, Sancesario G, De Persis C, Bernardi G, Calabresi P (2003) Activation of beta1adrenoceptors excites striatal cholinergic interneurons through a cAMP-dependent, protein kinase-independent pathway. J Neurosci 23:5272–5282.
- Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. Nature 338:582–585.
- Rang HP (2006) The receptor concept: pharmacology's big idea. Br J Pharmacol 147(Suppl 1):S9–S16.
- Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 450:383–387.
- Rivera A, Alberti I, Martin AB, Narvaez JA, de la Calle A, Moratalla R (2002) Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. Eur J Neurosci 16:2049–2058.
- Sans N, Prybylowski K, Petralia RS, Chang K, Wang YX, Racca C, Vicini S, Wenthold RJ (2003) NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. Nat Cell Biol 5:520–530.
- Schiffmann SN, Vanderhaeghen JJ (1993) Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J Neurosci 13:1080–1087.

- Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferre S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277–292.
- Schwarzschild MA, Agnati L, Fuxe K, Chen JF, Morelli M (2006) Targeting adenosine A2A receptors in Parkinson's disease. Trends Neurosci 29:647–654.
- Selmer I, Schindler M, Allen JP, Humphrey PP, Emson PC (2000) Advances in understanding neuronal somatostatin receptors. Regul Pept 90:1–18.
- Sergeeva OA (1998) Comparison of glycine- and GABA-evoked currents in two types of neurons isolated from the rat striatum. Neurosci Lett 243:9–12.
- Sergeeva OA, Haas HL (2001) Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. Neuroscience 104:1043–1055.
- Shen HY, Coelho JE, Ohtsuka N, et al. (2008) A critical role of the adenosine A2A receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A2A receptor knock-outs. J Neurosci 28:2970–2975.
- Sheng M, Sala C (2001) PDZ domains and the organization of supramolecular complexes. Annu Rev Neurosci 24:1–29.
- Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY (1994) Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368:144–147.
- Shigemoto R, Nakaya Y, Nomura S, Ogawa-Meguro R, Ohishi H, Kaneko T, Nakanishi S, Mizuno N (1993) Immunocytochemical localization of rat substance P receptor in the striatum. Neurosci Lett 153:157–160.
- Sieghart W (2006) Structure, pharmacology, and function of GABAA receptor subtypes. Adv Pharmacol 54:231–263.
- Simon MI, Strathmann MP, Gautam N (1991) Diversity of G proteins in signal transduction. Science 252:802–808.
- Sine SM, Engel AG (2006) Recent advances in Cys-loop receptor structure and function. Nature 440:448–455.
- Standaert DG, Landwehrmeyer GB, Kerner JA, Penney JB Jr., Young AB (1996) Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex and hippocampus. Brain Res Mol Brain Res 42:89–102.
- Standaert DG, Friberg IK, Landwehrmeyer GB, Young AB, Penney JB Jr. (1999) Expression of NMDA glutamate receptor subunit mRNAs in neurochemically identified projection and interneurons in the striatum of the rat. Brain Res Mol Brain Res 64:11–23.
- Stanic D, Brumovsky P, Fetissov S, Shuster S, Herzog H, Hokfelt T (2006) Characterization of neuropeptide Y2 receptor protein expression in the mouse brain. I. Distribution in cell bodies and nerve terminals. J Comp Neurol 499:357–390.
- Stefani A, Chen Q, Flores-Hernandez J, Jiao Y, Reiner A, Surmeier DJ (1998) Physiological and molecular properties of AMPA/Kainate receptors expressed by striatal medium spiny neurons. Dev Neurosci 20:242–252.
- Steinbusch HW, Sauren Y, Groenewegen H, Watanabe T, Mulder AH (1986) Histaminergic projections from the premammillary and posterior hypothalamic region to the caudate-putamen complex in the rat. Brain Res 368:389–393.
- Steward LJ, West KE, Kilpatrick GJ, Barnes NM (1993a) Labelling of 5-HT3 receptor recognition sites in the rat brain using the agonist radioligand [3H]meta-chlorophenylbiguanide. Eur J Pharmacol 243:13–18.
- Steward LJ, Bufton KE, Hopkins PC, Davies WE, Barnes NM (1993b) Reduced levels of 5-HT3 receptor recognition sites in the putamen of patients with Huntington's disease. Eur J Pharmacol 242:137–143.

- Strange PG (2001) Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. Pharmacol Rev 53:119–133.
- Sugiyama H, Ito I, Hirono C (1987) A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature 325:531–533.
- Svenningsson P, Lindskog M, Rognoni F, Fredholm BB, Greengard P, Fisone G (1998) Activation of adenosine A2A and dopamine D1 receptors stimulates cyclic AMP-dependent phosphorylation of DARPP-32 in distinct populations of striatal projection neurons. Neuroscience 84:223–228.
- Tomita S, Fukata M, Nicoll RA, Bredt DS (2004) Dynamic interaction of stargazin-like TARPs with cycling AMPA receptors at synapses. Science 303:1508–1511.
- Unwin N (2005) Refined structure of the nicotinic acetylcholine receptor at 4A resolution. J Mol Biol 346:967–989.
- Vincent JP, Mazella J, Kitabgi P (1999) Neurotensin and neurotensin receptors. Trends Pharmacol Sci 20:302–309.
- Vizuete ML, Traiffort E, Bouthenet ML, Ruat M, Souil E, Tardivel-Lacombe J, Schwartz JC (1997) Detailed mapping of the histamine H2 receptor and its gene transcripts in guinea-pig brain. Neuroscience 80:321–343.
- Wafford KA, Kathoria M, Bain CJ, Marshall G, Le Bourdelles B, Kemp JA, Whiting PJ (1995) Identification of amino acids in the N-methyl-Daspartate receptor NR1 subunit that contribute to the glycine binding site. Mol Pharmacol 47:374–380.
- Waldvogel HJ, Kubota Y, Fritschy J, Mohler H, Faull RL (1999) Regional and cellular localisation of GABA(A) receptor subunits in the human basal ganglia: An autoradiographic and immunohistochemical study. J Comp Neurol 415:313–340.
- Waldvogel HJ, Billinton A, White JH, Emson PC, Faull RL (2004) Comparative cellular distribution of GABAA and GABAB receptors in the human basal ganglia: immunohistochemical colocalization of the alpha 1 subunit of the GABAA receptor, and the GABABR1 and GABABR2 receptor subunits. J Comp Neurol 470:339–356.
- Waldvogel HJ, Baer K, Allen KL, Rees MI, Faull RL (2007) Glycine receptors in the striatum, globus pallidus, and substantia nigra of the human brain: an immunohistochemical study. J Comp Neurol 502:1012–1029.
- Waldvogel HJ, Baer K, Gai WP, Gilbert RT, Rees MI, Mohler H, Faull RL (2008) Differential localization of GABAA receptor subunits within the substantia nigra of the human brain: an immunohistochemical study. J Comp Neurol 506:912–929.
- Wang H, Pickel VM (2001) Preferential cytoplasmic localization of deltaopioid receptors in rat striatal patches: comparison with plasmalemmal mu-opioid receptors. J Neurosci 21:3242–3250.
- Wang H, Moriwaki A, Wang JB, Uhl GR, Pickel VM (1996) Ultrastructural immunocytochemical localization of mu opioid receptors and Leu5-enkephalin in the patch compartment of the rat caudate-putamen nucleus. J Comp Neurol 375:659–674.

Wank SA (1995) Cholecystokinin receptors. Am J Physiol 269:G628-G646.

- Watanabe T, Taguchi Y, Hayashi H, Tanaka J, Shiosaka S, Tohyama M, Kubota H, Terano Y, Wada H (1983) Evidence for the presence of a histaminergic neuron system in the rat brain: an immunohistochemical analysis. Neurosci Lett 39:249–254.
- Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS (2003) Trafficking of NMDA receptors. Annu Rev Pharmacol Toxicol 43:335–358.
- Wess J (2003) Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. Trends Pharmacol Sci 24:414–420.
- Wess J, Eglen RM, Gautam D (2007) Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov 6:721–733.
- White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH (1998) Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature 396:679–682.
- Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20:92–98.
- Wullner U, Standaert DG, Testa CM, Penney JB, Young AB (1997) Differential expression of kainate receptors in the basal ganglia of the developing and adult rat brain. Brain Res 768:215–223.
- Yamada M, Basile AS, Fedorova I, Zhang W, Duttaroy A, Cui Y, Lamping KG, Faraci FM, Deng CX, Wess J (2003) Novel insights into M5

muscarinic acetylcholine receptor function by the use of gene targeting technology. Life Sci 74:345–353.

- Zhang W, Yamada M, Gomeza J, Basile AS, Wess J (2002) Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. J Neurosci 22:6347–6352.
- Zhou FM, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4:1224–1229.
- Zhou FM, Wilson CJ, Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. J Neurobiol 53:590–605.
- Zukin SR, Young AB, Snyder SH (1974) Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system.. Proc Natl Acad Sci USA 71:4802–4807.

# The Striatal Skeleton: Medium Spiny Projection Neurons and their Lateral Connections

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### I. INTRODUCTION

Medium spiny projection neurons (MSNs) are the principal cell type of the striatum. By stereological methods, they account for more than 95% of striatal neurons in the rat, and their total unilateral number in the rat brain is estimated as 2.8 million (Oorschot, 1996) (see Chapter 3). In the human, the number is about 110 million (Schroder et al., 1975). The remaining 3–5% of striatal neurons includes cholinergic interneurons and several classes of gamma-amino butyric acid (GABA)-releasing interneurons (Kawaguchi, 1993; Kubota et al., 1993; Kawaguchi et al., 1995). The interneurons produce disproportionately strong effects despite being numerically in the minority (Koos and Tepper, 1999; Koos et al., 2004; Tepper et al., 2004). However, it is the MSNs that provide the substrate for the specific information processing operations of the striatum.

The MSNs are the only output neurons of the striatum and are also the major recipient of extrinsic input (Wilson and Groves, 1980; Somogyi et al., 1981). In effect they form

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. a single layer of cells interposed between the input and output side of the striatum. Thus, the physiological properties of the MSNs, their extrinsic inputs, and their interactions with other MSNs and interneurons are key determinants of the input–output operations performed by the striatum. In this chapter, we will review the anatomical and physiological characteristics of MSNs. Several other excellent reviews on MSNs and striatal function have been published recently (see Part B of this volume). We also point the interested reader to the recent review by Bolam et al. (2006).

## II. THE STRIATAL MEDIUM SPINY NEURON

## A. General Morphology of the Medium Spiny Neuron

The MSN has a characteristic morphology with a relatively uniform somatodendritic architecture (Wilson and Groves, 1980; Somogyi et al., 1981) as illustrated in Figure 5.1. The cell body is 12-20µm in diameter. The dendritic tree is usually formed by five or six primary dendrites which radiate from the cell body and divide once or twice to form secondary and tertiary dendrites extending within a roughly spherical volume of about 250-500 µm in diameter (DiFiglia et al., 1976; Wilson and Groves, 1980). The proximal dendrites are of small diameter and relatively spine-free, whereas the distal dendrites are densely spiny, starting about 20 µm from the soma and continuing to the tip of the dendrite (DiFiglia et al., 1976; Wilson and Groves, 1980). Each neuron gives rise to a main axon that originates close to the cell body and projects to target structures and also gives rise to local axon collaterals which divide repeatedly to form an extensive network that overlaps extensively with the dendritic tree (Wilson and Groves, 1980) (Fig. 5.1). The synaptic boutons of the local axon collaterals form synapses on other striatal neurons (Somogyi et al., 1981), and in particular with other MSNs, where they are located mostly at proximal and higher order dendrites (Wilson, 1994; Oorschot et al., 2002).

Although all MSNs share these common properties they are also separable into two roughly equal subpopulations, based on their axonal projection targets, the expression of genes for certain peptides, and the expression of dopamine receptors (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine and Bloch, 1995) (see also Chapter 1). About half of all MSNs project to the internal segment and half to the external segment of the globus pallidus, which in the rat corresponds to the striatonigral and striatopallidal neurons, respectively (Gerfen and Wilson, 1996). Early studies using in situ hybridization histochemistry demonstrated that the substance P/dynorphin-releasing striatonigral neurons carry predominantly the D1 dopamine receptor, whereas the enkephalin-containing striatopallidal neurons are regulated by the D2 receptor (Gerfen et al., 1990). While initial studies using single cell RT-PCR reported considerable co-localization of these dopamine receptor subtypes, however, refinement of this technique eventually confirmed this major subdivision of MSNs (Surmeier et al., 1996). The recent introduction of bacterial artificial chromosome (BAC) D1- and D2-labeled GFP mice (GENSAT project; Heintz, 2001; Gong et al., 2003) allowed for a more detailed physiological characterization of these two classes using modern recording techniques (Day et al., 2006; Gertler et al., 2008) (see Chapter 6).



**FIGURE 5.1** The morphology of striatal medium spiny projection neurons (MSNs). (A) Reconstruction of the dendritic arborization of a biocytinfilled MSN from the rat striatum. Dendrites are densely studded with spines except for the first  $20 \,\mu\text{m}$  of the primary dendrite. Thin line indicates the axon that leaves the striatum. (B) Corresponding arborization of the local axon collaterals of the neuron in A. Note that for most MSNs local axon collaterals overlap with the dendritic tree (modified from Wilson and Groves, 1980). (C) High-power electron microscopic image of the dendritic tree of an MSN (modified from Wilson, 1994).

Recent work using these D1- and D2-labeled GFP mice together with reconstructions of biocytin-filled MSNs revealed quantitative differences between the two classes of MSNs (Gertler et al., 2008). Although they possess the same qualitative characteristics, D1 MSNs have significantly greater total dendritic length and more branches than D2 MSNs. The difference is due to a different number of primary dendrites, as the mean tree length (i.e., total dendritic length/number of primary dendrites) was similar in the two classes of MSNs. Striatopallidal neurons fire more in response to current input (Day et al., 2006; Kreitzer and Malenka, 2007), most likely because of their fewer dendritic branches.

A second major subdividing factor for MSNs has been their relation to the patch and matrix compartments of the striatum, identified by the expression of opioid receptors and other markers (Gerfen, 1984; Graybiel, 1984) (see Chapter 1). Importantly, local axon collaterals and the dendrites of MSNs obey patch/matrix borders (Kawaguchi et al., 1989), suggesting a subdivision of the striatal skeleton into the two compartments.

#### **B.** Dendritic Spines

An important characteristic of the MSN is its very high density of dendritic spines. Although many different functions have been proposed for dendritic spines, they are commonly associated with plasticity of excitatory synapses and their morphology may determine the effectiveness of the excitatory inputs. In the striatum the dendritic spines are the primary recipients of input from two major extrinsic afferent sources. The MSNs receive excitatory synaptic input from pyramidal cells in all areas of the cerebral cortex (McGeorge and Faull, 1989) (see Chapter 1 for an overview) and from several intralaminar nuclei in the thalamus (Nauta et al., 1974; Dube et al., 1988) (see Chapter 22). The macroscopic, topographical organization of these glutamatergic projections is complex [for reviews, see Voorn et al., 2004 (rat); Haber, 2003 (primate)].

The glutamatergic inputs to MSNs terminate on the heads of dendritic spines (see Chapter 1). Although different types of spines have been described in cerebral cortex and striatum and examples of these different types can be found among the spines of MSNs, there is a continuum of intermediate forms, and quantitative analysis shows a unimodal distribution of major dimensions (Wilson et al., 1983b). Spine neck diameter range from less than  $0.1 \,\mu\text{m}$  to about  $0.5 \,\mu\text{m}$  and lengths range from  $0.35-3.80\,\mu\text{m}$ , with spine head diameters ranging from  $0.11-0.95\,\mu\text{m}$ . Densities of spines along a dendrite range as high as 46 per  $10\,\mu\text{m}$ . In computational models, the size range produces effects on the efficacy of synapses comparable to dendritic location (Wilson, 1984).

Although to date no differences have been identified between classes of MSNs in spine morphology, differential sensitivity of spine numbers to dopamine depletion has been shown. Dopamine depletion leads to a rapid and profound loss of spines and glutamatergic synapses on striatopallidal MSNs but not on striatonigral MSNs (Day et al., 2006).

#### C. Glutamate Receptor-Mediated Responses

Synaptic inputs to the MSNs from cortex and thalamus produce fast, monosynaptic excitatory postsynaptic potentials (EPSPs) that can be finely graded and probably represent the contribution of many individually small synaptic inputs (Wilson, 1986). The EPSP is mediated by glutamate acting on non-NMDA receptors (Calabresi et al., 1996) (see Chapters 6 and 12). Although they are present and can be activated pharmacologically, historically NMDA receptors have been difficult to activate in the acute slice in vitro, even with high-frequency stimulation (Herrling et al., 1983). This seems to be a result of the fairly polarized resting potential of MSNs and the uncontrolled severance of corticostriatal projections in most slice preparation. In vivo, a clear NMDA component is present in inputs from the cortex and thalamus (Kita, 1996), and NMDA receptors contribute to the depolarized states observed (Pomata et al., 2008) when strong excitatory input produces sufficient depolarization to overcome the voltage-dependent magnesium ion block of these channels. Similarly, in cortex-striatum-substantia nigra cultures, which regenerate the corticostriatal pathway and capture major aspects of the in vivo Up state dynamics, NMDA receptors have shown to control numerous aspects of MSN physiology (Kerr and Plenz, 2002; Kerr and Plenz, 2004). More specifically, despite their small dendritic diameter and shunts received from inhibitory inputs at their basal dendrites, slice culture and acute slice experiments demonstrated robust backpropagation of action potentials from the soma into MSN dendrites during rest, i.e., the Down state (Kerr and Plenz, 2002; Carter and Sabatini, 2004; Kerr and Plenz, 2004) as well as during synaptically driven Up states (Kerr and Plenz, 2002; Kerr and Plenz, 2004). Somatic depolarization from current injections (Carter and Sabatini, 2004) or synaptic depolarization during the Up state (Kerr and Plenz, 2004) allows calcium to enter through activated NMDA receptor during spike backpropagation (Fig. 5.2). Importantly,



**FIGURE 5.2** Spike backpropagation and dendritic calcium influx through the NMDA receptor pave the way for spike timing-dependent plasticity in MSNs. (A) Intracellular recording of an MSN showing spontaneous Up state transition and evoked spike at the time of a somatic, brief current injection (spike trigger; cortex-striatum-substantia nigra slice culture). (B) Intracellular calcium transients measured using Fura-2 are highest if the spike occurs early during the Up state and decreases for intermediate and late times. (C) Summary statistics of peak calcium transients with time from Up state onset. Note that transients are similar for spontaneous and evoked spikes from somatic current injections. Internal blockade of the NMDA receptor blocks the time dependence. (D) Extracellular, pulsatile application of NMDA close to the MSN tertiary dendrite evokes a supralinear calcium response when paired with a backpropagating burst of three spikes. Note the block of the supralinearity upon internal block of the NMDA receptors. (E) Change in the excitatory postsynaptic potential (EPSP) as a function of the relative time between glutamatergic inputs during the Down state and a backpropagating action potential. (F) Spike timing dependent plasticity rule obtained from averages of data in E. A–D: see Kerr and Plenz, 2004. E, F: see Pawlak and Kerr, 2008.

the amount of calcium that enters through the NMDA receptor into the dendrite decreases the longer the delay between Up state onset and somatic spiking [Fig. 5.2(A-D)].

The demonstration of these mechanisms paved the way for recent experiments establishing spike timing-dependent plasticity at corticostriatal synapses in MSNs (Pawlak and Kerr, 2008), since calcium entry through NMDA channels contributes to long-term potentiation (LTP) of corticostriatal afferents (Calabresi et al., 1992). Indeed, the increase in synaptic efficacy obtained is largest if spike backpropagation occurs shortly after a synaptic inputs (Fig. 5.2E,F). These experiments establish a clear temporal evaluation of cortical striatal inputs with respect to their ability to fire an action potential in the MSN. Importantly, this mechanism rewards specifically those inputs out of the potentially tens of thousands inputs to an MSNs that occurred just before the backpropagating spike and most likely contributed to the firing success. Conversely, inputs that occur right after a backpropagating action potential are not causally related to

firing success and are down regulated. This way, the relative timing between a backpropagating spike and the calcium influx through the NMDA channel combine to dynamically regulate the strength of corticostriatal inputs. Importantly, this regulation requires the presence of dopamine as a third factor (Pawlak and Kerr, 2008). Dopamine also seems to regulate the backpropagation into third and fourth-order MSN dendrites in striatonigral MSNs (Day et al., 2008). We note that Fino et al. (2005) reported bidirectional spike-timing dependent plasticity, but in the opposite direction to that reported by Pawlak and Kerr (reviewed by Wickens, 2009).

Shen et al. (2008) showed that dopamine plays a different role in D1 versus D2 MSNs. These authors used intrastriatal stimulation, which evokes not only glutamate release but also release of neuromodulators such as dopamine and acetylcholine, more so than cortical stimulation. In D1 neurons repeated theta-burst stimulation of presynaptic inputs before postsynaptic spikes caused LTP. This LTP was blocked by a D1 receptor antagonist, consistent with previous reports (Reynolds et al., 2001, Kerr and Wickens, 2001). Shen et al. also found that LTP did not occur in D1 MSNs in slices from dopamine-depleted animals, but could be restored by application of a D1 agonist. In D2 MSNs, on the other hand, timing dependent LTP was blocked by A2a adenosine receptor antagonists and LTD was blocked by D2 antagonists. In D2 MSNs from dopamine-depleted animals there was loss of bidirectional plasticity and LTP dominated spike-timing dependent plasticity. These and other findings show that a complex interplay exists between dopamine, adenosine, metabotropic glutamate, and endocannabinoid receptors that determine the final outcome. These interactions are discussed in more detail in Chapter 6.

### D. Neurophysiology of Medium Spiny Neurons

In awake animals, MSNs fire in brief episodes separated by longer periods of quiescence (Schultz and Romo, 1988; Kimura et al., 1990). The firing episodes are associated with initiation, execution, or termination of particular movements by the animal (Alexander, 1987; Schultz and Romo, 1988; Kimura et al., 1990). Similar episodic firing patterns also occur in immobilised, locally anaesthetised rats (Wilson and Groves, 1981) and in urethane-anaesthetised rats (Wilson, 1993) [Fig. 5.3(A,B)].

Intracellular recordings in awake (Wilson and Groves, 1981) and in urethane-anaesthetized rats (Wilson, 1993) revealed membrane potential transitions occurring continuously between a hyperpolarized Down state and a depolarised Up state (Wilson and Groves, 1981; Wilson and Kawaguchi, 1996) (Fig. 5.3A). These transitions are not intrinsic oscillations, which do not occur in MSNs, but are due to network properties. For example, Up state transitions are reduced by removal or deactivation of the cortex (Wilson et al., 1983a) and do not occur in brain slices in which coordinated cortical activity has been interrupted (Arbuthnott et al., 1985; Kawaguchi et al., 1989). Up state transitions do occur spontaneously in cortex-striatum co-cultures, in which there is intrinsic activity of the cortical explant (Plenz and Aertsen, 1996; Kerr and Plenz, 2002). Similarly, cortical stimulation in the intact animal can evoke depolarizing events very similar to the Up state transitions that occur spontaneously (Wilson, 1995a; Wilson and Kawaguchi, 1996). Thus, corticostriatal inputs are both necessary and sufficient for Up state transitions. On the other hand, although action potential firing only occurs during Up states, the Up state is not sufficient to cause action potential firing, which depends on the membrane potential in the Up state and the magnitude of the small amplitude membrane potential fluctuations that occur in the Up state, with many striatal neurons remaining silent in the Up state (Wickens and

С А 40 mV 2 nA –80 mV-100 ms 900 ms В D Е 0.5 s 0.8 nA 20 mV 1.0 s 0.7 nA 2.0 sJ 0.6 nA 3.0 s-0.5 nA 4.0 s 5.0 s 0.5 s 40 mV 0.4 nA 5.0 s 1 s

**FIGURE 5.3** (A) Spontaneous up and down transitions in the intracellular membrane potential of a MSN in the urethane anesthetized rat. (B) Corresponding suprathreshold spontaneous activity. (C) Non-linear membrane potential depolarizations and related spike delays in MSNs. Note increase in depolarization leading eventually to a spike during prolonged current injections close to spike threshold. (D) MSNs demonstrate a large range in delays over a fairly narrow range of suprathreshold current injections. (E). The pause between a preceding depolarization strongly effects the time to spiking in a subsequent depolarization.

Wilson, 1998). Recent intracellular studies in immobilized rat have shown yet another membrane potential dynamics of MSNs. Whereas during sleep the membrane potential is bistable in analogy to Up and Down states, during the wake state, potential, bi-stability is not dominant and the membrane potential tends to reside within an intermediate range of values between threshold and resting potential (Mahon et al., 2006).

In the absence of synaptic input, the MSN remains at a stable, hyperpolarized membrane potential dominated by an inwardly rectifying K<sup>+</sup> current, I<sub>Kir</sub> (Calabresi et al., 1987a; Uchimura et al., 1989; Wilson, 1992). This voltagesensitive potassium current is activated at resting membrane potential and becomes blocked as the membrane is depolarised, similar to the current described in starfish (Hagiwara and Takahashi, 1974). The current causes a low input resistance and a short membrane time constant at resting membrane potential, which act to shunt excitatory inputs, thereby maintaining the membrane potential in the hyperpolarised state. In contrast when a MSN is depolarized by a barrage of cortical afferent activity (Stern et al., 1997; Stern et al., 1998), IKir will begin to close. As closure occurs, the input resistance and time constant of the cell increase, permitting greater temporal and spatial summation of excitatory inputs (Nisenbaum and Wilson, 1995a, b). This current plays a major role in the sub-threshold behaviour of the cell and in the membrane potential trajectory during the Up state transition (Fig. 5.3C-E; for an extensive and detailed simulation on how intrinsic ion channels sculpture glutamatergic inputs in MSNs resulting in Up and Down state transitions, see Wolf et al., 2005).

A particular characteristic of the electrophysiological properties of the MSNs is that in response to near-threshold constant current, the membrane potential exhibits a gradual ramp-like depolarizing trajectory and a long-latency to spike discharge, after which relatively regular action potential firing occurs (Fig. 5.3C). During the ramp-like depolarisation, the slowly inactivating A-type K<sup>+</sup> channel I<sub>As</sub> (Nisenbaum et al., 1994) competes with inward Na<sup>+</sup> and Ca<sup>++</sup> currents, and acts to slow the rate of depolarisation, giving rise to the ramp potential and delayed spike discharge (Nisenbaum and Wilson, 1995b; Wilson, 1995b). The availability of this I<sub>As</sub> current to influence the membrane potential fluctuations seen in vivo depends on the recent history of the cell (Fig. 5.3E). The current is de-inactivated at the hyperpolarized potentials that occur in the Down state, and is available to reduce the response of the neuron to excitatory input. However, after prolonged depolarization in the Up state, the current inactivates and this makes the neuron more excitable (Nisenbaum et al., 1994).

## E. Dopaminergic Modulation of Ion Channels

Dopamine modulates various ion conductances in MSNs (see also Chapter 6). For example, dopamine and the specific D1 receptor agonist SKF 38393 (5 $\mu$ M) reduce I<sub>As</sub> (Kitai and Surmeier, 1993; Surmeier and Kitai, 1993). Conversely, the D2 receptor agonist quinpirole (5 $\mu$ M) enhances I<sub>As</sub> (Surmeier and Kitai, 1997). Due to the voltage-dependent activation and inactivation of I<sub>As</sub>, these D1-mediated effects of dopamine should depend upon the membrane potential range in which the neuron is operating. If in the Down state, or early in the Up state, then a considerable fraction of I<sub>As</sub> will be available. In this state, dopamine acting through D1 receptors should decrease the strength of this current. This should facilitate depolarisation in response to cortical inputs. Thus, dopamine acting via D1 receptors enables a transition from the Down state to the UP state (see also Chapter 21).

The effects of dopamine on the potassium channels discussed appear to oppose each other, in that  $I_{Kir}$  is increased while  $I_{As}$  is decreased. The former effect is to stabilise the Down state whereas the latter effect is to facilitate the transition to the UP state. The combination of these effects may be to make the MSNs reluctant to change states, but more snappy about doing so if their inputs are increased or decreased by a large enough amount (Gruber et al., 2003).

Slow and persistent Na<sup>+</sup> channels represented by  $I_{Na}$  are responsible for regenerative events underlying sub-threshold ramp depolarizations and action potential firing in MSNs. This current normally produces a depolarising prepotential, just before the action potential. The prepotential is sensitive to the sodium-channel blocker, TTX, but not to calcium channel blockers (Bargas et al., 1989). It is responsible for the later part of the slow rise in membrane potential seen during positive direct current injections (Bargas et al., 1989).

Dopaminergic modulation of Na<sup>+</sup> channels is a probable mechanism for the inhibitory effects reported in intracellular studies. As noted above, the amount of injected current required to reach the threshold voltage for action potential generation is increased by dopamine in a dosedependent way (Calabresi et al., 1987b). A D1 receptormediated reduction of the depolarising prepotential by dopamine was proposed as the mechanism underlying this inhibitory effect (Calabresi et al., 1987b; Calabresi et al., 1988). Voltage-clamp studies in dissociated striatal cells have confirmed that D1 receptor activation causes a reduction in peak Na<sup>+</sup> current, which may be with or without a shift in voltage dependence of inactivation (Surmeier et al., 1992a; Schiffmann et al., 1995; Schiffmann et al., 1998). The effect of these changes is likely to be to increase the delay of firing of MSNs.

Dopamine acting via D2 receptors has complex effects on  $Na^+$  currents. An increase in the amplitude of this current has been reported in a minority of cells (Surmeier et al., 1992b). These currents are also reduced in response to D2 receptor activation by means of a negative shift in voltage dependence of steady-state inactivation (Surmeier et al., 1992b). In cells in which a D2 receptor-mediated decrease in  $Na^+$  current was measured, the decrease was due to a shift in the voltage dependent inactivation towards more hyperpolarized potentials. This would make no difference at hyperpolarized Down state potentials but a big difference at more depolarised Up state potentials, where the effect would be to reduce the  $Na^+$  current in most cells.

MSNs express an extensive range of calcium currents, including L-, N-, P-, Q- and R-type  $Ca^{++}$  channels (Mermelstein et al., 1999). With maintained depolarisation, the depolarization-activated K<sup>+</sup> currents begin to inactivate, and inwardly-rectifying currents shut off. At this stage in the cycle of repetitive firing high-voltage activated  $Ca^{++}$  channels begin to activate (Bargas et al., 1991, 1994; Surmeier et al., 1995). The  $Ca^{++}$  channels have the effect of increasing the duration of the action potential and facilitating the entry of calcium into the cell. Dendritic entry of calcium is a function of both afferent activity and membrane potential (Kerr and Plenz, 2002). Although depolarization associated with  $Ca^{++}$  entry helps to maintain the depolarized state, the high voltage of activation of these channels suggests a primary role in controlling intracellular calcium (see Fig. 5.2).

Dopamine effects on  $Ca^{++}$  channels are complex. D1 receptor activation reduced N- and P/Q-type  $Ca^{++}$  currents but enhanced L-type currents (Surmeier et al., 1995) in dissociated cells. This was apparent in a much greater proportion of cells in brain slices (Hernandez-Lopez et al., 1997) arguing for a dendritic location, since significant amounts of dendrite is lost from dissociated cells. D1 receptor activation prolonged  $Ca^{++}$  plateau potentials in the presence of the potassium channel blocker, tetra-ethyl ammonium (TEA), an effect which was occluded by the calcium channel agonist BAY K8644, resulting in increased repetitive firing and prolonged AP duration.

On the other hand, D2 receptor stimulation in enkephalinexpressing MSNs suppresses Ca<sup>++</sup> currents through L-type Ca<sup>++</sup> channels (Hernandez-Lopez et al., 2000). Suppression is not mediated by inhibition of adenylate cyclase.

Although there is not yet sufficient information to achieve a complete coherent synthesis of all effects of dopamine on ion channels and striatal cell activity, it seems useful to attempt to put together what is known in relation to whole cell behaviour. The membrane potential trajectory in response to a depolarizing current pulse reflects the activation and inactivation of many of the currents modulated by dopamine. We start with the onset of a depolarizing current pulse, when the membrane begins to depolarise. As it does so, the I<sub>Kir</sub> is turning off. As the membrane depolarizes further, the fast and slow potassium currents begin to activate. The fast component is not known to be dopamine sensitive and is not further discussed here. As the membrane potential approaches threshold, the slow Na<sup>+</sup> current activates, while the I<sub>As</sub> begins to inactivate. As the cell begins to fire the L-type Ca<sup>++</sup> channels activate with each action potential.

Dopamine will influence this basic scenario in a number of ways. A dopamine-mediated increase of  $I_{Kir}$  increases the stability of the hyperpolarized state of the cell. The decrease of  $I_{Na}$  reduces the pre-potential. These two effects together produce a less excitable cell, in which it is more difficult to achieve a transition from the Down state to the Up state. Opposing these effects, the decrease in  $I_{As}$  and the increase in L channels mean that if the depolarized state is prolonged, D1 receptor activation increases excitability. These conclusions broadly agree with those of Calabresi et al. (1987b; see their Fig. 4) and Hernandez-Lopez et al. (1997; see their Fig. 1). Under conditions of prolonged depolarization, D1 receptor stimulation may thus lead to increased action potential firing, as observed *in vivo* (Gonon, 1997; West and Grace, 2002).

The effects of D2 receptor activation are more speculative at present, but essentially seem to be the reverse of the effects for the D1 receptor. Decreasing  $I_{Kir}$  (Uchimura and North, 1990) would be expected to decrease the stability of the Down state. Although an increase of  $I_{Na}$  (Surmeier and Kitai, 1997) would increase the excitability of cells in the Up state, this effect may be opposed by an increase in  $I_{As}$  and decrease in L-type Ca<sup>++</sup> channels leading to a delay in firing.

## III. ANATOMICAL CONNECTIVITY OF THE STRIATAL SKELETON

The synapses formed by the local axon collaterals of the MSNs have the appearance of inhibitory synapses, with symmetrical synaptic densities and large pleomorphic vesicles (Wilson and Groves, 1980). Some variability has been



FIGURE 5.4 Lateral synaptic transmission between MSNs. (A) Average postsynaptic potential in response to 200 presynaptic spikes in a MSN at three different steady-state resting potentials. Note reversal of the response towards spike threshold (sharp-intracellullar recordings; reproduced from Tunstall and Wickens, 2002). (B) Individual responses (arrow head) in a postsynaptic MSN (*post*) during prolonged spike firing from suprathreshold somatic current injection in the presynaptic MSN (*pre*). Note high failure rate in immature acute striatal slice. (C) Individual postsynaptic potentials in response to single spikes of the presynaptic neuron. Overplot of three responses each. Reciprocally connected pair of MSNs. (B,C: reproduced from Czubyako and Plenz, 2002). (D) Synaptic depression of the MSN to MSN synaptic connection during prolonged, precisely timed spike bursts in the presynaptic neuron. Voltage-clamp analysis of inhibitory postsynaptic currents (IPSC). Bottom: average IPSC current as a function of spike rank (reproduced from Koos et al., 2004).

reported in the proportion of striatal neurons that stain positively for glutamate decarboxylase (GAD), the synthesizing enzyme for GABA (Bolam et al., 1985; Kubota et al., 1987; Kita and Kitai, 1988). When conditions are optimized for detection of GAD, however, the great majority (>80%) of neurons with the morphological characteristics of MSNs stain positively for GAD (Kita, 1993). It has also been shown that GAD-positive boutons form synapses with the cell body and dendritic shafts of neurones identified as projection neurones by retrograde labeling from the substantia nigra (Aronin et al., 1986). Finally, immunohistochemical staining for GABA has identified numerous synapses between GABA-positive boutons and similarly staining dendrites (Pasik et al., 1988). Thus, the input to MSNs from other MSNs is GABAergic.

The striatopallidal and striatonigral terminations of the main axon have been known for some time to use the inhibitory neurotransmitter GABA (Precht and Yoshida, 1971; Yoshida and Precht, 1971) and produce inhibitory effects in the target nuclei (see also Chapter 13). It has, therefore, seemed probable that the local axon collaterals of MSNs should also be inhibitory.

### A. Quantitative Neuroanatomical Consideration of Local Connectivity

Two-dimensional drawings of MSNs make it seem inevitable that the extensive axon collaterals of a MSN would make synaptic contacts where they overlap with the dendrites of other nearby MSNs (see Fig. 5.1). This appearance in two-dimensional projections can be deceptive because a dense arborization in two dimensional projections is in reality a relatively sparse distribution of fibers when the axons and dendrites are opened out in three dimensions. This can be illustrated by estimates of the probability of connections based on realistic values of synapse density, extent of axonal and dendritic spread, and the volume of the region of overlap. The probability of a synapse between the local axonal collaterals of one MSN and the dendrites of another MSN located at a certain distance from the first can be estimated using statistical arguments. The probability of synaptic contact as a function of distance between somata can be calculated from the volume of the solid formed by the intersection of two spheres representing the dendritic and axonal arborizations of the respective neurons (Braitenberg and Schüz, 1991; Wickens and Oorschot, 2000). The number of postsynaptic sites in the volume that belong to the neuron in question (j), and the total number of synaptic sites in the volume (n) are calculated, and from this the fraction of the postsynaptic sites belonging to the receiving neuron is calculated. This gives the probability (**p**) that a postsynaptic site chosen at random will belong to the postsynaptic neuron ( $\mathbf{p} = \mathbf{j}/\mathbf{n}$ ). The number of contacts made in the same volume by the presynaptic neuron (k) is similarly determined. Finally, the probability of the presynaptic neuron making one, two or more synapses with the postsynaptic neuron is calculated from the cumulative hypergeometric distribution with parameters  $\mathbf{j}$ ,  $\mathbf{k}$  and  $\mathbf{n}$ (Wickens and Miller, 1997). Quantitative neuroanatomical studies have provided values for these parameters.

Ingham et al. (1998) determined a density of  $0.91 \,\mu m^{-3}$ synapses in the rat striatum and estimated that symmetrical synapses account for about 20% of the total number of synapses. The other sources of symmetrical synapses include GABA/parvalbumin, somatostatin and cholinergic interneurons, and dopaminergic afferents which are also predominantly of the symmetrical type. The proportion of symmetrical synapses that come from MSNs can be estimated to be about 1 in 6 (Wilson, 2000). Using these values, the number of synapses of MSNs per unit volume is on the order of  $0.038 \,\mu m^{-3}$ . The proportion of the synapses that belong to each individual neuron, on average, can be calculated from the density of medium-sized somata. According to Oorschot (1996) this number is 84,900 mm<sup>-3</sup>. From these values the average number of postsynaptic sites from one MSN is estimated as 448. Independent verification of these estimates is provided by counts of varicosities of identified striatonigral neurons in brain slices (Lee et al., 1997) that showed on average 749 synaptic boutons per cell (in a sample of five cells), and a sample of these studied by electron microscopy, confirmed that about 80% were involved in synaptic contacts. Based on this proportion, we can estimate 595 synapses per striatal cell, in good agreement with the estimate derived from quantitative neuroanatomy. Using these values the estimated probability of a synapse between the axons and dendrites of immediately adjacent neurons is p = 0.146 (assuming 448 synapses per cell and the diameter of the dendritic and axonal arborizations both to be  $400 \mu m$ ). It is important to note, however, that this value decreases rapidly with increasing distance between the neurons.

Paired recordings provide an independent experimental estimate of the probability of a connection. Using dual intracellular recording, Tunstall et al. (2002) found nine connected cells in a sample of 45 pairs of MSNs, corresponding to a probability of 0.1. With improved sensitivity for detecting a connection, we have recently detected 56 connected cells in a sample of 194 pairs (p = 0.14) of MSNs of which four were bidirectional (Shindou et al., 2005). These measures are in agreement with those obtained using similar techniques in other labs. In the ventral striatum, Taverna et al. (2004) found 13 connections in a sample of 38 pairs, corresponding to a probability of p = 0.17. Koos et al. (2004) found 39 connections in a sample of 325 pairs that were studied in one direction only, corresponding to a probability of p = 0.12. Venance et al. (2004) detected connections in 5/50 pairs in horizontally cut brain slices, and 7/22 pairs in sagittal slices, corresponding to unidirectional probabilities of p = 0.05 and p = 0.16, respectively. Of course, the probabilities cited above are likely to be an underestimate, due to the proximity of the cells to the cut surface, and that on average about 50% of the axonal and dendritic arborisations will be superficial to the recorded cell. For comparison, in organotypic slice cultures, where centrally located MSNs mature within a block of  $500\mu$ m thick striatum taken early during postnatal development, the connection probability of very close MSNs is about p = 0.25, providing an upper bound of connectivity in three dimensions (Czubayko and Plenz, 2002; Gustafson et al., 2006). Importantly, the estimates of synaptic connectivity given above all assume that the MSNs show no preference for particular target neurons.

The model, which is based on the assumption of a uniform distribution of synaptic contacts, predicts that multiple synaptic contacts between pairs of MSNs are highly improbable. To date there have been two anatomical reports of multiple synaptic contacts between the axon of one probable MSN and a single postsynaptic MSN (Wilson and Groves, 1980; Somogyi et al., 1982). These observations provide evidence that the MSNs may show a preference for making synaptic contact with particular postsynaptic MSNs. Such selectivity would have the effect of lowering the probability of a synaptic connection between a randomly chosen pair of neurons even further.

Paired-recordings also suggest connections between striatonigral and striatopallidal MSNs to be fewer than expected by chance, to contain fewer GABA<sub>A</sub> -receptors and to be saturated at physiological conditions leading to an overall  $\sim$ 50% smaller GABAergic input compared to those of connections between striatopallidal MSNs (Ade et al., 2008; Taverna et al., 2008). Such asymmetries in lateral connectivities need to be incorporated in future models of the striatal skeleton.

## IV. SYNAPTIC PHYSIOLOGY OF LATERAL INTERACTIONS

A fast synaptic transmission that utilizes the neurotransmitter GABA dominates the interaction between MSNs. At the electron microscopic level, MSN synapses reveal the typical morphology of an inhibitory synapse with symmetrical pre- and postsynaptic densities and small vesicles (Somogyi et al., 1981). These synapses predominantly contact the dendritic shaft (40%) and spine necks (50%) of other MSNs, as well as the cell body (10%) (Wilson and Groves, 1980) suggesting their dominant influence in dendritic processing (Plenz, 2003; Wilson, 2007). Their remote location from the soma in conjunction with the strong anomalous rectification at the resting membrane potential, might be responsible why this connection escaped early attempts of electrophysiological identification (Jaeger et al., 1994). Almost a decade later, with the introduction of stable paired recording techniques that allowed for extensive spike-triggered averaging (Fig. 5.4A; Tunstall et al., 2002) and visually identified whole-cell patch recordings from nearby identified MSNs (Fig. 5.4B,C; Czubayko and Plenz, 2002), the functional existence of this inhibitory synapse was confirmed for the dorsal striatum of the rat. Advanced statistical analysis and rigorous comparison with GABAergic synapses formed by fast spiking interneurons on MSNs (Koos et al., 2004; Gustafson et al., 2006) robustly established that MSN-MSN connections represent a standard inhibitory synapse with an average conductivity, a fast rise and decay constant, few multiple release sites, and average failure probability. Similar results were found for MSN connections in the nucleus accumbens (Taverna et al., 2004; Venance et al., 2004).

The sensitivity to the GABAA antagonists bicuculline and picrotoxin identified the MSN-MSN connection pharmacologically as an inhibitory synapse. However, whether the synapse solely functions inhibitory in regulation of MSN excitability has been hotly debated. Both Cl<sup>-</sup> as well as HCO3 ions act as carriers for GABA<sub>A</sub> channels under physiological conditions (Farrant and Kaila, 2007) and their gradients are easily disturbed under standard intracellular recording conditions. Recent perforated-patch recordings that maintained the intracellular [Cl-] of MSNs, located the reversal potential for the MSN-MSN connection between -60 and -45 mV (Fig. 5.4A; Koos et al., 2004; Bracci and Panzeri, 2006; Gustafson et al., 2006). The position of the reversal potential, more positive than the resting membrane potential, but nevertheless below action potential threshold, suggests diverse functional scenarios for this synapse in the context of dendritic integration and corticostriatal plasticity (see below).

MSNs often fire bursts of action potentials in vivo, which was suggested as a mechanism to increase information transmission in the face of unreliable synapses (see Fig. 5.3B; Lisman, 1997). The short-term plasticity for MSN-MSN postsynaptic currents displays significant postsynaptic depression of up to 60% during prolonged trains of action potentials (Fig. 5.4D; Koos et al., 2004; Tecuapetla et al., 2007) and so do postsynaptic potentials under physiological conditions even for high frequency bursts (Gustafson et al., 2006). The depression is slightly stronger than that reported for FS $\rightarrow$ MSN connections (Koos et al., 2004; Gustafson et al., 2006; Tecuapetla et al., 2007). The short-term plasticity is under robust neuromodulatory control of dopamine, somatostatin, and adenosine. Whereas D1 receptor activation enhances the short-term depression, D2 receptor stimulation decreases short-term depression (Tecuapetla et al., 2007). Thus, dopamine release in the striatum would favor burst transmission for striatopallidal neurons and support precisely timed synaptic transmission for striatonigral neurons, given the segregation of D1 and D2 receptors between the direct and indirect pathways (Gerfen et al., 1995; Surmeier et al., 1996). Of the four adenosine receptor subtypes, the  $A_{2A}$ receptor is highly expressed in the striatum, particularly on striatopallidal neurons (Dixon et al., 1996; Rosin et al., 2003) and its activation facilitates MSN to MSN synaptic transmission (Shindou et al., 2008). Equally, somatostatin released by striatal interneurons changes synaptic transmission between MSNs by acting presynaptically on GABA<sub>A</sub> terminals (Lopez-Huerta et al., 2008).

A second mode of interaction in the striatal skeleton has been established by the identification of electrical synapses between MSNs. The dye Lucifer Yellow, when injected intracellularly into one MSN, was found in numerous neighboring MSNs of the nucleus accumbens in vitro and in vivo, particularly in young tissue (Cepeda et al., 1989; Walsh et al., 1989; Onn and Grace, 1994). The selective spread to MSNs and its modulation by dopamine (O'Donnell and Grace, 1993), suggested the presence of gap-junctions between MSNs. Since then, smaller molecules such as biocytin have been shown to diffuse into neighboring MSNs from individually patched MSN (Venance et al., 2004). Although the presence of connexin36, which is responsible for the formation of electrical synapses, has been demonstrated in rat striatum (Venance et al., 2004), ultimately, precisely controlled paired whole-cell patch recordings were required to unequivocally demonstrate the presence of electrical synapses between MSNs in acute slices of rat striatum (Czubayko and Plenz, 2002; Venance et al., 2004). These electrical synapse are more frequent in young striatal tissue, reveal mostly symmetrical coupling with a mean coupling coefficient of 3% and act as low-pass filters (<5Hz) (Venance et al., 2004). In the absence of excitatory local connections, the formation of spatial clusters of electrically coupled MSNs early during striatal development could act as a cooperative mechanism to bind spatially neighbored neurons into similar functional operations.

## V. FUNCTIONAL IMPLICATIONS, MODELS AND OUTLOOK

Historically, the striatal skeleton has been interpreted as a winner-take-all network, in which the most active MSN

suppresses all neighbors (Wilson and Groves, 1980; Wickens et al., 1991). The lateral inhibition by reducing the number of neurons active at any given input favorably increases memory capacity for pattern recognition (Wilson, 2007). The dynamics, however, would require an all to all connectivity with high synaptic conductances (Maass, 2000). A more realistic simulation of the striatal skeleton that takes into account connection probabilities as well as synaptic physiologies leads to a striatal skeleton that is dominated by sparse, asymmetric connections of moderate strength and which excludes a winner-take-all dynamics. The skeleton, nevertheless, produces significant recurrent inhibition of striatal activity under physiological cortical input conditions (Wickens et al., 2007). It is likely to favor a winner-share-all dynamics (Fukai and Tanaka, 1997) that effectively maps input strength differences into output differences and it supports the idea of input selection among alternatives (Wickens et al., 1991).

These functional considerations in the previous paragraph view the striatum as an equilibrium steady-state input/output network. On the other hand, the dominance of asymmetric lateral interactions and the particular dynamics of its lateral connections suggest alternative, not mutually exclusive, functional views. First, asymmetric lateral inhibition has been established as the prototype for strengthening delayed, i.e., sequential inputs (Poggio and Reichardt, 1973; Plenz and Aertsen, 1994). Second, temporal shifts in the action potential occurrence in response to lateral inhibition might effectively control striatal plasticity that relies on spike-timing dependent plasticity during Up states (Fig. 5.4; Kerr and Plenz, 2002; Kerr and Plenz, 2004; Pawlak and Kerr, 2008). Third, preceding depolarizing GABA MSN input has been shown to facilitate or delay evoked suprathreshold action potential generation (Bracci and Panzeri, 2006; Gustafson et al., 2006). When viewed in this framework, lateral inhibition in the striatal skeleton would contribute to the learning of corticostriatal associations, rather than to the acute selection process of a particular action among alternatives.

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#### REFERENCES

Ade KK, Janssen MJ, Ortinski PI, Vicini S (2008) Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 28:1185–1197.

- Alexander GE (1987) Selective neuronal discharge in monkey putamen reflects intended direction of planned limb movements. Exp Brain Res 67:623–634.
- Arbuthnott GW, MacLeod N, Rutherford A (1985) The rat cortico-striatal pathway *in vitro*. J Physiology (London) 367:102P.
- Aronin N, Chase K, DiFiglia M (1986) Glutamic acid decarboxylase and enkephalin immunoreactive axon terminals in the rat neostriatum synapse with striatonigral neurons. Brain Res 365:151–158.
- Bargas J, Galarraga E, Aceves J (1989) An early outward conductance modulates the firing latency and frequency of neostriatal neurons of the rat brain. Exp Brain Res 75:146–156.
- Bargas J, Surmeier DJ, Kitai ST (1991) High- and low-voltage activated calcium currents are expressed by neurons cultured from embryonic rat neostriatum. Brain Res 541:70–74.
- Bargas J, Howe A, Eberwine J, Cao Y, Surmeier DJ (1994) Cellular and molecular characterization of Ca<sup>2+</sup> currents in acutely isolated, adult rat neostriatal neurons. J Neurosci 14:6667–6686.
- Bolam JP, Powell JF, Wu J-Y, Smith AD (1985) Glutamate decarboxylaseimmunoreactive structures in the rat neostriatum: A correlated light and electron microscopic study including a combination of Golgiimpregnation with immunocytochemistry. J Comp Neurol 237:1–20.
- Bolam JP, Bergman H, Graybiel A, Kimura M, Plenz D, Seung HS, Surmeier DJ, Wickens JR (2006) Molecules, microcircuits and motivated behaviour: Microcircuits in the striatum. In: Microcircuits: the interface between neurons and global brain function. Dahlem Workshop Report 93 (Grillner S ed). Cambridge, MA: The MIT Press.
- Bracci E, Panzeri S (2006) Excitatory GABAergic effects in striatal projection neurons. J Neurophysiol 95:1285–1290.
- Braitenberg V, Schüz A (1991) Anatomy of the cortex: Statistics and geometry. Berlin: Springer.
- Calabresi P, Misgeld U, Dodt HU (1987a) Intrinsic membrane properties of neostriatal neurons can account for their low level of spontaneous activity. Neuroscience 20:293–303.
- Calabresi P, Benedetti M, Mercuri NB, Bernardi G (1988) Endogenous dopamine and dopaminergic agonists modulate synaptic excitation in neostriatum: Intracellular studies from naive and catecholaminedepleted rats. Neuroscience 27:145–157.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1992) Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. Eur J Neurosci 4:929–935.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1996) The corticostriatal projection: From synaptic plasticity to dysfunctions of the basal ganglia. Trends Neurosci 19:19–24.
- Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G (1987b) Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurones in vitro: Evidence for D1 receptor involvement. Neuroscience 20:757–771.
- Carter AG, Sabatini BL (2004) State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. Neuron 44:483–493.
- Cepeda C, Walsh JP, Hull CD, Howard SG, Buchwald NA, Levine MS (1989) Dye-coupling in the neostriatum of the rat: I. Modulation by dopamine-depleting lesions. Synapse 4:229–237.
- Czubayko U, Plenz D (2002) Fast synaptic transmission between striatal spiny projection neurons. Proc Natl Acad Sci USA 99:15764–15769.
- Day M, Wokosin D, Plotkin JL, Tian X, Surmeier DJ (2008) Differential excitability and modulation of striatal medium spiny neuron dendrites. J Neurosci 28:11603–11614.

- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- DiFiglia M, Pasik P, Pasik T (1976) A Golgi study of neuronal types in the neostriatum of monkeys. Brain Res 114:245–256.
- Dixon AK, Gubitz AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC (1996) Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol 118:1461–1468.
- Dube L, Smith AD, Bolam JP (1988) Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum. J Comp Neurol 267:455–471.
- Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA(A) receptor signalling. Prog Brain Res 160:59–87.
- Fukai T, Tanaka S (1997) A simple neural network exhibiting selective activation of neuronal ensembles: from winner-take-all to winnersshare-all. Neural Comput 9:77–97.
- Fino E, Glowinski J, Venance L (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 25:11279–11287.
- Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461–464.
- Gerfen CR, Young WS 3rd (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161–167.
- Gerfen, C.R., Wilson, C.J. (1996). The basal ganglia. In: Handbook of Chemical Neuroanatomy, Vol. 12, Integrated Systems of the CNS, p. III: cerebellum, basal ganglia, olfactory system pp. 371-468.
- Gerfen CR, Keefe KA, Gauda EB (1995) D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. J Neurosci 15:8167–8176.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gertler TS, Chan CS, Surmeier DJ (2008) Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci 28:10814–10824.
- Gong S, Zheng C, Doughty ML, et al. (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425:917–925.
- Gonon F (1997) Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. J Neurosci 17:5972–5978.
- Graybiel AM (1984) Correspondence between the dopamine islands and striosomes of the mammalian striatum. Neuroscience 13:1157–1187.
- Gruber AJ, Solla SA, Surmeier JD, Houk JC (2003) Modulation of striatal single units by expected reward: A spiny neuron model displaying dopamine-induced bistability. J Neurophysiol 90:1095–1114.
- Gustafson N, Gireesh-Dharmaraj E, Czubayko U, Blackwell KT, Plenz D (2006) A comparative voltage and current-clamp analysis of feedback and feedforward synaptic transmission in the striatal microcircuit in vitro. J Neurophysiol 95:737–752.
- Haber SN (2003) The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.
- Hagiwara S, Takahashi K (1974) The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. J Membr Biol 18:61–80.

- Heintz N (2001) BAC to the future: the use of bac transgenic mice for neuroscience research. Nat Rev Neurosci 2:861–870.
- Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E (1997) D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca<sup>2+</sup> conductance. J Neurosci 17:3334–3342.
- Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca<sup>2+</sup> currents and excitability via a novel PLC[beta]1-IP3-calcineurin- signaling cascade. J Neurosci 20:8987–8995.
- Herrling PL, Morris R, Salt TE (1983) Effects of excitatory amino acids and their antagonists on membrane and action potentials of cat caudate neurones. J Physiol 339:207–222.
- Ingham CA, Hood SH, Taggart P, Arbuthnott GW (1998) Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18:4732–4743.
- Jaeger D, Kita H, Wilson CJ (1994) Surround inhibition among projection neurons is weak or nonexistent in the rat neostriatum. J Neurophysiol 72:2555–2558.
- Kawaguchi Y (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci 13:4908–4923.
- Kawaguchi Y, Wilson CJ, Emson PC (1989) Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. J Neurophysiol 62:1052–1068.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.
- Kerr JN, Plenz D (2004) Action potential timing determines dendritic calcium during striatal up-states. J Neurosci 24:877–885.
- Kerr JND, Plenz D (2002) Dendritic calcium encodes striatal neuron output during up-states. J Neurosci 22:1499–1512.
- Kerr JN, Wickens JR (2001) Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J Neurophysiol 85:117–124.
- Kimura M, Kato M, Shimazaki H (1990) Physiological properties of projection neurons in the monkey striatum to the globus pallidus. Exp Brain Res 82:672–676.
- Kita H (1993) GABAergic circuits of the striatum. Prog Brain Res 90:51–72.
- Kita H (1996) Glutamatergic and GABAergic postsynaptic responses of striatal spiny neurons to intrastriatal and cortical stimulation recorded in slice preparations. Neuroscience 70:925–940.
- Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in cat neostriatum: Their morphological types and populations. Brain Res 447:346–352.
- Kitai ST, Surmeier DJ (1993) Cholinergic and dopaminergic modulation of potassium conductances in neostriatal neurons. Adv Neurol 60:40–52.
- Koos T, Tepper JM (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nat Neurosci 2:467–472.
- Koos T, Tepper JM, Wilson CJ (2004) Comparison of IPSCs evoked by spiny and fast-spiking neurons in the neostriatum. J Neurosci 24:7916–7922.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.

- Kubota Y, Mikawa S, Kawaguchi Y (1993) Neostriatal GABAergic interneurones contain NOS, calretinin or parvalbumin. Neuroreport 5:205–208.
- Kubota Y, Inagaki S, Shimada S, Kito S, Wu JY (1987) Glutamate decarboxylase-like immunoreactive neurons in the rat caudate putamen. Brain Res Bull 18:687–697.
- Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426.
- Lee T, Kaneko T, Shigemoto R, Nomura S, Mizuno N (1997) Collateral projections from striatonigral neurons to substance P receptorexpressing intrinsic neurons in the striatum of the rat. J Comp Neurol 388:250–264.
- Lisman JE (1997) Bursts as a unit of neural information: making unreliable synapses reliable. Trends in Neurosci 20:38–43.
- Lopez-Huerta VG, Tecuapetla F, Guzman JN, Bargas J, Galarraga E (2008) Presynaptic modulation by somatostatin in the neostriatum. Neurochem Res 33:1452–1458.
- Maass W (2000) On the computational power of winner-take-all. Neural Comput 12:2519–2535.
- Mahon S, Vautrelle N, Pezard L, Slaght SJ, Deniau JM, Chouvet G, Charpier S (2006) Distinct patterns of striatal medium spiny neuron activity during the natural sleep-wake cycle. J Neurosci 26:12587–12595.
- McGeorge AJ, Faull RL (1989) The organisation of the projections from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- Mermelstein PG, Foehring RC, Tkatch T, Song WJ, Baranauskas G, Surmeier DJ (1999) Properties of Q-type calcium channels in neostriatal and cortical neurons are correlated with beta subunit expression. J Neurosci 19:7268–7277.
- Nauta NJW, Pritz MB, Lasek RJ (1974) Afferents to the rat striatum studied with horseradish peroxidase. An evaluation of a retrograde neuroanatomical research method. Brain Res 67:219–238.
- Nisenbaum ES, Wilson CJ (1995a) Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. J Neurosci 15:4449–4463.
- Nisenbaum ES, Wilson CJ (1995b) The role of potassium currents in the subthreshold responses of neostriatal spiny projection neurons. In: Molecular and Cellular Mechanisms of Neostriatal Function (Ariano MA, Surmeier DJ, eds), pp. 165–181: R.G. Landes Company.
- Nisenbaum ES, Xu ZC, Wilson CJ (1994) Contribution of a slowly inactivating potassium current to the transition to firing of neostriatal spiny projection neurons. J Neurophysiol 71:1174–1189.
- O'Donnell P, Grace AA (1993) Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. Synapse 13:135–160.
- Onn SP, Grace AA (1994) Dye coupling between rat striatal neurons recorded in vivo: compartmental organization and modulation by dopamine. J Neurophysiol 71:1917–1934.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereological study using the Cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Oorschot DE, Tunstall MJ, Wickens JR (2002) Local connectivity between striatal spiny projection neurons: A re-evaluation. In: Basal

Ganglia VII (Nicholson L, Faull RLM, eds), pp. 421–434. New York: Plenum Press.

- Pasik P, Pasik T, Holstein G, Hamori J (1988) GABAergic elements in the neuronal circuits of the monkey neostriatum: A light and electron microscopic immunocytochemical study. J Comp Neurol 270:157–170.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 28:2435–2446.
- Plenz D (2003) When inhibition goes incognito: feedback interaction between spiny projection neurons in striatal function. Trends Neurosci 26:436–443.
- Plenz D, Aertsen A (1994) The basal ganglia: Minimal coherence detection in cortical activity distributions. In: The Basal Gaglia V. New ideas and data on structure and function (Percheron G, McKenzie JS, Feger E, eds). New York: Plenum.
- Plenz D, Aertsen A (1996) Neural dynamics in cortex-striatum co-cultures – II. Spatiotemporal characteristics of neuronal activity. Neuroscience 70:893–924.
- Poggio T, Reichardt W (1973) Considerations on models of movement detection. Kybernetik 13:223–227.
- Pomata PE, Belluscio MA, Riquelme LA, Murer MG (2008) NMDA receptor gating of information flow through the striatum in vivo. J Neurosci 28:13384–13389.
- Precht W, Yoshida M (1971) Blockage of caudate-evoked inhibition of neurons in the substantia nigra by picrotoxin. Brain Res 32:229–233.
- Reynolds JNJ, Hyland BI, Wickens JR (2001) A cellular mechanism of reward-related learning. Nature 413:67–70.
- Rosin DL, Hettinger BD, Lee A, Linden J (2003) Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 61:S12–S18.
- Schiffmann SN, Lledo PM, Vincent JD (1995) Dopamine D1 receptor modulates the voltage-gated sodium current in rat striatal neurones through a protein kinase A. J Physiol 483:95–107.
- Schiffmann SN, Desdouits F, Menu R, Greengard P, Vincent JD, Vanderhaeghen JJ, Girault JA (1998) Modulation of the voltage-gated sodium current in rat striatal neurons by DARPP-32, an inhibitor of protein phosphatase. Eur J Neurosci 10:1312–1320.
- Schroder KF, Hopf A, Lange H, Thorner G (1975) Morphometrisch-statistische Strukturanalysen des Striatum, Pallidum und Nucleus subthalamacus beim Menschen. I Striatum. J Hirnforsch 16:333–350.
- Schultz W, Romo R (1988) Neuronal activity in the monkey striatum during the initiation of movements. Exp Brain Res 71:431–436.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Shindou T, Arbuthnott GW, Wickens JR (2008) Actions of Adenosine A2A Receptors on Synaptic Connections of Spiny Projection Neurons in the Neostriatal Inhibitory Network. J Neurophysiol 99:1884–1889.
- Shindou T, Ochi-Shindou M, Arbuthnott GW, Wickens JR (2005) Adenosine A2A receptor-mediated modulation of fast-spiking interneurons in the striatum. In: Society for Neuroscience. Washington, DC.
- Somogyi JP, Bolam JP, Smith AD (1981) Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscope study using the Golgi-peroxidase transport degeneration procedure. J Comp Neurol 195:567–584.
- Somogyi P, Priestley JV, Cuello AC, Smith AD, Takagi H (1982) Synaptic connections of enkephalin-immunoreactive nerve terminals in the neostriatum: a correlated light and electron microscopic study. J Neurocytol 11:779–807.

- Stern EA, Kincaid AE, Wilson CJ (1997) Spontaneous subthreshold membrane potential fluctuations and action potential variability of rat corticostriatal and striatal neurons in vivo. J Neurophysiol 77:1697–1715.
- Stern EA, Jaeger D, Wilson CJ (1998) Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo. Nature 394:475–478.
- Surmeier DJ, Kitai ST (1993) D1 and D2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. Prog Brain Res 99:309–324.
- Surmeier DJ, Kitai ST (1997) State-dependent regulation of neuronal excitability by dopamine. Nihon Shinkei Seishin Yakurigaku Zasshi 17:105–110.
- Surmeier DJ, Song WJ, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
- Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/ phosphatase cascade in rat neostriatal neurons. Neuron 14:385–397.
- Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST (1992a) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89:10178–10182.
- Taverna S, Ilijic E, Surmeier DJ (2008) Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's disease. J Neurosci 28:5504–5512.
- Taverna S, van Dongen YC, Groenewegen HJ, Pennartz CM (2004) Direct physiological evidence for synaptic connectivity between mediumsized spiny neurons in rat nucleus accumbens in situ. J Neurophysiol 91:1111–1121.
- Tecuapetla F, Carrillo-Reid L, Bargas J, Galarraga E (2007) Dopaminergic modulation of short-term synaptic plasticity at striatal inhibitory synapses. Proc Natl Acad Sci USA 104:10258–10263.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tunstall MJ, Oorschot DE, Kean A, Wickens JR (2002) Inhibitory interactions between spiny projection neurons in the rat striatum. J Neurophysiol 88:1263–1269.
- Uchimura N, North RA (1990) Actions of cocaine on rat nucleus accumbens neurones in vitro. Br J Pharmacol 99:736–740.
- Uchimura N, Cherubini E, North RA (1989) Inward rectification in rat nucleus accumbens neurons. J Neurophysiol 62:1280–1286.
- Venance L, Glowinski J, Giaume C (2004) Electrical and chemical transmission between striatal GABAergic output neurones in rat brain slices. J Physiol 559:215–230.
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468–474.
- Walsh JP, Cepeda C, Hull CD, Fisher RS, Levine MS, Buchwald NA (1989) Dye-coupling in the neostriatum of the rat: II. Decreased coupling between neurons during development. Synapse 4:238–247.
- West AR, Grace AA (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci 22:294–304.
- Wickens JR, Miller R (1997) A formalisation of the neural assembly concept: 1. Constraints on neural assembly size. Biol Cybern 77:351–358.
- Wickens JR, Wilson CJ (1998) Regulation of action potential firing in spiny neurons of the rat neostriatum, *in vivo*. J Neurophysiol 79:2358–2364.

- Wickens JR, Oorschot DE (2000) Neural dynamics and surround inhibition in the neostriatum: A possible connection. In: Brain Dynamics and the Striatal Complex (Miller R, Wickens JR, eds), pp. 141–150. Reading: Gordon and Breach.
- Wickens JR, Alexander ME, Miller R (1991) Two dynamic modes of striatal function under dopaminergic-cholinergic control: simulation and analysis of a model. Synapse 8:1–12.
- Wickens JR, Arbuthnott GW, Shindou T (2007) Simulation of GABA function in the basal ganglia: computational models of GABAergic mechanisms in basal ganglia function. Prog Brain Res 160:313–329.
- Wickens JR (2009) Synaptic plasticity in the basal ganglia. Behav Brain Res 199:119–128.
- Wilson CJ (1984) Passive cable properties of dendritic spines and spiny neurons. J Neurosci 4:281–297.
- Wilson CJ (1986) Postsynaptic potentials evoked in spiny neostriatal projection neurons by stimulation of ipsilateral and contralateral neocortex. Brain Res 367:201–213.
- Wilson CJ (1992) Dendritic morphology, inward rectification, and the functional properties of neostriatal neurons. In: Single Neuron Computation (McKenna T, Davis J, Zornetzer SF, eds), pp. 141–171. Academic Press: San Diego.
- Wilson CJ (1993) The generation of natural firing patterns in neostriatal neurons. Prog Brain Res 99:277–297.
- Wilson CJ (1994) Understanding the neostriatal microcircuitry: high-voltage electron microscopy. Microsc Res Tech 29:368–380.
- Wilson CJ (1995a) The contribution of cortical neurons to the firing pattern of striatal spiny neurons. In: Models of Information Processing in the Basal Ganglia (Houk JC, Davis JL, Beiser DG, eds), pp. 187–214: MIT Press.
- Wilson CJ (1995b) Dynamic modification of dendritic cable properties and synaptic transmission by voltage-gated potassium channels. J Comp Neurosci 2:91–115.
- Wilson CJ (2000) Striatal circuitry: Categorically selective, or selectively categorical? In: Brain Dynamics and the Striatal Complex (Miller R, Wickens J, eds), pp. 289-305
- Wilson CJ (2007) GABAergic inhibition in the neostriatum. Prog Brain Res 160:91–110.
- Wilson CJ, Groves PM (1980) Fine structure and synaptic connection of the common spiny neuron of the rat neostriatum: A study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615.
- Wilson CJ, Groves PM (1981) Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. Brain Res 220:67–80.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 16:2397–2410.
- Wilson CJ, Chang HT, Kitai ST (1983a) Disfacilitation and long-lasting inhibition of neostriatal neurons in the rat. Exp Brain Res 51:227–235.
- Wilson CJ, Groves PM, Kitai ST, Linder JC (1983b) Three-dimensional structure of dendritic spines in the rat neostriatum. J Neurosci 3:383–388.
- Wolf JA, Moyer JT, Lazarewicz MT, Contreras D, Benoit-Marand M, O'Donnell P, Finkel LH (2005) NMDA/AMPA ratio impacts state transitions and entrainment to oscillations in a computational model of the nucleus accumbens medium spiny projection neuron. J Neurosci 25:9080–9095.
- Yoshida M, Precht W (1971) Monosynaptic inhibition of neurons of substanita nigra by caudatonigral fibres. Brain Res 32:225–228.

# D1 and D2 Dopamine Receptor Modulation of Glutamatergic Signaling in Striatal Medium Spiny Neurons

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### I. INTRODUCTION

Dopamine (DA) has long been known to be a critical modulator of striatal processing of cortical and thalamic signals carried by glutamatergic synapses on the principal neurons of the striatum – medium spiny neurons (MSNs). DA regulation of these neurons is important for a wide array of psychomotor functions ascribed to the basal ganglia such as habit learning and the control of serial movement (Albin et al., 1989; Wickens et al., 2003; Schultz, 2006). In spite of its significance, an understanding of the physiological principles underlying MSN regulation has developed slowly (see also Chapter 5). When I started thinking about DA effects in the striatum some 20 years ago, Steve Kitai asked me a very simple question: "Is DA excitatory or inhibitory?" The conceptual framework of the question was built from classical synaptic transmission building blocks. We know now that this is wrong and that DA modulates cellular function not through ionotropic receptors that allow depolarizing (excitatory) or hyperpolarizing (inhibitory) current through them, but by activating through G-protein coupled receptors that change the way neurons respond to external signals, like the release of glutamate.

Another major obstacle to unraveling the DA puzzle in the striatum has been the lack of homogeneity in the MSN class; there are at least two major subsets of MSNs that differ in their expression of DA receptors (Gerfen, 1992; Surmeier et al., 1996) (see also Chapter 1). These subsets cannot be readily identified on the basis of their somatodendritic morphology or electrophysiological properties. Moreover, both cell types are imbedded in a rich neuronal network involving both MSNs and interneurons that is modulated by DA. This has made it extremely difficult to sort out what DA is doing directly and what it is doing indirectly through effects on network properties. The recent development of mouse lines in which neurons "report" their expression of D1 or D2 receptors by co-expressing enhanced green fluorescent protein (EGFP) promises to accelerate our pace of discovery. Another obstacle is that DA receptors are primarily found in dendrites that are inaccessible with electrodes (the principal tool of electrophysiologists), making direct study of their actions on glutamatergic signaling and dendritic excitability difficult. Optical techniques, like two photon laser scanning microscopy (2PLSM), are giving us access to these regions, and providing fundamental new insights into their physiology and modulation by DA.

This review largely focuses on what is known about how DA modulates postsynaptic properties that influence glutamatergic synaptic events and their integration by MSNs in the dorsal striatum. Only the actions of the principal DA receptors in this region (D1, D2 receptors) will be discussed. Even with this rather narrow focus, it is impossible to faithfully summarize what has become an enormous literature in the last decade. The reader is referred to several other recent reviews (Nicola et al., 2000; Surmeier, 2004; Arbuthnott and Wickens, 2006). Moreover, there is a rich literature characterizing the impact of glutamate on DA neurons and DA release that will not be addressed (Morari et al., 1998; David et al., 2005).

## II. THE "CLASSICAL" MODEL OF DOPAMINERGIC MODULATION

The now "classical" model of how DA shapes striatal activity was advanced almost two decades ago by Albin, Young, and Penny (Albin et al., 1989). In this model, D1 receptors excite MSNs of the "direct" striatonigral pathway, whereas D2 receptors inhibit MSNs of the "indirect" striatopallidal pathway. These were envisioned as acute, readily reversible effects. The evidence for this model stemmed almost entirely from indirect measures of neuronal activity (e.g., alterations in gene expression, glucose utilization, or receptor binding). Subsequent work has proven to be largely consistent with the general principles of this model, revealing that DA activation of G-protein coupled receptors (GPCRs) "excites" or "inhibits" MSNs by modulating the gating and trafficking of voltage-dependent and ligandgated (ionotropic) ion channels, essentially altering cellular excitability. The relationship between dopaminergic

and glutamatergic signaling has been the subject of a large number of studies, in part because of the apparent triadic relationship between spines where the vast majority of cortical glutamatergic synapses are formed and DA release sites (Bolam et al., 2000). As discussed below, there also are longer lasting alterations in glutamatergic synaptic strength induced by DA when there is a conjunction of pre- and postsynaptic activity. These lasting changes, rather than the acute effects of DA, are thought to underlie associative learning and action selection.

### III. MODULATION OF INTRINSIC EXCITABILITY AND GLUTAMATERGIC SIGNALING BY D1 RECEPTORS

Striatonigral MSNs express D1 receptors at high levels (Gerfen, 1992; Surmeier et al., 1996). These receptors are positively coupled to adenylyl cyclase (AC) through Golf (Herve et al., 1995). Elevation in cytosolic cAMP levels leads to the activation of protein kinase A (PKA) and phosphorylation of a variety of intracellular targets, like the dual function phosphoprotein DARPP-32 (Svenningsson et al., 2004), altering cellular function. A growing number of studies suggest that the D1/PKA cascade has direct effects on AMPA and NMDA receptor function and trafficking. For example, D1 receptor activation of PKA enhances surface expression of both AMPA and NMDA receptors (Snyder et al., 2000; Hallett et al., 2006). The precise mechanisms underlying the trafficking are still being pursued but the tyrosine kinase Fyn and the protein phosphatase STEP (striatal-enriched-phosphatase) appear to be important regulators of surface expression of glutamate receptors (Braithwaite et al., 2006). Trafficking and localization might also be affected by a direct interaction between D1 and NMDA receptors (Lee et al., 2002; Scott et al., 2006).

What is less clear is whether D1 receptor stimulation has rapid effects on glutamate receptor gating. Although PKA phosphorylation of the NR1 subunit is capable of enhancing NMDA receptor currents (Blank et al., 1997), the presence of this modulation in MSNs is controversial. In neurons where the engagement of dendritic voltagedependent ion channels has been minimized by dialyzing the cytoplasm with cesium ions, D1 receptor agonists have little or no discernible effect on AMPA or NMDA receptor mediated currents in dorsal striatum (Nicola and Malenka, 1998). However, in MSNs where this has not been done, D1 receptor stimulation rapidly enhances currents evoked by NMDA receptor stimulation (Cepeda et al., 1993). The difference between these results suggests that the effect of D1 receptors on NMDA receptor currents is indirect and mediated by voltage-dependent dendritic conductances that are eliminated by blocking K<sup>+</sup> channels and clamping dendritic voltage. Indeed, blocking L-type Ca<sup>2+</sup> channels, which open in the same voltage range as NMDA receptors (Mg<sup>2+</sup> unblock) attenuates the D1 receptor mediated enhancement of NMDA receptor currents (Liu et al., 2004).

This type of interaction between voltage-dependent ion channels and ionotropic receptors appears to be common in neurons. Far from the passive entities envisioned 20 years ago, neuronal dendrites are richly invested with voltagedependent ion channels that shape synaptic responses and plasticity. Although nearly all the studies of active dendrites to date have been in pyramidal neurons, there is evidence that similar mechanisms govern MSN dendrites (Carter and Sabatini, 2004; Kerr and Plenz, 2004). However, unlike pyramidal neurons, the dendrites of MSNs are too small to accommodate an electrode, so indirect measures have been used to understand how DA modulates the ion channels that invest MSN dendrites. More recently, the combination of imaging (most notably two photon laser scanning microscopy (2PLSM)) and patch clamp has been applied to MSN dendrites in organotypic culture and brain slices (Carter and Sabatini, 2004; Kerr and Plenz, 2004); this approach offers a powerful alternative to conventional approaches, particularly when applied to tissue in which phenotypically homogenous neuronal populations are fluorescently tagged.

Voltage-dependent Na<sup>+</sup> channels were the first wellcharacterized targets of the D1 receptor signaling pathway in MSNs. Confirming inferences drawn from earlier studies in tissue slices (Calabresi et al., 1987), voltage clamp work showed that D1 receptor signaling led to a reduction in Na<sup>+</sup> channel availability without altering the voltage-dependence of fast activation or inactivation (Surmeier et al., 1992). Subsequent work has shown that PKA phosphorylation of the pore-forming subunit of the Na<sup>+</sup> channel promotes activity dependent entry into a non-conducting, slow inactivated state that can be reversed only by membrane hyperpolarization (Carr et al., 2003). It is likely that the D1 receptor modulation is mediated by phosphorylation of somatic Nav1.1 channels, as Nav1.6 channels are not efficiently phosphorylated by PKA (Scheuer and Catterall, 2006). The coupling of the D1 receptor cascade to dendritic (as opposed to somatic) Nav1.1/Nav1.6 channels remains uncertain and the subcellular positioning of the scaffolding interactions necessary to bring about efficient phosphorylation of Na<sup>+</sup> channel subunits (Scheuer and Catterall, 2006) has not been mapped in MSNs.

When the somatic membrane potential is held for several hundred milliseconds near the up-state potential (~-60 mV) (Wickens and Wilson, 1998), D1 receptor stimulation has a quite different effect than when it is held at nominal down-state potentials ( $\sim -80 \,\mathrm{mV}$ ). At this up-state membrane potential, the personality of the MSN is transformed, as the constellation of ion channels governing activity is re-configured. Perhaps the most dramatic change is the closure or inactivation of Kir2, Kv1 and Kv4 K<sup>+</sup> channels that oppose the depolarizing influences of glutamate receptors. In this state, D1 receptor stimulation elevates (rather than lowers) the response to intrasomatic current injection (Hernandez-Lopez et al., 1997). The augmented response is attributable in part to enhanced opening of L-type Ca<sup>2+</sup> channels following PKA phosphorylation (Surmeier et al., 1995; Gao et al., 1997). L-type channels with a pore-forming Cav1.3 subunit are likely to be major targets of this modulation; these channels have a voltage threshold near  $-60 \,\mathrm{mV}$  and are anchored near glutamatergic synapses in spines through a scaffolding interaction with Shank (Olson et al., 2005). Enhanced opening of these channels and NMDA receptors (Cepeda et al., 1993; Levine et al., 1996; Snyder et al., 1998; Flores-Hernandez et al., 2002) accounts for the ability of D1 receptor stimulation to promote synaptically driven plateau potentials of MSNs (resembling up-states in vivo) in corticostriatal slices (Vergara et al., 2003), as in cortical pyramidal neurons (Tseng and O'Donnell, 2004). D1 receptor stimulation also reduces opening of Cav2 Ca<sup>2+</sup> channels that couple to SK K<sup>+</sup> channels (Vilchis et al., 2000), potentially further augmenting dendritic electrogenesis.

Taken together, these results suggest that D1 receptor signaling through PKA elevates the responsiveness of striatonigral neurons to sustained synaptic release of glutamate generating up-states but reduces the response to transient or uncoordinated glutamate release that fails to significantly depolarize the dendritic membrane for more than a few tens of milliseconds from the down-state.

#### Box 6.1 D1 and D2 MSNs Differ in Dendritic Morphology

MSNs have long been thought to be homogeneous in their somatodendritic morphology and physiology. However, recent studies using D1 and D2 BAC transgenic mice have revealed that D1 MSNs were less excitable than D2 MSNs over a broad range of developmental time points (Gertler et al., 2008). A straightforward explanation for the dichotomy in excitability of D1 and D2 MSNs is that they differ in surface area. To test this hypothesis, D1 and D2 MSNs were identified by epifluorescence in slices from BAC mice and then patched with electrodes containing biocytin (Horikawa and Armstrong, 1988). After filling, slices were processed and recorded MSNs were reconstructed, preserving as much of their three dimensional architecture as possible (Fig. 6.1A,B). Dendritic length and branching pattern were measured in a population of D1 and D2 MSNs. A three dimensional Sholl analysis was performed to determine the number of dendritic processes in concentric shells centered on the soma (Fig. 6.1D,E). D1 MSNs had more intersections than D2 MSNs from  $10-135\,\mu m$  from the soma. From the 3D Sholl analysis, the cumulative dendritic length within spheres of increasing diameter was measured and averaged to determine where branching diverged. Approximately 25 µm from the soma, the difference in cumulative dendritic length reached ~20% and remained constant (Fig. 6.1F). Total dendritic length was positively correlated with whole-cell capacitance, confirming the expected relationship between the electrical and anatomical measurements.

The difference in total dendritic length was attributable to a difference in the number of primary dendrites, as the mean tree length (i.e., total dendritic length/number of primary dendrites) was similar in the two types of MSNs. D1 MSNs had significantly more branch points and tips, but this was due to their having more primary dendrites. The mean number and length of dendritic segments as a function of branch order was not significantly different

between D1 and D2 MSNs. A convex hull analysis was used to estimate the three-dimensional space occupied by dendritic trees (this algorithm takes into account the three-dimensional space occupied by a set of dendritic processes, allowing for a more complex polygonal surface rendering than assuming a cubic or spherical distribution). D1 MSNs occupied significantly more space, though there was no significant difference in the dendritic trees from D1 and D2 MSNs. Taken together, the anatomical analyses showed that on average D1 MSNs have more primary dendrites than D2 MSNs.

A basic question is whether this difference in dendritic anatomy depends upon intrinsic (cell autonomous) or extrinsic (environmental) factors. A simple way to begin to examine this question is to see if the differences can be recapitulated in a simple system, such as a two-dimensional, dissociated corticostriatal culture where the normal striatal environment and the topography of cortical connections with MSNs has been disrupted. In the absence of cortex, MSN dendrites are aspiny and sparsely branching, demonstrating that this anatomical feature is not cell autonomous. However, in co-culture MSNs develop a relatively normal dendritic morphology, including spines (Segal et al., 2003) - showing that culturing itself did not prevent the elaboration of qualitatively normal dendritic morphology. To get at the quantitative features and to compare D1 and D2 MSNs, striata cultured from P0 D2 BAC mice and cerebral cortex from wild-type mice were maintained for three weeks in vitro. Cultures were then fixed; D2 MSNs were identified by eGFP expression and D1 MSNs were identified by immunoreactivity for D1 receptors. Although the average branching pattern of D1 and D2 MSNs differed from that seen in vivo, the total dendritic length was significantly greater in D1 MSNs, as found in vivo. This argues that the basic dichotomy between D1 and D2 MSNs is not dependent upon DA.



**FIGURE 6.1** D1 and D2 MSNs differ in dendritic anatomy. A–C. Striatal neurons from P35–P45 BAC transgenic mice were biocytin-filled, imaged, and reconstructed in 3D. A GABAergic interneuron is included for comparison. D. Fan-in diagrams displayed no apparent preferred orientation in either the D1 or D2 MSN populations. E. Dendrograms displaying in two dimensions the length, number, and connectivity of dendritic segments in sample neurons. F. 3D Sholl analysis of biocytin-filled and reconstructed neurons from P35–45 BAC transgenic mice. Data are shown as mean ( $\pm$  SEM) number of intersections at 1-µm eccentricities from the soma for 15 D1 and 16 D2 MSNs. D1 MSNs have a more highly branched dendritic tree, as indicated by the increased number of intersections and positive subtracted area (gray shading). B. Mean cumulative dendritic length at 1-µm eccentricities for D1 and D2 MSN populations. Note: The % difference between populations in cumulative total dendritic length increases and remains at ~20% (arrow and fit line) within 30µm from the soma. Inset: the total dendritic length in each cell/number of primary dendrites is not significantly different between populations (D1 MSN: median = 398.8µm, n = 15; D2 MSN: median = 400.5µm, n = 16). C. Whole-cell capacitance is positively correlated to total dendritic length ( $r_s = 0.45$ , P < 0.05). D. D1 MSNs have significantly more primary dendrites (D1 MSN: median = 8, n = 15; D2 MSN: median = 6, n = 16; P < 0.05), branch points (D1 MSN: median = 28, n = 15; D2 MSN: median = 19, n = 16; P < 0.05), tips (D1 MSN: median = 38, n = 16; D2 MSN: median = 28, n = 15; D2 MSN: median = 2878.3µm, n = 16; P < 0.001), and total dendritic length (D1 MSN: median = 3385.6µm, n = 15; D2 MSN: median = 2878.3µm, n = 16; P < 0.001).

### IV. MODULATION OF INTRINSIC EXCITABILITY AND GLUTAMATERGIC SIGNALING BY D2 RECEPTORS

D2 receptors are expressed at high levels in striatopallidal MSNs. D2 receptors couple to  $G_{i/o}$  proteins, leading to inhibition of adenylyl cyclase through  $G\alpha_i$  subunits (Stoof and Kebabian, 1984). In parallel, released  $G\beta\gamma$  subunits are capable of reducing Cav2 Ca<sup>2+</sup> channel opening and of stimulating phospholipase Cb isoforms, generating diacylglycerol (DAG) and protein kinase C (PKC) activation as well as inositol trisphosphate (IP3) liberation and the mobilization of intracellular Ca<sup>2+</sup> stores (Nishi et al., 1997; Hernandez-Lopez et al., 2000). D2 receptors also are capable of transactivating tyrosine kinases (Kotecha et al., 2002).

As with D1 receptor signaling, there are a number of studies showing that D2 receptor signaling alters glutamate receptor function in dorsal striatal MSNs. Activation of D2 receptors has been reported to decrease AMPA receptor currents of MSNs recorded in tissue slices (Cepeda et al., 1993). Subsequent work using acutely isolated neurons and voltage clamp techniques, support a direct action on dendritic AMPA receptors (Hernandez-Echeagaray et al., 2004). D2 receptor signaling leads to dephosphorylation of S845 of the GluR1 subunit, which should promote trafficking of AMPA receptors out of the synaptic membrane (Hakansson et al., 2006). D2 receptor stimulation also diminishes presynaptic release of glutamate (Bamford et al., 2004); however, it is not clear whether this is mediated by presynaptic or postsynaptic D2 receptors (Yin and Lovinger, 2006).

Studies of voltage-dependent channels are largely consistent with the proposition that D2 receptors act to reduce the excitability of striatopallidal neurons and their response to glutamatergic synaptic input. D2 receptor mediated mobilization of intracellular Ca<sup>2+</sup> leads to negative modulation of Cav1.3 Ca<sup>2+</sup> channels through a calcineurindependent mechanism (Hernandez-Lopez et al., 2000; Olson et al., 2005). D2 receptor activation also reduces opening of voltage-dependent Na<sup>+</sup> channels, presumably by a PKC-mediated enhancement of slow inactivation (Surmeier et al., 1992). In addition, D2 receptors promote the opening of K<sup>+</sup> channels (Greif et al., 1995). This coordinated modulation of ion channels provides a mechanistic foundation for the ability of D2 receptor agonists to reduce the responsiveness of MSNs in slices at up-state membrane potentials (Hernandez-Lopez et al., 2000).

Complementing this postsynaptic reduction in excitability, D2 receptor activation leads to diminished glutamate release (Calabresi et al., 1992; Hsu et al., 1995; Flores-Hernandez et al., 1997; Cepeda et al., 2001; Bamford et al., 2004). While there is no doubt about the phenomenon, the mechanism mediating this modulation is controversial. At issue is whether the D2 receptors involved are pre- or postsynaptic. Although this would seem to be an easy matter to resolve with immunocytochemical approaches, the commercially available D2 receptor antibodies are of questionable specificity in tissue that has been fixed appropriately for electron microscopy. Although it is not strong evidence, in BAC D2 GFP mice, there is no detectable cortical GFP expression, suggesting that, in rodents where the phenomenon has been studied, D2 receptors are not expressed by cortical pyramidal neurons. The most compelling evidence on the issue comes from the demonstration that the D2 receptor modulation is sensitive to deletion or blockade of CB1 receptors (Yin and Lovinger, 2006). This suggests that postsynaptic D2 receptor stimulation elevates endocannabinoid production (Giuffrida et al., 1999), leading to presynaptic inhibition by way of CB1 receptors. This matters because the presynaptic effects of D2 receptor stimulation are likely to be stronger near the source of the endocannabinoid signal - D2 MSNs. Recent work with BAC transgenic mice is consistent with this inference (Kreitzer and Malenka, 2007).

### V. DOPAMINERGIC MODULATION OF LONG-TERM SYNAPTIC PLASTICITY

As mentioned above, the place where DA is thought to exert its principal effects are in dendrites where glutamatergic synapses are formed. Although it modulates short-term network activity, DA's role in associative learning and habit formation is commonly thought to be in the regulation of corticostriatal synaptic plasticity. The best-studied form of synaptic plasticity in the striatum is long-term depression (LTD) (see also Chapter 12). When postsynaptic depolarization is paired with high-frequency stimulation (HFS) of glutamatergic fibers, a long-lasting reduction in synaptic strength of glutamatergic synapses is seen in most MSNs. Unlike LTD induced by low-frequency stimulation in the ventral striatum (Brebner et al., 2005), LTD induction in the dorsal striatum is not NMDA dependent. This form of LTD (HFS-LTD) is initiated postsynaptically, but expressed through a presynaptic reduction in glutamate release. There is a general agreement that striatal LTD requires activation of Cav1.3 L-type Ca<sup>2+</sup> channels, G<sub>q</sub>-linked mGluR1/5 receptors, and the generation of endocannabinoids (ECs). ECs exert their effect presynaptically by acting at CB1 receptors (Lovinger et al., 1993; Centonze et al., 2001; Kreitzer and Malenka, 2005) (see Chapter 9). There is less agreement that activation of D2 receptors is necessary for LTD induction. Activation of D2 receptors is a very potent stimulus for EC production (Giuffrida et al., 1999) and the ability of D2 receptors to activate PLC (Hernandez-Lopez et al., 2000) certainly is consistent with a direct involvement in EC production. However, attempts to test for the

Box 6.2 MSN Dendrites are Active

Striatopallidal (D2) or striatonigral (D1) MSNs were visually identified using 2PLSM excitation of eGFP as previously described (Day et al., 2006). Somatic whole-cell current recordings were made with electrodes filled with Alexa 568  $(50\mu M)$  and Fluo-4  $(200\mu M)$ . The Alexa 568 enabled detailed visualization of distal dendrites and spines while the Ca<sup>2+</sup>sensitive indicator Fluo-4 reported Ca2+ transients induced in these regions by the somatically generated bAPs. After eGFP phenotyping and patching, the internal solution was allowed to equilibrate for 20 minutes. 2PLSM line scanning was then performed between 45 and 130µm from the soma. Estimates of the Ca2+ transient were generated by eliciting six bAPs (equally spaced at 5-second intervals), and then averaging the responses. Spacing in this way allowed ample time for the Ca<sup>2+</sup> to return to basal levels and remain there for several seconds prior to the onset on the next bAP in the series. The bAPs were evoked by injecting a 2ms, 2nA current pulse into the soma (Fig. 6.2A, right panel). To control for photodamage, dendritic processes were only illuminated during a 0.5 second window that bracketed the initiation of the bAP. Measurements were taken concurrently from a spine and the parent dendrite close to the base of the spine. In all cases, if a Ca<sup>2+</sup> transient was detected in the spine, it was also detected in the dendrite. The maximum amplitude of the bAP-evoked spine Ca<sup>2+</sup> transients was determined by calculating  $\Delta$ F/Fo for each transient (image panels), averaging the results (black traces), and then fitting the decay phase of data with a single exponential (grey lines). The key finding in these initial experiments was that in D1 MSNs, single bAPs frequently failed to evoke a detectable Ca<sup>2+</sup> transient at dendritic sites more than 60µm from the soma (Fig. 6.2A), whereas in D2 MSNs, dendritic Ca2+ transients were readily detected at this distance and beyond (Fig. 6.2B).

To more closely examine the disparity in the bAP-evoked  $Ca^{2+}$  transients between the two populations of MSNs, bAPevoked  $Ca^{2+}$  transients from each cell type were scanned at varying distances from the soma (Fig. 6.2C). Here, the amplitudes of bAP-evoked  $Ca^{2+}$  transients from each scan point in each cell type were normalized to the most proximal location scanned and then plotted as a function of distance from the necessity of D2 receptor expression using bacterial artificial chromosome (BAC) mice have met with mixed results (Wang et al., 2006; Kreitzer and Malenka, 2007). Kreitzer and Malenka (2007) reported that LTD was inducible only in striatopallidal MSNs, using a minimal local striatal stimulation. However, our group and Lovinger's found that HFS-LTD was inducible in both striatonigral and striatopallidal MSNs when using macroelectrode stimulation of the cortex (Wang et al., 2006), consistent with the high probability of induction seen in previous work (Calabresi et al., 2007). We have reproduced the Kreitzer and Malenka finding using minimal local striatal stimulation,

soma (n = 6 each). These findings show differences in somato/ dendritic excitability between MSNs, with the D2 MSNs showing less attenuation of bAP-evoked Ca2+ transients in distal spines and dendrites than D1 MSNs. To test the possibility that the loss in bAP response was attributable to declining dendritic Ca<sup>2+</sup> channel density, D1 MSNs were loaded with Cs<sup>+</sup> (to improve voltage control of distal dendrites) and the somatic membrane briefly stepped to a depolarized potential. In this situation, there was no detectable attenuation of the Ca<sup>2+</sup> transient with distance from the soma (Fig. 6.2D), arguing that the loss of the bAP-evoked Ca<sup>2+</sup> transient was not due to diminished Ca<sup>2+</sup> channel density. Further evidence that this phenomenon does not simply reflect diminished Ca<sup>2+</sup> channel density in distal dendrites is that strong depolarization (1 sec) and trains of APs ( $10 \times 10$  Hz) consistently evoked Ca<sup>2+</sup> transients in distal process of all MSNs tested.

Although single bAPs were not propagated efficiently into the distal dendrites of D1 MSNs, bursts of somatic action potentials were able to evoke  $Ca^{2+}$  transients in more distant dendritic regions. Three spike bursts (50 Hz) delivered at a theta frequency reliably evoked shaft and spine  $Ca^{2+}$  transients in both D1 and D2 MSN dendrites 100-120 µm from the soma (Fig. 6.2E). The  $Ca^{2+}$  signals evoked by successive bursts summed in a sub-linear fashion (Fig. 6.2E,F). This sublinearity was more pronounced in D1 MSNs than in D2 MSNs (Fig. 6.2F). Moreover, consistent with the response to single bAPs, the relative elevation in  $Ca^{2+}$  evoked by somatically generated theta bursts were smaller in amplitude and area in D1 MSNs (Fig. 6.2F).

In spite of the ability to see dendritic  $Ca^{2+}$  transients with repetitive somatic spikes, the imaging studies to date make it look as though distal MSN dendrites are not efficiently activated this way. As mice mature, MSNs dendrites become even less excitable (unpublished observations). This raises the possibility that synaptically driven regenerative events in distal dendrites are the gating signal for the induction of synaptic plasticity, rather than somatic spikes. At this point in time, answering this question appears to require a combination of electrophysiology, 2PLSM and 2PLU of glutamate.



FIGURE 6.2 BAP-evoked Ca<sup>2+</sup> transients are readily detected in the distal dendrites and spines of the D2 population of MSNs. (A, B) 2PLSM images of MSNs in 275 µm thick corticostriatal slices from a (A) BAC D1 and (B) BAC D2 mouse. Neurons were visualized with Alexa Fluor 568 (50µM) by filling through the patch pipette (patch pipettes are graved-out for presentation). Maximum projection images of the somas and dendritic fields (left panels A & B) and high magnification projections of dendrite segments from the regions outlined by the boxes are shown (top right panels A & B). BAP-evoked  $Ca^{2+}$  transients were detected by line scanning through the spine in the region indicated by the yellow line. Fluorescence traces were generated from the pseudocolor image (lower panels A & B) by calculating  $\Delta$ F/Fo (top black trace). The fluorescence image,  $\Delta F/Fo$  trace, action potential (middle trace) and current pulse (bottom trace) are shown in temporal registration. (C) Maximum projection image of a soma and dendritic branch from a D2 MSN. Line scans were acquired at 2 eccentricities, 120 and 60 µm, as indicated by the red arrows. (D) Graph of the change in amplitude with distance from the soma calculated by normalizing scans taken at distal points to the most proximal scan point in each MSN. The magnitude of the  $Ca^{2+}$  transients decrements more in the D1 MSNs (D1 MSNs = open blue circles; D2 MSNs = open green circles). This decrementation is not seen in MSNs loaded with  $Cs^+$  based internals (open orange circles). The points were scaled to represent the number of cells scanned at each point (smallest points = 1 cell; largest points = 4 cells). The data, fit from the median distance of the most proximal point, shows that the magnitude of the  $Ca^{2+}$  transients decrements more in the D1 MSNs (n = 11, blue line) vs. the D2 MSNs (n = 6, green line) [Kruskal–Wallis ANOVA, P < 0.01]. (E) Maximum projection image of the soma and dendritic field of a D2 MSN. A high magnification image of the dendritic segment outlined in the yellow box is shown in the inset. Scale bars in B apply to both images. The pseudocolor image,  $\Delta F/Fo$  trace, action potential (middle trace) and current pulse (bottom trace) are shown in temporal registration. Arrows indicate the timing of current pulses delivered to initiate APs. (F) Average peak  $\Delta$ F/Fo values after each of the five pulses constituting the theta burst bAP protocol. Values are from distal dendritic spines ( $100-120 \mu m$  from the soma), and normalized to the maximum peak  $\Delta F/F_0$  value measured in a proximal spine (60–80 $\mu$ m) of the same dendrite in response to the first burst of the same theta burst protocol. The area under the  $\Delta$ F/Fo plot was calculated for each cell type in response to the entire theta burst protocol; in line with larger peak Ca<sup>2+</sup> transients, the box plots to the right demonstrate significantly larger Ca<sup>2+</sup> transient areas in the D2 vs. the D1 MSNs [Kruskal-Wallis ANOVA, P < 0.05]. From Day et al., 2008. (see Color Plate Section to view the color version of this figure)

suggesting that the method of induction is important. This result underscores the difficulties inherent in stimulation paradigms that do not activate just glutamatergic fibers, but also a heterogeneous population of dopaminergic, cholinergic and interneuronal fibers that might influence the induction of plasticity. An example of how we have attempted to sort this out is given below.

One strategy for gaining better control over which fibers are activated in studies of plasticity is to develop in vitro preparations that preserve connectivity between nuclei. Consider the glutamatergic synapses formed on MSNs. Most reviews have focused almost entirely on the cortical innervation of MSNs, leaving the thalamic input to a virtual footnote. Studies using nominal white matter or cortical stimulation of coronal brain slices typically assume that the glutamatergic fibers being stimulated are of cortical origin, but very few of these fibers are left uncut in this preparation (Kawaguchi et al., 1989). The thalamic innervation of MSNs is similar in magnitude to that of the cerebral cortex, perhaps constituting as much as 40% of the total glutamatergic input to MSNs, terminating on both shafts and spines (Wilson, 2004a) (see Chapter 22). Anatomical studies suggest that the intralaminar thalamic nuclei target primarily striatonigral neurons in primate striatum (however, this might not be the case in rodents; Bacci et al., 2004), whereas "motor" nuclei [(ventroanterior (VA) and ventrolateral (VL) nuclei] project primarily to striatopallidal neurons (Smith et al., 2004; Hoshi et al., 2005). This apparent dichotomy between motor and "associative" inputs is consistent with recent studies suggesting that cortical input to striatopallidal neurons comes largely from pyramidal neurons contributing to descending motor control circuits, whereas the input to striatonigral neurons comes from cortical neurons whose axons are largely intra-telencephalic (Lei et al., 2004). Recently, several studies have shown that parahorizontal slices can preserve both cortical and thalamic connectivity, allowing each to be selectively stimulated (Smeal et al., 2007; Ding et al., 2008). However, these preparations have not been used to date to study the rules governing the induction of plasticity at these two types of synapse.

Much less is known about the mechanisms controlling induction of long-term potentiation (LTP) than LTD. Studies in tissue slices have argued that LTP induced by HFS of corticostriatal glutamatergic inputs (HFS-LTP) depends upon co-activation of D1 and NMDA receptors (Kerr and Wickens, 2001; Centonze et al., 2003). As noted above, D1 receptor stimulation enhances NMDA receptor currents both directly and indirectly by enhancing L-type Ca<sup>2+</sup> channels located nearby (Surmeier et al., 1995; Liu et al., 2004), although "boosting" by L-type channels appears not to be necessary for LTP induction (Calabresi et al., 2000). There was some question about the physiological relevance of LTP in MSNs, but this issue has been resolved by the demonstration that it is readily inducible in vivo (Mahon et al., 2004). The discrepancy presumably stemmed from the difficulty in depolarizing MSN dendrites enough to overcome Mg<sup>2+</sup> block of NMDA receptors with focal stimulation in a brain slice. How HFS-LTP is expressed has not been carefully examined. As with HFS-LTD, the dependence of a nominally wide-spread form of synaptic plasticity upon a receptor with restricted distribution is puzzling. BAC transgenic mice in which D1 and D2 receptor expressing MSNs are labeled should be helpful in sorting this issue out.

As it is apparent from the presentation thus far, there are several obstacles that have slowed progress toward a sound understanding of the DA modulation of synaptic plasticity in the striatum. Cellular heterogeneity has been one of the biggest of these in our view. The development of D1 and D2 receptor BAC transgenic mice has made this aspect of the problem tractable. Another issue is the induction protocol. Until very recently, plasticity studies have not attempted to engage the postsynaptic membrane and dendrites in a physiological way during the induction of synaptic plasticity (e.g.,  $Cs^+$  loading cells and voltage clamping).

Why is this important? Most learning theories postulate that changes in synaptic strength reflect the precise temporal relationship between presynaptic and postsynaptic activity. Hebb's classic postulate asserts that excitatory synaptic activity that consistently leads to postsynaptic spiking induces a strengthening or potentiation of the active synapses. An unstated corollary is that synaptic activity that follows postsynaptic activity (and hence cannot be causally linked to spiking) should be weakened or depressed. Dendrites are an integral part of this learning equation, forming the conduit between the axon initial segment where spikes are initiated and synaptic sites where plasticity is induced. DA receptors richly invest dendrites of MSNs (Hersch et al., 1995), putting them in a position to modulate this linkage. The extended Hebbian postulate has been tested in several types of neurons by examining how the temporal relationship between presynaptic and postsynaptic spiking influences lasting changes in synaptic strength (Dan and Poo, 2004; Kampa et al., 2007; Sjostrom et al., 2008). Spike-timing-dependent plasticity (STDP) of this sort depends upon back-propagating action potentials (bAPs) that serve to depolarize synaptic regions before, during, or after glutamate release. At most synapses, Hebb's postulate appears to be correct. That is, when presynaptic activity precedes postsynaptic spiking, LTP is induced, whereas reversing the order induces LTD (Sjostrom and Nelson, 2002; Letzkus et al., 2006; Nevian and Sakmann, 2006; Pawlak and Kerr, 2008).

Using perforated-patch recordings (to preserve intracellular signaling mechanisms) and minimal local electrical stimulation of glutamatergic afferent fibers in tissue slices from BAC transgenic mice, we have used STDP protocols to examine the rules governing the induction of plasticity at striatonigral and striatopallidal MSN synapses (Shen et al., 2008). These studies have revealed a set of rules that are largely consistent with those inferred from studies using conventional induction protocols (see above), but pushed us beyond our current conceptual model by showing that DA controls the induction of Hebbian synaptic plasticity in a receptor and cell-type specific manner.

Specifically, D1 receptor signaling in striatonigral MSNs was necessary for the induction of Hebbian longterm potentiation, whereas D2 receptor signaling in striatopallidal MSNs was necessary for the induction of Hebbian long-term depression. More importantly, our studies demonstrate that DA, in concert with adenosine and glutamate, makes STDP at MSN glutamatergic synapses bidirectional and Hebbian (Shen et al., 2008). In striatopallidal MSNs (Fig. 6.3A), repeated pairing of a synaptic stimulation with a postsynaptic spike later (positive timing) resulted in LTP of the synaptic response (Fig. 6.3B). In contrast, preceding synaptic stimulation with a short burst of postsynaptic spikes (negative timing) induced LTD (Fig. 6.3C). The timing-dependent LTP relies upon activation of NMDA and A2a receptors, as blocking them disrupts the potentiation of synaptic response in striatopallidal MSNs (Fig. 6.3D). As with conventional LTD, timing-dependent LTD is disrupted by antagonizing mGluR5, CB1, or D2 receptors (Fig. 6.3D). The bidirectionality of STDP appeared to be controlled by a balanced interaction between "opponent" GPCR signaling cascades controlling the induction of LTP and LTD (Nevian and Sakmann, 2006; Seol et al., 2007; Tzounopoulos et al., 2007). D2 and A2a receptor signaling cascades have long been known to oppose one another at several levels (Schwarzschild et al., 2006; Fuxe et al., 2007). In the STDP paradigm, elevating D2 receptor stimulation by bath application of quinpirole resulted in a robust LTD even when postsynaptic activity followed presynaptic activity, a protocol that would normally induce LTP. In contrast, elevating A2a receptor signaling by bath application of CGS21680 restored LTP, even when presynaptic activity followed postsynaptic activity (Fig. 6.3D).

In striatonigral MSNs (Fig. 6.4A), pairing presynaptic activity with a trailing postsynaptic spike induced robust LTP (Fig. 6.4B). As in striatopallidal MSNs, STDP LTP was dependent upon NMDA receptors (Fig. 6.4D). However, when presynaptic activity followed postsynaptic spiking, EPSP amplitude did not change. In light of the opponent signaling hypothesis, we reasoned that this failure could be due to the activation of the GPCR responsible for LTP induction. To test this hypothesis, D1 receptors were blocked by SCH23390. In the absence of D1 receptor activity, pairing postsynaptic spiking with a presynaptic volley led to a robust LTD (Fig. 6.4C). Moreover, the CB1 receptor antagonist AM251 blocked the LTD, establishing a mechanistic parallel to LTD in striatopallidal MSNs. To determine whether attenuating D1 receptor signaling altered the timing dependence of plasticity, the effects of the positive timing protocol (presynaptic activity followed by postsynaptic activity) were re-examined. In control conditions, this protocol induced a robust LTP (Fig. 6.4B). Blocking D1 receptors not only prevented LTP induction, it led to the induction of LTD (Fig. 6.4D).

The recognition that DA is not essential for all forms of synaptic plasticity in MSNs, resolves the apparent paradox posed by the segregation of DA receptors in the two MSN populations. The finding that STDP plasticity at MSN glutamatergic synapses is Hebbian is consistent with a recent study (Pawlak and Kerr, 2008), but conflicts with another (Fino et al., 2005). The discrepancy could be attributable to the engagement of GABAergic interneurons in the striatum, confounding modifications in the strength of glutamatergic synapses (Fino et al., 2008). Indirect, modulatory influences of other striatal interneurons also have been implicated in the induction of plasticity at glutamatergic synapses when large regions of the striatum are stimulated (Wang et al., 2006; Calabresi et al., 2007). Our reliance upon focal stimulation near synaptic sites minimized the involvement of these interneurons and helped to resolve how DA receptors expressed by postsynaptic MSNs shaped the induction process.

These studies suggest that while DA makes STDP in striatal MSNs bidirectional and Hebbian, it is not necessary for the induction of synaptic plasticity. This stands in contrast to previous work asserting that DA is essential



**FIGURE 6.3** Striatopallidal MSNs displayed bidirectional STDP dependent upon D2 and A2a receptors. A, Upper, single cell RT-PCR (scRT-PCR) amplicons from an individual BAC D2 eGFP-labeled neuron confirmed co-expression of enkephalin and D2 receptor mRNA. M, marker; SP, substance P; ENK, enkephalin. Bottom, two-photon laser scanning microscopic image of eGFP-labeled MSNs in a slice from a BAC D2 mouse. B, LTP induced in eGFP labeled striatopallidal MSN by a positive timing pairing. Plots show EPSP amplitude and input resistance as a function of time in a single cell. The dashed line shows the average EPSP amplitude before induction. The induction was performed at the vertical bar. Filled symbol shows the averages of 12 trials ( $\pm$  SEM). The averaged EPSP traces before and after induction are showed at the top. C, LTD induced by a negative timing pairing. Plots and EPSP traces as in B. D, Schematic illustration shows that activation of A2a and NMDA receptors leads to LTP and activation of D2 and mGluR5 receptors and Cav1.3 channels leads to LTD. Moreover, A2a and D2 receptor activation oppose each other in inducing plasticity. Glu, glutamate; EC, endocannabinoid. From Shen et al., 2008.

for plasticity and that striatal DA depletion in Parkinson's disease models eliminates both LTD and LTP (Calabresi et al., 2007; Kreitzer and Malenka, 2007). To test this hypothesis, BAC mice were rendered parkinsonian by unilateral 6-OHDA lesions, sacrificed a week later and slices prepared from their brains. What we found was consistent

with the work in unlesioned brains. That is, in striatopallidal MSNs pairing pre- and postsynaptic activity induced LTP, regardless of the order of presentation; in contrast, in striatonigral MSNs, pairing pre- and postsynaptic activity induced LTD, again regardless of order. Thus, synaptic plasticity is not lost in PD models, but it ceases to be



**FIGURE 6.4** Striatonigral MSNs displayed bidirectional STDP dependent upon D1 receptors. A, Upper, scRT-PCR amplicons from an individual eGFP-labeled neuron from a BAC D1 mouse confirmed co-expression of substance P and D1 receptor mRNA. M, marker; SP, substance P; ENK, enkephalin. Bottom, two-photon image of eGFP-labeled MSNs in a slice from a BAC D1 mouse. B, LTP induction in labeled striatonigral neuron by a positive timing pairing protocol (+5 ms) coupled with postsynaptic depolarization to -70 mV. EPSP amplitude and input resistance of the recorded cell were plotted as a function of time. The dashed line shows the average of EPSP amplitude before induction. The induction was performed at the vertical bar. Filled symbol shows the averages of 12 trials ( $\pm$  SEM). The averaged EPSP traces before and after induction are shown at the top. C, In the presence of SCH23390, a negative timing pairing revealed a robust LTD. Plots and EPSP traces are from a single cell as in B. D, Schematic drawing shows that activation of D1 and NMDA receptors evokes LTP and activation of mGluR5 receptor and Cav1.3 channels evokes LTD. Moreover, D1 and mGluR5 receptor activation oppose each other in inducing plasticity. Glu, glutamate; EC, endocannabinoid. From Shen et al., 2008.

bidirectional and dependent upon the timing of pre- and postsynaptic activity (Shen et al., 2008).

In addition to reconciling a discordant literature on the role of DA in the modulation of glutamatergic synaptic plasticity, these studies establish a parallel between the shortterm and long-term effects of DA on MSNs. As reviewed above, the short-term effects of DA are receptor specific, tending to diminish the excitability of striatopallidal MSNs through D2 receptors and to increase the excitability of striatonigral MSNs through D1 receptors. Now it is clear that the effects of DA on synaptic plasticity are also receptor and cell-type specific. That is, by promoting LTD, D2 receptors diminish the excitatory synaptic input to striatopallidal MSNs, decreasing their activity; conversely, by promoting LTP, D1 receptors increase the excitatory synaptic input to striatonigral MSNs, enhancing their activity.

## VI. THE INDIRECT PLAYERS – STRIATAL INTERNEURONS

In thinking about how DA influences MSN activity, it is impossible to ignore the contribution of interneurons. Most, if not all, of the different types of striatal interneurons (see Chapter 8) express DA receptors (Tepper et al., 2004). Reviewing this literature is beyond our scope, but a few comments are called for particularly in the context of D2 receptor signaling. The best characterized of the interneurons is the giant, aspiny, acetylcholine (ACh)-releasing interneuron (see Chapter 7). In primates, cholinergic interneurons are important determinants of associative and motor learning (Graybiel et al., 1994), which are presumably mediated by alterations in the strength of MSN glutamatergic synapses. D2 receptor signaling diminishes ACh release both by reducing autonomous interneuron spiking and by inhibiting Ca<sup>2+</sup> entry necessary for exocytosis (Maurice et al., 2004; Salgado et al., 2005).

ACh has a plethora of intrastriatal targets, including DA terminals, glutamatergic terminals and MSNs (Dodt and Misgeld, 1986; Zhou et al., 2002; Perez-Rosello et al., 2005). There are five muscarinic receptors that have been identified. M1-like receptors (M1, M3 and M5) are coupled to Gq/11, mobilization of intracellular Ca<sup>2+</sup> stores and activation of phospholipase C (PLC) and protein kinase C (PKC) signaling. M2-like receptors (M2 and M4) are coupled to G<sub>i/o</sub> proteins that inhibit AC isoforms and reduce the opening of voltage-dependent Cav2 Ca<sup>2+</sup> channels. Within the striatum, M1 and M4 receptors are the major muscarinic receptors expressed in MSNs (Bernard et al., 1992; Yan et al., 2001). Nicotinic ACh receptors are expressed on glutamatergic and DA terminals but are absent in MSNs (Zhou et al., 2002).

M1 receptors are highly expressed in both direct and indirect pathway MSNs (Yan et al., 2001). Unlike D1 and D2 DA receptors, there is little evidence that M1 receptor activation modulates postsynaptic glutamatergic synapses. In contrast, M1 receptor activation elevates postsynaptic excitability by modulating voltage-dependent ion channels. M1 receptor activation reduces the opening of Kv4 channels (A-type potassium channels) throughout the somatodendritic membrane (Akins et al., 1990; Day et al., 2008). The reduction of Kv4 channel current might be mediated by PKC (Nakamura et al., 1997). In addition, M1 receptor activation coupled to PLCB and PKC leads to membrane depletion of PIP2, decreasing the opening of KCNQ (M-channel) and Kir2 (inward-rectifying potassium channel) channels (Shen et al., 2005; Shen et al., 2007). M1 receptor activation also modulates MSNs by modulating Cav channels (Howe and Surmeier, 1995). M1 receptor activation decreases the opening of somatic Cav1.3 and Cav2 Ca<sup>2+</sup> channels (Howe and Surmeier, 1995; Olson et al., 2005; Perez-Rosello et al., 2005). These effects on somatic Ca<sup>2+</sup> channels appear to work in concert with the suppression of KCNQ and Kir2 K<sup>+</sup> channels by diminishing the opening of  $Ca^{2+}$ -dependent K<sup>+</sup> (SK) channels that regulate repetitive spiking. The coordinated modulation of K<sup>+</sup> and Ca<sup>2+</sup> channels leads to increased excitability in both the dendritic and somatic regions, enhancing synaptic integration and the translation of that input to spiking.

M2-like receptors are located both presynaptically and postsynaptically. M2/3 receptors are expressed on presynaptic glutamatergic terminals (Alcantara et al., 2001), whereas M4 receptors are expressed postsynaptically in MSNs and have higher expression levels in striatonigral neurons than in striatopallidal neurons (Yan et al., 2001). M4 receptor activation inhibits Cav2 Ca<sup>2+</sup> channels (as D2 receptors do) and therefore shapes the spiking and up-state transitions in MSNs (Howe and Surmeier, 1995; Perez-Rosello et al., 2005). Presynaptically, M2/3 receptors reduce the release probability at glutamatergic synapses, tuning them to repetitive cortical activity rather than a single isolated spike (Calabresi et al., 1998; Barral et al., 1999; Alcantara et al., 2001; Pakhotin and Bracci, 2007).

How cholinergic interneurons and DA regulation of them factors into long-term synaptic plasticity has yet to be worked out. Our work is consistent with the proposition that M1 muscarinic receptors have a role in opposing the induction of LTD; conversely, work by Calabresi et al. (1999) and unpublished work from our group is consistent with the contention that M1 receptor stimulation is necessary for LTP induction. Testing this proposition and sorting out how this signaling interacts with DA and adenosine in the control of plasticity is a challenge that awaits.

### VII. DOPAMINERGIC MODULATION OF GLUTAMATERGIC SIGNALING IN PARKINSON'S DISEASE

The relationship between DA and glutamate in Parkinson's disease (PD) has long been the subject of speculation. Since

the debut of the now "classical" model nearly 20 years ago, there have been few direct tests of its predictions and precious little hard data generated on how the physiology of MSNs adapts to DA depletion, largely because it has been difficult to distinguish striatonigral and striatopallidal neurons. Furthermore, in animal with lesions of the DA system, there are a variety of changes that are not encompassed by this model. For example, behavioral supersensitivity to DA receptor agonists develops quickly, not as a consequence of significant alterations in DA receptor expression but rather as a result of altered functional coupling (Mileson et al., 1991). There is evidence of alterations in glutamate receptor expression (Dunah et al., 2000), perhaps as a consequence of alterations in the rules governing synaptic plasticity (see below). There also are changes in short-term synaptic integration and dendritic morphology, at least in some medium spiny neurons (Nisenbaum et al., 1986; Ingham et al., 1998; Dunah et al., 2000; Pang et al., 2001; Tseng et al., 2001; Gubellini et al., 2002; Picconi et al., 2003). Furthermore, there are alterations in cortical activity in the lesion models (Tseng et al., 2001; Mallet et al., 2006). This makes unraveling the global adaptations to the loss of DA in PD a much more difficult and complicated task than one would have guessed from the classical model.

In spite of the daunting nature of the problem, some headway has been made in characterizing striatal adaptations in PD models using D1 and D2 receptor BAC transgenic mice. The first study of DA depletion using these animals revealed a stark asymmetry between striatopallidal and striatonigral MSNs in their response to the loss of DA (Day et al., 2006). DA depletion led to the loss of glutamatergic synapses and spines in striatopallidal MSNs (Fig. 6.5). In contrast, DA depletion had no discernible morphological or physiological effect on synaptic function in neighboring striatonigral MSNs (but see below). In parallel with the elimination of glutamatergic synaptic contacts, the dendritic trees of striatopallidal neurons shrank, suggesting that the overall loss in glutamatergic synaptic input was even more profound. Unlike other adaptations in PD models (Zigmond and Hastings, 1998), the extent of the loss did not appear to be significantly different one month following DA depletion, suggesting that the regulatory processes controlling synapse elimination are complete within days and dependent upon the loss of DA, not the death of DA neurons. Although spine and glutamatergic synapse loss following DA depletion had been seen in animal models of Parkinson's disease and in Parkinson's disease patients (McNeill et al., 1988; Starr, 1995; Ingham et al., 1998; Dunah et al., 2000), the speed, selectivity and magnitude of the loss was not expected.

Some of the determinants of the synaptic pruning have been identified. Genetic deletion or pharmacological blockade of L-type Cav1.3 Ca<sup>2+</sup> channels prevents the loss of spines and synapses following DA depletion. As noted above, these channels are strategically positioned at spiny glutamatergic synapses (Olson et al., 2005). L-type channels contribute to the rise in intraspine Ca<sup>2+</sup> concentration particularly in response to back-propagating action potentials (bAPs) (Carter and Sabatini, 2004). DA depletion, by eliminating the D2 receptor "brake" on somatodendritic excitability (Mallet et al., 2006), could enhance intraspine Ca<sup>2+</sup> entry. Falling DA levels also increase interneuron ACh release and M1 muscarinic receptor activity in striatopallidal MSNs, further elevating dendritic responsiveness to glutamatergic input (Shen et al., 2005; Shen et al., 2007). Thus, by increasing dendritic excitability and  $Ca^{2+}$ entry associated with excitatory glutamatergic input, DA depletion appears to trigger a homeostatic mechanism aimed at normalizing activity (measured by  $Ca^{2+}$  entry).

To directly pursue this question, BAC D2 mice were DA depleted for 5 days using reserpine and the bAPevoked Ca<sup>2+</sup> transient was mapped in the dendrites of D2 MSNs (Day et al., 2008). As described above, the amplitude of the fluorescence change ( $\Delta$ F/Fo) at distal dendritic sites was normalized by the proximal fluorescence signal. In D2 MSNs from DA-depleted mice, the relative amplitude of bAP-evoked Ca<sup>2+</sup> transient in dendritic shafts and spines fell less steeply with distance from the soma than in untreated neurons (Fig. 6.6A). At distal dendritic locations (100 and 150µm from the soma), DA depletion significantly increased the relative amplitude of the Ca2+ transient evoked by a single bAP (Fig. 6.6B). In fact, in all of the neurons examined following DA-depletion, bAPassociated Ca<sup>2+</sup> transients were detectable as far out on the dendrites as we were capable of imaging ( $\sim 150 \mu m$  from the soma). The simplest interpretation of these results is that the loss of spines and dendritic surface area following DA depletion diminished the capacitative load of the dendrites, improving bAP invasion into distal regions. Although consistent with theoretical and experimental examination of other neurons (Wilson et al., 1992), this hypothesis was tested in an anatomically accurate model of an MSN; to the end, NEURON simulations were conducted in which the surface area of spiny dendrites was decreased and the effects on the bAP examined. These simulations



FIGURE 6.5 Dopamine depletion causes a reduction in spine density in the D2 receptor expressing - but not D1 receptor expressing - MSNs. (A) 2PLSM projection shows EGFP labeled MSNs in a slice from a BAC D1 EGFP mouse. Green signals (500-550nm) were acquired from EGFP labeled D1 BAC neurons (Fig. 6.3A right panel & Fig. 6.3D using 810nm excitation, while EGFP labeled D2 BAC neurons (Fig. 6.3B right panel & Fig. 6.3D) required 900nm excitation. Amplicons from an individual EGFP labeled neuron (scRT-PCR, Fig. 6.3A&B left panels) show coexpression of SP (616bp) and D1 receptor (234bp) mRNAs. (B) 2PLSM projection shows EGFP labeled MSNs in a slice from a BAC D2 GFP mouse. Single cell reverse transcription-polymerase chain reaction (scRT-PCR) studies from these EGFP labeled neurons shows coexpression of ENK (477bp) and D2 receptor (264bp) mRNAs. (C) Following DA depletion (reserpine, 5 days), EGFP labeled MSNs from BAC D1 mice appear normal (projections acquired as per Fig. 6.2). (D) EGFP labeled MSNs from BAC D2 mice show a reduction in the number of spines. (E) Traces taken from a control BAC D1 (top) and a DA depleted BAC D1 (bottom) show that mEPSCs are similar in frequency and amplitude. (F) Cumulative probability plots illustrate the invariance in the inter-event interval of mEPSCs between the control BAC D1 and the DA depleted BAC D1. (G) Recordings taken from a control (top) and DA depleted BAC D2 (bottom) show a reduction in mEPSC frequency. (H) Cumulative probability plots of the DA depleted D2 BAC shows an increase in interevent interval compared to BAC D2 controls. (I, left panel) Box plots showing reserpine DA depletion produces a decrease in spine density in the D2 MSN population measured with 2PLSM (wild type median = 9, n = 11; BAC D2 control median = 8.7, n = 6; DA depleted D1 median = 8, n = 7; DA depleted D2 median = 4.5, n = 5; Kruskal-Wallis ANOVA/Mann–Whitney test P < 0.01). (I, right panel) Box plots showing DA depletion produces a decrease in mEPSC frequency in the D2 MSN population (BAC D1 control median = 1.9, n = 11; BAC D1 DA depleted median = 1.8, n = 12; BAC D2 control median = 2.0, n = 11; BAC D2 DA depleted median = 0.8, n = 7; Kruskal-Wallis ANOVA/Mann-Whitney test P < 0.001). From Day et al., 2006.

corroborated the inference that spine loss enhances dendritic bAP invasion, showing enhanced bAP propagation, enhanced opening of voltage-dependent  $Ca^{2+}$  channels and an elevation in bAP evoked change in intracellular  $Ca^{2+}$  concentration at distal dendritic locations. A second explanation is that DA depletion facilitates an increase in ACh tone as has been long hypothesized to underlie some of the disorders seen in patients with PD. We tested this prospect and found that in BAC D2 MSNs, bath application of the muscarinic antagonist scopolamine (20  $\mu$ M) significantly suppressed the bAP-evoked Ca<sup>2+</sup> transient in the DA-depleted mice as compared to untreated controls. This finding suggests that cholinergic tone is elevated in the DA-depleted mice leading to a down-regulation of Kv4 channels. The down-regulation of Kv4 channels could be sufficient to enhance dendritic excitability in the



**FIGURE 6.6** DA depletion enhances excitability in distal dendrites in D2 MSNs. (A) Maximum projection image of a D2 MSN soma and dendrite from a DA-depleted BAC D2 mouse (left). The traces show the bAP-evoked  $Ca^{2+}$  transient recorded at four different eccentricities along this dendrite (45, 60, 100, 150 µm, right). (B) Plot of the amplitude of the bAP-evoked  $Ca^{2+}$  transient normalized to the most proximal recording in each cell (red diamonds, line). For comparison, the fit line from the D2 untreated MSNs (Fig. 6.1D, green line) is added to the plot. The box plot demonstrates the increase in the amplitude of the normalized bAP-evoked  $Ca^{2+}$  in the distal regions of the DA-depleted D2 MSN dendrites compared to control (untreated D2 = 0.24, n = 4; DA-depleted D2 = 0.6, n = 4; Kruskal-Wallis ANOVA, P < 0.05). From Day et al., 2008. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

DA-depleted D2 MSNs or it could synergize with the decrease in spine density to render the dendrites even more excitable. We also considered the possibility that the decrease in the decrement of the bAP-evoked  $Ca^{2+}$  transients seen following reserpine treatment reflected a D2-mediated disinhibition  $Ca^{2+}$  channels. To test this hypothesis, we compared the amplitude of bAP-evoked  $Ca^{2+}$  transients in untreated BAC D2 MSNs recorded before and after bath application of the D2 antagonist

sulpiride (10  $\mu$ M). We did not detect any significant differences in the distal dendrites before and after D2 blockade indicating that tonic DA tone is an unlikely contributor to differences in dendritic excitability seen following DA depletion.

The loss of D2 receptor stimulation will also handicap the induction of LTD that might serve to normalize global activity without eliminating synapses (Wang et al., 2006; Calabresi et al., 2007; Kreitzer and Malenka, 2007). Recent work by our group (Shen et al., 2008) has revealed that the loss of D2 receptor stimulation not only prevents the induction of LTD in D2 MSNs, but it also promotes LTP induction through adenosine A2a receptor signaling mechanisms. This interaction is mediated by an antagonism between the signaling mechanisms promoting LTD (D2 receptor dependent) and those promoting LTP (A2a receptor dependent). The loss of D2 receptor signaling disrupts the balance between these two processes, leading to strengthening of synaptic connections in inappropriate situations. This maladaptive response to DA depletion, together with the elevation in dendritic excitability attributable to down-regulation of Kir2 and Kv4 K<sup>+</sup> channels might provide an explanation for the anomalous increase in glutamatergic mEPSC frequency seen in several studies of MSNs in PD models (Galarraga et al., 1987; Gubellini et al., 2002; Picconi et al., 2004).

As mentioned above, while the majority of the glutamatergic synapses formed on dendritic spines are of cortical origin, many are not (Wilson, 2004b). Preliminary studies from our group suggest that it is corticostriatal, rather than thalamostriatal, synapses that are lost following DA depletion. However, this hypothesis has yet to be rigorously tested.

### VIII. FUNCTIONAL IMPLICATIONS FOR THE PATHOPHYSIOLOGY IN PARKINSON'S DISEASE

Several lines of evidence point to the importance of striatopallidal MSNs in the expression of Parkinson's disease motor symptoms (Baik et al., 1995; Wichmann and DeLong, 2003). Perhaps the most compelling of these is the finding that the activity of neurons in structures controlled by striatopallidal neurons is dramatically altered in people suffering from Parkinson's disease and in animal models of the disease. Neurons in the globus pallidus and in the reciprocally connected subthalamic nucleus begin to discharge in anomalous rhythmic bursts that are often synchronized. Silencing this abnormal patterning with lesions or deep brain stimulation provides dramatic relief from motor symptoms (Gross et al., 1999; Hutchison et al., 2004). Computer simulations grounded in experimental observation suggest that this rhythmic bursting is an intrinsic property of the pallidosubthalamic circuitry that is normally suppressed by striatopallidal GABAergic inhibition (Terman et al., 2002). Ineffectively timed or patterned striatopallidal activity could "release" this circuitry, allowing it to display activity patterns like those seen in Parkinson's disease. Because striatopallidal medium spiny neurons depend upon highly convergent glutamatergic synaptic inputs from cortical and thalamic motor command centers (Wilson and Kawaguchi, 1996), the loss of a substantial portion of this input should profoundly disrupt movement related, patterned activity and in so doing limit their ability to control the emergence of synchronous bursting in the pallidosubthalamic circuit. The failure to control the pallidosubthalamic circuit should lead to unwanted movements and the cardinal symptom of Parkinson's disease – the inability to translate thought into efficient movement.

### IX. CONCLUDING REMARKS

Although we are still some way from a secure grasp of how DA shapes the activity of striatal circuits, some tentative conclusions can be drawn. Acting principally through D2 receptors, DA reduces glutamate release as well as the postsynaptic responsiveness of striatopallidal MSNs to released glutamate. This short-term modulation is complemented by D2 receptor dependent promotion of longterm depression of glutamatergic synaptic transmission. Our grasp of how DA modulates striatonigral MSNs is less secure. Acting principally at postsynaptic D1 receptors in striatonigral MSNs, DA appears to depress weak, asynchronous synaptic signals but to augment the response to strong, coordinated glutamatergic input, promoting NMDA receptor opening and up-state transitions. In addition, D1 receptor signaling facilitates long-term potentiation of glutamatergic signaling, enhancing network connections which are consistently active during important environmental events that trigger phasic DA release.

What this means for striatal function is far from clear. Alterations in DA signaling can have profound effects on cognitive and motor function, being implicated in disorders like schizophrenia, dystonia, Tourette's syndrome, drug abuse and Parkinson's disease. One possible role of striatally released DA is to promote cortically driven action selection. This conjecture is based upon the popular model asserting that activity in striatopallidal MSNs and the indirect pathway serves to suppress action, whereas activity in striatonigral MSNs serves to promote action (Mink, 2003). Basal striatal DA levels produced by autonomous activity in SNc DA neurons should act primarily at high affinity D2 receptors expressed by striatopallidal MSNs, preventing them from responding too readily to uncoordinated cortical activity. When SNc DA neurons transiently spike at high frequency in response to environmental cues, low affinity D1 receptors should be activated, transiently enhancing the responsiveness of striatonigral MSNs to properly coordinated cortical "action commands"; in parallel, this burst of DA cell activity will suppress tonic activity in cholinergic interneurons, potentially synergizing with postsynaptic D2 receptors on striatopallidal MSNs to prevent cortical gluta-matergic signals shared with striatonigral MSNs from generating a state-transition, spiking and inappropriate action suppression. In this way, DA might gate the responsiveness of striatal output pathways to shared cortical glutamatergic action commands, preventing co-activation of incompatible action programs.

#### REFERENCES

- Akins PT, Surmeier DJ, Kitai ST (1990) Muscarinic modulation of a transient K<sup>+</sup> conductance in rat neostriatal neurons. Nature 344:240–242.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alcantara AA, Mrzljak L, Jakab RL, Levey AI, Hersch SM, Goldman-Rakic PS (2001) Muscarinic m1 and m2 receptor proteins in local circuit and projection neurons of the primate striatum: anatomical evidence for cholinergic modulation of glutamatergic prefrontostriatal pathways. J Comp Neurol 434:445–460.
- Arbuthnott GW, Wickens J (2006) Space, time and dopamine. Trends Neurosci 30:62–69.
- Bacci JJ, Kachidian P, Kerkerian-Le Goff L, Salin P (2004) Intralaminar thalamic nuclei lesions: widespread impact on dopamine denervationmediated cellular defects in the rat basal ganglia. J Neuropathol Exp Neurol 63:20–31.
- Baik JH, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, Le Meur M, Borrelli E (1995) Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 377:424–428.
- Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, Schmauss C, Zakharenko SS, Zablow L, Sulzer D (2004) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 42:653–663.
- Barral J, Galarraga E, Bargas J (1999) Muscarinic presynaptic inhibition of neostriatal glutamatergic afferents is mediated by Q-type Ca<sup>2+</sup> channels. Brain Res Bull 49:285–289.
- Bernard V, Normand E, Bloch B (1992) Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. J Neurosci 12:3591–3600.
- Blank T, Nijholt I, Teichert U, Kugler H, Behrsing H, Fienberg A, Greengard P, Spiess J (1997) The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. Proc Natl Acad Sci USA 94:14859–14864.
- Bolam JP, Hanley JJ, Booth PA, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196(Pt 4):527–542.
- Braithwaite SP, Paul S, Naim AC, Lombroso PJ (2006) Synaptic plasticity: one STEP at a time. Trends Neurosci 29:452–458.
- Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, Phelps L, Phillips AG, Wang YT (2005) Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science 310:1340–1343.

- Calabresi P, De Murtas M, Mercuri NB, Bernardi G (1992) Chronic neuroleptic treatment: D2 dopamine receptor supersensitivity and striatal glutamatergic transmission. Ann Neurol 31:366–373.
- Calabresi P, Centonze D, Gubellini P, Bernardi G (1999) Activation of M1-like muscarinic receptors is required for the induction of corticostriatal LTP. Neuropharmacology 38:323–326.
- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopaminemediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G (1987) Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons in vitro: evidence for D1 receptor involvement. Neuroscience 20:757–771.
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998) Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. Eur J Neurosci 10:3020–3023.
- Calabresi P, Centonze D, Gubellini P, Marfia GA, Pisani A, Sancesario G, Bernardi G (2000) Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog Neurobiol 61:231–265.
- Carr DB, Day M, Cantrell AR, Held J, Scheuer T, Catterall WA, Surmeier DJ (2003) Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. Neuron 39:793–806.
- Carter AG, Sabatini BL (2004) State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. Neuron 44:483–493.
- Centonze D, Picconi B, Gubellini P, Bernardi G, Calabresi P (2001) Dopaminergic control of synaptic plasticity in the dorsal striatum. Eur J Neurosci 13:1071–1077.
- Centonze D, Grande C, Saulle E, et al. (2003) Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 23:8506–8512.
- Cepeda C, Buchwald NA, Levine MS (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc Natl Acad Sci USA 90:9576–9580.
- Cepeda C, Hurst RS, Altemus KL, et al. (2001) Facilitated glutamatergic transmission in the striatum of D2 dopamine receptor-deficient mice. J Neurophysiol 85:659–670.
- Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23–30.
- David HN, Ansseau M, Abraini JH (2005) Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudateputamen and nucleus accumbens of "intact" animals. Brain Res Brain Res Rev 50:336–360.
- Day M, Wokosin D, Plotkin JL, Tian X, Surmeier DJ (2008) Differential excitability and modulation of striatal medium spiny neuron dendrites. J Neurosci 28:11603–11614.
- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- Ding J, Peterson JD, Surmeier DJ (2008) Corticostriatal and thalamostriatal synapses have distinctive properties. J Neurosci 28:6483–6492.
- Dodt HU, Misgeld U (1986) Muscarinic slow excitation and muscarinic inhibition of synaptic transmission in the rat neostriatum. J Physiol 380:593–608.
- Dunah AW, Wang Y, Yasuda RP, Kameyama K, Huganir RL, Wolfe BB, Standaert DG (2000) Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate

receptors in a rat 6-hydroxydopamine model of Parkinson's disease. Mol Pharmacol 57:342–352.

- Fino E, Glowinski J, Venance L (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 25:11279–11287.
- Fino E, Deniau JM, Venance L (2008) Cell-specific spike-timing-dependent plasticity in GABAergic and cholinergic interneurons in corticostriatal rat brain slices. J Physiol 586:265–282.
- Flores-Hernandez J, Galarraga E, Bargas J (1997) Dopamine selects glutamatergic inputs to neostriatal neurons. Synapse 25:185–195.
- Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, et al. (2002) Dopamine enhancement of NMDA currents in dissociated mediumsized striatal neurons: role of D1 receptors and DARPP-32. J Neurophysiol 88:3010–3020.
- Fuxe K, Marcellino D, Genedani S, Agnati L (2007) Adenosine A2A receptors, dopamine D2 receptors and their interactions in Parkinson's disease. Mov Disord 22:1990–2017.
- Galarraga E, Bargas J, Martinez-Fong D, Aceves J (1987) Spontaneous synaptic potentials in dopamine-denervated neostriatal neurons. Neurosci Lett 81:351–355.
- Gao T, Yatani A, Dell'Acqua ML, Sako H, Green SA, Dascal N, Scott JD, Hosey MM (1997) cAMP-dependent regulation of cardiac L-type Ca2<sup>+</sup> channels requires membrane targeting of PKA and phosphorylation of channel subunits. Neuron 19:185–196.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285–320.
- Gertler TS, Chan CS, Surmeier DJ (2008) Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci 28:10814–10824.
- Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358–363.
- Graybiel AM, Aosaki T, Flaherty AW, Kimura M (1994) The basal ganglia and adaptive motor control. Science 265:1826–1831.
- Greif GJ, Lin YJ, Liu JC, Freedman JE (1995) Dopamine-modulated potassium channels on rat striatal neurons: specific activation and cellular expression. J Neurosci 15:4533–4544.
- Gross CE, Boraud T, Guehl D, Bioulac B, Bezard E (1999) From experimentation to the surgical treatment of Parkinson's disease: prelude or suite in basal ganglia research? Prog Neurobiol 59:509–532.
- Gubellini P, Picconi B, Bari M, et al. (2002) Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. J Neurosci 22:6900–6907.
- Hakansson K, Galdi S, Hendrick J, Snyder G, Greengard P, Fisone G (2006) Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. J Neurochem 96:482–488.
- Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW (2006) Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. J Neurosci 26:4690–4700.
- Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS (2004) Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? Eur J Neurosci 19:2455–2463.
- Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E (1997) D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca<sup>2+</sup> conductance. J Neurosci 17:3334–3342.
- Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal

medium spiny neurons reduce L-type Ca<sup>2+</sup> currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. J Neurosci 20:8987–8995.

- Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Herve D, Rogard M, Levi-Strauss M (1995) Molecular analysis of the multiple G<sub>olf</sub> alpha subunit mRNAs in the rat brain. Brain Res Mol Brain Res 32:125–134.
- Horikawa K, Armstrong WE (1988) A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. J Neurosci Methods 25:1–11.
- Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL (2005) The cerebellum communicates with the basal ganglia. Nat Neurosci 8:1491–1493.
- Howe AR, Surmeier DJ (1995) Muscarinic receptors modulate N-, P-, and L-type Ca<sup>2+</sup> currents in rat striatal neurons through parallel pathways. J Neurosci 15:458–469.
- Hsu KS, Huang CC, Yang CH, Gean PW (1995) Presynaptic D2 dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. Brain Res 690:264–268.
- Hutchison WD, Dostrovsky JO, Walters JR, Courtemanche R, Boraud T, Goldberg J, Brown P (2004) Neuronal oscillations in the basal ganglia and movement disorders: evidence from whole animal and human recordings. J Neurosci 24:9240–9243.
- Ingham CA, Hood SH, Taggart P, Arbuthnott GW (1998) Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18:4732–4743.
- Kampa BM, Letzkus JJ, Stuart GJ (2007) Dendritic mechanisms controlling spike-timing-dependent synaptic plasticity. Trends Neurosci 30:456–463.
- Kawaguchi Y, Wilson CJ, Emson PC (1989) Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. J Neurophysiol 62:1052–1068.
- Kerr JN, Wickens JR (2001) Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J Neurophysiol 85:117–124.
- Kerr JN, Plenz D (2004) Action potential timing determines dendritic calcium during striatal up-states. J Neurosci 24:877–885.
- Kotecha SA, Oak JN, Jackson MF, Perez Y, Orser BA, Van Tol HH, MacDonald JF (2002) A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. Neuron 35:1111–1122.
- Kreitzer AC, Malenka RC (2005) Dopamine modulation of statedependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25:10537–10545.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Lee FJ, Xue S, Pei L, Vukusic B, Chery N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F (2002) Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 111:219–230.
- Lei W, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.
- Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timingdependent plasticity depend on dendritic synapse location. J Neurosci 26:10420–10429.

- Levine MS, Altemus KL, Cepeda C, et al. (1996) Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. J Neurosci 16:5870–5882.
- Liu JC, DeFazio RA, Espinosa-Jeffrey A, Cepeda C, de Vellis J, Levine MS (2004) Calcium modulates dopamine potentiation of N-methyl-D-aspartate responses: electrophysiological and imaging evidence. J Neurosci Res 76:315–322.
- Lovinger DM, Tyler EC, Merritt A (1993) Short- and long-term synaptic depression in rat neostriatum. J Neurophysiol 70:1937–1949.
- Mahon S, Deniau JM, Charpier S (2004) Corticostriatal plasticity: life after the depression. Trends Neurosci 27:460–467.
- Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 26:3875–3884.
- Maurice N, Mercer J, Chan CS, Hernandez-Lopez S, Held J, Tkatch T, Surmeier DJ (2004) D2 dopamine receptor-mediated modulation of voltage-dependent Na<sup>+</sup> channels reduces autonomous activity in striatal cholinergic interneurons. J Neurosci 24:10289–10301.
- McNeill TH, Brown SA, Rafols JA, Shoulson I (1988) Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. Brain Res 455:148–152.
- Mileson BE, Lewis MH, Mailman RB (1991) Dopamine receptor "supersensitivity" occurring without receptor up-regulation. Brain Res 561:1–10.
- Mink JW (2003) The Basal Ganglia and involuntary movements: impaired inhibition of competing motor patterns. Arch Neurol 60:1365–1368.
- Morari M, Marti M, Sbrenna S, Fuxe K, Bianchi C, Beani L (1998) Reciprocal dopamine-glutamate modulation of release in the basal ganglia. Neurochem Int 33:383–397.
- Nakamura TY, Coetzee WA, Vega-Saenz De Miera E, Artman M, Rudy B (1997) Modulation of Kv4 channels, key components of rat ventricular transient outward K<sup>+</sup> current, by PKC. Am J Physiol 273:H1775–H1786.
- Nevian T, Sakmann B (2006) Spine Ca<sup>2+</sup> signaling in spike-timingdependent plasticity. J Neurosci 26:11001–11013.
- Nicola SM, Malenka RC (1998) Modulation of synaptic transmission by dopamine and norepinephrine in ventral but not dorsal striatum. J Neurophysiol 79:1768–1776.
- Nicola SM, Surmeier J, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 23:185–215.
- Nisenbaum ES, Stricker EM, Zigmond MJ, Berger TW (1986) Long-term effects of dopamine-depleting brain lesions on spontaneous activity of type II striatal neurons: relation to behavioral recovery. Brain Res 398:221–230.
- Nishi A, Snyder GL, Greengard P (1997) Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J Neurosci 17:8147–8155.
- Olson PA, Tkatch T, Hernandez-Lopez S, et al. (2005) G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca<sup>2+</sup> channels is dependent on a Shank-binding domain. J Neurosci 25:1050–1062.
- Pakhotin P, Bracci E (2007) Cholinergic interneurons control the excitatory input to the striatum. J Neurosci 27:391–400.
- Pang Z, Ling GY, Gajendiran M, Xu ZC (2001) Enhanced excitatory synaptic transmission in spiny neurons of rat striatum after unilateral dopamine denervation. Neurosci Lett 308:201–205.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 28:2435–2446.
- Perez-Rosello T, Figueroa A, Salgado H, Vilchis C, Tecuapetla F, Guzman JN, Galarraga E, Bargas J (2005) Cholinergic control of firing pattern

and neurotransmission in rat neostriatal projection neurons: role of CaV2.1 and CaV2.2  $Ca^{2+}$  channels. J Neurophysiol 93:2507–2519.

- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci 6:501–506.
- Picconi B, Gardoni F, Centonze D, Mauceri D, Cenci MA, Bernardi G, Calabresi P, Di Luca M (2004) Abnormal Ca<sup>2+</sup>-calmodulindependent protein kinase II function mediates synaptic and motor deficits in experimental parkinsonism. J Neurosci 24:5283–5291.
- Salgado H, Tecuapetla F, Perez-Rosello T, Perez-Burgos A, Perez-Garci E, Galarraga E, Bargas J (2005) A reconfiguration of CaV2 Ca<sup>2+</sup> channel current and its dopaminergic D2 modulation in developing neostriatal neurons. J Neurophysiol 94:3771–3787.
- Scheuer T, Catterall WA (2006) Control of neuronal excitability by phosphorylation and dephosphorylation of sodium channels. Biochem Soc Trans 34:1299–1302.
- Schultz W (2006) Behavioral theories and the neurophysiology of reward. Annu Rev Psychol 57:87–115.
- Schwarzschild MA, Agnati L, Fuxe K, Chen JF, Morelli M (2006) Targeting adenosine A2a receptors in Parkinson's disease. Trends Neurosci 29:647–654.
- Scott L, Zelenin S, Malmersjo S, et al. (2006) Allosteric changes of the NMDA receptor trap diffusible dopamine 1 receptors in spines.. Proc Natl Acad Sci USA 103:762–767.
- Segal M, Greenberger V, Korkotian E (2003) Formation of dendritic spines in cultured striatal neurons depends on excitatory afferent activity. Eur J Neurosci 17:2573–2585.
- Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, Huganir RL, Lee HK, Kirkwood A (2007) Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. Neuron 55:919–929.
- Shen W, Hamilton SE, Nathanson NM, Surmeier DJ (2005) Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. J Neurosci 25:7449–7458.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Shen W, Tian X, Day M, Ulrich S, Tkatch T, Nathanson NM, Surmeier DJ (2007) Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. Nat Neurosci 10:1458–1466.
- Sjostrom PJ, Nelson SB (2002) Spike timing, calcium signals and synaptic plasticity. Curr Opin Neurobiol 12:305–314.
- Sjostrom PJ, Rancz EA, Roth A, Hausser M (2008) Dendritic excitability and synaptic plasticity. Physiol Rev 88:769–840.
- Smeal RM, Gaspar RC, Keefe KA, Wilcox KS (2007) A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents. J Neurosci Methods 159:224–235.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Snyder GL, Fienberg AA, Huganir RL, Greengard P (1998) A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J Neurosci 18:10297–10303.
- Snyder GL, Allen PB, Fienberg AA, Valle CG, Huganir RL, Nairn AC, Greengard P (2000) Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J Neurosci 20:4480–4488.

- Starr MS (1995) Glutamate/dopamine D1/D2 balance in the basal ganglia and its relevance to Parkinson's disease. Synapse 19:264–293.
- Stoof JC, Kebabian JW (1984) Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 35:2281–2296.
- Surmeier DJ (2004) Microcircuits in the striatum: Cell types, intrinsic membrane properties and neuromodulation. In: Microcircuits: The Interface between Neurons and Global Brain Function (Grillner S, Graybiel AM, eds), pp. 105–126. Berlin: MIT Press.
- Surmeier DJ, Song WJ, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
- Surmeier DJ, Bargas J, Hemmings HC Jr., Nairn AC, Greengard P (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/ phosphatase cascade in rat neostriatal neurons. Neuron 14:385–397.
- Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89:10178–10182.
- Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol 44:269–296.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Terman D, Rubin JE, Yew AC, Wilson CJ (2002) Activity patterns in a model for the subthalamopallidal network of the basal ganglia. J Neurosci 22:2963–2976.
- Tseng KY, O'Donnell P (2004) Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 24:5131–5139.
- Tseng KY, Kasanetz F, Kargieman L, Riquelme LA, Murer MG (2001) Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions. J Neurosci 21:6430–6439.
- Tzounopoulos T, Rubio ME, Keen JE, Trussell LO (2007) Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron 54:291–301.
- Vergara R, Rick C, Hernandez-Lopez S, Laville JA, Guzman JN, Galarraga E, Surmeier DJ, Bargas J (2003) Spontaneous voltage

oscillations in striatal projection neurons in a rat corticostriatal slice. J Physiol 553:169–182.

- Vilchis C, Bargas J, Ayala GX, Galvan E, Galarraga E (2000) Ca<sup>2+</sup> channels that activate Ca<sup>2+</sup>-dependent K<sup>+</sup> currents in neostriatal neurons. Neuroscience 95:745–752.
- Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, Tkatch T, Lovinger DM, Surmeier DJ (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50:443–452.
- Wichmann T, DeLong MR (2003) Functional neuroanatomy of the basal ganglia in Parkinson's disease. Adv Neurol 91:9–18.
- Wickens JR, Wilson CJ (1998) Regulation of action-potential firing in spiny neurons of the rat neostriatum in vivo. J Neurophysiol 79:2358–2364.
- Wickens JR, Reynolds JN, Hyland BI (2003) Neural mechanisms of reward-related motor learning. Curr Opin Neurobiol 13:685–690.
- Wilson CJ (2004a) Basal ganglia. In: The synaptic organization of the brain (Shepherd GM ed), pp. 361–414. Oxford: Oxford UP.
- Wilson CJ (2004b) Basal ganglia. In: The Synaptic Organization of the Brain, 5th edn. (Shepherd GM ed). USA: Oxford University Press.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 16:2397–2410.
- Wilson CJ, Mastronarde DN, McEwen B, Frank J (1992) Measurement of neuronal surface area using high-voltage electron microscope tomography. Neuroimage 1:11–22.
- Yan Z, Flores-Hernandez J, Surmeier DJ (2001) Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. Neuroscience 103:1017–1024.
- Yin HH, Lovinger DM (2006) Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 103:8251–8256.
- Zhou FM, Wilson CJ, Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. J Neurobiol 53:590–605.
- Zigmond MJ, Hastings TG (1998) Neurochemical responses to lesions of dopaminergic neurons: implications for compensation and neuropathology. Adv Pharmacol 42:788–792.

# The Cholinergic Interneurons of the Striatum: Intrinsic Properties Underlie Multiple Discharge Patterns

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#### I. INTRODUCTION

Cholinergic interneurons in the striatum were first identified as giant interneurons by Kölliker (1896). They are very rare, accounting for only about 1–2% of the neurons in the mammalian striatum (Kemp and Powell, 1971; Bolam et al., 1984; Phelps et al., 1985), and this creates a strong sampling bias against them when attempting to record their activity intracellularly either *in vivo* or blindly in slice preparations. The first intracellular recording studies of these neurons were published from the laboratory of S.T. Kitai, and included a small number of cells recorded *in vivo* over many years (Bishop et al., 1982; Wilson et al. 1990). Although rare, once impaled these cells proved to be very resilient *in vivo* and could be studied for over 2 hours. These initial studies demonstrated that these cells discharge tonically *in vivo* at a rate of 3–10 spikes/s, and continue to

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. fire spontaneously in ipsilaterally-decorticate animals. In addition, they were shown to exhibit a broad action potential followed by a deep afterhyperpolarization. The cells were tentatively identified as cholinergic using intracellular staining methods which demonstrated that they have the same cytological features as well as synaptic distribution and morphology as the choline acetyltransferase (ChAT)containing cells of the striatum (Wilson et al., 1990). Subsequent studies identified them conclusively by double labeling (Kawaguchi, 1992). The neurons possess a large soma (often  $>50 \mu m$  along its long axis); 2–4 large primary tapering aspiny dendrites that bifurcate repeatedly but infrequently and that can extend over a range of 1 mm; and an axon that arises from one of the primary dendrites and that branches densely and profusely over a large portion of the striatum (Chang and Kitai, 1982; DiFiglia and Carey, 1986; DiFiglia, 1987; Kawaguchi, 1992, 1993) (Fig. 7.1A).


FIGURE 7.1 Morphological and physiological characteristics of the cholinergic interneurons. A. 2D projection of multiple focal length images of a cholinergic interneuron filled with biocytin and stained by the nickel enhanced ABC-DAB reaction. Several dendrites can be seen emanating from the soma and bifurcating into higher order processes. The axonal arborizations can be seen both in the vicinity of the cell body and at two additional patches distanced a few hundred micrometers from the soma. White arrowhead indicates the axonal tortuosity. Inset: IR-DIC image of a different cholinergic interneuron and neighboring striatal cells. Three primary dendrites are visible as well as the recording pipette approaching from the right side. B. Current clamp recording from a cholinergic interneuron in whole cell configuration. A long depolarizing pulse leads to a speed up in the cell's firing rate, accompanied by spike accommodation followed by spike frequency adaptation. When the pulse is over the cell exhibits a long-lasting afterhyperpolarization (sAHP). A long hyperpolarizing pulse leads to a hyperpolarizing rectification response followed by a prominent sag in the voltage. When the pulse is over there is a rebound speed up, followed by a relaxation back to rhythmic tonic discharge. Note that each action potential is followed by a prominent medium-sized afterhyperpolarization (mAHP) and that these are briefer and shallower during the depolarizing pulse.

In an early recording study in slices only 11 cells from over 350 recorded were found to be cholinergic interneurons (Jiang and North, 1991). That study described a prominent sag in voltage in response to hyperpolarizing current injections that was shown to result from the hyperpolarizationactivated, cyclic nucleotide-gated cation (HCN) inward current. This study also reported that 40% of the cells were spontaneously active *in vitro*, when recorded using conventional sharp electrodes.

The advent of visualized infra-red differential interference contrast (IR-DIC) patch clamp recordings corrected and reversed the bias against isolating cholinergic interneurons in slices. Thanks to their large somata they are easily discernable against the background of the other smaller spiny neurons and they are easily "patched" (Fig. 7.1A, inset). Numerous studies have since contributed to the systematic characterization of the physiology of these cells. The first of these studies, which also positively identified the cholinergic cells with ChAT-immunoreactivity, was published by Kawaguchi (1993) in which he described most of the salient features of these cells' responses to



taneous discharge of the cholinergic interneurons. A. several seconds of discharge depicting a transition from single-spiking (left) to bursting (right). B. During the broad action potential emitted by the cholinergic interneurons calcium entry by way of Q-type high voltage activated (HVA) calcium current activates the calcium- (and voltage-) activated BK current, which repolarizes the membrane potential. C. Cycle of rhythmic single spiking: The N-type HVA calcium current is activated during the action potential. This current selectively activates the SK current, which generates the mAHP. A-type potassium currents contribute to the mAHP and possibly to the slow ramp up to action potential threshold. The HCN current is responsible for depolarizing the cell into the voltage range in which persistent sodium (NaP) currents bring the cell to action potential threshold. D. During rhythmic bursting there is subthreshold calcium entry through L-type calcium channels, which activate the sAHP current (I<sub>sAHP</sub>), which leads to spike frequency adaptation during the burst and ultimately to the termination of the burst. E. The inward rectifying potassium current (IRK) induces a rapid hyperpolarization of the membrane potential and in conjunction with the hyperpolarization-activated depolarizing HCN current can lead to subthreshold oscillations that are sometimes accompanied by action potentials.

current injections, as depicted in Figure 7.1B. The cells respond to depolarizing pulses with increased firing that undergoes spike frequency adaptation (and spike threshold accommodation) during the pulse. The action potentials are very broad (see Fig. 7.2A) and are followed by an after-hyperpolarization, called the mAHP that lasts 100–200 milliseconds. At the end of a long depolarizing pulse the

membrane potential undergoes a long-lasting AHP, called the sAHP, lasting several seconds long. When the cells are hyperpolarized with a current pulse they exhibit a rapid hyperpolarization followed by the above-mentioned sag due to HCN currents. After the hyperpolarizing pulse there is a rebound of increased firing, but then the cells relax back to spontaneous discharge (Fig. 7.1B). Other firing patterns and membrane dynamics exhibited regularly by these cells include rhythmic bursting and subthreshold oscillations (Fig. 7.2) as well as irregular firing (Bennett and Wilson, 1999; Wilson, 2005).

The focus of the present chapter will be to describe the membrane currents and calcium dynamics that underlie the various firing patterns of the cholinergic interneurons; how they are affected by various neurotransmitters; and how they exert their influence on the striatal network. We will discuss how the firing patterns observed in vitro are related to the discharge properties of the tonically active neurons (TANs) of the striatum in the awake-behaving primate. We will conclude with a hypothesis concerning the function of the cholinergic interneurons, with their unique firing patterns, within the striatal network.

## II. AUTONOMOUS FIRING PATTERNS IN CHOLINERGIC INTERNEURONS

The spontaneous discharge of cholinergic interneurons is best studied using extracellular recordings or perforated patch recordings. It is usually short-lived in whole cell recordings, presumably because the contents of the cells are corrupted by the composition of the pipette. The argument that spontaneous activity is autonomous in nature is based on two kinds of evidence. The first is its insensitivity to blockade of a wide range of synaptic transmitter receptors, including alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), N-methyl-D-aspartate (NMDA),  $\gamma$ -aminobutyric acid-A (GABA<sub>A</sub>) receptors, muscarinic and dopaminergic receptors that are present and in these cells (Bennett and Wilson, 1999). The second and more persuasive evidence for the autonomous nature of the firing comes from the elucidation of specific mechanisms underlying spontaneous activity (Bennett et al., 2000). The neurons display two types of rhythmical discharge - tonic single spiking and bursting – as well as irregular firing, all of which are autonomous in origin. Figure 7.2A depicts a trace in which a cholinergic interneuron switches patterns from single spiking to bursting.

## A. Biophysical Mechanism of Autonomous Firing

#### 1. Rhythmic Single-Spiking

Spontaneous single spiking is depicted in Figure 7.1B (after the hyperpolarizing pulse) and in the first part of the trace in Figure 7.2A. Close-ups of a single action potential and a single cycle of this rhythmic firing are depicted in Figures 7.2B and 7.2C, respectively. The threshold for action potential generation is in the -45 to -50 mV range. Tetrodotoxin (TTX)-sensitive (fast-activating and fast-inactivating) sodium currents (Maurice et al., 2004) and delayed-rectifier potassium currents (Song et al., 1998) are present in these cells and are the action potential mechanism. The long duration action potential is accompanied by inward Ca<sub>v</sub>2.1 (primarily of the Q-type) and Ca<sub>V</sub>2.2 (N-type) calcium currents. The action potential would be wider still were it not for the activation of the big-conductance calcium- and voltage-dependent (BK) potassium current, which is activated selectively by the Ca<sub>v</sub>2.1 current, and repolarizes the action potential (Fig. 7.2B) (Bennett et al., 2000; Goldberg and Wilson, 2005).

The Ca<sub>V</sub>2.2 calcium channels are functionally coupled to the small-conductance calcium-activated (SK) potassium current (I<sub>SK</sub>) and give rise to the prominent mAHP that follows each action potential and hyperpolarize the voltage below -60 mV (Bennett et al., 2000; Goldberg and Wilson, 2005). A hyperpolarization-de-inactivated A-type potassium current that is mediated by Kv4.2 and Kv4.1 channels becomes activated by the action potential, and presumably contributes to the shaping of the mAHP, but it may also contribute to a gradual recovery from the mAHP as it inactivates (Song et al., 1998; Hattori et al., 2003).

The main current responsible for depolarizing the cells from the depths of the mAHP is the HCN current that is activated by the mAHP. It depolarizes the cells into the voltage range of a subthreshold persistent-sodium current (Bennett et al., 2000). The persistent-sodium current may be attributable to a high abundance of Na<sub>v</sub>1.6 channels expressed in these neurons (Maurice et al., 2004). Moreover, the voltage-dependence of fast sodium inactivation in these neurons, as in other pace-making neurons of the basal ganglia (Surmeier et al., 2005), is quite depolarized (in comparison, for example, to cortical pyramidal neurons). This property also contributes to a larger sodium window-current in these neurons (Maurice et al., 2004). The persistent sodium current drives the membrane voltage to threshold and thus destabilizes the resting potential. This destabilization is evident as a TTX-sensitive negative slope conductance that is evident in the steady-state I-V curve of the cells at -60 mV (Bennett et al., 2000). The negative slope in the steady-state I-V curve means that the oscillatory mechanism can generate rhythmic firing at arbitrarily low frequencies, and therefore accounts for the ability of these cells to maintain tonic slow firing.

The cycle of the rhythmic single spiking is generated as follows: (a) at threshold, a sodium action current depolarizes the cells, leading to an influx of calcium via  $Ca_V 2$  calcium channels; (b) the  $Ca_V 2.1$  current activates BK channels which participate (along with voltage sensitive potassium currents) in repolarizing the cells, and the  $Ca_V 2.2$  current activates I<sub>SK</sub> that induces the ensuing mAHP; (c) HCN currents depolarize the cells to approximately -60 mV, after which the persistent sodium current slowly returns the membrane potential to the action potential threshold (Fig. 7.2C).

## 2. *Rhythmic Bursting and Subthreshold Oscillations*

An example of bursting in cholinergic interneurons is depicted in Figure 7.2D. Unlike principal neurons of the thalamus, bursting in cholinergic interneurons cannot be triggered or abolished simply by changing the average membrane potential. The bursting mechanism in cholinergic interneurons does not depend on inactivation of low threshold inward currents, and these cells express no appreciable low threshold Cav3 (T-type) currents (Bennett et al., 2000) that underlie bursting in the thalamus. Other calcium currents underlie bursting in cholinergic interneurons. The first clue as to the mechanism of bursting comes from noting that the mAHPs following each action potential in a burst are smaller and briefer than when the same cell is in the single spiking mode (compare the mAHPs in the beginning and end of Fig. 7.2A), suggesting that perhaps one way to induce bursting is to dramatically reduce the mAHPs. Application of apamin, which blocks I<sub>SK</sub> and thus reduces mAHPs, causes all cholinergic interneurons to burst (Bennett et al., 2000; Goldberg and Wilson, 2005) suggesting a shared mechanism for intrinsic and apamininduced bursting. However, there is an important difference between the two: while TTX-treatment abolishes apamin-induced bursting and establishes a resting potential, in intrinsically bursting cholinergic interneurons, TTX only abolished action potentials, while the cells continue to exhibit subthreshold oscillations (Wilson, 2005). A close look at the calcium dynamics of these cells clarifies this difference.

Direct measurement of the calcium concentration inside the cholinergic interneurons, using wide-field fluorescent calcium imaging, demonstrates an important difference between rhythmic single spiking and rhythmic bursting. In the rhythmic single spiking mode there is no subthreshold entry of calcium into the cells. Instead, the calcium concentration rises only after each action potential and then relaxes back to baseline during the mAHP. In contrast, when the neurons burst there is considerable subthreshold calcium entry that accumulates in the cell body during the voltage ramp-up to the burst (Goldberg et al., 2009). This slow subthreshold build up of calcium is critical for intrinsic bursting, as it is required to activate the apamin-insensitive current that underlies the sAHP  $(I_{sAHP})$ .  $I_{sAHP}$  is necessary for terminating the burst, as a reduction in this current causes bursting cells to switch to single spiking (Goldberg and Wilson, 2005). The effect of  $I_{\text{sAHP}}$  is observable also before the burst ends, in the elongation of the final inter-spike interval (ISI) in the burst (Fig. 7.2D).  $I_{sAHP}$  is sensitive to caffeine as well as other blockers of calcium release from intracellular stores, suggesting that calcium influx via Ca<sub>V</sub>1 (L-type) calcium channels, may engage calcium-induced calcium-release (CICR) to activate  $I_{sAHP}$  (Goldberg and Wilson, 2005). The affinity of  $I_{sAHP}$  to the calcium that activates it is so high, that very small increases in intracellular calcium are sufficient to activate it. As a result I<sub>sAHP</sub> is activated substantially even at voltages as low at -70 mV (Wilson and Goldberg, 2006), which is at the lower end of the activation curve of the L-type (particularly  $Ca_V 1.3$ ) currents (Lipscombe et al., 2004).

The effect of TTX on apamin-induced *vs.* intrinsic bursting can now be understood. During intrinsic bursting the calcium entry is subthreshold and thus persists after TTX, continuing to activate  $I_{sAHP}$  that is required for bursting. In contrast, by reducing the mAHPs and causing a rapid succession of action potentials, apamin-treatment can only admit calcium entry that is strictly action potential-triggered, and therefore because this entry is lost after TTX, there is no calcium to activate  $I_{sAHP}$  and consequently no bursting.

An interesting question is what causes the mAHPs to get smaller during intrinsic bursting relative to the singlespiking regime. This would require some endogenous down-regulation of  $I_{SK}$  that underlies the mAHP, or of the Ca<sub>v</sub>2.2 current that activates this current. One possibility is that the slow buildup of calcium entering through  $Ca_V l$  channels induces calcium-dependent inactivation of the  $Ca_V 2.2$  current (Liang et al., 2003) thereby decreasing the mAHPs during the burst, but this has not been shown.

The slow hyperpolarization that follows the termination of the burst, which is induced by I<sub>sAHP</sub>, engages two opposing hyperpolarization-activated currents that are activated below -60 mV and above the potassium reversal potential: the outward inwardly-rectifying potassium (IRK) current and the inward HCN current. In this voltage range the IRK current produces an outward current that increases with hyperpolarization (Hagiwara et al., 1978) and is present in cholinergic cells (Wilson, 2005). This property induces yet another more hyperpolarized negative conductance region in the I-V curve of the cells that is TTXinsensitive. But this negative conductance region is present only in the instantaneous I-V curve, not in the steady-state curve. This negative conductance region creates a voltage instability that gives rise to regenerative hyperpolarizations that follow the burst (Fig. 7.2D). This mechanism is also responsible for the hyperpolarizing rectification seen in response to hyperpolarizing current injections (Fig. 7.1A). Due to their all-or-none nature, the depth and time-course of these regenerative hyperpolarizations are not directly related to the size of I<sub>sAHP</sub> that evoked them. Thus, while these hyperpolarizations usually follow bursts of action potentials, in cells in which the IRK current is especially large, single action potentials are able to trigger them, giving rise to one form of irregular single spiking, as depicted in Figure 7.2E (Wilson, 2005). Moreover, as is evident from that figure, the subthreshold oscillations are sustainable even in the absence of action potentials altogether.

In summary, the cycle of intrinsic rhythmic bursting is as follows: (a) during and prior to the rapid succession of action potentials that make up the burst, there is a subthreshold build-up in the cells of calcium entering through  $Ca_V l$  channels; (b) the  $Ca_V l$  current presumably induces CICR from intracellular stores that activates the slowlyactivating  $I_{sAHP}$ ; (c)  $I_{sAHP}$  terminates the burst and triggers an sAHP; (d) IRK currents amplify the sAHP into a rapid and prolonged hyperpolarization, during which calcium concentrations drop; (e) HCN currents, followed by persistent sodium currents, depolarizes the cells so that they approach action potential threshold and so that calcium begins to build up in the cells.

Analysis of bursting in the cholinergic interneurons reveals two autonomous mechanisms for subthreshold oscillations that share the same inward HCN current, but that differ in their outward current. One requires subthreshold accumulation of calcium, which activates  $I_{sAHP}$ , while the other does not and depends on the activation of the IRK current instead. For this reason, these two oscillatory mechanisms are, in principle, independent of each other. However, during rhythmic bursting it is likely that both outward currents participate in the cycle. This is visible in Figure 7.2D in which the initial hyperpolarization following the burst is gradual, due to  $I_{sAHP}$  and then there is an inflection point after which the voltage rapidly hyperpolarizes, due to IRK current.

#### 3. Transitions Between Firing Patterns and the Mechanism of Selective Coupling Between Calcium and Potassium Currents

The two forms of endogenous rhythmic discharge – single spiking and bursting - are mutually exclusive: at any moment only one can be exhibited. Which firing pattern is expressed at any moment depends on which of the two calcium-activated potassium currents –  $I_{SK}$  or  $I_{SAHP}$  – is more dominant.  $I_{SAHP}$ promotes bursting, through its ability to slowly accumulate during the burst up to the point when it shuts down the bursting. I<sub>SK</sub>, on the other hand, promotes single spiking as the mAHP it induces repolarizes the cells long enough to prevent accumulation of IsAHP. The ability of the cells to endogenously switch between firing patterns implies that the balance between these currents is dynamic, and raises the question at to how it is achieved. One possibility is that this balance is under the control of neuromodulators which can alter the properties of the potassium channels directly or the calcium channels to which they are coupled.

As mentioned above,  $I_{SK}$  and  $I_{sAHP}$  are activated selectively by calcium influx via Ca<sub>v</sub>2.2 and Ca<sub>v</sub>1 channels, respectively (Goldberg and Wilson, 2005). Hence, the cholinergic interneurons' ability to maintain a specific firing pattern must depend on their ability to maintain this selective coupling between calcium sources and potassium currents. One mechanism for such calcium compartmentalization requires spatial segregation of either the various calcium channels or one of the two potassium channels. The segregation can be between various subcellular compartments (e.g., Sah and Bekkers, 1996), or can be at the microscopic level with calcium channels tethered to the potassium ones (e.g., Marrion and Tavalin, 1998). Goldberg and co-workers (Goldberg et al., 2009) recently tested this possibility with wide-field imaging of calcium transients throughout the somato-dendritic field of the cholinergic interneurons. They found that all calcium entry is present throughout all subcellular compartments. Moreover, a pharmacological block of specific calcium channels affects all regions equally. These findings rule out calcium compartmentalization by subcellular localization of calcium channels. Instead they found that individual compartments can express multiple time-scales of calcium decay, which is consistent with the presence of multiple buffering systems within all subcellular compartments. The model they propose to explain how the cells sustain multiple time scales is that of non-equilibrium calcium dynamics, wherein before steady-state is reached a cascade of calcium buffers binds sequentially to the calcium ions, each interaction giving rise to a different time scale of calcium decay (Markram et al., 1998). They propose that the selective coupling of calcium to potassium channels should be reinterpreted as a selective activation of SK channels by fast calcium transients and as selective activation of sAHP currents by slow calcium transients. Hence, fast voltage transients, such as action potentials, give rise to faster calcium transients, and therefore preferentially activate ISK whereas slow transients, such as the subthreshold activity, preferentially activate I<sub>SAHP</sub>. Because Ca<sub>v</sub>1.3 channels have a more hyperpolarized halfactivation than Ca<sub>v</sub>2.2 channels (Lipscombe et al., 2004), action potentials preferentially admit calcium via the latter, while subthreshold activity admits calcium via the former. Thus, the selectivity of  $I_{SK}$  to  $Ca_V 2.2$  block is a by-product of its selectivity to fast calcium transients which tend to admit calcium via these channels. Similarly, the selectivity of I<sub>sAHP</sub> to Ca<sub>V</sub>1 block is a by-product of its selectivity to slow calcium dynamics.

#### 4. Irregular Firing

In addition to the rhythmic discharge patterns addressed above the cholinergic interneurons also exhibit irregular firing. There can be various mechanisms for irregular firing. The first two are implicit in Figure 7.2. The transitions (especially if they are frequent) between rhythmic single spiking and rhythmic bursting, give rise to one form of irregular firing. Subthreshold oscillations in which the number of action potentials emitted in each cycle is stochastic is another (Fig. 7.2E). Another form of irregularity is tonic firing in which the distribution of ISIs is wider than in the case of rhythmic single spiking (see Fig. 7.4B) (Bennett and Wilson, 1999). There are at least two mechanisms that can account for this irregularity: the first is sodium-dependent and the second is calcium-dependent. In most cholinergic interneurons the TTX-sensitive negative conductance region in the steady-state I-V curve passes a few picoamperes negative to the zero current line, guaranteeing the presence of a robust inward current that brings the cells to action potential threshold, as discussed above. However, if this region happens to be closer (particularly if it is tangential) to the zero line, it can give rise to an effect of lingering, in which the time spent by the voltage to reach action potential threshold is very sensitive to noise, and the ISIs can vary significantly. The calcium-dependent mechanism is related to the fact that all cholinergic interneurons support subthreshold calcium entry (e.g., in response to depolarizing current injections). During tonic firing the irregular single-spiking cells can be distinguished from the regular ones in that the former exhibit slow subthreshold calcium modulation (in addition to the action potential triggered calcium entry) while the latter do not (Goldberg et al., 2009). The subthreshold calcium fluctuations introduce variability in the ISI distribution.

## B. Influence of Neurotransmitters on Autonomous Firing

The physiological effects of various neurotransmitters on cholinergic interneurons have been reviewed by Pisani and colleagues (2003a, 2007). In the following section we will focus on how these neurotransmitters and their receptors might affect the firing patterns exhibited by these cells.

#### 1. Ionotropic vs. Metabotropic Receptors

Cholinergic interneurons receive glutamatergic input from both the cortex and the intralaminar nuclei of the thalamus (centre-median and parafascicular in primates and the parafascicular proper in the rat). The cortical inputs are confined to the distal dendrites whereas thalamic synapses are also present at the soma and proximal dendrites (Wilson et al., 1990; Lapper and Bolam, 1992; Kawaguchi, 1993; Bennett and Wilson, 1998; Thomas et al., 2000). Thus, thalamic glutamatergic inputs are presumably more influential on cholinergic activity than cortical ones (Sidibè and Smith, 1999). Cholinergic interneurons also receive GABAergic input mediated by GABAA receptors (Chang and Kitai, 1982; DiFiglia and Carey, 1986; DiFiglia, 1987; Bolam, 1989; Bennett and Wilson, 1998) from the GABAergic striatal interneurons (Sullivan et al., 2008) and possibly from axon collaterals of spiny neurons as well. Because the cholinergic interneurons are pacemakers the effect of such a fast and weak ionotropic synaptic input (Kawaguchi 1993)

is confined to either advancing or delaying the next action potential: a glutamatergic excitatory postsynaptic potential advances, while a GABAergic inhibitory postsynaptic potential (IPSP) delays it (Bennett and Wilson, 1998).

Stronger or sustained fast synaptic input or activation of metabotropic receptors may give rise to more dramatic effects such as silencing the cells, or perhaps inducing transitions between firing patterns. For example, muscarinic IPSPs can be elicited in cholinergic interneurons by focal stimulation of striatal slices, and application of muscarine or its agonists to these cells can silence them - both effects are mediated by M<sub>2</sub> class receptors (Calabresi et al., 1998c; Bonsi et al., 2008), that inhibit adenylate cyclase. These results suggest that cholinergic interneurons may be mutually inhibitory in addition to their mutual polysynaptic inhibition via GABAergic interneurons (Sullivan et al., 2008). Virtually all cholinergic interneurons express M<sub>2</sub> class receptors whose activation has been shown to reduce Ca<sub>v</sub>2 currents in the soma and proximal dendrites (Yan and Surmeier, 1996; Thomas et al., 2000). Due to the dense cholinergic innervation (Bolam et al., 1984; Phelps et al., 1985) and the presumed volume transmission of acetylcholine (ACh) (Contant et al., 1996; Descarries et al., 1997; Descarries and Mechawar, 2000; Jones et al., 2001; Koós and Tepper, 2002) in the striatum, activation of these receptors could be expected to affect action potential width and reduce the mAHPs, through the above-mentioned coupling of these calcium currents to the various potassium currents. Indeed, activation of muscarinic receptors has been shown to reduce mAHPs and induce irregular firing in cholinergic interneurons (Ding et al., 2006).

Activation of group I metabotropic glutamate receptor, which are present in virtually all cholinergic interneurons (Tallaksen-Greene et al., 1998; Bell et al., 2002), depolarizes the cholinergic interneurons by suppressing potassium currents and inducing an effective inward current (Takeshita et al., 1996). Group II metabotropic glutamate receptors can affect the excitability of the cholinergic interneurons indirectly by reducing the synaptic potentials elicited in them. In particular, activation of these receptors reduced the cholinergic IPSP, as well as the release of ACh from these cells by suppression of  $Ca_v 2.1$  currents (Pisani et al., 2002, 2003a).

Finally, 5-hydroxytryptamine (5-HT, serotonin), which is released in the striatum by fibers arising from somata in the raphe nucleus (Steinbusch, 1981), induces an inward current, that is caused by closure of potassium channels, and that depolarizes the cells and causes a three-fold increase in the spontaneous firing rate of cholinergic interneurons. In addition, the mAHP and sAHP exhibited by these cells are both significantly reduced by serotonin. These effects are mediated by the 5- $HT_{2,6,7}$  (metabotropic) receptors. It remains to be determined how activation of this receptor closes the potassium channels and modulates the potassium currents that underlie the AHPs, or perhaps the calcium currents that activate them (Blomeley and Bracci, 2005; Bonsi et al., 2007).

#### 2. Catecholamines and Other Neuromodulators

The striatum has the highest expression of dopamine receptors in the brain. The D<sub>1</sub>-type includes D<sub>1</sub> and D<sub>5</sub> receptors, that activate adenylyl cyclase and the D<sub>2</sub>-type includes the  $D_2$ ,  $D_3$ , and  $D_4$  receptors that inhibit it. The cholinergic interneurons mostly express the D<sub>2</sub> receptor and the D<sub>5</sub> receptor (Yan et al., 1997). Activation of D<sub>1</sub>-like receptors has been shown, on the one hand, to evoke a transient slow depolarization (and increased spiking) in the cholinergic interneurons by evoking an inward current (Aosaki et al., 1998). This depolarization may be partially dependent on activation of HCN currents (Pisani et al., 2003b; but see Deng et al., 2007). On the other hand, activation of these receptors has been shown to increase the mAHP, which would presumably slow down the cells once they fire (Bennett and Wilson, 1998). Dopamine acting by way of D<sub>2</sub> receptors down-regulates the persistent sodium (Maurice et al., 2004) and HCN (Deng et al., 2007) currents thereby lowering the autonomous firing rate of these neurons in the single spiking mode. As mentioned above, muscarinic receptors down-regulate Cav2 channels. Similarly, activation of D<sub>2</sub> receptors reduces Ca<sub>v</sub>2.2 currents in cholinergic interneurons (Yan et al., 1997), as does activation of adenosine A<sub>1</sub> receptors (Song et al., 2000). In addition to the importance of these channels to vesicular release of ACh, reduction of the Ca<sub>V</sub>2.2 current in cholinergic interneurons reduces the mAHP (due to its coupling to SK currents) and causes them to burst (Goldberg and Wilson, 2005). Thus, it is possible that activation of any of these receptors could induce a transition to bursting. Indeed, after strong electrical stimulation of the SNc in anesthetized rats, cholinergic interneurons transition into a state of persistent bursting that lasts several minutes (Reynolds et al., 2004). It is possible that this stimulation releases large amounts of dopamine in the striatum, which presumably activates  $D_2$  receptors, thereby inducing a transition to bursting by down-regulating Cav2.2 channels. Finally, activation of presynaptic  $D_2$  receptors reduces GABA and ACh release onto cholinergic interneurons via down-regulation of  $Ca_V 2.2$  channels (Pisani et al., 2000; Momiyama and Koga, 2001), giving rise to an additional second-order mechanism by which dopamine can affect the precise timing or disinhibit the firing of cholinergic interneurons.

Because activation of D<sub>2</sub> receptors decreases the excitability of cholinergic interneurons - through its downregulation of the persistent sodium, HCN and Ca<sub>v</sub>2.2 currents (the latter is also required for vesicular release of ACh) - loss of dopamine, as in parkinsonism, would be expected to lead to an effective increase in the firing of cholinergic interneurons, and to an increased release of ACh. Indeed, the drop in dopamine in parkinsonism disrupts the so-called dopamine-acetylcholine balance leading to an increased release of ACh, which exacerbates the symptoms of the disease (McGeer et al., 1961; Barbeau, 1962; Lehmann and Langer, 1983; DeBoer et al., 1996). Thus, the common view is that the reduction in D<sub>2</sub> receptor activation is responsible for the increase in ACh release. However, Surmeier and co-workers have presented compelling evidence for an alternative mechanism, which is related to loss of self-regulation of ACh release in cholinergic interneurons. Under normal physiological conditions, activation of M<sub>4</sub> muscarinic autoreceptors down-regulates Cav2.2 channels, thereby selflimiting vesicular release of ACh. A drop in dopamine, leads to an attenuation of the coupling between the muscarinic autoreceptors and the calcium channels, leading to an effective increase in striatal ACh levels (Ding et al., 2006).

Activation of  $\beta_1$ -adrenoreceptors, which are prevalent in the striatum (Pazos et al., 1985; Aoki et al., 1987) and present in particular in cholinergic interneurons, depolarizes their (average) membrane potential and increases their firing rate. This effective depolarization was caused in a large portion of the neurons tested due to closure of potassium currents, but in some cells seemed to involve activation of the HCN current (Pisani et al., 2003b). The prevalence of these adrenergic receptors is puzzling in light of the fact that the catecholamine innervation of the striatum is primarily dopaminergic rather then adrenergic (Lindvall and Björkland, 1974). This discrepancy has been suggested to be resolved by the finding that the affinity of dopamine to noradrenergic receptors may be higher than their affinity to norepinephrine (Zhang et al., 1999; Pisani et al., 2003b). Finally, histamine by way of H<sub>1</sub>-receptor activation depolarizes and speeds up cholinergic interneurons, again due to a mix of ionic currents (Bell et al., 2000).

Because cholinergic interneurons are autonomous pacemakers they will inevitably fire action potentials. Hence the role of the synaptic input is not to bring the neurons to action potential threshold as in striatal spiny neurons (see Chapter 5), but rather to influence the timing of action potentials for weak inputs, or to affect the pattern of the discharge for strong ones. Thus, the influence of ionotropic synaptic inputs is restricted to the precise timing of the action potentials. Activation of metabotropic receptors (Fig. 7.3A), on the other hand, can have a more persistent effect on their discharge. The effect can be on the rate of the discharge as in the case of metabotropic glutamate or serotonin receptors that down-regulate potassium currents, thereby depolarizing the cells and speeding up their discharge. Similarly, D<sub>2</sub> receptors down-regulate the persistent sodium current thereby slowing them down. The most important effects of transmitters may be to alter the firing pattern itself, as in the case of M<sub>2</sub> class receptors that down-regulate Ca<sub>v</sub>2.2 channels leading to irregular firing or D<sub>2</sub> receptors that down-regulate the same channels, and lead, under certain circumstances, to reduced mAHPs and burst firing.

## III. INFLUENCE OF THE CHOLINERGIC INTERNEURONS ON THE STRIATAL NETWORK

The striatum contains one of the highest levels of ACh in the brain, and it is all provided endogenously by the cholinergic interneurons (Mesulam et al., 1992; Contant et al., 1996). Through their dense local innervation they influence striatal circuitry (see Chapter 1) in a variety of ways: ranging from postsynaptic effects on neuronal excitability of striatal neurons; through presynaptic effects on synaptic transmission; and synaptic plasticity. Nicotinic receptors exist on axons of dopaminergic neurons and act directly on fast-spiking GABAergic interneurons (see Chapter 8). However, most of the known effects of ACh in the striatum are mediated by muscarinic receptors (Koós and Tepper, 2002; Zhou et al., 2002; Wilson, 2004).

### A. Neuronal Excitability

Nearly all spiny neurons express  $M_1$  receptors (Bernard et al., 1992; Hersch et al., 1994; Yan et al., 2001), and these are known to induce an effective inward current by suppression of several potassium currents including: the inactivating A-type current, the IRK current, and the Kv7/KCNQ/M current (Akins et al., 1990; Kitai and Surmeier, 1993; Hsu et al., 1996; Gabel and Nisenbaum, 1999; Galarraga et al.,



**FIGURE 7.3** Input to and output of the cholinergic interneurons. A. The excitability of the cholinergic interneurons can be enhanced (left side) by down-regulation of potassium currents as a result of the activation of certain metabotropic receptors and can be tempered (right side) by down-regulation of persistent sodium (NaP) current, and the HCN and N-type calcium channels, as a result of the activation of other receptors. B. Diagram of known synaptic interactions between cholinergic interneurons and neighboring neurons as well as afferent input to the striatum (refer to text for explanations). DA – dopamine; glu – glutamate; m/nAChR – muscarinic/nicotinic ACh receptors.

1999; Shen et al., 2005, 2007) (see Chapters 5 and 6). These receptors also down-regulate  $Ca_V 1$  and  $Ca_V 2$  currents in spiny neurons (Howe and Surmeier, 1995), which due to their coupling to calcium-activated potassium currents (Vilchis et al., 2000) also increase the excitability of these cells, by reducing their AHPs and increasing their evoked firing rate (Perez-Rosello et al., 2005).

ACh, acting via nicotinic receptors, can depolarize the normally quiescent fast-spiking GABAergic interneurons and cause them to fire (Koós and Tepper, 2002) (see also Chapter 8). Because these interneurons can strongly inhibit spiny neurons (Koós and Tepper, 1999), this provides a mechanism for cholinergic interneurons to inhibit spiny cells. However, these interneurons possess an additional mechanism by which ACh can temper this effect: ACh weakens the GABAergic inhibition of the spiny neurons by activating presynaptic muscarinic receptors (Koós and Tepper, 2002; Perez-Rosello et al., 2005). The GABAergic interneurons also provide a mechanism of lateral inhibition among cholinergic interneurons: action potentials in one cholinergic interneuron create polysynaptic inhibitory postsynaptic currents (IPSCs) in itself and in other cholinergic interneurons, and transiently suppress ongoing discharge in these neurons. The IPSCs are polysynaptic and are nictonic-receptor-dependent because they are mediated by the GABAergic interneurons (Sullivan et al., 2008).

#### **B.** Synaptic Transmission

Just as ACh can weaken GABAergic transmission, it has been found to regulate other forms of synaptic transmission in the striatum. ACh, acting through  $M_1$  class receptor, facilitates inward currents in spiny neurons via NMDA, but not AMPA receptors. This facilitation is TTX-insensitive indicating a postsynaptic site of action (Pisani et al., 1997; Calabresi et al., 1998a). Presynaptic inhibition involving muscarinic receptors is known to affect glutamatergic transmission, as well (Akaike et al., 1988; Barral et al., 1999). In general, to first approximation, postsynaptic muscarinic effects on spiny neurons are mediated by  $M_1$ class receptors, whereas pre-synaptic effects are mediated by  $M_2$  class receptors, the latter usually attenuating transmission (Calabresi et al., 1998b; Calabresi et al., 2000). Pakhotin and Bracci (2007) have shown the direct presynaptic effect of a single action potential emitted by a cholinergic interneuron on glutamatergic transmission to neighboring spiny neurons and cholinergic interneurons, within a radius of  $100\mu$ m. Each action potential reduced by up to 30% the size of glutamatergic excitatory postsynaptic currents induced by stimulation of afferent cortical fibers. This reduction was shown to be mediated by muscarinic receptors.

Acetylcholine also has important effects on dopamine release. Activation of nicotinic receptor on terminals of dopaminergic neurons (Jones et al., 2001), promotes the release of dopamine - a mechanism that has been proposed to underlie the hedonic nature of smoking, especially among schizophrenics. However, nicotine at levels achieved by smokers seems to desensitize the receptors leading to a decrease in dopamine release (Di Chiara and Imperato, 1988; Zhou et al., 2001, 2002). The nictonic effect either facilitates or suppresses dopamine release depending on the firing pattern of the dopaminergic neurons. When the firing rate is slow and tonic the desensitization tends to occur, but when dopaminergic neurons burst, the nicotinic effect leads to an enhanced dopamine release (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008). Because the level of ACh in the striatum is determined by the firing rate of the cholinergic interneurons, the nicotinic effect on dopamine release will also depend on their firing pattern, and it has been suggested that tonic firing in the 3-10 spikes/s range, which is typical for these neurons in vivo (Wilson et al., 1990), is optimal for minimizing the desensitization of the nicotinic receptors (Zhou et al., 2002).

#### C. Synaptic Plasticity

The most studied synapses in the striatum are the glutamatergic synapses on spiny cells (for reviews of the main issues, see Calabresi et al., 2000; Pisani et al., 2007; Kreitzer and Malenka, 2008) (see also Chapter 12). ACh affects synaptic plasticity in the striatum in various ways. Initially, it was found that muscarinic receptors were required only for longterm potentiation (LTP). Through their down-regulation of postsynaptic potassium currents, discussed above,  $M_1$  class receptors promote postsynaptic depolarization which in conjunction with the high-frequency stimulation protocol for synaptic plasticity leads to LTP. Blockade of  $M_2$  class receptors also promotes LTP (Calabresi et al., 1998b, 1999; Pisani et al., 2007). More recently, however,  $M_1$  receptors have been also implicated in long-term depression (LTD) of the glutamatergic synapses. LTD has been known to depend on activation of D<sub>2</sub> dopamine receptors (Calabresi et al., 1997). This activation increases postsynaptic endocannabinoid release, which activates its presynaptic CB1 receptors (Giuffrida et al., 1999; Gerdeman et al., 2002; Ronesi et al., 2004; Kreitzer and Malenka, 2005, 2008; Wang et al., 2006) which in turn lead to a reduction in the probability of glutamate release (Choi and Lovinger, 1997). Wang and co-workers have proposed that the mechanism by which D<sub>2</sub> receptor activation leads to endocannabinoid release involves several intermediate steps. First, as discussed above, activation of D<sub>2</sub> receptors on cholinergic interneurons reduces ACh release. Secondly, reduction in ACh leads to a compromised activation of M1 class receptors, which are normally potent down-regulators of Ca<sub>V</sub>1.3 channels. Thus, finally, this disinhibition of Ca<sub>V</sub>1.3 channels, increases the calcium entry which induces calcium-dependent release of endocannabinoids (Wang et al., 2006).

Therefore, to first approximation,  $M_1$  class receptors are the primary players in conveying the influence of cholinergic interneurons on synaptic plasticity in glutamatergic striatal synapses: enhancement of  $M_1$  class receptor activation favors LTP, and inhibition of these receptors favors LTD (Centonze et al., 1999; Pisani et al., 2007). However,  $M_2$  class receptors also have an indirect influence on LTD. In their capacity as autoreceptors on cholinergic interneurons, they lead to self-inhibition of ACh release (Calabresi et al., 1998c), which leads presumably to a reduced activity of  $M_1$  class receptors and hence to LTD (Bonsi et al., 2008). Finally, nicotinic receptors affect synaptic plasticity in glutamatergic synapses, through their above-mentioned enhancement of dopamine release which via  $D_2$  receptor activation leads to LTD (Partridge et al., 2002).

The release of ACh from cholinergic interneurons influences neuronal excitability, synaptic transmission and synaptic plasticity at its neighboring cells in the striatal network in multiple ways (Fig. 7.3B). The ability of the cell to exhibit variegated firing patterns suggests that its influence at any given moment could depend on the current firing pattern it exhibits. The ongoing nature of these neurons' discharge maintains an ambient level of ACh in the striatum. It is likely that ACh release from a burst of rapid action potentials is more efficient at silencing neighboring cells; at weakening glutamatergic input; or at inducing plasticity than the slower rate during tonic firing. On the other hand, bursts are also intermittent by nature. Perhaps the importance of burst firing lies in its parsing time into periods of high vs. low ACh output which could represent different modes of striatal processing. An important question in this respect relates to the *population* discharge of cholinergic interneurons: do they fire largely independently or is their discharge coordinated? Similarly, how is their discharge related to the timing of other inputs to the striatal network, notably the dopaminergic input. Finally, how is the cooperativity at a given moment among cholinergic interneurons and between them and other elements of the striatal network dependent on the firing pattern of these interneurons? These questions open important avenues for further research. Answering some of these questions requires work in intact animals. The next section outlines the identification of cholinergic interneurons in awake behaving primates and the firing patterns that they exhibit.

## IV. THE CHOLINERGIC INTERNEURONS ARE THE TONICALLY ACTIVE NEURONS OF THE STRIATUM

Extracellular recording in the striatum of awake behaving primates reveals the presence of tonically active neurons (TANs) that possess particularly broad action potentials (Anderson, 1977; Kimura et al., 1984; Aosaki et al., 1994b). TANs were shown to be spatially distributed within the striatum in a fashion that most closely resembles the distribution of ChAT-immunoreactive neurons in these animals (Aosaki et al., 1995), indicating that the TANs recorded in these animals are none other than the cholinergic interneurons of the striatum.

#### A. The Pause Response

TANs recorded in awake behaving monkeys were initially shown not to be responsive to movement per se (Crutcher and DeLong, 1984). Instead, when the animal is presented with a primary reward (e.g., a drop of liquid) TANs respond with a pause in their tonic firing that lasts a few hundred milliseconds (Kimura et al., 1984; Apicella et al., 1997). A subsequent study showed that that these neurons acquire this pause in response to sensory stimuli that become associated with the reward, and subsequently lose it when this association is extinguished (Aosaki et al., 1994b). In this sense, the pause response is a neural correlate of classical conditioning. It is now widely accepted that the pause response of the TANs is related to the detection of the motivational significance of an external stimulus, both rewarding and aversive (Ravel et al., 1999; Apicella, 2002; Morris et al., 2004). The pause response is sometimes flanked by a preceding and/or a subsequent excitation. The temporal structure of the pause response observed in primates is reminiscent of the sAHP observed in response to a depolarizing pulse in rat cholinergic interneurons recorded in vivo (Reynolds et al., 2004): the depolarizing pulse is sufficient to trigger the sAHP, regardless of whether it elicits action potentials, and then the cells often fire a rebound of action potentials after the sAHP (Fig. 7.4A). Because it outlives the typical duration of IPSPs generated in these neurons (Bennett and Wilson, 1998), and due to its stereotypic time-course, the pause is likely to be fashioned by the IRK currents that drive the regenerative hyperpolarization in these neurons (Wilson, 2005). It is absent in dopamine-depleted animals (Aosaki et al., 1994a) or when thalamic input is interrupted (Matsumoto et al., 2001). The pause in the cholinergic interneurons is time locked to an increase in firing of the dopaminergic neurons of the SNc. Responses of both of these neurons are related to the probability of reward: the dopaminergic neurons respond to reward mismatch, while the pause, reports the timing of the expected reward (or its omission) (Fiorillo et al., 2003; Morris et al., 2004).

The uniformity and temporal alignment of the pause response among TANs that are distributed widely within the striatum led Graybiel and colleagues to suggest that these neurons form synchronous cell assemblies, presumably to modulate the GABAergic output of the striatum in a spatially uniform fashion (Graybiel et al., 1994). Bergman and co-workers tested this idea by recording extracellularly in awake behaving primates the simultaneous discharge of several TANs that were a few millimeters apart (see Chapter 38). They found that the cross-correlograms of pairs of TANs that paused in response to reward presentation had significant zero-lag peaks, indicating that TANs do function as a distributed synchronous network (Raz et al., 1996).

# **B.** Spontaneous Firing Patterns and Synchronization of TANs

Aosaki and colleagues described the statistics of the TANs' spontaneous firing patterns in primates (Aosaki et al., 1994b). Their analysis of the TANs' ISI histograms demonstrated the existence of multiple firing patterns in their spontaneous discharge. The first discharge pattern is characterized by a skewed unimodal ISI histogram. The second pattern is characterized by a bimodal ISI distribution in which the first modal peaks at an interval that is smaller than the modal of the unimodal distribution, while the second mode occurs at a larger interval. Rhythmically bursting neurons in which bursts are separated by relatively long





**FIGURE 7.4** Driven and spontaneous firing patterns in cholinergic interneurons are similar to the firing patterns of the tonically active neurons (TANs) of primate striatum observed in response to external stimuli (reward) or occurring spontaneously. A. left: the response of a cholinergic interneuron recorded *in vivo* from an anesthetized rat to a depolarizing current injection includes a rapid firing of several action potentials followed by a sAHP, after which there is a rebound firing and a return to background firing at 3–10 spikes/s (from Reynolds et al., 2004, with permission). right: peri-stimulus time histogram of the response of a TAN to primary liquid reward, exhibits a pause response flanked by excitations (from Raz et al., 1996, with permission). B. left: regular and irregular single spiking and burst firing recorded in cell-attached mode from cholinergic interneurons recorded in rat slices, give rise to narrow and broad unimodal, as well as bimodal interspike interval (ISI) distributions, respectively (from Bennett and Wilson, 1998, with permission). right: ISI distributions of TANs are either broad unimodal or bimodal distributions (from Aosaki et al., 1994b, with permission).

pauses and whose intraburst firing is rapid can give rise to such bimodal ISI distributions. It has been suggested that these two firing patterns represent two different classes of TANs (Apicella, 2002), however, this is unlikely as some TANs alternate between these two firing patterns (Aosaki et al., 1994b). These two firing patterns correspond to the single-spiking/irregular and the bursting firing patterns seen in cholinergic interneurons recorded from rat slices (Bennett and Wilson, 1999) as can be seen in the similarity in their ISI histograms (Fig. 7.4B).

Primates treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exhibit most of the cardinal symptoms of parkinsonism: including akinesia, bradykinesia, cogwheel rigidity and in some species tremor (Burns et al., 1983). Extracellular recording of the spontaneous firing of TANs in MPTP-treated primates demonstrate that the cells remain synchronized but their firing rates become oscillatory in the 10–20 Hz range (Raz et al., 1996; Goldberg et al., 2004).

#### V. SUMMARY AND CONCLUSIONS

Cholinergic interneurons exhibit multiple discharge patterns including two forms of rhythmic discharge - single spiking and bursting – as well as irregular firing. The mechanism that prevents the existence of a stable resting potential in these cells, and thus guarantees that they will always discharge, relies on the presence of a robust persistent sodium current that repeatedly brings the cells to action potential threshold. Thus, even in the absence of synaptic input the cholinergic interneurons fire autonomously. A host of high-voltage activated calcium currents, expressed by the cells, which are selectively coupled to specific calcium-activated potassium currents participates in selecting among the various firing patterns. The Ca<sub>v</sub>2.2 current, which activates ISK, gives rise to mAHPs that promote regular single spiking. Ca<sub>V</sub>1 currents that enter the cells even in the subthreshold voltage range tend to disrupt the regularity of this firing. During burst firing the Ca<sub>V</sub>1 currents are responsible for activating  $I_{sAHP}$  that terminates the burst and triggers the ensuing prominent hyperpolarization, which is often amplified by the action of the IRK current (Bennett and Wilson, 1999; Bennett et al., 2000; Maurice et al., 2004; Goldberg and Wilson, 2005; Wilson, 2005; Wilson and Goldberg, 2006; Goldberg et al., 2009).

While the basic mechanisms of discharge are intrinsic to the cholinergic interneurons, synaptic input can exert an important influence on the discharge of these neurons. Weak ionotropic inputs, resulting from glutamatergic inputs from the intralaminar nuclei or from the cortex, or from GABAergic inputs from striatal neurons, can nudge the timing of individual action potentials. Persistent activation of synaptic inputs or activation of metabotropic inputs can influence the firing rate, induce transitions among the firing patterns or even silence these cells. The most dramatic example of this is the pause response exhibited by the primate tonically active cholinergic interneurons in response to primary reward or stimuli associated with reward (Aosaki et al., 1994b). This response requires intact input from the SNc and the intralaminar nuclei (Aosaki et al., 1994a; Matsumoto et al., 2001). It is currently unclear what is the precise mechanism of the pause. While it may result from feed-forward inhibition by way of striatal GABAergic interneurons, its temporal structure is most certainly sculpted by the regenerative nature of the IRK-induced hyperpolarization exhibited in cholinergic interneurons.

The extended and dense axonal arborization of the network of cholinergic interneurons, combined with their shared pause responses in primates, led to the proposal that these neurons act in concert to modulate activity of the whole striatal network (Graybiel et al., 1994). TANs have been shown to discharge and pause synchronously (Raz et al., 1996). Future work needs to determine to what extent this synchrony is controlled by the firing pattern of the cholinergic interneurons. Bursting may correspond to a higher degree of synchrony than during single-spiking. This is observed in MPTP-treated primates wherein the firing is more synchronized, more burstlike and oscillatory relative to healthy animals.

Synchronized bursting could also form a useful substrate for directing learning and synaptic plasticity in the striatum. Bursting effectively divides time into periods of firing, which are accompanied by high levels of ACh in the striatum and periods (several seconds) of quiescence, in which ACh levels presumably drop due to the fast action of ACh esterase. LTP, that requires ACh release and activation of M<sub>1</sub> class receptors, would be favored during burst firing, whereas the period between bursts when ACh levels and M1 class receptor activation drop would favor LTD (Centonze et al., 1999; Pisani et al., 2007; Bonsi et al., 2008). Moreover, the reduction in ACh levels would reduce activation of nicotinic receptors on dopamine releasing axons, which has been shown to accentuate the frequency-dependence of dopamine release in the striatum (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Cragg, 2006; Exley and Cragg, 2008). Thus, during the quiescent phase of the burst, where ACh levels drop, dopaminergic input would have a freer hand in inducing LTD by activating  $D_2$  receptors, according to the precise temporal firing pattern of the dopaminergic neurons. In this context, the pause response, which is concurrent with increased firing of dopaminergic neurons of the SNc in primates (Morris et al., 2004) may represent a period of enhanced LTD and reduced or blocked learning by the striatum. This makes sense, because the intensity of the pause increases as the association learned by classical conditioning is strengthened (Aosaki et al., 2004b). Thus in their pause the cholinergic interneurons may be signaling that the current association is already well-known and therefore the striatal network should refrain from any form of LTP, but should rather promote LTD throughout the striatum (Wilson, 2004).

#### REFERENCES

- Akaike A, Sasa M, Takaori S (1988) Muscarinic inhibition as a dominant role in cholinergic regulation of transmission in the caudate nucleus. J Pharmacol Exp Ther 246:1129–1136.
- Akins PT, Surmeier DJ, Kitai ST (1990) Muscarinic modulation of a transient K<sup>+</sup> conductance in rat neostriatal neurons. Nature 344:240–242.
- Anderson ME (1977) Discharge patterns of basal ganglia neurons during active maintenance of postural stability and adjustment to chair tilt. Brain Res 143:325–338.
- Aoki C, Joh TH, Pickel V (1987) Ultrastructural localization of β-adrenergic receptor-like immunoreactivity in the cortex and neostriatum of rat brain. Brain Res 437:264–282.
- Aosaki T, Graybiel AM, Kimura M (1994a) Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. Science 265:412–415.
- Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M (1994b) Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. J Neurosci 14:3969–3984.
- Aosaki T, Kimura M, Graybiel AM (1995) Temporal and spatial characteristics of tonically active neurons of the primate's striatum. J Neurophysiol 73:1234–1252.
- Aosaki T, Kiuchi K, Kawaguchi Y (1998) Dopamine D1-like receptor activation excites rat striatal large aspiny neurons in vitro. J Neurosci 18:5180–5190.
- Apicella P, Legallet E, Trouche E (1997) Responses of tonically discharging neurons in the monkey striatum to primary rewards delivered during different behavioral states. Exp Brain Res 116:456–466.
- Apicella P (2002) Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. Eur J Neurosci 16:2017–2026.
- Barbeau A (1962) The pathogenesis of Parkinson's disease: a new hypothesis. Can Med Ass J 87:802–807.
- Barral J, Galarraga E, Bargas J (1999) Muscarinic presynaptic inhibition of neostriatal glutamatergic afferents is mediated by Q-type Ca2<sup>+</sup> channels. Brain Res Bull 49:285–289.
- Bell MI, Richardson PJ, Lee K (2000) Histamine depolarizes cholinergic interneurons in the rat striatum via a H1-receptor mediated action. Br J Pharmacol 131:1135–1142.

- Bell MI, Richardson PJ, Lee K (2002) Functional and molecular characterization of metabotropic glutamate receptors expressed in rat striatal cholinergic interneurons. J Neurochem 81:142–149.
- Bennett BD, Wilson CJ (1998) Synaptic regulation of action potential timing in neostriatal cholinergic interneurons. J Neurosci 18:8539–8549.
- Bennett BD, Wilson CJ (1999) Spontaneous activity of neostriatal cholinergic interneurons in vitro. J Neurosci 19:5586–5596.
- Bennett BD, Callaway JC, Wilson CJ (2000) Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. J Neurosci 20:8493–8503.
- Bernard V, Normand E, Bloch B (1992) Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. J Neurosci 12:3591–3600.
- Bishop GA, Chang HT, Kitai ST (1982) Morphological and physiological properties of neostriatal neurons: an intracellular horseradish peroxidase study in the rat. Neuroscience 7:179–191.
- Blomeley C, Bracci E (2005) Excitatory effects of serotonin on rat striatal cholinergic interneurones. J Physiol 569(3):715–721.
- Bolam JP (1989) Cholinergic neurons in the striatum and basal forebrain receive direct synaptic input form GABA-containing axon terminals. Neurosci Lett Suppl 36:S9.
- Bolam JP, Wainer BH, Smith AD (1984) Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. Neuroscience 12:711–718.
- Bonsi P, Cuomo D, Ding J, Sciamanna G, Ulrich S, Tscherter A, Bernardi G, Surmeier DJ, Pisani A (2007) Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. Neuropsychopharmacology 32:1840–1854.
- Bonsi P, Martella G, Cuomo D, Platania P, Sciamanna G, Bernardi G, Wess J, Pisani A (2008) Loss of muscarinic autoreceptor function impairs long-term depression but not long-term potentiation in the striatum. J Neurosci 28:6258–6263.
- Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci USA 80:4546–4550.
- Calabresi P, Saiardi A, Pisani A, Baik JH, Centonze D, Mercuri NB, Bernardi G, Borrelli E (1997) Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. J Neurosci 17:4536–4544.
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998a) Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. Eur J Neurosci 10:2887–2895.
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998b) Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. Eur J Neurosci 10:3020–3023.
- Calabresi P, Centonze D, Pisani A, Sancesario G, North RA, Bernardi G (1998c) Muscarinic IPSPs in rat striatal cholinergic interneurones. J Physiol 510:421–427.
- Calabresi P, Centonze D, Gubellini P, Bernardi G (1999) Activation of M1-like muscarinic receptors is required for the induction of corticostriatal LTP. Neuropharm 38:323–326.
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (2000) Acetylcholine-mediated modulation of striatal function. Trends Neurosci 23:120–126.
- Cetonze D, Gubellini P, Bernardi G, Calabresi P (1999) Permissive role of interneurons in corticostriatal synaptic plasticity. Brain Res Rev 31:1–5.

- Chang HT, Kitai ST (1982) Large neostriatal neurons in rat: an electron microscopic study of gold-toned Golgi-stained cells. Brain Res Bull 8:631–643.
- Choi S, Lovinger DM (1997) Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. Proc Natl Acad Sci USA 94:2665–2670.
- Contant C, Umbriaco D, Garcia S, Watkins KC, Descarries L (1996) Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. Neuroscience 71:937–947.
- Cragg SJ (2006) Meaningful silences: how dopamine listens to the ACh pause. Trends Neurosci 29:125–131.
- Crutcher MD, DeLong MR (1984) Single cell studies of the primate putamen. II. Relations to direction of movement and pattern of muscular activity. Exp Brain Res 53:244–258.
- DeBoer P, Heeringa MJ, Abercrombie ED (1996) Spontaneous release of acetylcholine in striatum is preferentially regulated by inhibitory dopamine D2 receptors. Eur J Pharmacol 317:257–262.
- Deng P, Zhang Y, Xu ZC (2007) Involvement of I(h) in dopamine modulation of tonic firing in striatal cholinergic interneurons. J Neurosci 27:3148–3156.
- Descarries L, Gisiger V, Steriade M (1997) Diffuse transmission by acetylcholine in the CNS. Prog Neurobiol 53:603–625.
- Descarries L, Mechawar N (2000) Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. Prog Brain Res 125:27–47.
- Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85:5274–5278.
- DiFiglia M, Carey J (1986) Large neurons in the primate neostriatum examined with the combined Golgi-electron microscopic method. J Comp Neurol 244:36–52.
- DiFiglia M (1987) Synaptic organization of cholinergic neurons in the monkey neostriatum. J Comp Neurol 255:245–258.
- Ding J, Guzman JN, Tkatch T, Chen S, Goldberg JA, Ebert PJ, Levitt P, Wilson CJ, Hamm HE, Surmeier DJ (2006) RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. Nat Neurosci 9:832–842.
- Exley R, Cragg SJ (2008) Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. Br J Pharmacol 153(Suppl. 1):S283–S297.
- Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299:1898–1902.
- Gabel LA, Nisenbaum ES (1999) Muscarinic receptors differentially modulate the persistent potassium current in striatal spiny projection neurons. J Neurophysiol 81:1418–1423.
- Galarraga E, Hernández-López S, Reyes A, Miranda I, Bermudez-Rattoni F, Vilchis C, Bargas J (1999) Cholinergic modulation of neostriatal output: a functional antagonism between different types of muscarinic receptors. J Neurosci 19:3629–3638.
- Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451.
- Giuffrida A, Parsons LH, Kerr TM, Rodríguez de Fonseca F, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358–363.
- Goldberg JA, Rokni U, Boraud T, Vaadia E, Bergman H (2004) Spike synchronization in the cortex-basal ganglia networks of Parkinsonian primates reflects global dynamics of the local field potentials. J Neurosci 24:6003–6010.

- Goldberg JA, Wilson CJ (2005) Control of spontaneous firing patterns by the selective coupling of calcium currents to calcium-activated potassium currents in striatal cholinergic interneurons. J Neurosci 25:10230–10238.
- Goldberg JA, Teagarden MA, Foehring RC, Wilson CJ (2009) Non-equilibrium calcium dynamics regulate the autonomous firing pattern of rat striatal cholinergic interneurons. J Neurosci 29(26):8396–8407.
- Graybiel AM, Aosaki T, Flaherty AW, Kimura M (1994) The basal ganglia and adaptive motor control. Science 265:1826–1831.
- Hagiwara S, Miyazaki S, Moody W, Patlak J (1978) Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. J Physiol 279:167–185.
- Hattori S, Murakami F, Song WJ (2003) Quantitative relationship between Kv4.2 mRNA and A-type K+ current in rat striatal cholinergic interneuron's during development. J Neurophysiol 90:175–183.
- Hersch SM, Gutekunst CA, Rees HD, Heilman CJ, Levey AL (1994) Distribution of m1-m4 muscarinic receptors proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. J Neurosci 14:3351–3363.
- Howe AR, Surmeier DJ (1995) Muscarinic receptors modulate N-, P-, and L-type calcium currents in rat striatal neurons through parallel pathways. J Neurosci 15:458–469.
- Hsu KS, Yang CH, Huang CC, Gean PW (1996) Carbachol induces inward current in neostriatal neurons through M1-like muscarinic receptors. Neuroscience 73:751–760.
- Jiang Z-G, North RA (1991) Membrane properties and synaptic responses of rat striatal neurons in vitro. J Physiol 443:533–553.
- Jones IW, Bolam JP, Wonnacott S (2001) Presynaptic localization of the nicotinic acetylcholine receptor β2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurons. J Comp Neurol 439:235–247.
- Kawaguchi Y (1992) Large aspiny cells in the matrix of the rat neostriatum in vitro: physiological identification, relation to the compartments and excitatory postsynaptic currents. J Neurophysiol 67:1669–1682.
- Kawaguchi Y (1993) Physiological, morphological and histochemical characterization of three classes of interneurons in the rat neostriatum. J Neurosci 13:4908–4923.
- Kemp JM, Powell TP (1971) The synaptic organization of the caudate nucleus. Philos Trans R Soc Lond B Biol Sci 262:403–412.
- Kimura M, Rajkowski J, Evarts E (1984) Tonically discharging putamen neurons exhibit set-dependent responses. Proc Natl Acad Sci USA 81:4998–5001.
- Kitai ST, Surmeier DJ (1993) Cholinergic and dopaminergic modulation of potassium conductances in neostriatal neurons. Adv Neurol 60:40–52.
- Kölliker A (1896) Handbuch der Gewebelehre des Menchen, Bd. II. Leipzig: Kengelman.
- Koós T, Tepper JM (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nat Neurosci 2:467–472.
- Koós T, Tepper JM (2002) Dual cholinergic control of fast-spiking interneurons in the neostriatum. J Neurosci 22:529–535.
- Kreitzer AC, Malenka RC (2005) Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25:10537–10545.
- Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543–554.

- Lapper SR, Bolam JP (1992) Input form the fontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. Neuroscience 51:533–545.
- Liang H, DeMaria CD, Erickson MG, Mori MX, Alseikhan BA, Yue DT (2003) Unified mechanisms of Ca<sup>2+</sup> regulation across the Ca<sup>2+</sup> channel family. Neuron 39:951–960.
- Lehmann J, Langer SZ (1983) The striatal cholinergic interneuron: synaptic target of dopaminergic terminals? Neuroscience 10:1105–1120.
- Lindvall O, Björkland A (1974) The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol Scand 412(Suppl.):1–48.
- Lipscombe D, Helton TD, Xu W (2004) L-type calcium channels: the low down. J Neurophysiol 92:2633–2641.
- Markram H, Roth A, Helmchen E (1998) Competitive calcium binding: implications for dendritic calcium signaling. J Comput Neurosci 5:331–348.
- Marrion NV, Tavalin SJ (1998) Selective activation of  $Ca^{2+}$ -activated K<sup>+</sup> channels by co-localized  $Ca^{2+}$  channels in hippocampal neurons. Nature 395:900–905.
- Matsumoto N, Minamimoto T, Graybiel AM, Kimura M (2001) Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events. J Neurophysiol 85:960–976.
- Maurice N, Mercer J, Chan CS, Hernandez-Lopez S, Held J, Tkatch T, Surmeier DJ (2004) D2 dopamine receptor-mediated modulation of voltage-dependent Na<sup>+</sup> channels reduces autonomous activity in striatal cholinergic interneurons. J Neurosci 24:10289–10301.
- McGeer PL, Boulding JE, Gibson WC, Foulkes RG (1961) Drug-induced extrapyramidal reactions. J Am Med Ass 177:665–670.
- Mesulam MM, Mash D, Hersh L, Bothwell M, Geula C (1992) Cholinergic innervation of the human striatum, globus pallidus, subthalamic nucleus, substantia nigra, and red nucleus. J Comp Neurol 323:252–268.
- Momiyama T, Koga E (2001) Dopamine D(2)-like receptors selectively block N-type Ca(2+) channels to reduce GABA release onto rat striatal cholinergic interneurons. J Physiol 533:479–492.
- Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H (2004) Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron 43:133–143.
- Pakhotin P, Bracci E (2007) Cholinergic interneurons control the excitatory input to the striatum. J Neurosci 27:391–400.
- Partridge JG, Apparsundaram S, Gerhardt GA, Ronesi J, Lovinger DM (2002) Nicotinic acetylcholine receptors interact with dopamine in induction of striatal long-term depression. J Neurosci 22:2541–2549.
- Pazos A, Probst A, Palacios JM (1985) β-adrenoreceptor subtypes in the human brain: autoradiographic localization. Brain Res 358:324–328.
- Perez-Rosello T, Figueroa A, Salgado H, Vilchis C, Tecuapetla F, Guzman JN, Galarraga E, Bargas J (2005) Cholinergic control of firing pattern and neurotransmission in rat neostriatal projection neurons: role of Ca<sub>v</sub>2.1 and Ca<sub>v</sub>2.2 Ca<sup>2+</sup> channels. J Neurophysiol 93:2507–2519.
- Phelps PE, Houser CR, Vaughn JE (1985) Immunocytochemical localization of choline acetyltransferase within the rat neostriatum: a correlated light and electron microscopic study of cholinergic neurons and synapses. J Comp Neurol 238:286–307.
- Pisani A, Calabresi P, Centonze D, Bernardi G (1997) Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. Br J Pharmacol 120:1007–1014.
- Pisani A, Bonsi P, Centonze D, Calabresi P, Bernardi G (2000) Activation of D2-like dopamine receptors reduces synaptic inputs to striatal cholinergic interneurons. J Neurosci 20 RC69(1-6).

- Pisani A, Bonsi P, Catania MV, Giuffrida R, Morari M, Marti M, Centonze D, Bernardi G, Kingston AE, Calabresi P (2002) Metabotropic glutamate 2 receptors modulate synaptic inputs and calcium signals in striatal cholinergic interneurons. J Neurosci 22:6176–6185.
- Pisani A, Bonsi P, Centonze D, Gubellini P, Bernardi G, Calabresi P (2003a) Targeting striatal cholinergic interneurons in Parkinson's disease: Focus on metabotropic glutamate receptors. Neuropharmacology 45:45–56.
- Pisani A, Bonsi P, Centonze D, Martorana A, Fusco F, Sancesario G, De Persis C, Bernardi G, Calabresi P (2003b) Activation of β1-adrenoreceptors excites striatal cholinergic interneurons through cAMP-dependent, protein kinase-independent pathway. J Neurosci 23:5272–5282.
- Pisani A, Bernardi G, Ding J, Surmeier DJ (2007) Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30:545–553.
- Ravel S, Legallet E, Apicella P (1999) Tonically active neurons in the monkey striatum do not preferentially respond to appetitive stimuli. Exp Brain Res 128:531–534.
- Raz A, Feingold A, Zelanskaya V, Vaadia E, Bergman H (1996) Neuronal synchronization of tonically active neurons in the striatum of normal and parkinsonian primates. J Neurophysiol 76:2083–2088.
- Reynolds JNJ, Wickens JR (2004) The corticostriatal input to giant aspiny interneurons in the rat: a candidate pathway for synchronizing the response to reward-related cues. Brain Res 1011:115–128.
- Reynolds JN, Hyland BI, Wickens JR (2004) Modulation of an afterhyperpolarization by the substantia nigra induces pauses in the tonic firing of striatal cholinergic interneurons. J Neurosci 24:9870–9877.
- Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7:583–584.
- Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679.
- Sah P, Bekkers JM (1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: Implications for the integration of long-term potentiation. J Neurosci 16:4537–4542.
- Shen W, Hamilton SE, Nathanson NM, Surmeier DJ (2005) Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. J Neurosci 25:7449–7458.
- Shen W, Tian X, Day M, Ulrich S, Tkatch T, Nathanson NM, Surmeier DJ (2007) Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. Nat Neurosci 10:1458–1466.
- Sidibè M, Smith Y (1999) Thalamic inputs to striatal interneurons in monkeys: synaptic organization and co-localization of calcium-binding proteins. Neuroscience 89:1189–1208.
- Song W-J, Tkatch T, Baranauskas G, Ichinohe N, Kitai ST, Surmeier DJ (1998) Somatodendritic depolarization-activated potassium currents in rat neostriatal cholinergic interneurons are predominantly of the A type and attributable to coexpression of Kv4.2 and Kv4.1 subunits. J Neurosci 18:3124–3137.
- Song W-J, Tkatch T, Surmeier DJ (2000) Adenosine receptor expression and modulation of Ca(2+) channels in rat striatal cholinergic interneurons. J Neurophysiol 83:322–332.
- Steinbusch HW (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6:557–618.
- Sullivan MA, Chen H, Morikawa H (2008) Recurrent inhibitory network among striatal cholinergic interneurons. J Neurosci 28:8682–8690.

- Surmeier DJ, Mercer JN, Chan CS (2005) Autonomous pacemakers in the basal ganglia: who needs excitatory synapses anyway? Curr Opin Neurobiol 15:312–318.
- Tallaksen-Greene SJ, Kaatz KW, Romano C, Albin RL (1998) Localization of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified populations of striatal neurons. Brain Res 780:210–217.
- Takeshita Y, Harata N, Akaike N (1996) Suppression of K<sup>+</sup> conductance by metabotropic glutamate receptor in acutely dissociated large cholinergic neurons in rat caudate putamen. J Neurophysiol 76:1545–1558.
- Thomas TM, Smith Y, Levey AI, Hersch SM (2000) Cortical inputs to m2-immunoreactive striatal interneurons in rat and monkey. Synapse 37:252–261.
- Vilchis C, Bargas J, Ayala GX, Galvan E, Galarraga E (2000) Ca<sup>2+</sup> channels that activate Ca<sup>2+</sup>-dependent K<sup>+</sup> currents in neostriatal neurons. Neuroscience 95:745–752.
- Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, Tkatch T, Lovinger DM, Surmeier DJ (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50:443–452.
- Wilson CJ (2004) Basal Ganglia. In: The Synaptic Organization of the Brain, 5th edn (Shepherd GM ed), pp. 361–413. New York: Oxford University Press.
- Wilson CJ (2005) The mechanism of intrinsic amplification of hyperpolarizations and spontaneous bursting in striatal cholinergic interneurons. Neuron 45:575–585.

- Wilson CJ, Chang HT, Kitai ST (1990) Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J Neurosci 10:508–519.
- Wilson CJ, Goldberg JA (2006) Origin of the slow afterhyperpolarization and slow rhythmic bursting in striatal cholinergic interneurons. J Neurophysiol 95:196–204.
- Yan Z, Surmeier DJ (1996) Muscarinic (m2/m4) receptors reduce N- and P-type Ca<sup>2+</sup> currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G-protein pathway. J Neurosci 16:2592–2604.
- Yan Z, Song W-J, Surmeier DJ (1997) D2 dopamine receptors reduce Ntype Ca<sup>2+</sup> currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. J Neurophysiol 77:1003–1015.
- Yan Z, Flores-Hernandez J, Surmeier DJ (2001) Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. Neuroscience 103:1017–1024.
- Zhang W, Klimek V, Farley JT, Zhu M-Y, Ordway GA (1999) A2C adrenoreceptors inhibit adenylyl cyclase in mouse striatum: potential activation by dopamine. J Pharmacol Exp Ther 289:1286–1292.
- Zhang H, Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7:581–582.
- Zhou F-M, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4:1224–1229.
- Zhou F-M, Wilson CJ, Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. J Neurobiol 53:590–605.

# **GABAergic Interneurons of the Striatum**

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## I. INTRODUCTION

One of the areas in which our understanding of the structure and function of the striatum is undergoing the most rapid expansion and change is in the identification and characterization of striatal interneurons, their synaptic connectivity and the role that they play in the organization and control of striatal output. First shown by strong uptake of radiolabeled GABA (Bolam et al., 1983), and subsequently by immunoreactivity for GABA and/or GAD (Bolam et al., 1985; Cowan et al., 1990; Kita, 1993), a population of striatal GABAergic interneurons comprising only a few percent of all the striatal neurons was identified. These interneurons have become the focus of a large number of anatomical and, more recently, electrophysiological studies. Results from these experiments, most of them performed over the past 10 years, have led to the current view that although few in number, striatal GABAergic interneurons play a predominant role in regulating spike timing

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. in the spiny output neurons through feedforward inhibition (for reviews, see Tepper et al., 2004, 2008). Although Golgi studies have suggested that there are as many as 9 morphologically distinct neuron types in the rodent striatum (e.g., Chang et al., 1982), the current consensus view is that in addition to the spiny projection neurons that comprise up to 97.7% of striatal neurons in rodents (Rymar et al., 2004), the striatum is composed of one type of cholinergic interneuron (see Chapter 7), and three types of neurochemically and electrophysiologically distinct GABAergic interneurons (Kawaguchi, 1993; Kawaguchi et al., 1995; Tepper and Bolam, 2004; Tepper et al., 2008). However, recent physiological studies in striatum, and by analogy with findings from the cortex and hippocampus over the past 15 years, this view is almost certain to underestimate the true diversity of striatal GABAergic interneurons. This review will focus on the anatomy and physiology of the three well-known GABAergic interneuronal cell types of the rodent striatum, as well as recent advances in striatal neuroanatomy and neurophysiology which suggest that a re-evaluation of the number of distinct striatal GABAergic interneuron subtypes is overdue.

# II. PARVALBUMIN-IMMUNOREACTIVE INTERNEURONS

## A. Neurocytology

Parvalbumin-immunoreactive (PV+) striatal interneurons are medium to large sized, averaging 16-18µm in diameter. These were the striatal GABAergic neurons originally identified by their high affinity uptake of GABA (Bolam et al., 1983) and subsequently by the strongest immunoreactivity for GAD<sub>67</sub> and GABA of any striatal neuron (Bolam et al., 1985; Cowan et al., 1990; Kita, 1993; Kubota et al., 1993). PV+ neurons do not express any of the other markers that identify the other types of striatal GABA interneurons, i.e., calretinin, nitric oxide synthase (NOS), somatostatin (SOM) or neuropeptide Y (NPY). Striatal PV+ interneurons issue 5-8 aspiny, often varicose, dendrites that taper rapidly and branch relatively sparsely to form a restricted dendritic arborization only 200-300 µm in diameter. The axon issues from the soma or proximal dendrite and branches repeatedly forming an extremely dense, highly varicose arborization that overlaps and extends well beyond the limits of the dendritic field of the cell of origin. The axonal arborization is the densest of all the striatal neurons (cf Fig. 8.1B). A subset of PV+ interneurons exhibits a more extended dendritic arborization and somata well in excess of 20µm in diameter (Kawaguchi, 1993; Koós and Tepper, 1999; Tepper and Bolam, 2004). The proportion of striatal neurons that are PV+ interneurons is 0.7% based on unbiased stereological cell counting (Rymar et al., 2004) and PV+ interneurons exhibit a dorsal to ventral and medial to lateral gradient of expression (Luk and Sadikot, 2001).

#### **B.** Afferents and Efferents

PV+ interneurons receive a substantial monosynaptic input from the cortex but in contrast to the spiny cells, which receive only one or two synapses from each cortical afferent (Kincaid et al., 1998), single cortical axons make multiple contacts with PV+ interneurons (Ramanathan et al., 2002). This may account, in part, for the greater responsivity of PV+ interneurons to cortical stimulation (Parthasarathy and Graybiel, 1997) compared to spiny neurons (Mallet et al., 2005). In contrast to this powerful cortical input, PV+ interneurons appear to receive few synaptic inputs from thalamus (Kita, 1993).

Striatal PV+ interneurons also receive GABAergic input from PV+ globus pallidus neurons that specifically target interneurons in the striatum (Bevan et al., 1998). In addition, there is a cholinergic input from striatal cholinergic interneurons (Chang and Kita, 1992), and a dopaminergic input, presumably from substantia nigra (Kubota et al., 1987).

By far the predominant target of the PV+ interneuron is the spiny projection neuron with about 50% of striatal PV+ boutons synapsing pericellularly, on the soma or proximal dendrites of spiny neurons (Kita, 1993; Bennett and Bolam, 1994a). Other PV+ boutons (some of which likely originated in globus pallidus) were observed making synapses with spiny dendrites as well as with varicose dendrites (Kita, 1993) belonging to PV+ and nitric oxide synthase (NOS)-immunoreactive interneurons (Bevan et al., 1998).

### C. Basic Membrane Properties

The earliest descriptions of the electrophysiological properties of the PV+ interneuron were provided by Kawaguchi (1993) who used visually guided whole cell recordings in slices from young (16-20 days) rats and targeted somata larger than those of spiny cells to attempt to record from interneurons. The first class of interneuron he described had the shortest duration action potentials of all striatal neurons (0.29  $\pm$  0.04 ms) at half amplitude), a rapid  $(1.3 \pm 0.27 \,\mathrm{ms})$  time to peak and brief spike afterhyperpolarization and the lowest input resistance of all GABAergic interneurons ( $86 \pm 38 \text{ ms}$ ). These interneurons were, like spiny neurons, hyperpolarized (resting membrane potential  $\sim -80 \,\mathrm{mV}$ ) and displayed no spontaneous activity at rest. Of 11 neurons with these electrophysiological characteristics tested, 10 were immunopositive for PV (Kawaguchi, 1993).

Subsequent experiments in acute slices from older rats revealed a class of fast-spiking (FS) interneurons with identical properties including a nearly linear IV response, low input resistance, hyperpolarized resting membrane potential and narrow action potentials with a rapid, large amplitude and brief duration spike afterhyperpolarization (Koós and Tepper, 1999, 2002; Bracci et al., 2002, 2003; Taverna et al., 2007; cf Fig. 8.1C).

PV+ interneurons in striatum have been shown to exhibit gap junctions at the electron microscopic level



**FIGURE 8.1** Striatal PV+ fast-spiking interneurons. A. Typical morphology of an immunocytochemically striatal GABAergic PV+ interneuron stained with an antibody against parvalbumin from adult rat striatum. B. Drawing tube reconstruction of a biocytin labeled rat striatal FS interneuron that was presynaptic to a spiny neuron (not shown). Note the extremely dense axonal arborization (red) that extends beyond the simple, aspiny and compact dendritic arbor (black). C. 1. Typical response of mouse striatal FS interneuron to intracellular injection of de- and hyperpolarizing current pulses. Note the low input resistance, the linear IV relation and the characteristic non-linear current-spiking relation where the minimum suprathreshold stimulus elicits a short burst of three spikes (black trace), the next greater current injection elicits a 120 ms burst of non-accommodating spikes followed by a silent period followed by intermittent spiking (green trace) and a slightly larger current injection evokes longer periods of intermittent spiking. Sufficiently greater current injection elicits sustained firing in excess of 200 spikes/sec (not shown). C2. Note extremely narrow action potential and deep and rapid onset spike AHP. D. Striatal FS interneurons are silent at rest (3). As they are depolarized small subthreshold membrane oscillations occur (2) that eventually give rise to intermittent spiking (red arrows in 1). E. Left two panels show a pair of FS interneurons that are electrotonically coupled. Intracellular current pulses injected into FS # 1 (right lower panel) induces much smaller, electrotonic responses in FS #2 (upper right, black traces) distorted by the membrane capacitance of the intervening dendrites. F. Spiking induced by intracellular depolarization in a pair of electrotonically coupled FS interneurons is nearly synchronous due to the effects of the coupling. All recordings were obtained from neurons in acute brain slices in vitro. Sources: B: Modified from Tepper and Bolam, 2004; D: Modified from Koós and

(Kita et al., 1990, Kita, 1993). In the first report of paired recordings between striatal FS interneurons, clear evidence of electrotonic coupling was found (Koós and Tepper, 1999; cf Fig. 8.1E). The coupling ratio ranged between 3 and 20%, and although the coupling was not strong enough to induce spiking per se, it was sufficient to synchronize depolarization-induced spiking in electrically coupled neurons such that the variability between spikes in the two neurons

in response to current injection, when they did occur, was extremely small (cf Fig. 8.1F). This suggests that groups of FS interneurons may form an inhibitory syncytium capable of exerting powerful and synchronous inhibitory control over spike timing in a large number of spiny neurons, thereby influencing the temporal relationship of their spike trains leading to the formation of behaviorally relevant functional pools of cells (Koós and Tepper, 1999). (154 )

Electrotonic coupling with similar properties has been observed between pairs of PV+ fast spiking interneurons in cortex and hippocampus (Freund and Buzsáki, 1996; Galaretta and Hestrin, 1999, 2001, 2002), although the highest coupling ratios in the cortical interneurons appear larger than in striatum, but this could be an artifact of the relatively small striatal sample size.

#### **D.** Firing Characteristics

One of the most characteristic and consistently reported properties of striatal PV+ FS interneurons cells is a non-linear spiking response to intracellular depolarization. Lower amplitude stimuli of increasing strength produce only passive depolarizing responses but at a certain level, a tiny increase in current results in the appearance of short bursts of spikes interrupted by periods of no spiking (Kawaguchi, 1993; Koós and Tepper, 1999, 2002; Kubota and Kawaguchi, 2000; Narushima et al., 2006). Stronger depolarizing pulses in striatal FS interneurons elicit high, sustained firing rates (>200 spikes/sec) with little spike frequency adaptation (Koós and Tepper, 1999, 2002; Plotkin et al., 2005; cf Fig. 8.1C).

During the non-spiking periods intercalated between episodic firing, prominent subthreshold membrane oscillations can be observed (Koós and Tepper, 1999, 2002; Bracci et al., 2002, 2003; Taverna et al., 2007; cf Fig. 8.1C1, D1). The oscillations are clearly voltage dependent and are not present when the neuron is hyperpolarized or at rest. They are 2-3 mV in amplitude and exhibit a peak in power near 40 Hz (Bracci et al., 2003). The oscillations are able to induce episodes of firing and appear to be responsible for the stuttering, intermittent firing pattern of striatal FS interneurons. The oscillations and the intermittent firing pattern were resistant to blockade of Ca<sup>2+</sup> channels, SK channels or intracellular Ca<sup>2+</sup> chelation. The oscillations were, however, completely eliminated by TTX, and it has been suggested that they are due to an interaction between a persistent Na<sup>+</sup> conductance and voltage gated K<sup>+</sup> conductances (Bracci et al., 2003).

Whole cell recordings from striatal FS interneurons in slices from mice reveal that they express virtually identical properties to those described for rat FS interneurons (Centonze et al., 2003; Narushima et al., 2006; Tecuapetla et al., 2009b). These electrophysiological characteristics are very similar to those of parvalbumin-expressing fast spiking interneurons of adult mouse cortex (Galaretta et al., 1999, 2001, 2002) and hippocampal basket cells (Freund and Buzsáki, 1996). The short duration action potential, lack

of spike frequency adaptation and large spike afterhyperpolarizations are likely related to the expression of Kv 3.1, a slowly inactivating delayed rectifier channel that exhibits rapid activation and deactivation kinetics, and that is selectively expressed in striatum in PV+ FS interneurons (Lenz et al., 1994).

Interestingly, in another study in which PV+ FS interneurons were identified in striatal slices of young (12-18 day old) BAC transgenic mice engineered to express enhanced green fluorescent protein (EGFP) controlled by the PV promoter (Freiman et al., 2006), although fluorescent (PV+) fast spiking interneurons were in several ways qualitatively similar to FS interneurons previously described, they did not exhibit the intermittent firing or subthreshold oscillations seen in juvenile and adult rats or in adult mice, and also exhibited a more depolarized resting membrane potential  $(-63 \,\mathrm{mV})$  and wider action potential duration (0.70 ms at half amplitude) than reported by others (Freiman et al., 2006). As these neurons were unquestionably PV+ FS interneurons, these differences may be attributable to the younger age of the mice, lower (room) temperature recording, and/or other aspects of the recording conditions. There is also the intriguing possibility that there are actually several subtypes of striatal PV+ interneurons that each have distinct physiological properties, as is known to be the case, for example, in the amygdala (e.g., Woodruff and Sah, 2007).

#### E. Synaptic Connectivity

Synaptic responses in spiny neurons resulting from spiking of FS interneurons were first reported by Plenz and Kitai (1998) in one out of four paired recordings of an FS interneuron and a spiny cell in an organotypic co-culture of cortex, striatum and substantia nigra. This recording showed that a single spike in the presynaptic FS interneuron elicited an IPSP several mV in amplitude. Soon after, paired whole cell recordings of FS interneurons and spiny neurons were obtained from acute slices of striatum from adult rats. These revealed unusually strong unitary IPSPs in the spiny cells from single spikes in FS interneurons. IPSPs were blocked completely by bath application of bicuculline indicating that the FS-spiny neuron synaptic response is mediated predominantly or exclusively by GABA<sub>A</sub> receptors. The synaptic connections were always unidirectional, from the interneuron to the spiny cell but never in the other direction, and were observed in approximately 25% of the paired recordings when the two cells were within 250µm of each other. The synaptic connection was remarkably

reliable and for most pairs exhibited a failure rate of less than 1% (Koós and Tepper, 1999, 2002).

Early estimates of the convergence values of FS interneurons and spiny cells were based on the measured volume of the axonal arborization of four biocytin-filled FS interneurons  $(6.65 \pm 1.19 \times 10^{-3} \text{ mm})$  assuming 3dimensional isotropic density, a cell density in striatum of 84,900 cells/mm<sup>3</sup> (Oorschot, 1996) that corresponds to  $541 \pm 101$  spiny cells within the volume of the axonal arbor of a FS interneuron. Assuming that all spiny cells within the arborization are innervated and if FS interneurons make up 5% of the cells in striatum, the upper limit of convergence is 27 FS interneurons per spiny cell  $(0.05 \times 541)$ . The lower limit was estimated by assuming that only 25% of the spiny cells are innervated (the probability of finding a synaptically connected pair) and only 3% of the striatal cells are FS interneurons and was calculated to be four interneurons per spiny cell (541  $\times$  0.25  $\times$  0.03; (Koós and Tepper, 1999). However, in subsequent reports from other labs and more recent paired recordings from our lab where the pre- and postsynaptic neurons were in closer proximity to one another, the incidence of connectivity was significantly higher, with a lower limit around 50% (Taverna et al., 2007). In terms of convergence, the increase in the lower limit of synaptic connectivity is offset by more accurate estimates of the number of PV+ neurons from stereological cell counts that suggest that the proportion of PV+ neurons in the rat striatum is actually only 0.7% (Rymar et al., 2004). This would give a lower limit of convergence of 2 FS interneurons per spiny cell  $(541 \times 0.5 \times 0.007)$  and an upper limit of 4 FS interneurons per spiny cell. Note that this assumes that all FS interneurons are PV +, an assumption that may prove incorrect (see below).

Average unitary FS-Spiny IPSPs recorded in hyperpolarized spiny neurons were over 0.4 mV in amplitude, and when measured when the spiny cell was just subthreshold, averaged greater than 1 mV in amplitude. Temporal summation in response to bursts of 2–5 spikes in the FS interneuron within 10–50 ms led to compound IPSPs that could be up to 7 mV in amplitude. The effectiveness of these IPSPs was evident by the ability of unitary IPSPs to significantly delay the timing of depolarization-evoked spikes in spiny neurons and, in the case of short bursts of presynaptic spikes, to completely block spiking in the spiny neuron (Koós and Tepper 1999).

When compared to IPSPs arising from the local axon collaterals of spiny neurons (Czubayko and Plenz, 2002; Tunstall et al., 2002; Tepper et al., 2004; Tecuapetla et al., 2009) (see Chapter 5), the feedforward interneuronal IPSP appeared significantly larger and had a significantly lower failure rate than the collateral IPSP under a number of different experimental conditions (Guzman et al., 2003; Koós et al., 2004; Tecuapetla et al., 2005, 2009; Gustafson et al., 2006). Quantal analysis revealed that whereas individual FS-Spiny and Spiny-Spiny synapses were in fact biophysically similar, the differences in average IPSP/C amplitude and failure rate could be attributable to a more proximal location and larger number of synapses formed by FS inputs to spiny neurons than from collateral inputs from other spiny neurons (Koós et al., 2004).

#### F. In Vivo Recordings

Each of the striatal interneurons make up such a small proportion of the cells in the striatum that in vivo recordings from unambiguously identified GABAergic interneurons are relatively rare. Intracellular recordings from a neuron identified post-hoc by horseradish peroxidase staining as a likely PV+FS interneuron based on the large soma size and varicose dendritic arborization exhibited EPSPs in response to cortical stimulation. The EPSPs gave rise to short bursts of high frequency spikes (Kita, 1993), as would be predicted from the in vitro responses of striatal PV+FS interneurons.

More recently, extracellular single unit recording studies in vivo in anesthetized rats putatively identified FS interneurons on the basis of their short duration action potentials and a short, high frequency (>300Hz) burst of 3-5 spikes, very similar to that reported by Kita (1993), in response to cortical stimulation (Mallet et al., 2005, 2006). These neurons exhibited spontaneous firing rates of around 0.5 spikes/second during slow wave sleep and 3.5 spikes/second at other times. When neurons with these characteristics were juxtacellularly stained with biocytin and then tested for parvalbumin immunoreactivity, all neurons tested were immunopositive for parvalbumin, thus unequivocally identifying them as PV+FS interneurons (Mallet et al., 2005, 2006). Comparison of the responses of spiny neurons to those of FS interneurons following cortical stimulation showed the FS interneurons to be more responsive during periods of cortical desynchronization than during slow wave sleep whereas the opposite was true for spiny neurons (Mallet et al., 2005). On average FS interneurons were more responsive to cortical stimulation than spiny neurons (Mallet et al., 2006), consistent with results from previous immediate early gene expression experiments (Parthasarathy and Graybiel, 1997). Local application of picrotoxin increased the spiking of spiny neurons in response to cortical stimulation, particularly under conditions favoring the activity of FS interneuronal activity strongly suggesting that strong feedforward inhibition of spiny neurons by FS interneurons normally occurs in vivo as well as in vitro (Koós and Tepper, 1999, 2002; Koos et al., 2004; Mallet et al., 2005).

Presumed FS interneurons have also been identified in vivo in unanesthetized behaving rats. Tetrode recordings revealed a population of neurons that were tonically active with average firing rates of 5-30Hz spikes/sec during waking that displayed narrow duration waveforms and high frequency bursts during slow wave sleep, and an anatomical distribution very similar to that reported for PV+ FS interneurons (Berke et al., 2004; Berke, 2008). The presumed FS interneurons were more active when the animals were awake than during slow wave sleep, consistent with the results from the anesthetized animals. These neurons were found to be entrained by high voltage spindle activity that occurred principally while rats were immobile but interestingly, even nearby FS interneurons failed to exhibit correlated firing while rats were performing a radial maze task (Berke, 2008), suggesting that feedforward inhibition of individual spiny neurons may be comprised of inputs from neurons with very different firing rates and/or behavioral correlates.

### G. Pharmacology

Striatal FS interneurons are innervated by striatal cholinergic interneurons (Chang and Kita, 1992), and express both nicotinic and muscarinic receptors. The two types of cholinergic receptors have opposing effects on the feedforward inhibition mediated by FS interneurons.

Local or bath application of nicotinic cholinergic agonists in vitro depolarizes striatal FS interneurons by up to 40 mV, evoking episodes of irregular bursty firing in the normally silent neurons, thereby increasing feedforward inhibition of spiny neurons (Koós and Tepper, 2002; Fig. 8.2). The cholinergic excitation persists during bath application of carbachol and is insensitive to high concentrations of the Type 1 nicotinic receptor antagonist, methyllycaconitine (MLA), but can be completely blocked by mecamylamine. This profile strongly suggests that the receptor responsible is one of the heteromeric, non-desensitizing nicotinic receptor subtypes (Alkondon and Albuquerque, 1993). It has been suggested that disfacilitation of FS interneurons as their nicotinic excitation is transiently reduced during the brief and stereotyped pause in striatal cholinergic interneurons that accompanies behaviorally relevant stimuli (Aosaki et al., 1994) may play a role in relaying the pause to the spiny neurons with high temporal fidelity (Koós and Tepper, 2002).

Acetylcholine also acts through pirenzapine-sensitive muscarinic receptors located on axon terminals of striatal FS interneuron to presynaptically inhibit GABA release and reduce the feedforward inhibition of spiny cells by FS interneurons as illustrated in Figure 8.2E,F. (Koós and Tepper, 2002). The balance of this dual cholinergic regulation of FS interneurons may depend on behavioral state and the level of interneuronal activity with the direct excitatory nicotinic effects predominating when FS neurons are relatively inactive during periods of cortical synchrony and the presynaptic inhibitory effects predominating during cortical desynchronization when the FS interneurons are highly active.

FS interneurons are also excited by dopamine (Bracci et al., 2002). The excitation is accompanied by a decrease in membrane conductance and, like nicotinic stimulation, is sufficient to generate spiking. These effects were blocked by SCH-23390 but not by quinpirole, suggesting that they were mediated by a D1-like dopamine receptor. Subsequent experiments revealed that SCH-23390-sensitive dopamineinduced excitation persisted in D<sub>1</sub> knockout mice, indicating that it is mediated by dopamine D<sub>5</sub> receptors that have been shown to be co-expressed with parvalbumin in striatal FS interneurons (Centonze et al., 2003). Interestingly, unlike D<sub>1</sub> expressing direct pathway spiny neurons that express presynaptic  $D_1$  receptors on their axon terminals that facilitate GABA release (Misgeld et al., 2007), or striatopallidal neurons that express presynaptic D2 receptors that inhibit GABA release (Tecuapetla et al., 2009), the evidence for presynaptic modulation of the FS to spiny neuron synapse by dopamine, particularly by the D<sub>5</sub> receptor known to be expressed by these neurons, is considerably weaker and more equivocal (Bracci et al., 2002; Guzman et al., 2003; Tecuapetla et al., 2007).

### III. SOMATOSTATIN/NOS/NEUROPEPTIDE Y INTERNEURONS

#### A. Neurocytology

The discovery of somatostatin (SOM)-immunoreactive interneurons in striatum was followed by demonstrations that SOM, NPY and NADPH-diaphorase/nitric oxide synthase (NOS) were all co-expressed in the same neuronal population (Vincent and Johansson, 1983; Chesselet and Graybiel, 1986) and that these neurons were distinct from



**FIGURE 8.2** A. Synaptic connections between FS interneurons and spiny neurons in rat exhibit large and variable amplitude and extremely low failure rates. B. Single IPSPs in FS interneurons elicit IPSPs in spiny cells and delay the occurrence of spikes in spiny neurons evoked by intracellular current pulses (black trace). Presynaptic spike doublets evoked IPSPs that summate effectively and cause even greater delay in elicited spiny cell spikes (green trace). C1. FS interneurons are potentially depolarized by pressure application of ACh, which is completely blocked by mecamylamine (MEC). C2. In contrast, ACh-induced depolarization is not blocked by the Type 1 nicotinic antagonist, methyllycaconitine (MLA) indicating that the receptor is one of the non-desensitizing heteromeric subtypes. D. Bath application of carbachol depolarizes a FS interneuron and evokes intermittent spiking, effects that were sustained for the duration of the bath application. E. Synaptic transmission between FS interneurons and spiny cells is subject to powerful muscarinic inhibition. F. Correlation between the mean amplitude depression and the CV of the depression shows that the effect is mediated presynaptically. Sources: B. Modified from Koós and Tepper, 1999; C–F. Modified from Koós and Tepper, 2002. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

those that expressed parvalbumin or calretinin (Kubota et al., 1993). At first these interneurons did not appear to be GABAergic because unlike the other striatal GABAergic interneurons, somatostatin-immunoreactive neurons did not appear to express GAD mRNA (Chesselet and Robbins, 1989) or immunoreactivity for GABA or  $GAD_{67}$  (Kubota et al., 1993). However, subsequent immunocytochemical labeling following colchicine treatment revealed that all NOS-reactive cells were strongly immunopositive for  $GAD_{67}$  (Kubota et al., 1993) and intracellular labeling followed by electron microscopic post-embedding immunogold labeling for GABA or GAD showed that their synaptic boutons were strongly GABA immunopositive (Kubota and Kawaguchi, 2000).

SOM/NPY interneurons have medium-large somata, 15–25  $\mu$ m in diameter, and are the second largest cell in the striatum after the cholinergic interneuron. The neurons emit 2–5 thick, rapidly tapering aspiny primary dendrites that branch within 50  $\mu$ m of the cell body, become varicose, and give rise to a relatively simple and unbranched dendritic arborization up to 600  $\mu$ m in diameter (Difiglia and Aronin, 1982; Vincent and Johannson, 1983; Vincent et al., 1983; Kawaguchi, 1993; Aoki and Pickel, 1988; see Fig. 8.3). The axonal arborization of SOM/NPY interneurons is the sparsest of any striatal neuron and is also the longest, tending to course in straight lines for up to 1 mm. Some of these neurons appeared to give rise to two main axons (Kawaguchi, 1993). Unbiased stereological counts of striatal neurons immunostained for SOM (21,300) and NPY (14,355) differ (Rymar et al., 2004), consistent with the results of a double and triple labeling study that showed that only about 80% of SOM neurons also expressed NPY and that only 73% of neurons immunoreactive for SOM, NOS, NADPH-diaphorase or NPY expressed all four peptides (Figueredo-Cardenas et al., 1996). Therefore the proportion of striatal neurons comprised of PLTS cells would be somewhere between 0.55 and 0.8%. It is unclear if the different combinations of co-expression of SOM, NOS, NADPH-diaphorase and NPY are associated with different electrophysiological and/or morphological phenotypes.

#### **B.** Afferents and Efferents

Striatal PLTS neurons receive a monosynaptic excitatory input from the cortex (Kawaguchi, 1993) as well as a dopaminergic innervation, presumably from substantia nigra (Kubota et al., 1988; Li et al., 2002). They are also the target, along with PV+ FS interneurons, of PV+ afferents from globus pallidus (Bevan et al., 1998) and cholinergic inputs from striatal cholinergic interneurons.

#### C. Basic Membrane Properties

SOM/NPY interneurons were first described by Kawaguchi in whole cell recordings from young rats (Kawaguchi, 1993). These cells were initially distinguished from spiny neurons and from the other GABAergic interneurons by the presence of a low threshold  $Ca^{2+}$  spike (*LTS*) that could be elicited by intracellular depolarization or synaptic stimulation delivered at the resting membrane potential, and by the expression of long-lasting depolarizing plateau potentials (P) that occurred following depolarizing current injections, sufficiently strong excitatory synaptic stimulation or as a rebound upon cessation of a hyperpolarizing current injection and were termed PLTS neurons (Kawaguchi, 1993; cf Fig. 8.3D,E). PLTS cells were further characterized by a very high input resistance, more than seven times greater than that of the FS interneurons (638  $\pm$  245 M $\Omega$ ), a resting membrane potential more than 20 mV more depolarized than that of the FS interneurons  $(-56.4 \pm 15.7 \text{ mV})$ , and long duration action potential ( $1.0 \pm 0.41$  ms at half amplitude; Kawaguchi, 1993; Kubota and Kawaguchi, 2000; Centonze et al., 2002).

Although Kawaguchi (1993) originally termed these cells PLTS interneurons, more recently most authors,

including Kawaguchi and colleagues, have dropped the P from the electrophysiological acronym for the striatal NPY-NOS immunoreactive neurons that display an LTS and plateau potentials, and refer to them simply as LTS neurons (e.g., Kawaguchi et al., 1995; Kubota and Kawaguchi, 2000; Centonze et al., 2002).

## **D.** Synaptic Connectivity

The only available data on the connectivity of PLTS neurons comes from a few recordings of synaptically connected PLTS interneurons and spiny neurons. These recordings show that intracellular stimulation of the PLTS neuron that evokes a single spike elicits an IPSC in the spiny cell that shows very little amplitude variability, perhaps suggesting that each PLTS neuron makes only one or a very limited number of synapses with target spiny neurons (Koós, 2000; Tepper and Bolam, 2004; Tepper et al., 2008; Fig. 8.3G), a suggestion that is consistent with the sparse and longitudinal morphology of the PLTS axonal arborization (Fig. 8.3F).

A recent in vitro study showed that stimulation of striatal cholinergic interneurons, or a single cholinergic neuron leads to activation of a recurrent network that results in IPSCs in the stimulated cholinergic neuron (Sullivan et al., 2008). Pharmacological studies showed that the IPSCs were GABA<sub>A</sub> IPSCs. Furthermore, the IPSCs were eliminated by repetitive stimulation and/or by antagonists of nicotinic receptor  $\beta 2$  subunits, suggesting that the cholinergic interneuron activated a striatal GABAergic neuron via a \(\beta2\)-subunit containing nicotinic receptor that then synapsed back onto the cholinergic interneuron and inhibited it. FS interneurons, but not spiny neurons are known to express nicotinic receptors; however these are non-desensitizing unlike the receptor mediating the recurrent feedback to the cholinergic interneuron. Thus, the GABAergic intermediary is some type of striatal GABAergic interneuron other than the FS interneuron. Although this interneuron is still unidentified, there are cholinergic inputs to the PLTS interneuron (Vuillet et al., 1992), whose other properties make it an excellent candidate for the GABAergic interneuron responsible for the recurrent inhibition in the cholinergic interneuron (Sullivan et al., 2008).

### E. Spontaneous Activity

There are scant data on the spontaneous activity of PLTS interneurons. Despite their depolarized resting membrane



FIGURE 8.3 Striatal SOM/NOS/NPY GAB-Aergic PLTS interneurons. A,B,C. Fluorescent immunocytochemistry of mouse striatum with an antibody against NOS. D. Typical PLTS neuron recorded in slice from an adult mouse displays the very high input resistance, depolarized resting membrane potential, sag in response to hyperpolarizing current injections and the LTS and sustained plateau depolarizations (arrow) following relaxation of hyperpolarizing current injections that are characteristic of PLTS neurons. E. The same neuron, held hyperpolarized at -80 mV, responds to a depolarizing current injection with a plateau depolarization (arrow). F. Drawing tube reconstruction of PLTS neurons from rat striatum filled with biocytin after recording shows that sparsely branching, mostly linear axonal arborization previously described for these neurons. Soma and dendrites are in black and the axonal arborization is in red. G. Paired recording of the cell reconstructed in F with a spiny neuron showing a monosynaptic connection with low failure rate and little amplitude variability. H. Typical PLTS neuron recorded in slices from adult rat striatum exhibits same characteristics illustrated in the mouse PLTS neuron shown in D. Compare with the LTS neuron illustrated in I. The LTS neuron recorded from a slice of adult rat striatum has approximately one fourth the input resistance of the PLTS neuron, lacks the prolonged plateau potentials, and has a narrower duration spike with a biphasic AHP. J. Short burst of 3 spikes in a presynaptic LTS interneuron delays or completely aborts depolarization-evoked spiking in a postsynaptic spiny neuron. Sources: F. Modified from Tepper et al., 2008; G, I: Modified from Tepper and Bolam, 2004; J. Modified from Koós and Tepper, 1999. To view a color version of this image please visit http://www.elsevierdirect .com/companion/9780123747679

potential, they are not spontaneously active in vitro, and there is no way to identify them from extracellular recordings in vivo.

### F. Pharmacology

PLTS interneurons are depolarized by bath application of dopamine, an effect that is blocked by the D1-like antagonist, SCH-23390. The depolarization is sufficient to trigger spiking and is associated with a decrease in membrane conductance (Centonze et al., 2002). More than 75% of striatal SOM+ neurons are also immunoreactive for the D<sub>5</sub> dopamine receptor (Rivera et al., 2002) whereas most show no D<sub>1</sub> mRNA expression and those that do express only low levels of the message (Le Moine et al., 1991). Thus, like the PV+ interneurons, the excitatory effects of dopamine on PLTS interneurons are mediated by D<sub>5</sub> receptors.

### **IV. LTS NEURONS**

Another striatal interneuron that expresses low threshold Ca<sup>2+</sup> spikes has also been described, and termed an LTS interneuron (Koós and Tepper, 1999). The principal differences between the PLTS and LTS neurons were the absence of the persistent plateau depolarizations in the LTS neuron, either upon depolarization or following the cessation of a hyperpolarizing current injection, and a significantly lower input resistance (>600 M $\Omega$  for PLTS [Kawaguchi, 1993] vs. <200 M\Omega for LTS). In addition, these authors also occasionally observed PLTS neurons with the same characteristics described by Kawaguchi (1993) in the same slices as those in which LTS neurons were recorded. Only a few examples of this cell type were recorded, and none was recovered so their morphology and peptide expression remain unknown. Although it is possible that the LTS neurons and the PLTS neurons represent the extremes of a distribution of properties of a single neuronal type as pointed out by Koós and Tepper (1999), the differences were significant enough to classify the LTS as a different cell type, as can readily be seen by comparing panels H and I in Figure 8.3.

#### A. Synaptic Connectivity

Like the PV+ FS interneurons, paired recordings between LTS interneurons and spiny neurons revealed that LTS interneurons exerted a particularly strong inhibitory effect on spiny neurons. Small depolarizing pulses to LTS neurons evoked brief, high frequency (>200 Hz) bursts of spikes riding on an LTS. These bursts were capable of delaying or aborting depolarization-induced spiking in postsynaptic spiny neurons (Koós and Tepper, 1999; Fig. 8.3J). These characteristics suggest that along with PV+ FS interneurons, LTS cells play a significant role in mediating powerful feedforward inhibition of spiny neurons.

#### V. CALRETININ INTERNEURONS

Calretinin (CR)-expressing interneurons are medium sized  $(12-20\mu m \text{ in diameter})$ . They issue a small number of smooth, aspiny dendrites that branch sparingly and taper into very thin processes (Bennett and Bolam, 1993). All the anatomical information we have is from immunocytochemical studies as there have been no recordings or biocytin labeling, so little is known about the detailed neurocytology of rat CR+ neurons. Examples are shown in Figure 8.4. CR+ interneurons make up a similar proportion of neurons in the rodent striatum as the PV+, FS and SOM/NPY GABAergic interneurons, about 0.8% (Rymar et al., 2004). However, in primates including humans, the proportion of CR+ neurons is much greater and outnumbers that of PV+ and SOM/NPY interneurons by 3 or 4 to 1 (Wu and Parent, 2000). Furthermore, in the human striatum, there are four morphologically distinct types of neurons that express CR (Prensa et al., 1998). Neonatal hypoxia results in the neurogenesis of CR+ striatal interneurons in rats that persists for at least 5 months after induction. Interestingly the neurogenesis appears limited to the CR+ interneurons since there is no neurogenesis of striatal neurons that express markers for any of the other striatal interneurons or projection neurons (Yang et al., 2008).

Almost nothing is known about the neurophysiology of the calretinin interneurons. There have been no recordings of these cells in vitro to date, and no way to identify them from in vivo recordings.

## VI. OTHER GABAERGIC INTERNEURONS: TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS

In addition to the classically recognized striatal interneurons described above, there appears to be (at least one) additional population of neurons in the striatum. Originally identified in the striatum of adult monkeys by tyrosine



**FIGURE 8.4** Striatal calretinin intereurons. A. 2-dimensional projection of 40 deconvolved 1  $\mu$ m optical sections through mouse striatum immunostained for CR shows a single medium sized, aspiny CR+ interneuron. B. Medium sized mouse striatal CR+ interneuron. Arrows point to large axonal varicosities, presumably synaptic boutons. C. Single 60 $\mu$ m section through mouse striatum immunostained for CR shows a medium sized aspiny CR+ interneuron with two primary dendrites visible. D. Single section through primate striatum shows a medium sized aspiny interneuron immunoreactive for CR. Note the varicose dendrites and invaginated nuclear envelope (arrow). Panel C courtesy of Paul Bolam, modified from Bennett and Bolam, 1994b.

hydroxylase (TH) immunocytochemistry (Dubach et al., 1987), striatal TH-immunoreactive (TH+) neurons have subsequently been reported in a number of other species including rat (Tashiro et al., 1989a,b; Meredith et al., 1999), mouse (Mao et al., 2001; Petroske et al., 2001), monkeys (Betarbet et al., 1997; Mazloom and Smith, 2006) and man (Cossette et al., 2005; Huot and Parent, 2007).

In addition to TH, others have reported the existence of striatal neurons immunoreactive for l-aromatic acid decarboxylase (AADC) in normal rats (Tashiro et al., 1989a; Mura et al., 1995, 2000; Meredith et al., 1999), as well as neurons immunoreactive for dopamine itself. Investigators who have examined two or more of these markers generally agree that there are more TH<sup>+</sup> neurons than AADC<sup>+</sup> or DA<sup>+</sup> neurons in the striatum of rodents (Mura et al., 1995, 2000; Meredith et al., 1999), suggesting that not all striatal TH<sup>+</sup> neurons are dopaminergic, and further, that there are *at least two* distinct populations of striatal TH<sup>+</sup> neurons.

It is difficult to summarize the literature on the morphology and incidence of the striatal TH+ neurons because the description of both are surprisingly variable, ranging from "in the tens of thousands" (Dubach et al., 1987) to a low of about of about 1 TH+ neuron per section in humans (Huot et al., 2007). Other estimates range from to several 10s of neurons per 30–60 $\mu$ m section in rat (e.g., DA+ neurons in Mura et al., 2000) or monkey (TH + /DAT+ neurons in Tandé et al., 2006) to very large numbers in some studies (>450,000 TH+ neurons/striatum in primate; Palfi et al., 2002).

Reports concerning the somatic size and neurocytology of striatal TH+ neurons are equally disparate with cells being reported as small as  $6-12 \mu m$  in diameter (Meredith et al., 1999; Jollivet et al., 2004), 8-12µm (Dubach et al., 1987; Mazloom and Smith, 2006) or up to 20µm in diameter (Tashiro et al., 1989b). There is further disagreement about the nuclear envelope, which is often used to identify neurons as projection neurons or interneurons since the spiny projection neurons always have smooth, noninvaginated nuclei whereas the PV+, CR+, and NOS/NPY GABAergic interneurons described above consistently show invaginated nuclei (Bolam et al., 1983). Some investigators claim that striatal TH+ neurons have the invaginated nuclei of interneurons (e.g., Dubach et al., 1987; Mazloom and Smith, 2006) while others claim that they possess the smooth nuclear envelopes of spiny projection neurons (Meredith et al, 1999). While most of the papers cited above report that striatal TH+ neurons exhibit smooth, sometimes varicose aspiny dendrites characteristic of other striatal interneurons, one claims that striatal TH+ neurons are spiny, express substance P or enkephalin, and are therefore spiny projection neurons (e.g., Darmopil et al., 2008).

In addition, striatal TH+ neurons are often described as immunoreactive for the dopamine transporter (DAT) as well as GABA or  $GAD_{67}$ , suggesting that they are both dopaminergic and GABAergic (e.g., Betarbet et al., 1997; Cossette et al., 2005; Mazloom and Smith, 2006; Tandé et al., 2006; Huot and Parent, 2007). In primates, 8% of the striatal TH+ neurons colocalized CR+ (Mazloom and Smith, 2006).

Some investigators argue that TH+ neurons are not present in the striatum of control animals at all, but only appear after 6-OHDA or MPTP lesions of the nigrostriatal system in rat (Meredith et al., 1997; Lopez-Real et al., 2003; Darmopil et al., 2008) or monkey (Mazloom and (162



**FIGURE 8.5** Striatal EGFP-TH interneurons. A. Low magnification fluorescence photomicrograph of a section through the striatum of a BAC transgenic mouse that expresses EGFP under the control of the TH promoter. Arrows point to some of the EGFP-TH+ neurons visible. B, C. Higher magnification photomicrographs of striatal EGFP-TH+ neurons. D. Drawing tube reconstruction of a striatal Type II EGFP-TH+ neuron filled with biocytin after recording in vitro. Note the varicose dendrites (black) and axonal arborization (red). E. Responses to current injections in a Type I EGFP-TH+ neuron. The extreme spike frequency adaptation leading to complete spike failure, the sag in response to hyperpolarizing current pulses and the high input resistance (upper inset) and wide duration action potential (lower inset) are all characteristic of this, the most common of the striatal EGFP-TH+ neurons. F. Some Type I neurons exhibit spontaneous activity in vitro. G. Local electrical stimulation (arrow) elicits GABA<sub>A</sub> IPSPs sufficient to block spiking (left panel, red trace) as well as glutamatergic EPSPs sufficient to elicit spiking (middle panel, red trace) in Type I neurons. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

Smith, 2006), while still others argue that they are not present at all in control *or* lesioned rat or mouse striatum, but only in primates (Dubach et al., 1987; Betarbet et al., 1997; Yang et al., 2008).

It is obviously difficult to know what to make of these disparate findings. Some of the discrepancies can almost certainly be attributed to species differences, while others may be the result of different technical artifacts associated with fixation, preservation and the vagaries of immunocytochemistry. It is clearly difficult to pick out TH-immunostained somata in the striatal neuropil from the background of dense staining of TH+ nigrostriatal dopaminergic axons and terminals, and this undoubtedly contributes to the discrepant results. There is, however, a general consensus, at least based on the most recent primate data, that striatal TH+ neurons do exist, and represent a novel class or classes of striatal interneuron that also express the dopamine transporter (DAT) as well as GAD, and are therefore likely both dopaminergic and GABAergic. These neurons appear to be distinct from the other, previously defined striatal PV+, CR+ or NOS/NPY+ GABAergic interneurons (Betarbet et al., 1997; Cossette et al., 2005; Huot and Parent, 2007).

#### A. Striatal EGFP-TH+ Interneurons

Until recently, there has been no way to identify striatal TH+ neurons in brain slices in order to obtain recordings from them that would allow description of their electrophysiological properties, afferent or efferent connectivity, or detailed morphology of the dendritic or axonal arborization. With the advent of strains of mice genetically engineered to express enhanced green fluorescent protein (EGFP) under the control of cell-type specific promoters such as TH, ChAT or PV (Gong et al., 2007), one can identify almost any cell type desired in a brain slice and then use IR-DIC optics to patch that neuron cell and record.

Using this approach in mice engineered to express EGFP in neurons that express TH, we have identified a population of EGFP-TH+ neurons in normal mouse striatum (Ibanez-Sandoval et al., 2007, 2008). These neurons have been classified into four electrophysiologically different cell types that are clearly distinct from striatal spiny neurons, the cholinergic interneuron, or the PV+ or NOS/NPY GABAergic interneurons. Injection of fluorescent-labeled beads into substantia nigra and GP do not result in retrograde labeling of the EGFP-TH+ neurons, demonstrating that they are striatal interneurons.

Two of these cell types, Types II and III are a type of FS interneuron, whereas Type I neurons are characterized by a very strong spike frequency adaptation that leads to complete spike failure after 100 ms or so in response to strong depolarizing current injections. Type IV neurons fire low threshold spikes, but do not display the prolonged plateau depolarizations that characterize the NOS/NPY PLTS neuron described by Kawaguchi and others (Kawaguchi, 1993, Kawaguchi et al., 1995). This cell type is very similar to the LTS neuron described by Koós and Tepper (1999).

All the EGFP-TH+ interneurons are well integrated into the functional architecture of the striatum. Local stimulation elicits EPSPs and IPSPs and paired recordings show that the most common efferent target is the spiny projection neurons. Evoked spiking in striatal EGFP-TH+ neurons produces potent IPSP/Cs in spiny neurons sufficient to delay spikes evoked by intracellular depolarization. The IPSP/Cs are blocked by picrotoxin or bicuculline demonstrating that the EGFP-TH+ neurons are also GABAergic (Ibanez-Sandoval et al., 2008). Their dopaminergic nature has yet to be conclusively verified.

#### **VII. SUMMARY AND CONCLUSIONS**

Striatal GABAergic interneurons participate in a powerful feedforward inhibitory circuit that is likely the primary mechanism by which the spike timing of the striatal output neurons is controlled. Of the three types of striatal GABAergic interneurons that have been recognized for some time, we have the most information about the PV+ FS interneurons and the NOS/NPY+ PLTS interneurons, since they have been identified, recorded and intracellularly labeled in vitro. Although we tend to consider the PV+ fast spiking interneuron as *the* striatal fast spiking interneuron, there are clues from various studies in striatum of rodents and primates, both older and more recent, that there may well be more than a single type of striatal FS interneuron, and perhaps even more than one type of PV+ striatal fast spiking interneuron.

Similarly, it is unclear if the neuron originally described by Kawaguchi (1993) as the PLTS interneuron is *the* striatal GABAergic interneuron that fires low threshold  $Ca^{2+}$ spikes and whether or not all such neurons express the same neurochemical makeup. Recent data from several sources suggest otherwise.

We know very little about the physiological role and detailed neurocytology of the CR+ interneuron, since as of this writing, no one has yet succeeded in recording from these cells and filling them. This will undoubtedly change in the very near future as soon as a BAC transgenic mouse that expresses EGFP selectively in CR+ neurons is created. Nevertheless, the finding that there are 4 morphologically distinct types of CR+ neurons in human striatum indicates the possibility that there is more than one functional subtype of striatal CR+ GABAergic interneuron.

A BAC transgenic mouse that expresses EGFP selectively in neurons with the TH promoter has recently allowed the description of four electrophysiologically distinct subtypes of striatal EGFP-TH+ interneurons, three of which display electrophysiological characteristics that are completely unlike those of any previously described striatal neuron.

It is becoming clear that the striatum enjoys a much richer diversity of GABAergic interneurons than was once thought. We are only now beginning to discover the functional differences between the varieties of these interneurons. As new molecular, anatomical and physiological techniques continue to become available, particularly more strains of transgenic mice that selectively label neurons that express calretinin and other peptides, receptors and neuroactive agents expressed by striatal neurons with fluorescent markers such as EGFP, it is almost certain that the number of functionally different striatal GABAergic interneurons, currently numbering at least 8, will continue to grow.

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#### REFERENCES

- Alkondon M, Albuquerque EX (1993) Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. J Pharmacol Exp Ther 265:1455–1473.
- Aoki C, Pickel VM (1988) Neuropeptide Y-containing neurons in the rat striatum: ultrastructure and cellular relations with tyrosine hydroxylase-containing terminals and with astrocytes. Brain Res 459:205–225.
- Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M (1994) Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. J Neurosci 14:3969–3984.

- Bennett BD, Bolam JP (1993) Characterization of calretinin-immunoreactive structures in the striatum of the rat. Brain Res 609:137–148.
- Bennett BD, Bolam JP (1994a) Synaptic input and output of parvalbuminimmunoreactive neurons in the neostriatum of the rat. Neuroscience 62:707–719.
- Bennet BD and Bolam JP (1994b) Localization of calcium binding proteins in the neostriatum, In: The Basal Ganglia IV – New Ideas and Data on Structure and Function (Percheron G, McKenzie JS, Fegér J, eds) Adv Behav Biol 41:21–34.
- Betarbet R, Turner R, Chockkan V, DeLong MR, Allers KA, Walters J, Levey AI, Greenamyre JT (1997) Dopaminergic neurons intrinsic to the primate striatum. J Neurosci 17:6761–6768.
- Berke JD (2008) Uncoordinated firing rate changes of striatal FS interneurons during behavioral task performance. J Neurosci 28:10075–10080.
- Berke JD, Okatan M, Skurski J, Eichenbaum HB (2004) Oscillatory entrainment of striatal neurons in freely moving rats. Neuron 43:883–896.
- Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bolam JP, Clarke DJ, Smith AD, Somogyi P (1983) A type of aspiny neuron in the rat neostriatum accumulates [3H] gamma-aminobutyric acid: combination of Golgi-staining, autoradiography, and electron microscopy. J Comp Neurol 213:121–134.
- Bolam JP, Powell JF, Wu JY, Smith AD (1985) Glutamate decarboxylaseimmunoreactive structures in the rat neostriatum: a correlated light and electron microscopic study including a combination of Golgi impregnation with immunocytochemistry. J Comp Neurol 237:1–20.
- Bracci E, Centonze D, Bernardi G, Calabresi P (2002) Dopamine excites fast-spiking interneurons in the striatum. J Neurophysiol 87:2190–2194.
- Bracci E, Centonze D, Bernardi G, Calabresi P (2003) Voltage-dependent membrane potential oscillations of rat striatal fast-spiking interneurons. J Physiol 549:121–130.
- Chang HT, Kita H (1992) Interneurons in the rat striatum: relationships between parvalbumin neurons and cholinergic neurons. Brain Res 574:307–311.
- Chang HT, Wilson CJ, Kitai ST (1982) A Golgi study of rat neostriatal neurons: light microscopic analysis. J Comp Neurol 208:107–126.
- Chesselet MF, Robbins E (1989) Characterization of striatal neurons expressing high levels of glutamic acid decarboxylase messenger RNA. Brain Res 492:237–244.
- Centonze D, Bracci E, Pisani A, Gubellini P, Bernardi G, Calabresi P (2002) Activation of dopamine D1-like receptors excites LTS interneurons of the striatum. Eur J Neurosci 15:2049–2052.
- Centonze D, Grande C, Usiello A, et al. (2003) Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. J Neurosci 23:6245–6254.
- Chesselet MF, Graybiel AM (1986) Striatal neurons expressing somatostatin-like immunoreactivity: evidence for a peptidergic interneuronal system in the cat. Neuroscience 17:547–571.
- Cossette M, Levesque D, Parent A (2005) Neurochemical characterization of dopaminergic neurons in human striatum. Parkinsonism Relat Disord 11:277–286.
- Cowan RL, Wilson CJ, Emson PC, Heizmann CW (1990) Parvalbumincontaining GABAergic interneurons in the rat neostriatum. J Comp Neurol 302:197–205.

- Czubayko U, Plenz D (2002) Fast synaptic transmission between striatal spiny projection neurons. Proc Natl Acad Sci USA 99:15764–15769.
- Darmopil S, Muneton-Gomez VC, de Ceballos ML, Bernson M, Moratalla R (2008) Tyrosine hydroxylase cells appearing in the mouse striatum after dopamine denervation are likely to be projection neurones regulated by 1-DOPA. Eur J Neurosci 27:580–592.
- DiFiglia M, Aronin N (1982) Ultrastructural features of immunoreactive somatostatin neurons in the rat caudate nucleus. J Neurosci 2:1267–1274.
- Dubach M, Schmidt R, Kunkel D, Bowden DM, Martin R, German DC (1987) Primate neostriatal neurons containing tyrosine hydroxylase: immunohistochemical evidence. Neurosci Lett 75:205–210.
- Figueredo-Cardenas G, Morello M, Sancesario G, Bernardi G, Reiner A (1996) Colocalization of somatostatin, neuropeptide Y, neuronal nitric oxide synthase and NADPH-diaphorase in striatal interneurons in rats. Brain Res 735:317–324.
- Freiman I, Anton A, Monyer H, Urbanski MJ, Szabo B (2006) Analysis of the effects of cannabinoids on identified synaptic connections in the caudate-putamen by paired recordings in transgenic mice. J Physiol 575:789–806.
- Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6:347–470.
- Galarreta M, Hestrin S (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72–75.
- Galarreta M, Hestrin S (2001) Electrical synapses between GABA-releasing interneurons. Nat Rev Neurosci 2:425–433.
- Galarreta M, Hestrin S (2002) Electrical and chemical synapses among parvalbumin fast-spiking GABAergic interneurons in adult mouse neocortex.. Proc Natl Acad Sci USA 99:12438–12443.
- Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci 27:9817–9823.
- Gustafson N, Gireesh-Dharmaraj E, Czubayko U, Blackwell KT, Plenz D (2006) A comparative voltage and current-clamp analysis of feedback and feedforward synaptic transmission in the striatal microcircuit in vitro. J Neurophysiol 95:737–752.
- Guzman JN, Hernandez A, Galarraga E, Tapia D, Laville A, Vergara R, Aceves J, Bargas J (2003) Dopaminergic modulation of axon collaterals interconnecting spiny neurons of the rat striatum. J Neurosci 23:8931–8940.
- Huot P, Parent A (2007) Dopaminergic neurons intrinsic to the striatum. J Neurochem 101:1441–1447.
- Ibanez-Sandoval O, Abercrombie ED, Koós T, Tepper JM (2007) Electrophysiology and morphology of a striatal dopaminergic neuron in mouse. Soc Neurosci Abstr 33:515.22.
- Ibanez-Sandoval O, Tecuapetla F, Shah F, Altinbilek B, Koós T, Tepper JM (2008) Synaptic connections of striatal dopaminergic neurons. Soc Neurosci Abstr 37:179.16.
- Jollivet C, Montero-Menei CN, Venier-Julienne MC, Sapin A, Benoit JP, Menei P (2004) Striatal tyrosine hydroxylase immunoreactive neurons are induced by L-dihydroxyphenylalanine and nerve growth factor treatment in 6-hydroxydopamine lesioned rats. Neurosci Lett 362:79–82.
- Kawaguchi Y (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci 13:4908–4923.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.

- Kincaid AE, Zheng T, Wilson CJ (1998) Connectivity and convergence of single corticostriatal axons. J Neurosci 18:4722–4731.
- Kita H (1993) GABAergic circuits of the striatum. Prog Brain Res 99:51–72.
- Kita H, Kosaka T, Heizmann CW (1990) Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. Brain Res 536:1–15.
- Koós T (2000) The Role of GABAergic Interneurons in Neostriatal Function, Ph.D. dissertation, Rutgers The State University of New Jersey-Newark, (Publication No. AAT 9967086).
- Koós T, Tepper JM (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nat Neurosci 2:467–472.
- Koós T, Tepper JM (2002) Dual cholinergic control of fast-spiking interneurons in the neostriatum. J Neurosci 22:529–535.
- Koós T, Tepper JM, Wilson CJ (2004) Comparison of IPSCs evoked by spiny and fast-spiking neurons in the neostriatum. J Neurosci 24:7916–7922.
- Kubota Y, Inagaki S, Kito S, Shimada S, Okayama T, Hatanaka H, Pelletier G, Takagi H, Tohyama M (1988) Neuropeptide Y-immunoreactive neurons receive synaptic inputs from dopaminergic axon terminals in the rat neostriatum. Brain Res 458:389–393.
- Kubota Y, Inagaki S, Kito S, Wu JY (1987) Dopaminergic axons directly make synapses with GABAergic neurons in the rat neostriatum. Brain Res 406:147–156.
- Kubota Y, Mikawa S, Kawaguchi Y (1993) Neostriatal GABAergic interneurones contain NOS, calretinin or parvalbumin. Neuroreport 5:205–208.
- Kubota Y, Kawaguchi Y (2000) Dependence of GABAergic synaptic areas on the interneuron type and target size. J Neurosci 20:375–386.
- Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.
- Lenz S, Perney TM, Qin Y, Robbins E, Chesselet MF (1994) GABA-ergic interneurons of the striatum express the Shaw-like potassium channel Kv3.1. Synapse 18:55–66.
- Li JL, Kaneko T, Mizuno N (2002) Synaptic association of dopaminergic axon terminals and neurokinin-1 receptor-expressing intrinsic neurons in the striatum of the rat. Neurosci Lett 324:9–12.
- Lopez-Real A, Rodriguez-Pallares J, Guerra MJ, Labandeira-Garcia JL (2003) Localization and functional significance of striatal neurons immunoreactive to aromatic L-amino acid decarboxylase or tyrosine hydroxylase in rat Parkinsonian models. Brain Res 969:135–146.
- Luk KC, Sadikot AF (2001) GABA promotes survival but not proliferation of parvalbumin-immunoreactive interneurons in rodent neostriatum: an in vivo study with stereology. Neuroscience 104:93–103.
- Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 26:3875–3884.
- Mallet N, Le Moine C, Charpier S, Gonon F (2005) Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo. J Neurosci 25:3857–3869.
- Mao L, Lau YS, Petroske E, Wang JQ (2001) Profound astrogenesis in the striatum of adult mice following nigrostriatal dopaminergic lesion by repeated MPTP administration. Brain Res Dev Brain Res 131:57–65.
- Mazloom M, Smith Y (2006) Synaptic microcircuitry of tyrosine hydroxylase-containing neurons and terminals in the striatum of 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine-treated monkeys. J Comp Neurol 495:453–469.
- Meredith GE, Farrell T, Kellaghan P, Tan Y, Zahm DS, Totterdell S (1999) Immunocytochemical characterization of catecholaminergic

neurons in the rat striatum following dopamine-depleting lesions. Eur J Neurosci 11:3585–3596.

- Misgeld U, Drew G, Yanovsky Y (2007) Presynaptic modulation of GABA release in the basal ganglia. Prog Brain Res 160:245–259.
- Mura A, Jackson D, Manley MS, Young SJ, Groves PM (1995) Aromatic L-amino acid decarboxylase immunoreactive cells in the rat striatum: a possible site for the conversion of exogenous L-DOPA to dopamine. Brain Res 704:51–60.
- Mura A, Linder JC, Young SJ, Groves PM (2000) Striatal cells containing aromatic L-amino acid decarboxylase: an immunohistochemical comparison with other classes of striatal neurons. Neuroscience 98:501–511.
- Narushima M, Uchigashima M, Hashimoto K, Watanabe M, Kano M (2006) Depolarization-induced suppression of inhibition mediated by endocannabinoids at synapses from fast-spiking interneurons to medium spiny neurons in the striatum. Eur J Neurosci 24:2246–2252.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a stereological study using the cavalieri and optical disector methods. J Comp Neurol 366:580–599.
- Palfi S, Leventhal L, Chu Y, Ma SY, Emborg M, Bakay R, Deglon N, Hantraye P, Aebischer P, Kordower JH (2002) Lentivirally delivered glial cell line-derived neurotrophic factor increases the number of striatal dopaminergic neurons in primate models of nigrostriatal degeneration. J Neurosci 22:4942–4954.
- Parthasarathy HB, Graybiel AM (1997) Cortically driven immediateearly gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. J Neurosci 17:2477–2491.
- Petroske E, Meredith GE, Callen S, Totterdell S, Lau YS (2001) Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. Neuroscience 106:589–601.
- Plenz D, Kitai ST (1998) Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fastspiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. J Neurosci 18:266–283.
- Plotkin JL, Wu N, Chesselet MF, Levine MS (2005) Functional and molecular development of striatal fast-spiking GABAergic interneurons and their cortical inputs. Eur J Neurosci 22:1097–1108.
- Prensa L, Gimenez-Amaya JM, Parent A (1998) Morphological features of neurons containing calcium-binding proteins in the human striatum. J Comp Neurol 390:552–563.
- Ramanathan S, Hanley JJ, Deniau JM, Bolam JP (2002) Synaptic convergence of motor and somatosensory cortical afferents onto GABAergic interneurons in the rat striatum. J Neurosci 22:8158–8169.
- Rivera A, Alberti I, Martin AB, Narvaez JA, de la Calle A, Moratalla R (2002) Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. Eur J Neurosci 16:2049–2058.
- Rymar VV, Sasseville R, Luk KC, Sadikot AF (2004) Neurogenesis and stereological morphometry of calretinin-immunoreactive GABAergic interneurons of the neostriatum. J Comp Neurol 469:325–339.
- Sullivan MA, Chen H, Morikawa H (2008) Recurrent inhibitory network among striatal cholinergic interneurons. J Neurosci 28:8682–8690.
- Tashiro Y, Kaneko T, Sugimoto T, Nagatsu I, Kikuchi H, Mizuno N (1989a) Striatal neurons with aromatic L-amino acid decarboxylaselike immunoreactivity in the rat. Neurosci Lett 100:29–34.
- Tashiro Y, Sugimoto T, Hattori T, Uemura Y, Nagatsu I, Kikuchi H, Mizuno N (1989b) Tyrosine hydroxylase-like immunoreactive neurons in the striatum of the rat. Neurosci Lett 97:6–10.

- Taverna S, Canciani B, Pennartz CM (2007) Membrane properties and synaptic connectivity of fast-spiking interneurons in rat ventral striatum. Brain Res 1152:49–56.
- Tecuapetla F, Carrillo-Reid L, Guzman JN, Galarraga E, Bargas J (2005) Different inhibitory inputs onto neostriatal projection neurons as revealed by field stimulation. J Neurophysiol 93:1119–1126.
- Tecuapetla F, Carrillo-Reid L, Bargas J, Galarraga E (2007) Dopaminergic modulation of short-term synaptic plasticity at striatal inhibitory synapses. Proc Natl Acad Sci USA 104:10258–10263.
- Tecuapetla F, Koós T, Tepper JM, Kabbani N, Yekel M (2009) Differential dopaminergic modulation of neostriatal synaptic connections of striatopallidal axon collaterals. J. Neurosci. 29:8977–8990.
- Tepper JM, Bolam JP (2004) Functional diversity and specificity of neostriatal interneurons. Curr Opin Neurobiol 14:685–692.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tepper JM, Wilson CJ, Koos T (2008) Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons. Brain Res Rev 58:272–281.
- Tunstall MJ, Oorschot DE, Kean A, Wickens JR (2002) Inhibitory interactions between spiny projection neurons in the rat striatum. J Neurophysiol 88:1263–1269.

- Vincent SR, Johansson O (1983) Striatal neurons containing both somatostatin- and avian pancreatic polypeptide (APP)-like immunoreactivities and NADPH-diaphorase activity: a light and electron microscopic study. J Comp Neurol 217:264–270.
- Vincent SR, Staines WA, Fibiger HC (1983) Histochemical demonstration of separate populations of somatostatin and cholinergic neurons in the rat striatum. Neurosci Lett 35:111–114.
- Vuillet J, Dimova R, Nieoullon A, Kerkerian-Le Goff L (1992) Ultrastructural relationships between choline acetyltransferase- and neuropeptide Y-containing neurons in the rat striatum. Neuroscience 46:351–360.
- Wu Y, Parent A (2000) Striatal interneurons expressing calretinin, parvalbumin or NADPH-diaphorase: a comparative study in the rat, monkey and human. Brain Res 863:182–191.
- Yang Z, You Y, Levison SW (2008) Neonatal hypoxic/ischemic brain injury induces production of calretinin-expressing interneurons in the striatum. J Comp Neurol 511:19–33.

# Endocannabinoid Signaling in the Striatum

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## I. INTRODUCTION: THE ENDOCANNABINOID SYSTEM

Endocannabinoid juxtacrine and paracrine signaling is widespread throughout the brain and body, representing one of the most prevalent lipid/fatty acid-based intercellular communication systems in mammals (Pacher et al., 2006). The cannabinoid part of the name is derived from the cannabis sativa plant and the drugs, marijuana and hashish among others, made from this plant. The receptors for these drugs are the major targets of a group of lipidderived signaling molecules known as the eCBs. Two arachidonoyl-containing fatty acids, arachidonoylethanolamide (AEA or anandamide) and 2-arachydonoyl glycerol (2-AG) are thought to produce the majority of eCB signaling. These two compounds are synthesized from arachidonate-containing membrane lipids via separate pathways consisting of several enzyme-catalyzed steps (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). Once AEA and 2-AG are produced they can escape the plasma membrane, and are released from cells to act on neighboring cells. Because of their highly hydrophobic nature these compounds are not likely stored inside of vesicles, and there is no evidence for vesicular involvement in of eCB production and release is that the compounds are made "on-demand" following calcium influx into cells and/ or activation of metabotropic receptors (particularly receptors that couple to Gq-type G-proteins). Release is thought to occur automatically following synthesis. However, there is some evidence for pools of pre-synthesized eCB and regulated release, particularly in neurons of the central nervous system (Ronesi et al., 2004; Edwards et al., 2006; Adermark and Lovinger, 2007b). The mechanisms controlling eCB release are not yet fully understood, and it is still not clear how the hydrophobic eCBs cross what is thought to be a hydrophilic extracellular environment to produce actions on nearby cells.

eCB release (Wilson and Nicoll, 2001). The simplest model

The intercellular signaling functions of eCBs are mediated by CB receptors that were originally identified as targets for  $\Delta$ -9-THC, the psychoactive ingredient of cannabis-derived drugs (Matsuda et al., 1990; Herkenham et al., 1991). The CB1 receptor is the main mediator of eCB actions in the brain, and is responsible for the majority of the intoxicating effects of natural and synthetic cannabinoid drugs (Pacher et al., 2006). Endocannabinoids can also activate the CB2 receptor, that is mainly found in the periphery but is apparently also present in the CNS (Munro et al., 1993, Van Sickle et al., 2005; Gong et al., 2006). In addition, AEA acts as a weak partial agonist at the TRPV1 vannilloid receptor-channel, and also has lower affinity interactions with other receptors and signaling molecules in the body and brain (Van Der Stelt and Di Marzo, 2004; Oz, 2006). The CB1 and CB2 receptors are class I G-proteincoupled receptors that activate Gi/o-type G-proteins. Activation of these G-protein subtypes normally produces inhibition of adenylyl cyclase, inhibition of voltage-gated calcium channels, and activation of certain potassium channels. Other intracellular signaling pathways, such as increased phosphorylation/activation of the multifunctional ERK kinase cascade can also result from CB1 activation (Wartmann et al., 1995; Davis et al., 2003).

Within the nervous system, the major function of eCBs appears to be mediation of "retrograde" signaling. Unlike traditional synaptic transmission, in which neurotransmitter release from the presynaptic axon terminal leads to postsynaptic receptor activation, retrograde signaling involves postsynaptic release of a compound that then acts on presynaptic receptors. In the case of eCB CNS actions, localization of synthetic enzymes for 2-AG suggests a postsynaptic locus of synthesis, while CB1 receptors are localized almost exclusively on presynaptic neuronal elements (Katona et al., 2001; Kofalvi et al., 2005; Katona et al., 2006; Uchigashima et al., 2007). There is also abundant physiological evidence for a retrograde signaling function of eCBs. The two major neurophysiological actions involving eCBs are short-term and long-term synaptic depression (reviewed in Chevaleyre and Castillo, 2003; Lovinger, 2008). Short-term depression (STD) can be produced by activation of postsynaptic metabotropic glutamate or acetylcholine receptors, and by depolarization of postsynaptic membrane potential. This latter, depolarization-induced synaptic depression is termed depolarization-induced suppression of excitation (DSE) or inhibition (DSI) depending on whether the net effect is to decrease glutamatergic excitatory or GABAergic inhibitory synaptic transmission. Long-term depression (LTD) is produced by repetitive synaptic activation that leads to a CB1-dependent, longlasting decrease in neurotransmitter release (Chevaleyre et al., 2006; Lovinger, 2008). Postsynaptic manipulations that alter eCB production can produce or inhibit STD and LTD (see Chevaleyre et al., 2006) for review). Maintained expression of STD and LTD involves activation of presynaptic CB1 receptors leading to a transient (STD) or long-lasting (LTD) decrease in the probability of neurotransmitter release (reviewed in Chevaleyre et al., 2006;

Lovinger, 2008). Thus, the predominant model of eCB signaling is that the compounds are synthesized and released from postsynaptic elements, traverse the synapses in a retrograde direction, and act on presynaptic CB1 receptors to decrease transmission. In the case of STD, including DSE and DSI, the depression appears to persist only as long as the eCBs are present in the synapse and CB1 receptors are activated. Inhibition of presynaptic voltage-gated calcium channels probably accounts for the presynaptic depression in STD. In LTD, the synaptic depression appears to outlast CB1 activation, and thus the receptor is thought to give rise to signaling within the presynaptic terminal that leads to a persistent decrease in neurotransmitter release probability (Chevaleyre et al., 2006; Lovinger, 2008).

Once released from postsynaptic cells, eCBs can persist at levels high enough to activate receptors for 10s of sec to several min (Robbe et al., 2001; Chevaleyre and Castillo, 2003; Kim and Alger, 2004; Ronesi et al., 2004; Makara et al., 2005; Szabo et al., 2006; Sheinin et al., 2008). Termination of the eCB synaptic signal is thought to involve enzymes that degrade AEA and 2-AG (Kim and Alger, 2004; Makara, 2005; Szabo et al., 2006) as well as cellular reuptake of the molecules (although the presence and identity of any transporter molecule that mediates this reuptake is controversial; see Lovinger, 2007, for discussion of this issue). At present the identification of molecular tools to examine transport and degradation are still at an early stage, and thus not much is known about which processes predominate at different synapses. In addition, identification of which eCB mediates the different forms of STD and LTD at different synapses is not nearly complete, although there is strong evidence for 2-AG mediation of DSI, STD and LTD at some synapses (Chevaleyre and Castillo, 2003; Kim and Alger, 2004; Makara et al., 2005; Uchigashima et al., 2007; Sheinin et al., 2008).

## II. ENDOCANNABINOIDS AND CANNABINOID RECEPTORS IN THE STRIATUM

#### A. The CB1 Receptor

CB1 is one of the most abundant GPCRs in the CNS and is expressed at high levels throughout the extended striatum but within discrete neuronal populations. This expression pattern is retained phylogenetically with similar expression patterns in rodents, canines and primates (Herkenham et al., 1001; Glass and Felder, 1997). The first studies of

CB1 expression used receptor binding to grossly map the distribution of CB1 in the brain (Herkenham et al., 1900; Herkenham et al., 1991; Jansen et al., 1992; Mailleux and Vanderhaeghen, 1992; Thomas et al., 1997). Cortical projections showed low CP55,940 binding; corpus callosum binding was lower than binding to the anterior commissure, while projections from the striatum to globus pallidus/ external pallidum (GP/GPe), substantia nigra pars reticulata (SNr) and entopeduncular nucleus/internal pallidum (GPi) displayed the highest binding (Herkenham et al., 1991) (see Chapter 1 for an overview of basal ganglia anatomy, and Chapter 5 for a description of striatal projection neurons). Quantitative receptor binding also revealed a dorsolateral to ventromedial gradient in striatal CB1 receptor density, with low binding in the nucleus accumbens (NAc), and other parts of the "extended striatum" such as central amygdala and bed nucleus of stria terminalis (BNST) (Herkenham et al., 1990; Herkenham et al., 1991; Jansen et al., 1992). CB1 receptor binding was dense in the SNr but less so in the ventral tegmental area (VTA). Ibotenate lesions of the striatum abolished receptor binding in the SNr while 6-hydroxy-dopamine lesions of substantia nigra did not alter binding, indicating that the source of CB1 was striatal (Herkenham et al., 1991).

Following the cloning of the CB1 receptor, in situ hybridization histochemistry (ISHH) was used to detect receptor-coding mRNA which produced results similar to radioligand binding, with mRNA absent from striatal projection areas, as expected. CB1 mRNA is expressed throughout the basal ganglia, with the notable exceptions of the dopaminergic neurons (Herkenham et al., 1991; Matsuda et al., 1993) and the GP. mRNA is frequently detected at different levels in distinct subsets of neurons within each region but in situ hybridization studies revealed moderate but uniform levels within the striatum with a dorsolateral to ventromedial gradient of expression. CB1 mRNA is low or nearly undetectable in many other subregions of the extended striatum (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Marsicano and Lutz, 1999).

Double labeling ISHH techniques have been employed to identify CB1-expressing neuronal subtypes. Parvalbumin (PV) and GAD67+ interneurons in the striatum express CB1 mRNA, but there is less evidence for expression in cholinergic, calretinin-containing and somatostatin-containing interneurons (Marsicano and Lutz, 1999; Hohmann and Herkenham, 2000; Martin et al., 2008) (see also Chapters 7 and 8, for striatal interneurons). CB1+ cells co-express GAD65 and 75% of these cells also express calbindin 28. Immunolocalization studies have recapitulated receptor binding for the most part (Fig. 9.1). Initial studies using commercially available antisera agreed with receptor binding but poor fidelity between lots has been noted for commercial sources (Grimsey et al., 2008). The most reliable antibodies have been generated by Dr. Kenneth Mackie's laboratory against C-terminus epitopes, although these differ as well in their ability to recognize a presumed oligomeric form (recognized by AbL73) or surface receptor clusters (AbL15), suggesting that the epitope may be partially blocked in situ by the signaling complex (Grimsey et al., 2008).

Messenger RNA and protein studies provided insight into the function of CB1 since the mRNA is often found far from the protein itself. Protein expression is similar to mRNA with notable exceptions. mRNA is not detected in



**FIGURE 9.1** CB1 immunoreactivity in the rostromedial (A,  $\sim$ Bregma + 1.10) and caudal (C,  $\sim$ Bregma -0.82) striatum detected by immunofluorescence using the L15 C-terminus antibody. A gradient of decreasing immunoreactivity is observed from the dorsolateral (DLS) to ventromedial (VMS) striatal subregions with a parallel pattern and topology in the globus pallidus (GP). DAPI stained nuclei from the same sections are shown in B and D for orientation. Higher magnification image showing striosome-like immunoreactivity is shown in panel E, with the corresponding image of DAPI staining in panel F indicating that the cells are evenly distributed. Immunoreactivity appears to be mostly in fibers and puncta with no apparent somata. Note the network of brightly-stained axons coursing through the less immunoreactive adjacent regions (E). Scale bar in A, 200  $\mu$ m, applies to images A–D. Scale bar in E, 50  $\mu$ m.

the GP while CB1+ fibers are rich in this region, showing a topographical organization that parallels DLS receptor binding, with projections to the dorsolateral GP having high levels of CB1 immunoreactivity and receptor binding (Fig. 9.1A,C; Tsou et al., 1998; Julian et al., 2003). This gradient shows higher CB1 levels in the dorsolateral striatum (DLS), with gradually declining levels in a dorsolateral to ventromedial gradient with projections radiating through the GP at Bregma -0.82 (Fig. 9.1C). Levels of expression are low in the limbic striatum and virtually undetectable in CeA (Katona et al., 2001). Both striatopallidal and striatonigral medium spiny neurons express CB1 (Tsou et al., 1998; Hohmann and Herkenham, 2000; Julian et al., 2003; Martin et al., 2008), although CB1 is estimated to be higher in striatonigral (i.e., D1 dopamine receptorexpressing, D1+) neurons (Martin et al., 2008). CB1 is also detected on glutamatergic afferents into the extended striatum (Uchigashima et al., 2007; Massi et al., 2008).

Ultrastructural localization studies in the striatum are not in complete agreement, however. Rodriguez and colleagues (2001) and Pickel and colleagues (2004) found CB1 is partially co-expressed with mu-opioid receptors and D2 receptors and on somata and dendrites in rats (Rodriguez et al., 2001; Pickel et al., 2004). Similar localization was reported in NAc, where CB1 was also associated with glutamatergic synapses (Pickel et al., 2004). Immunofluorescence reveals a striosome-like distribution in the DLS of mice that tapers in a lateral to medial gradient (Fig. 9.1A,C) (Julian et al., 2003; Villares, 2007; Martin et al., 2008). Immunofluorescence in mice using the L15 antiserum did not reveal any somatic CB1 in striatum (Fig. 9.1), but a study by Matyas and colleagues (2006) found low level somatodendritic staining in the medial striatum at the EM level (Matyas et al., 2006). Some studies have attributed somatic immunoreactivity to CB1 being translated while EM studies show it associated with the membrane in cell bodies and dendrites (Rodriguez et al., 2001; Pickel et al., 2004). Anatomical studies clearly support presynaptic CB1 in regulating neurotransmitter release but the role of somatodentritic CB1 is uncertain.

High levels of CB1 immunoreactivity are found in the substantia nigra pars reticulata (SNr) and, as discussed above, CB1 appears to be primarily on striatal efferents (Herkenham et al., 1991). Striatal topology and functional grouping is maintained at the level of projections to the rodent SNr, with dense CB1+ fibers terminating in the lateral SNr in an inverted teardrop pattern (Julian et al., 2003). The medial SNr receives projections from the NAc (Deniau et al., 1996),

while the lateral SNr projections are sensory-motor (Deniau et al., 1994). Similarly, CB1 mRNA is not detected in VTA DA neurons but protein is present on both excitatory and inhibitory afferent projections and in local interneurons where is regulates feed forward inhibition (Melis et al., 2004a; Melis et al., 2004b; Riegel and Lupica, 2004).

Figure 9.2 illustrates current knowledge about the location of CB1 receptors on synaptic terminals at synapses onto postsynaptic elements of striatal medium spiny neurons (MSNs) (Fig. 9.2A), and the axon terminals of the MSNs themselves (Fig. 9.2B).

### **B.** The CB2 Receptor

CB2 has traditionally been considered a cannabinoid receptor of the immune system, with brain expression limited to microglia (Cabral et al., 2008), however, CB2 was recently identified on neurons in the CNS using both PCR and immunodetection with multiple sources of antibody (Van Sickle et al., 2005; Gong et al., 2006). CB2 expression was localized to postsynaptic sites, dendritic structures and cell bodies within the cortex, amygdala, striatum and SNr. Expression of CB2 is estimated to be some 30-fold lower in brain than in spleen (Gong et al., 2006).

## C. TRPV1

The transient receptor potential vannilloid 1 (TRPV1) is a temperature sensitive receptor-channel that is activated by capsaicin, the hydrophobic, pungent compound found in peppers and other "hot" foods (Caterina et al., 1997). This receptor-channel can also be activated by AEA, although it is a relatively weak partial agonist at TRPV1 (reviewed in Van Der Stelt and Di Marzo, 2004). Mezey et al. (2000) showed tyrosine hydroxylase (TH) and VR1 colabeling in SNc by in situ hybridization and immunochemistry (Mezey et al., 2000). These authors also mentioned observing TRPV1 immunoreactivity in the striatum and CeA and these observations in striatum were subsequently corroborated by immunodetection (Maccarrone et al., 2008). In situ autoradiography for TRPV1 also provided evidence for expression of these receptors in striatum (Tzavara et al., 2006).

## D. Endocannabinoids in Striatum

Both AEA and 2-AG are present in striatum at levels comparable to or exceeding that of other brain regions. Tissue eCBs have been measured using fresh or frozen striatal


FIGURE 9.2 CB1 Receptors on axon terminals of afferents innervating striatal MSNs and terminals of MSNs themselves. (A) Schematic diagram of a striatal MSN including soma, dendrites and dendritic spines. Predominant sites of innervation are shown for cortical glutamatergic, MSN-MSN GABAergic, fast-spiking interneuron (FSN)-MSN GABAergic, and lowthreshold spiking interneuron (LTSN)-MSN GABAergic synapses, with presence or absence of CB1 receptors on axon terminals indicated. (B) Schematic diagram of striatonigral synapses onto neurons in SN pars reticulata (direct pathway), indicating CB1 receptor presence on GABAergic terminals at these synapses. (C) Schematic diagram of striatopallidal synapses onto neurons in GP (indirect pathway), indicating CB1 receptor presence on GABAergic terminals at these synapses.

extracts, striatal brain slices, and organotypic slice cultures of striatum, mainly taken from rat brain (Stella et al., 1997; Giuffrida et al., 1999; Marsicano et al., 2002; Jung et al., 2005; Ade and Lovinger, 2007; Rademacher et al., 2008). Chromatographic separation of the eCBs followed by mass spectroscopy to identify individual compounds is the method of choice, since eCBs are generally present at low concentrations in tissue. Measurements in striatum from different preparations have produced general agreement that tissue concentrations of 2-AG (nmol/mg tissue) are several orders of magnitude higher than those of AEA (pmol/mg tissue). Indeed, AEA levels are usually just above detectability in a single striatal slice. However, this difference in concentration may not accurately reflect the eCB pools involved in intercellular communication, as there is likely to be a metabolic pool of 2-AG, but not AEA. Measurements of whole tissue eCB content are useful, but we are most interested in examining eCB release from cells, as that process will determine the paracrine and juxtacrine actions of these neuromodulators.

#### E. Biosynthetic Enzymes

Compared to our current knowledge of CB1 expression and distribution, relatively little is known about the expression of the biosynthetic enzymes for eCBs. Frequently the expression parallels CB1 but the pre/postsynaptic distribution of the biosynthetic enzymes may provide insight into the differential roles of 2-AG and anandamide in different neural pathways.

#### 1. 2-AG: DAGL a/b

Uchigashima and colleagues (2007) performed an extensive anatomical and physiological study of DAGLa in the striatum (Uchigashima et al., 2007). DAGLa distribution was compared with VGLUT, CB1, mGluR5, D1 and D2 immunoreactivity. DAGLa immunoreactivity was speckled with membrane and vesicle localization. Expression was highest in the DLS and located in post-synaptic structures in a gradient with the highest levels in extrasynaptic spines, decreasing to the soma. This expression pattern was also observed for mGluR5 in the striatum while only low levels of mGluR1 were detected. DAGLa was detected at both symmetric and asymmetric synapses at the EM level but slightly higher in D1- than D2-expressing neurons. There was no overlap between VGlut and DAGLa, indicating that 2AG is not synthesized in excitatory terminals.

Matyas et al. (2008) used ISHH to localize DAGLa in midbrain and found high levels of expression in the SNc and VTA, with lower levels in other midbrain regions, including SNr (Matyas et al., 2006). Immunostaining revealed a punctate pattern in the neuropil associated with three putative synaptic populations by EM. The first is presumably glutamatergic while the second and third populations likely represent MSNs and striatal interneurons. This pattern of immunoreactivity was complementary to CB1 staining, which was found at both glutamatergic and GABAergic axon terminals in the VTA.

#### 2. Anandamide: NAPE-PLD and Other Enzymes

Anandamide is believed to be produced primarily from (NAPE). n-acetylphosphatidyl ethanolamine Many n-acyltransferase enzymes have been proposed to mediate production of the NAPE but the suggested rate-limiting enzyme, NAPE-phospholipase D (PLD), remains a controversial subject. A NAPE-PLD enzyme has been cloned that can catalyze AEA formation when heterologously expressed in cells (Okamoto et al., 2004). This enzyme shows limited distribution in brain, with the highest levels of expression in the thalamus and hippocampus, and enzyme activity as well as NAPE-PLD mRNA expression was detected in basal ganglia, but there is no specific information on expression in the striatum (Morishita et al., 2005). Morishita and colleagues (2005) measured NAPE-PLD expression and activity in homogenates from various brain regions and noted moderate levels in the basal ganglia but the techniques employed did not allow for cellular resolution. After careful characterization of the antisera, immunohistochemical analysis Egertová et al. (2008) showed low immunoreactivity in the caudal striatum with slightly higher, but still low, levels in the amygdala complex (Egertova et al., 2003).

Unfortunately, it is not clear that this form of NAPE-PLD is required for AEA synthesis in vivo since NAPE-PLD-/- mice show no difference in AEA levels, but NAPE-PLD is required for Ca++ dependent synthesis of longer chain NAEs (Leung et al., 2006). ABHD4 or PLC coupled to a tyrosine or an inositol phosphatase can also catalyze AEA production (Liu et al., 2006; Simon and Cravatt, 2006; Simon and Cravatt, 2008), and sequential PLA2 and lysoPLD activities have also been suggested to participate in AEA synthesis (Sun et al., 2004). These enzymes are certainly present in many brain regions, including striatum, and thus the AEA involved in retrograde signaling in this region may proceed through pathways other than those mediated by NAPE-PLD.

#### F. Degrading Enzymes

#### 1. Fatty Acid Amido-Hydrolase (FAAH)

The FAAH enzyme catalyzes breakdown of AEA and other ethanolamides to arachidonic acid and ethanolamine.

FAAH activity and mRNA expression are roughly parallel with high levels in neocortical structures (cortex, hippocampus) and cerebellum, with moderate levels in striatum (Thomas et al., 1997, Hillard, 2000). There is a developmental peak in whole brain mRNA at 10 days of age (Thomas et al., 1997). FAAH is expressed homogeneously in striatum, likely MSNs, with weaker hybridization in the GP, but is also found in non-neuronal cells (Thomas et al., 1997). There is prominent FAAH mRNA in lateral, basolateral, and basomedial amygdala but FAAH mRNA is low or absent in the CeA (Gulyas et al., 2004).

FAAH immunoreactivity is similar to mRNA expression in the basal ganglia. FAAH is detected in perivascular regions, in glia and in white matter (Thomas et al., 1997; Egertova et al., 2003) with diffuse somatodendritic localization in the striatum but fibers were apparent in GP (Romero et al., 2002; Egertova et al., 2003). Interestingly, Egertova et al. (2003) show that white matter immunoreactivity in GP is associated with oligodendroglia (Egertova et al., 2003). Romero and colleagues (2002) detected intense FAAH immunoreactivity in SNr and SNc using a previously characterized antibody (Tsou et al., 1998), however, a subsequent study using a different antibody failed to detect FAAH in the SN (Egertova et al., 2003). This is surprising since there is considerable agreement between these two studies in other brain regions. FAAH is primarily localized to postsynaptic smooth ER (50%) but can also be detected in mitochondrial membranes (Gulyas et al., 2004). FAAH is notably absent in the CeA, paralleling the distribution of CB1 receptors.

#### 2. Monoacylglycerol Lipase (MGL)

Lipases have been extensively characterized in the periphery where they regulate fatty acid levels, catalyzing the breakdown of monoacylglycerides to free fatty acids and glycerol. MGL activity was recognized as early as 1976, when it was initially purified (Tornqvist and Belfrage, 1976). The gene encoding MGL was originally cloned from a mouse adipocyte library and, not surprisingly, Northern blots detected a high level of this enzyme in brain (Karlsson et al., 1997). Subsequently, Dinh et al. (2002) cloned MGL from brain , and demonstrated that it can catalyze the breakdown of 2-AG (Dinh et al., 2002). The distribution in different brain regions has been examined, and mRNA is high in deep layers of cortex and low in striatum, with moderate expression in the NAc shell.

## III. CB1 RECEPTOR FUNCTION IN THE STRIATUM

Physiological and neurochemical studies have generally confirmed the predominant presynaptic role of CB1 receptors in striatum and other CNS regions (Chevaleyre et al., 2006; Lovinger, 2008). In brain slice electrophysiological experiments, application of CB1 agonists inhibits GABAergic (Szabo et al., 1998; Hoffman and Lupica, 2001; Centonze et al., 2004; Adermark and Lovinger, 2007a; Adermark and Lovinger, 2007b; Uchigashima et al., 2007) and glutamatergic (Gerdeman and Lovinger, 2001; Hoffman and Lupica, 2001; Huang et al., 2001; Robbe et al., 2001) synaptic transmission onto striatal medium spiny neurons (Fig. 9.2). Similar results have been observed in ventral striatum/nucleus accumbens and the BNST (Hoffman and Lupica, 2001; Robbe et al., 2001; Grueter et al., 2006). The CB1-mediated depression of synaptic transmission is accompanied by changes in evoked and spontaneous synaptic responses that are indicative of presynaptic inhibition. The ratio of responses to pairs of evoked glutamateric EPSCs or GABAergic IPSCs is altered in the presence of CB1 agonist, indicating that inhibition most likely involves decreased probability of glutamate and GABA release (Gerdeman and Lovinger, 2001; Hoffman and Lupica, 2001; Huang et al., 2001; Robbe et al., 2001). Measurement of spontaneous and miniature EPSCs and IPSCs, as well as asynchronous Sr<sup>2+</sup>-enhanced PSCs, also supports a presynaptic site of CB1-mediated synaptic inhibition (Gerdeman and Lovinger, 2001; Huang et al., 2001; Robbe et al., 2001). The frequency of sPSCs and mPSCs is reduced by agonist treatment, in the absence of changes in the amplitude or other properties of these synaptic currents, consistent with a decrease in neurotransmitter release with no change in postsynaptic responsiveness. These effects are blocked by CB1 antagonists, demonstrating the specificity of the agonist effects.

There is little physiological evidence that CB1 receptor activation has postsynaptic effects on striatal neurons. Huang and coworkers (2001) reported that application of WIN produced hyperpolarization of MSNs in striatal slices, and this could be a response to activation of a G protein-activated inwardly rectifying potassium (GIRK) current. However, GIRK expression is quite low in striatum (Karschin et al., 1996), and there is little evidence in the literature that Gi/o-linked GPCRs activate GIRK channels in striatal MSNs. Thus, the mechanism underlying this hyperpolarization is unclear, and it is also not clear if this effect was mediated by CB1 receptors or an off-target effect of the agonist (see Oz, 2006 for discussion of nonspecific cannabinoid effects). No such hyperpolarization were observed when CB1 agonists were applied to MSNs in the NAc, and the agonists did not produce any change in the resting membrane properties of these neurons (Hoffman and Lupica, 2001). Szabo et al. (1998) did not observe any effect of CB1 activation on postsynaptic voltage-gated calcium channels in MSNs.

Neurochemical studies also indicate that CB1 activation inhibits neurotransmitter release in striatal slices. Examination of stimulus-induced efflux of radiolabeled GABA and glutamate indicates that CB1 activation decreases release of these neurotransmitters (Kofalvi et al., 2005). Mixed effects on striatal dopamine release have been reported (Cadogan et al., 1997; Kathmann et al., 1999; Szabo et al., 1999; Sidlo et al., 2008). Most of these experiments were performed using striatal slices, and thus it is not clear that the effects are due to direct actions of CB1 receptors on presynaptic terminals.

The mechanisms coupling presynaptic CB1 receptors to inhibition of neurotransmitter release have not been worked out in great detail at striatal synapses. However, we can infer something about the mechanisms of CB1-mediated presynaptic inhibition from studies of other synapses. Inhibition of voltage-gated calcium channels, and thus calcium entry into terminals, is the major contributor to presynaptic depression at synapses in the auditory brainstem and cerebellum (Kreitzer and Regehr, 2001; Kushmerick et al., 2004). This is a common mechanism of presynaptic depression by GPCRs that is likely to contribute to CB1 actions in striatum. Additional evidence for inhibitory actions of CB1 on vesicle release itself has been obtained, and these mechanisms may also play at role at striatal synapses. For example, at glutamatergic striatal synapses CB1 activation reduces the frequency of mEPSCs independent of afferent stimulation (Gerdeman and Lovinger, 2001; Huang et al., 2001; Robbe et al., 2001), suggesting that there may be inhibition downstream of voltage-gated calcium channels. Similar results have recently been obtained for mIPSCs recorded in dorsal striatum (Adermark et al., 2009b). Hoffman and Lupica (2001) reported that effects of CB1 activation on mIPSCs were variable, with most cells showing no change (Hoffman and Lupica, 2001).

Application of CB1 antagonists generally does not have any effect on synaptic transmission evoked by low frequency stimulation of glutamatergic afferents onto striatal MSNs (Gerdeman and Lovinger, 2001). Thus, "resting" synaptic eCB levels do not appear to be sufficient to activate CB1 and produce an inhibitory "tone". Some or all of the CB1 antagonists have been touted as inverse agonists, mainly based on data from heterologous expression systems (c.f. Pan et al., 1998). However, it is not clear that they act in this manner when expressed at normal levels within the CNS, and they certainly do not seem to do so at synapses within the striatum. In cases where the CB1 antagonists have been shown to increase synaptic transmission these effects are prevented by postsynaptic chelation of calcium (Zhu and Lovinger, 2005; Foldy et al., 2006). This finding is most consistent with the idea that tonic CB1 receptor activation is due to ongoing eCB synthesis.

Recent studies have revealed that TRPV1 is present in striatum (discussed above). Work from Centonze and Maccarrone suggests a unique interaction between anandamide signaling and 2-AG production involving TRPV1 in striatum. Their findings support a scenario in which AEA acts on presumed intracellular TRPV1 receptors to inhibit DAG lipase activity and thus suppress 2-AG synthesis. One physiological consequence of this action appears to be a reduction in 2-AG/CB1-mediated inhibition of GABAergic transmission (Maccarrone et al., 2008). These investigators have also shown that TRPV1 activation potentiates glutamatergic synaptic transmission at synapses onto MSNs, and that this potentiation exhibits rapid desensitization (Musella et al., 2009).

## IV. ENDOCANNABINOID-MEDIATED SYNAPTIC PLASTICITY IN THE STRIATUM

#### A. Short-Term Depression

The previous discussion indicates that cannabinoid drugs and agonists modulate synaptic transmission at synapses within the striatum. In addition different types of eCBmediated synaptic plasticity have been observed in different striatal subregions. Several laboratories have examined DSE, DSI and short-term synaptic depression in slices from striatum and nucleus accumbens. In general it has proven difficult to elicit robust DSE or DSI in synapses onto striatal MSNs. Narushima et al. (2006) has observed that postsynaptic depolarization produces eCB-mediated DSI (lasting 20–30 sec) of GABAergic transmission at synapses between dorsal striatal fast-spiking interneurons and MSNs (Narushima et al., 2007). DSI at striatal synapses is constitutively enhanced by tonic cholinergic transmission involving M1 mAChRs on striatal MSNs (Narushima et al., 2007; Uchigashima et al., 2007), while activation of group I mGluRs enhances both DSI and DSE (Uchigashima et al., 2007). These investigators observed only very weak DSE of glutamatergic transmission in the same neurons in the absence of receptor activation, while Kreitzer and Malenka (2005) reported that they could not elicit DSE in dorsal striatal MSNs (Kreitzer and Malenka, 2005). Endocannabinoid-mediated synaptic depression produced by postsynaptic depolarization alone has not been reported in other striatal regions.

Activation of GPCRs has been shown to elicit eCBmediated short term synaptic depression in striatum. D2 dopamine receptor activation inhibits glutamatergic synaptic transmission in striatum, but this inhibitory action seems to be prominent only when synapses are very active (e.g. in the presence of elevated extracellular potassium concentrations or when afferents are activated by pairs or short trains of stimulation) (Cepeda et al., 1993; Flores-Hernandez et al., 1997; Bamford, 2004; Yin and Lovinger, 2006). While this effect may be mediated in part by presynaptic D2 receptors, involvement of CB1 receptors has also been demonstrated using receptor antagonists (Yin and Lovinger, 2006). The D2/eCB-mediated synaptic depression is relatively short lasting, only persisting for 5-10min after D2 agonist is removed from the preparation (Yin and Lovinger, 2006). D2 receptor activation has also been reported to stimulate eCB production and increase eCB levels in striatum (Giuffrida et al., 1999), and to produce short-term inhibition of GABAergic transmission onto medium spiny neurons (Centonze et al., 2004). Neurotensin, acting through NTR1 receptors also produces eCB-mediated short-term synaptic depression (Yin et al., 2008a). This effect also requires activation of group I mGluRs and D2 dopamine receptors, and the role of the NT receptor is most likely stimulation of postsynaptic eCB production. This may be a direct effect or secondary to increased dopamine or glutamate release (Okuma et al., 1983; Chapman et al., 1992; Diaz-Cabiale et al., 2002; Matsuyama et al., 2003).

Activation of group I mGluRs also produces eCBmediated short-term depression in striatal brain regions. In dorsal striatum, Kreitzer and Malenka (2005) found that the group I mGluR agonist DHPG produces short-term depression if applied when the MSN membrane potential was held at -70 mV, but longer lasting depression when the membrane potential was -50 mV.

#### **B.** Long-Term Depression

Perhaps the best characterized form of eCB-mediated synaptic plasticity in striatum is LTD. Indeed, the striatum was the first brain region in which it was recognized that eCBs are necessary for LTD (Gerdeman et al., 2002; Robbe et al., 2002). A form of LTD elicited by high frequency afferent stimulation had been known since the early 1990s to occur at glutamatergic corticostriatal synapses (Calabresi et al., 1992a; Calabresi et al., 1992b; Lovinger et al., 1993; Walsh, 1993) (see also Chapter 12) (Fig. 9.3A), and it was later realized that expression of this LTD involves a presynaptic decrease in neurotransmitter release probability (Choi and Lovinger, 1997a; Choi and Lovinger, 1997b). The need for postsynaptic induction mechanisms in striatal LTD suggested the involvement of a retrograde signal linking these mechanisms to presynaptic expression. The identification of eCBs as the likely retrograde messenger in this HFS-induced LTD (Gerdeman et al., 2002), as well as LTD induced by more moderate stimulus frequencies in ventral striatum/nucleus accumbens (Robbe et al., 2002), spurred investigation of eCB-dependent LTD in a number of brain regions. It is now appreciated that eCB-dependent LTD occurs throughout the brain (see Chevaleyre et al., 2006; Lovinger, 2008 for review).

Evidence for eCB-LTD at GABAergic synapses in the dorsolateral striatum is beginning to emerge (Adermark and Lovinger, 2009a,b). Recent studies indicate that LTD is easier to elicit at GABAergic synapses on dorsal striatal MSNs than at glutamatergic synapses onto the same neurons (Fig. 9.3B). Afferent stimulation at low frequencies (e.g. 1Hz for 1min) elicits eCB-LTD at GABAergic striatal synapses (Fig. 9.3B), while moderate-to-high frequency stimulation is needed to induce eCB-LTD at glutamatergic synapses (Choi and Lovinger, 1997b, Gerdeman, 2002 et al.; Ronesi et al., 2004; Kreitzer and Malenka, 2005; Ronesi and Lovinger, 2005; Wang et al., 2006) (Fig. 9.3A). The relative ease of induction of eCB-LTD at GABAergic striatal synapses may be due in part or in full to the higher expression of CB1 receptors on GABAergic, as opposed to glutamatergic, axon terminals in striatum (Uchigashima et al., 2007). The long-lasting decrease in inhibitory GABAergic transmission within striatum can initiate a long-lasting disinhibition (or DLL) of excitatory synaptic input to striatal medium spiny neurons (Adermark and Lovinger, 2009a) (Fig. 9.3C). Thus, long-term changes in the net output of striatal projection neurons can be shaped by DLL that produces a net increase in output and eCB-LTD at glutamatergic synapses that produces a net decrease in output, as well as long-term potentiation of glutamatergic synapses that produces a net increase in MSN activation (Fig. 9.3C). Together, these forms of long-lasting synaptic plasticity sculpt net striatal output in a manner that depends on the frequency of afferent input (Adermark and Lovinger, 2009a).

Several cellular and molecular mechanisms have been implicated in eCB-LTD at striatal glutamatergic synapses. The glutamatergic drive produced by afferent activation depolarizes MSNs through AMPA receptor activation, and also activates postsynaptic group I mGluRs (Calabresi et al., 1992b; Choi and Lovinger, 1997b; Gubellini et al., 2001; Sung et al., 2001). Depolarization of the MSNs activates CaV1.3-type voltage-gated calcium channels, a subtype of dihydropyridine-sensitive "L-type" calcium channels that have a relatively low voltage threshold for activation (Wang et al., 2006). The influx of calcium through these channels, perhaps in combination with release of calcium from postsynaptic intracellular stores, appears to contribute to eCB synthesis. Activation of the group I mGluRs probably also contributes to eCB synthesis (Jung et al., 2005).

Induction of eCB-LTD at glutamatergic synapses by high frequency afferent activation also requires release of the neurotransmitter dopamine, and activation of the D2 dopamine receptors (Calabresi et al., 1992a; Tang et al., 2001; Jung et al., 2005; Kreitzer and Malenka, 2005). The D2 receptor role appears to be modulatory, increasing the probability of LTD induction (Kreitzer and Malenka, 2005; Pawlak and Kerr, 2008). Activation of group I mGluRs or L-type calcium channels induces LTD independently of D2 receptors activation (Kreitzer and Malenka, 2005; Adermark and Lovinger, 2007a).

However, the cellular location of the D2 receptors that participate in LTD induction, as well as the signaling pathways influenced by these receptors that bias synapses toward LTD, have not yet been fully resolved. The most straightforward hypothesis is that postsynaptic D2 receptors on MSNs contribute to eCB production. As mentioned above, D2 receptor activation can increase extracellular levels of eCBs in striatum in vivo (Giuffrida et al., 1999), and can also induce eCB-dependent short-term depression (Yin and Lovinger, 2006). However, D2 receptors are only abundantly expressed in a subset of MSNs, those that project to the globus pallidus internal segment via the "indirect pathway" (Gerfen et al., 1990). Thus, if postsynaptic D2 receptors on MSNs are needed for HFS-induced eCB-LTD, then LTD might not be observed in the MSNs with many



FIGURE 9.3 Endocannabinoid-mediated long-term synaptic depression (LTD) of GABAergic and glutamatergic transmission produces frequencydependent inversion of net striatal output. (A) LTD at glutamatergic synapses is produced by high frequency afferent stimulation (HFS) ( $4 \times 100$  Hz, 1 sec duration trains, delivered 1/10 sec). Top: representative excitatory postsynaptic currents (EPSCs) recorded before and after HFS showing depressed EPSC amplitude and increased paired-pulse facilitation. Bottom: Graph shows normalized EPSC amplitude before and after HFS. (B) LTD at GABAergic synapse produced by sustained low-frequency stimulation (LFS) (1Hz, 1 min duration). Graph shows normalized IPSC amplitude before and after LFS in slices treated with the CB1 antagonist AM251 after LFS (black symbols, black line), and in slices treated with AM251 throughout the experiments (gray symbols, dashed line). Note the prolonged decrease in IPSC amplitude (LTD) in the post-LFS treatment group, and the prevention of LTD when AM251 is present continuously. (C) Long-term changes in net striatal output vary with stimulus frequency. Ca, Field potential recording data showing population spike (PS) amplitude over time course of an experiment in which the GABA<sub>A</sub> receptor antagonist picrotoxin was applied to the slice. Note the increase in PS amplitude indicating a net inhibitory action of GABA<sub>A</sub> receptors. Cb, Representative traces showing field potentials during the baseline recording period, after 1 min 1 Hz stimulation, and after high frequency (100 Hz) stimulation. Taken from the experiments shown in the graph in Cc. Cc, Changes in PS amplitude in response to LFS (1 Hz) and HFS stimulation when NMDA receptors are blocked with 50µM AP-5. Note the persistent increase in net striatal output after LFS, and the decrease after HFS. Cd, Blockade of CB1 receptors with AM251 (3µM) prevents both the LFS-induced PS increase and the HFS-induced decrease, indicating involvement of eCB-LTD in both types of plasticity. Ce, Application of picrotoxin (50 µM) prevents LFS-induced PS increase, but not the HFS-induced decrease, indicating that LFS has a net disinhibitory action. Cf, Stimulation at different frequencies and durations produces either long-lasting disinhibition (increased PS), no change, or a slight LTD (decreased PS). Overall, net striatal output is inverted by long-lasting disinhibition or LTD due to endocannabinoid actions at GABAergic or glutamatergic synapses, respectively (reprinted from Adermark and Lovinger, 2009a).

fewer D2 receptors (i.e., the striatonigral "direct pathway" MSNs that express mainly D1 receptors) (Gerfen et al., 1990). However, experiments in rat brain slices have generally shown that LTD is observed in a large majority of neurons when induced using the high frequency stimulation protocol (Calabresi et al., 1992a; Calabresi et al., 1992b; Partridge et al., 2000). Furthermore, HFS-induced LTD can be observed at glutamatergic synapses on both striatopallidal and striatonigral neurons (Wang et al., 2006; Adermark and Lovinger, 2007a), and D2 antagonists prevent LTD in both neuronal subtypes (Wang et al., 2006). More recent studies indicate that LTD is preferentially induced in striatopallidal neurons under some conditions (Kreitzer and Malenka, 2007), including induction with a protocol using timing of postsynaptic EPSPs and presynaptic action potentials (so-called spike-timing dependent plasticity, Shen et al., 2008). Thus, the presence of postsynaptic D2 receptors may enhance the likelihood of LTD induction. Nonetheless, the finding that D2-dependent LTD is observed in striatonigral neurons indicates that dopamine activation of these receptors likely participates in LTD via another mechanism. One such mechanism involves D2 receptor-mediated suppression of the activity of tonicallyactive cholinergic striatal interneurons (Wang et al., 2006). The acetylcholine released from these interneurons inhibits the CaV1.3 calcium channel via activation of type1 mACh receptors (Howe and Surmeier, 1995). Suppression of interneuron activity by D2 receptors would relieve this inhibition and free CaV1.3 channels to participate more strongly in eCB production and LTD induction. Indeed, blockade of LTD induction by D2 antagonists can be reversed by an M1R antagonist (Wang et al., 2006), and mAChR antagonists enhance the magnitude of LTD even in the absence of D2 blockade (Calabresi et al., 1992b; Bonsi et al., 2008). Thus, ACh-DA interactions appear to regulate eCB production and synaptic plasticity, and the molecules involved in these interactions may provide new targets for therapies aimed at treating disorders of the basal ganglia.

Long-term depression mediated by eCBs and CB1 receptors has not been observed in all of the striatal-like brain regions. In both the dorsomedial (caudate equivalent in rodent) and dorsolateral (putamen equivalent) striatal eCB-LTD can be observed (Partridge et al., 2000). However, LTD is more easily induced in dorsolateral striatum in adult rat (Partridge et al., 2000). It is tempting to speculate that the stronger expression of CB1 receptors in dorsolateral striatum underlies the subregional LTD difference. It is still not clear which eCB mediates striatal LTD.

To date, there has not been a convincing demonstration that inhibition of synthesis of either eCB prevents striatal LTD induction. The DAG lipase inhibitor THL does not prevent LTD in the dorsolateral striatum, despite the fact that this drug increases 2-AG levels and prevents DSI and short-term depression in striatum (Ade and Lovinger, 2007; Uchigashima et al., 2007). It should be noted that DAG lipase inhibition has been shown to prevent eCB-LTD in other brain regions (Chevaleyre et al., 2006). Unfortunately, it is not yet clear which enzymes are involved in AEA synthesis, and no specific inhibitors of synthesis of this eCB have been developed. Thus, it is not yet possible to fully assess the role of AEA in eCB-LTD. Involvement of AEA in striatal LTD has been suggested by the finding that a FAAH inhibitor rescues LTD after dopamine depletion (Kreitzer and Malenka, 2007). The first appearance of LTD during development is correlated with an increase in striatal AEA expression, and direct application of AEA to brain slices earlier in development allows for LTD induction (Ade and Lovinger, 2007). Studies in our laboratory using intracellular postsynaptic application of AEA also indicate that this eCB can participate in LTD induction (Adermark and Lovinger, 2009a,b). Nonetheless, the evidence to date is not sufficient to allow us to determine which eCB has a predominant role in striatal LTD induction.

As mentioned above, expression and maintenance of eCB-LTD appears to involve a decrease in probability of neurotransmitter release (Choi and Lovinger, 1997a,b; Kreitzer and Malenka, 2005; Wang et al., 2006; Adermark and Lovinger, 2007a). The observation that the majority of CB1 receptors are expressed on presynaptic terminals reinforces the idea of a strong presynaptic component in LTD expression. At this point, little is known about the changes in presynaptic terminal function that underlie this decreased probability of release. It is clear that continued activation of CB1 receptors does not account for the persistence of eCB-LTD, as CB1 antagonists can only prevent or reverse LTD when given during, or for the first few minutes after, repetitive afferent stimulation (Ronesi et al., 2004). Once LTD is fully established the depression of transmission becomes independent of eCBs and CB1 activation (Fig. 9.3B). Evidence from studies in hippocampus indicates that maintained LTD expression may involve decreased cAMP-dependent signaling, perhaps set into motion by CB1 inhibition of adenylyl cyclase (Chevaleyre et al., 2007). We have also observed that inhibition of protein translation converts LTD to short-term depression (Yin et al., 2006a; Adermark and Lovinger, 2009a.b).

Interestingly, inhibiting translation in the postsynaptic neuron alone is not sufficient to prevent LTD, suggesting that the relevant translation event takes place at a site other than the postsynaptic cell. Clearly, future research will need to focus on the presynaptic mechanisms that support maintained eCB-LTD expression. These might include long-lasting changes in the function of presynaptic calcium channels, or long-lasting decreases in vesicle docking, fusion rate or degree of fusion. Clearly, there is a great deal still to be learned about the presynaptic mechanisms underlying expression of striatal eCB-LTD.

## V. ENDOCANNABINOID ROLES IN STRIATUM-DEPENDENT BEHAVIOR

Different regions of the dorsal striatum have been shown to be necessary for the learning and execution of goal-directed actions and habits (Balleine and Dickinson, 1998; Corbit and Balleine, 2003; Killcross and Coutureau, 2003; Yin and Knowlton, 2004; Yin et al., 2004; Yin et al., 2005a,b; Yin et al., 2006b) (see Chapter 32). Goal-directed actions are actions whose performance is aimed at obtaining a particular outcome, and as such they are dependent on the expected value of the outcome and on the causal relation between executing the action and obtaining the outcome. They allow us to respond in an efficient way to changing situations, but they are effortful and in some situations inefficient. For example, in situations where the behavior is repeated regularly for a long time without major changes in the incentive value of the outcome, or situations where one cannot manipulate the probability of obtaining an outcome irrespective of the strategy employed, rules and habits can be advantageous. However, habitual behavior when taken to an extreme is associated with loss of control and with maladaptive behavior, such as drug seeking in addiction or compulsivity.

Although the dorsal striatum in rodents is not divided clearly into caudate and putamen, it does have a mediallateral gradient of connectivity which is similar (but not identical) to the caudate (ventromedial), and putamen (dorsolateral) connectivity in primates (McFarland and Haber, 2000; Voorn et al., 2004). The medial portion of the dorsal striatum, which extends ventrally to the limits of accumbens has been shown to receive most of its input from the associative areas of the cortex (like the caudate), while the dorsolateral striatal region receives input from the sensorimotor areas of the cortex (like the putamen) (Voorn et al., 2004) (see Chapter 29, for maps of corticostriatal projections in the rat). The associative cortico-basal ganglia circuits involving the dorsomedial striatum (Yin et al., 2005a; Yin et al., 2005b) have been shown to support the learning and performance of goal-directed behavior, but do not affect habit formation. In contrast, the dorsolateral or sensorimotor striatum (Yin et al., 2004) has been shown to support the formation of habits (Fig. 9.4A) (see Chapter 32).

Although it is increasingly clear that the dorsolateral region of the striatum is involved in habit formation, much less is known about the molecular bases of habit formation. Dopaminergic signaling in the dorsolateral or sensorimotor striatum seems to be involved in habit formation (Porrino et al., 2004; Takahashi et al., 2007). The dopaminergic projections to striatum follow an interesting gradient with dopaminergic neurons projecting from the substantia nigra pars compacta (A9) targeting more the dorsolateral striatum, and dopaminergic neurons projecting from the ventral tegmental area (A10) targeting more the ventromedial striatum, nucleus accumbens (Moore et al., 2001), and frontal cortices (Fig. 9.4B). Consistently, lesions of the nigrostriatal input to the dorsolateral striatum (Faure et al., 2005), and infusion of dopamine into the ventral medial prefrontal cortex seem to impair habits and favor goaldirected behavior (Hitchcott et al., 2007). Furthermore, the dopamine transporter (DAT), the main target of cocaine and amphetamine, is highly expressed in the dorsolateral striatum (involved in habit formation), and less expressed in more medial and ventral regions of the striatum and in the pre-frontal cortex (Matsumoto et al., 2003; Arbuthnott and Wickens, 2007). Consistently, sensitization with amphetamine, which acts on DAT, favors a shift from goaldirected to habitual behavior (Nelson and Killcross, 2006; Nordquist et al., 2007).

As mentioned earlier, eCB release in the striatum has been shown to be modulated by dopamine signaling (Giuffrida et al., 1999; Kreitzer and Malenka, 2005; Yin and Lovinger, 2006). Interestingly, eCB and CB1-dependent striatal LTD is more prevalent in the dorsolateral striatum where CB1 expression is higher (Herkenham et al., 1991; Partridge et al., 2000; Gerdeman and Lovinger, 2001; Gerdeman et al., 2002).

Signaling through the CB1 receptor has been implicated in reward and addiction (Casadio et al., 1999; Cossu et al., 2001; De Vries et al., 2001; Di Marzo et al., 2001; Gerdeman et al., 2003; Wang et al., 2003; Sanchis-Segura et al., 2004; Houchi et al., 2005; Caille et al., 2007; Hansson et al., 2007). The medial-lateral gradient of striatal CB1 expression, with the highest expression in the



**FIGURE 9.4** Investigating the role of endocannabinoids in goal-directed actions and habit formation in mice. (A) Mice are trained with two reinforcers. In the figure, the task is exemplified with one of the reinforcers, cheese, being delivered in the operant box contingent upon lever pressing, while the other reinforcer, sugar water, is being delivered freely to the mouse in the home cage. (B) Devaluation is performed in two days: Day 1, the mouse is given the reinforcer, cheese, previously earned by lever pressing (devalued condition); Day 2, the mouse receives the reinforcer, sugar water, previously freely available in its home cage (valued condition). The order of the conditions is randomized. Immediately after each feeding session (1 h duration) the mouse goes through a 5 min extinction test in the operant chamber, with the training lever extended. The number of presses on the training lever under the valued and the devalued conditions are compared. If the mouse presses more under the valued versus devalued condition, then the behavior is goal-directed behavior. However, if the mouse presses both levers equally his behavior is classified as habitual. (C) Decreased predisposition for habit formation in CB1 mutant mice (left) and in C57Bl6/J mice injected with CB1 antagonist during random interval training, but not during testing (right). (Left panel) Normalized lever pressing during the valued versus the devalued condition for WT, Cb1 + / - and Cb1 - / - mice. (Right panel). Normalized lever pressing during the valued condition for mice injected with saline, 3 mg/kg AM251 or 6 mg/kg AM251.

dorsolateral striatum (Herkenham et al., 1991; Gerdeman et al., 2003), a subregion shown to be necessary for habit formation, raises the possibility that that eCB signaling in dorsolateral striatum is necessary for habit formation. Consistently, amphetamine sensitization, which predisposes for habit formation (Nelson and Killcross, 2006), depends on eCB signaling through CB1 receptors in the dorsal striatum (Corbille et al., 2007).

Indeed, recent studies have shown that eCB signaling through CB1 receptors is necessary for habit formation (Hilario et al., 2007). Mice with genetically targeted mutations in the *Cb1* gene (Zimmer et al., 1999) were shown to have decreased predisposition for habit formation when trained on a random interval schedule, previously shown by the authors to promote habitual behavior (Fig. 9.4). When tested using a devaluation test by sensory-specific satiety (Hilario et al., 2007), WT mice showed insensitivity to change in value of the outcome and thus habitual behavior, while both  $Cb1^{+/-}$ , and  $Cb1^{-/-}$  mutants showed sensitivity to sensory-specific satiety, suggesting that their actions were still goal-directed (Fig. 9.4C).

The role of eCBs in habit formation was confirmed using acute pharmacological blockade of CB1 receptors (Hilario et al., 2007), which suggests that the lack of habit formation in *Cb1* mutant animals is not caused by chronic developmental or behavioral abnormalities, or changes in feeding behavior and reward perception (Di Marzo et al., 2001; Sanchis-Segura et al., 2004; Caille et al., 2007). These studies confirmed that mice injected with the CB1 antagonist AM251 specifically during training but not during testing (Fig. 9.4C), were impaired in habit formation.

In addition to the role of eCB signaling in amphetamine sensitization (Corbille et al., 2007) and habit formation (Hilario et al., 2007), there is also evidence that eCB signaling may be important for skill learning (Hilario and Costa, unpublished results).  $Cb1^{-/-}$  mice are impaired in skill learning in the rotarod in relation to WT littermates. Furthermore, acute blockade of CB1 in mice also seems to affect skill learning in the rotarod. Interestingly, the striatum has been shown to be involved in skill learning in the rotarod in mice (Costa et al., 2004; Yin et al., 2008b).

The finding that eCB signaling is necessary for habit formation opens new lines of questioning, such as where and how CB1 signaling operates to promote habit formation. As previously described, eCBs in the brain can function as retrograde messengers, modulating the release of different neurotransmitters, and producing short-term and long-term depression of excitatory and inhibitory transmission (Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001; Gerdeman et al., 2002; Yin and Lovinger, 2006). Although CB1 receptors are one of the most-abundant G-protein coupled receptors in the brain and are expressed almost ubiquitously, the dorsolateral striatum could be one of the regions where CB1 signaling is critical for learning and behavior. In the dorsolateral striatum CB1 receptors could serve to decrease "competing" glutamatergic inputs to medium spiny neurons by inducing depression at these synapses (Huang et al., 2001; Gerdeman et al., 2001; Gerdeman et al., 2002). However, CB1 receptor activation is also important for the depression of inhibitory inputs in the dorsolateral striatum (Adermark and Lovinger, 2009a), suggesting it could potentially reduce lateral inhibition between MSNs, or reduce inhibition of MSNs by fast-spiking interneurons. Interestingly, a combination of depression of "competing" excitatory inputs and reduction in lateral inhibition could facilitate the firing of groups of neurons that are preferentially connected, like a cell assembly (Carrillo-Reid et al., 2008), with less interference from the cortex and competing cell assemblies in the striatum. The decrease in presynaptic release probability involved in the expression of striatal eCB-LTD is manifested as a decrease in frequency of spontaneous excitatory postsynaptic currents, but also by an increase in paired pulse facilitation (a second afferent stimulation given within a certain time window of the first produces a larger response). Therefore, another interesting possibility is that eCB signaling through CB1 receptors acts as a filter to increase signal to noise, since after the induction of presynaptic depression the postsynaptic neuron would listen preferentially to bursts of inputs rather than single inputs.

CB1 is also expressed heavily in the distal terminals of the MSNs from the direct and indirect pathway, which synapse onto the substantia nigra pars reticulata and the globus pallidus, respectively (Sanudo-Pena et al., 1999). Therefore, since MSNs are inhibitory projection neurons, it is possible that eCB signaling through CB1 receptor activation is necessary to disinhibit basal ganglia nuclei downstream of the striatum. Another intriguing possibility is that CB1mediated signaling modulates the strength of excitatory and inhibitory synaptic inputs onto dopaminergic neurons (Szabo et al., 2002; Lupica and Riegel, 2005). It has been shown that eCBs are released in to response drugs of abuse (Caille et al., 2007), and that the transient increases in dopamine release by drugs of abuse are mediated by CB1 receptors (Cheer et al., 2007). Since CB1 receptor blockade diminishes the effects of several drugs of abuse on dopamine release (Cheer et al., 2007), one possibility is that eCB-mediated inhibition of GABA release onto dopamine neurons is necessary for dopaminergic neurons to increase firing and release dopamine onto downstream targets like the dorsolateral striatum, where dopamine has been shown to be necessary for habit formation (Szabo et al., 2002; Faure et al., 2005; Nelson and Killcross, 2006).

Precisely how eCBs modulate striatal information processing in vivo and interact with other neurotransmitter systems, such as glutamate, acetylcholine, and dopamine, is still a matter for much needed research. If eCBs are indeed involved in the balance of the neural mechanisms that underlie our vulnerability to develop habits, drug seeking behaviors, compulsions, or even other striatal-based pathologies, their understanding is of the utmost importance to the formulation of more adequate treatments. The drug Rimonabant, a CB1 antagonist, has been employed in the treatment of addiction (Cahill and Ussher, 2007), and has been proposed to function by reducing the levels of dopamine in the motivation centers of the brain, which are triggered by addictive drugs. This drug class has been shown to induce a decrease in drug rewarding effects, to reduce the influence of drug-associated stimuli, and to lower the relapse rates of drugs such as opioids, cocaine, nicotine, ethanol and amphetamine (De Vries et al., 2001; Le Foll et al., 2008). It has also been proposed that manipulations of eCB signaling through CB1 could be beneficial in other striatal involving disorders like Parkinson's disease (Kreitzer and Malenka, 2007; Garcia-Arencibia et al., 2008). In the future, it will be important to investigate the brain regions and cell types where CB1 signaling is required for its effects, to not only define how eCBs contribute to normal behavior, but to also understand how therapies can be customized to specific pathologies.

#### REFERENCES

- Ade KK, Lovinger DM (2007) Anandamide regulates postnatal development of long-term synaptic plasticity in the rat dorsolateral striatum. J Neurosci 27:2403–2409.
- Adermark L, Lovinger DM (2007a) Combined activation of L-type Ca2+ channels and synaptic transmission is sufficient to induce striatal long-term depression. J Neurosci 27:6781–6787.
- Adermark L, Lovinger DM (2007b) Retrograde endocannabinoid signaling at striatal synapses requires a regulated postsynaptic release step. Proc Natl Acad Sci USA 104:20564–20569.
- Adermark L, Lovinger DM (2009a) Frequency-dependent inversion of net striatal output by endocannabinoid-dependent plasticity at different synaptic inputs. J Neurosci 29(5):1375–1380.

- Adermark L, Talani G, Lovinger DM (2009b) Endocannabinoid-dependent plasticity at GABAergic and glutamatergic synapses in the striatum is regulated by synaptic activity. Eur J Neurosci 29:32–41.
- Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30:62–69.
- Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37:407–419.
- Bamford NS, Robinson S, Palmiter RD, Joyce JA, Moore C, Meshul C (2004) Dopamine modulates release from corticostriatal terminals. J Neurosci 24(43):9541–9552.
- Bonsi P, Martella G, Cuomo D, Platania P, Sciamanna G, Bernardi G, Wess J, Pisani A (2008) Loss of muscarinic autoreceptor function impairs long-term depression but not long-term potentiation in the striatum. J Neurosci 28:6258–6263.
- Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F (2008) CB2 receptors in the brain: role in central immune function. Br J Pharmacol 153:240–251.
- Cadogan AK, Alexander SP, Boyd EA, Kendall DA (1997) Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. J Neurochem 69:1131–1137.
- Cahill K, Ussher M (2007) Cannabinoid type 1 receptor antagonists (rimonabant) for smoking cessation. Cochrane Database Syst Rev Issue 3. Art. No.: CD005353. DOI: 10.1002/14651858.CD005353.pub3.
- Caille S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH (2007) Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. J Neurosci 27:3695–3702.
- Calabresi P, Maj R, Mercuri NB, Bernardi G (1992a) Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. Neurosci Lett 142:95–99.
- Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992b) Longterm synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:4224–4233.
- Carrillo-Reid L, Tecuapetla F, Tapia D, Hernandez-Cruz A, Galarraga E, Drucker-Colin R, Bargas J (2008) Encoding network states by striatal cell assemblies. J Neurophysiol 99:1435–1450.
- Casadio A, Martin KC, Giustetto M, Zhu H, Chen M, Bartsch D, Bailey CH, Kandel ER (1999) A transient, neuron-wide form of CREBmediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. Cell 99:221–237.
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824.
- Centonze D, Battista N, Rossi S, Mercuri NB, Finazzi-Agro A, Bernardi G, Calabresi P, Maccarrone M (2004) A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal GABAergic Transmission. Neuropsychopharmacology 29:1488–1497.
- Cepeda C, Buchwald NA, Levine MS (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc Natl Acad Sci USA 90:9576–9580.
- Chapman MA, See RE, Bissette G (1992) Neurotensin increases extracellular striatal dopamine levels in vivo. Neuropeptides 22:175–183.
- Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci 27:791–795.

- Chevaleyre V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38:461–472.
- Chevaleyre V, Heifets BD, Kaeser PS, Sudhof TC, Castillo PE (2007) Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1alpha. Neuron 54:801–812.
- ChevaleyreV, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29:37–76.
- Choi S, Lovinger DM (1997a) Decreased frequency but not amplitude of quantal synaptic responses associated with expression of corticostriatal long-term depression. J Neurosci 17:8613–8620.
- Choi S, Lovinger DM (1997b) Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. Proc Natl Acad Sci USA 94:2665–2670.
- Corbille AG, Valjent E, Marsicano G, Ledent C, Lutz B, Herve D, Girault JA (2007) Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. J Neurosci 27:6937–6947.
- Corbit LH, Balleine BW (2003) The role of prelimbic cortex in instrumental conditioning. Behav Brain Res 146:145–157.
- Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M, Fratta W (2001) Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav Brain Res 118:61–65.
- Costa RM, Cohen D, Nicolelis MA (2004) Differential corticostriatal plasticity during fast and slow motor skill learning in mice. Curr Biol 14:1124–1134.
- Davis MI, Ronesi J, Lovinger DM (2003) A predominant role for inhibition of the adenylate cyclase/protein kinase A pathway in ERK activation by cannabinoid receptor 1 in N1E-115 neuroblastoma cells. J Biol Chem 278:48973–48980.
- De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, Vanderschuren LJ, Schoffelmeer AN (2001) A cannabinoid mechanism in relapse to cocaine seeking. Nat Med 7:1151–1154.
- Deniau JM, Menetrey A, Charpier S (1996) The lamellar organization of the rat substantia nigra pars reticulata: segregated patterns of striatal afferents and relationship to the topography of corticostriatal projections. Neuroscience 73:761–781.
- Deniau JM, Menetrey A, Thierry AM (1994) Indirect nucleus accumbens input to the prefrontal cortex via the substantia nigra pars reticulata: a combined anatomical and electrophysiological study in the rat. Neuroscience 61:533–545.
- Devane WA, Hanus L, Breuer A, et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949.
- Di Marzo V, Goparaju SK, Wang L, et al. (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410:822–825.
- Diaz-Cabiale Z, Fuxe K, Narvaez JA, Finetti S, Antonelli T, Tanganelli S, Ferraro L (2002) Neurotensin-induced modulation of dopamine D2 receptors and their function in rat striatum: counteraction by a NTR1like receptor antagonist. Neuroreport 13:763–766.
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 99:10819–10824.
- Edwards D, Kim J, Alger BE (2006) Multiple mechanisms of endocannabinoid response initiation in hippocampus. J Neurophysiol 95:67–75.

- Egertova M, Cravatt BF, Elphick MR (2003) Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119:481–496.
- Egertová M, Simon GM, Cravatt B, Elphick MR (2008) Localization of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: A new perspective on N-acylethanolamines as neural signaling molecules. J Comp Neurol. 506(4):604–615.
- Faure A, Haberland U, Conde F, El Massioui N (2005) Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation. J Neurosci 25:2771–2780.
- Flores-Hernandez J, Galarraga E, Bargas J (1997) Dopamine selects glutamatergic inputs to neostriatal neurons. Synapse 25:185–195.
- Foldy C, Neu A, Jones MV, Soltesz I (2006) Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. J Neurosci 26:1465–1469.
- Garcia-Arencibia M, Ferraro L, Tanganelli S, Fernandez-Ruiz J (2008) Enhanced striatal glutamate release after the administration of rimonabant to 6-hydroxydopamine-lesioned rats. Neurosci Lett 438:10–13.
- Gerdeman G, Lovinger DM (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85:468–471.
- Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM (2003) It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends Neurosci 26:184–192.
- Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358–363.
- Glass M, Felder CC (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J Neurosci 17:5327–5333.
- Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, Uhl GR (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071:10–23.
- Grimsey NL, Goodfellow CE, Scotter EL, Dowie MJ, Glass M, Graham ES (2008) Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies are not all created equal! J Neurosci Methods 171:78–86.
- Grueter BA, Gosnell HB, Olsen CM, Schramm-Sapyta NL, Nekrasova T, Landreth GE, Winder DG (2006) Extracellular-signal regulated kinase 1-dependent metabotropic glutamate receptor 5-induced longterm depression in the bed nucleus of the stria terminalis is disrupted by cocaine administration. J Neurosci 26:3210–3219.
- Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, Calabresi P (2001) Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology 40:839–846.
- Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 20:441–458.

- Hansson AC, Bermudez-Silva FJ, Malinen H, et al. (2007) Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. Neuropsychopharmacology 32:117–126.
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583.
- Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936.
- Hilario MRF, Clouse E, Yin HH, Costa RM (2007) Endocannabinoid signaling is critical for habit formation. Frontiers in Integrative Neuroscience 1:6 doi: 10.3389/neuro.07/006.2007.
- Hillard CJ (2000) Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat 61(1–2):3–18.
- Hitchcott PK, Quinn JJ, Taylor JR (2007) Bidirectional modulation of goal-directed actions by prefrontal cortical dopamine. Cereb Cortex 17:2820–2827.
- Hoffman AF, Lupica CR (2001) Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. J Neurophysiol 85:72–83.
- Hohmann AG, Herkenham M (2000) Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a doublelabel in situ hybridization study. Synapse 37:71–80.
- Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M (2005) CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. Neuropsychopharmacology 30:339–349.
- Howe AR, Surmeier DJ (1995) Muscarinic receptors modulate N-, P-, and L-type Ca2+ currents in rat striatal neurons through parallel pathways. J Neurosci 15:458–469.
- Huang CC, Lo SW, Hsu KS (2001) Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. J Physiol 532:731–748.
- Jansen EM, Haycock DA, Ward SJ, Seybold VS (1992) Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. Brain Res 575:93–102.
- Julian MD, Martin AB, Cuellar B, Rodriguez De Fonseca F, Navarro M, Moratalla R, Garcia-Segura LM (2003) Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. Neuroscience 119:309–318.
- Jung KM, Mangieri R, Stapleton C, Kim J, Fegley D, Wallace M, Mackie K, Piomelli D (2005) Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. Mol Pharmacol 68:1196–1202.
- Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C (1997) cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 272:27218–27223.
- Karschin C, Dissmann E, Stuhmer W, Karschin A (1996) IRK(1-3) and GIRK(1-4) inwardly rectifying K+ channel mRNAs are differentially expressed in the adult rat brain. J Neurosci 16:3559–3570.
- Kathmann M, Bauer U, Schlicker E, Gothert M (1999) Cannabinoid CB1 receptor-mediated inhibition of NMDA- and kainate-stimulated noradrenaline and dopamine release in the brain. Naunyn Schmiedebergs Arch Pharmacol 359:466–470.
- Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, Freund TF (2001) Distribution of CB1 cannabinoid receptors in the amygdala

and their role in the control of GABAergic transmission. J Neurosci 21:9506–9518.

- Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26:5628–5637.
- Killcross S, Coutureau E (2003) Coordination of actions and habits in the medial prefrontal cortex of rats. Cereb Cortex 13:400–408.
- Kim J, Alger BE (2004) Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. Nat Neurosci 7:697–698.
- Kofalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA, Sperlagh B (2005) Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J Neurosci 25:2874–2884.
- Kreitzer AC, Malenka RC (2005) Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25:10537–10545.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Kreitzer AC, Regehr WG (2001) Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. J Neurosci 21(20):RC174.
- Kushmerick C, Price GD, Taschenberger H, et al. (2004) Retroinhibition of presynaptic Ca2+ currents by endocannabinoids released via postsynaptic mGluR activation at a calyx synapse. J Neurosci 24:5955–5965.
- Le Foll B, Forget B, Aubin HJ, Goldberg SR (2008) Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. Addict Biol 13:239–252.
- Leung D, Saghatelian A, Simon GM, Cravatt BF (2006) Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. Biochemistry 45:4720–4726.
- Liu J, Wang L, Harvey-White J, et al. (2006) A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA 103:13345–13350.
- Lovinger DM (2007) Endocannabinoid liberation from neurons in transsynaptic signaling. J Mol Neurosci 33:87–93.
- Lovinger DM (2008) Presynaptic modulation by endocannabinoids. Handb Exp Pharmacol 184:435–477.
- Lovinger DM, Tyler EC, Merritt A (1993) Short- and long-term synaptic depression in rat neostriatum. J Neurophysiol 70:1937–1949.
- Lupica CR, Riegel AC (2005) Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. Neuropharmacology 48:1105–1116.
- Maccarrone M, Rossi S, Bari M, et al. (2008) Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. Nat Neurosci 11:152–159.
- Mailleux P, Vanderhaeghen JJ (1992) Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons. Neurosci Lett 148:173–176.
- Makara JK, Mor M, Fegley D, et al. (2005) Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. Nat Neurosci 8:1139–1141.
- Marsicano G, Lutz B (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 11:4213–4225.
- Marsicano G, Wotjak CT, Azad SC, et al. (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534.

- Martin AB, Fernandez-Espejo E, Ferrer B, et al. (2008) Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. Neuropsychopharmacology 33:1667–1679.
- Massi L, Elezgarai I, Puente N, Reguero L, Grandes P, Manzoni OJ, Georges F (2008) Cannabinoid receptors in the bed nucleus of the stria terminalis control cortical excitation of midbrain dopamine cells in vivo. J Neurosci 28:10496–10508.
- Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327:535–550.
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564.
- Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE, Weinberger DR (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116:127–137.
- Matsuyama S, Fukui R, Higashi H, Nishi A (2003) Regulation of DARPP-32 Thr75 phosphorylation by neurotensin in neostriatal neurons: involvement of glutamate signalling. Eur J Neurosci 18:1247–1253.
- Matyas F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF (2006) Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. Neuroscience 137:337–361.
- Matyas F, Urban GM, Watanabe M, Mackie K, Zimmer A, Freund TF, Katona I (2008) Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. Neuropharmacology 54:95–107.
- McFarland NR, Haber SN (2000) Convergent inputs from thalamic motor nuclei and frontal cortical areas to the dorsal striatum in the primate. J Neurosci 20:3798–3813.
- Mechoulam R, Ben-Shabat S, Hanus L, et al. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90.
- Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M (2004a) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 24:10707–10715.
- Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004b) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24:53–62.
- Mezey E, Toth ZE, Cortright DN, et al. (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci USA 97:3655–3660.
- Moore AE, Cicchetti F, Hennen J, Isacson O (2001) Parkinsonian motor deficits are reflected by proportional A9/A10 dopamine neuron degeneration in the rat. Exp Neurol 172:363–376.
- Morishita J, Okamoto Y, Tsuboi K, Ueno M, Sakamoto H, Maekawa N, Ueda N (2005) Regional distribution and age-dependent expression of N-acylphosphatidylethanolamine-hydrolyzing phospholipase D in rat brain. J Neurochem 94:753–762.
- Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61–65.
- Musella A, De Chiara V, Rossi S, Prosperetti C, Bernardi G, Maccarrone M, Centonze D (2009) TRPV1 channels facilitate glutamate transmission in the striatum. Mol Cell Neurosci 40:89–97.

- Narushima M, Uchigashima M, Hashimoto K, Watanabe M, Kano M (2006) Depolarization-induced suppression of inhibition mediated by endocannabinoids at synapses from fast-spiking interneurons to medium spiny neurons in the striatum. Eur J Neurosci 24(8):2246–2252.
- Narushima M, Uchigashima M, Fukaya M, Matsui M, Manabe T, Hashimoto K, Watanabe M, Kano M (2007) Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. J Neurosci 27:496–506.
- Nelson A, Killcross S (2006) Amphetamine exposure enhances habit formation. J Neurosci 26:3805–3812.
- Nordquist RE, Voorn P, de Mooij-van Malsen JG, Joosten RN, Pennartz CM, Vanderschuren LJ (2007) Augmented reinforcer value and accelerated habit formation after repeated amphetamine treatment. Eur Neuropsychopharmacol 17:532–540.
- Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279:5298–5305.
- Okuma Y, Fukuda Y, Osumi Y (1983) Neurotensin potentiates the potassium-induced release of endogenous dopamine from rat striatal slices. Eur J Pharmacol 93:27–33.
- Oz M (2006) Receptor-independent actions of cannabinoids on cell membranes: focus on endocannabinoids. Pharmacol Ther 111:114–144.
- Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58:389–462.
- Pan X, Ikeda SR, Lewis DL (1998) SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca2+ currents by reversal of tonic CB1 cannabinoid receptor activity. Mol Pharmacol 54:1064–1072.
- Partridge JG, Tang KC, Lovinger DM (2000) Regional and postnatal heterogeneity of activity-dependent long-term changes in synaptic efficacy in the dorsal striatum. J Neurophysiol 84:1422–1429.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 28:2435–2446.
- Pickel VM, Chan J, Kash TL, Rodriguez JJ, MacKie K (2004) Compartment-specific localization of cannabinoid 1 (CB1) and muopioid receptors in rat nucleus accumbens. Neuroscience 127:101–112.
- Porrino LJ, Lyons D, Smith HR, Daunais JB, Nader MA (2004) Cocaine self-administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci 24:3554–3562.
- Rademacher DJ, Meier SE, Shi L, Ho WS, Jarrahian A, Hillard CJ (2008) Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. Neuropharmacology 54:108–116.
- Riegel AC, Lupica CR (2004) Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. J Neurosci 24:11070–11078.
- Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ (2001) Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J Neurosci 21:109–116.
- Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA 99:8384–8388.
- Roberto M, Cruz M, Bajo M, Schweitzer P (2008) Cannabinoids and ethanol interaction on inhibitory and excitatory transmission in central amygdala neurons. Society for Neuroscience Abstracts 431.22.

- Rodriguez JJ, Mackie K, Pickel VM (2001) Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat caudate putamen nucleus. J Neurosci 21:823–833.
- Romero J, Hillard CJ, Calero M, Rabano A (2002) Fatty acid amide hydrolase localization in the human central nervous system: an immunohistochemical study. Brain Res Mol Brain Res 100:85–93.
- Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679.
- Ronesi J, Lovinger DM (2005) Induction of striatal long-term synaptic depression by moderate frequency activation of cortical afferents in rat. J. Physiol. 562(Pt 1):245–256.
- Sanchis-Segura C, Cline BH, Marsicano G, Lutz B, Spanagel R (2004) Reduced sensitivity to reward in CB1 knockout mice. Psychopharmacology (Berl) 176:223–232.
- Sanudo-Pena MC, Tsou K, Walker JM (1999) Motor actions of cannabinoids in the basal ganglia output nuclei. Life Sci 65:703–713.
- Sheinin A, Talani G, Davis MI, Lovinger DM (2008) Endocannabinoidand mGluR5-dependent short-term synaptic depression in an isolated neuron/bouton preparation from the hippocampal CA1 region. J Neurophysiol 100:1041–1052.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Sidlo Z, Reggio PH, Rice ME (2008) Inhibition of striatal dopamine release by CB1 receptor activation requires nonsynaptic communication involving GABA, H2O2, and KATP channels. Neurochem Int 52:80–88.
- Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/ beta-hydrolase 4 in this pathway. J Biol Chem 281:26465–26472.
- Simon GM, Cravatt BF (2008) Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. J Biol Chem 283:9341–9349.
- Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388:773–778.
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215:89–97.
- Sun YX, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, Ueda N (2004) Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. Biochem J 380:749–756.
- Sung KW, Choi S, Lovinger DM (2001) Activation of group I mGluRs is necessary for induction of long-term depression at striatal synapses. J Neurophysiol 86:2405–2412.
- Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85:395–403.
- Szabo B, Muller T, Koch H (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens in vitro. J Neurochem 73:1084–1089.
- Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15:2057–2061.
- Szabo B, Urbanski MJ, Bisogno T, Di Marzo V, Mendiguren A, Baer WU, Freiman I (2006) Depolarization-induced retrograde

synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoylglycerol. J Physiol 577:263–280.

- Takahashi Y, Roesch MR, Stalnaker TA, Schoenbaum G (2007) Cocaine exposure shifts the balance of associative encoding from ventral to dorsolateral striatum. Front Integr Neurosci 1:1 doi: 10.3389/neuro.07/011.2007.
- Tang K, Low MJ, Grandy DK, Lovinger DM (2001) Dopamine-dependent synaptic plasticity in striatum during in vivo development. Proc Natl Acad Sci USA 98:1255–1260.
- Thomas EA, Cravatt BF, Danielson PE, Gilula NB, Sutcliffe JG (1997) Fatty acid amide hydrolase, the degradative enzyme for anandamide and oleamide, has selective distribution in neurons within the rat central nervous system. J Neurosci Res 50:1047–1052.
- Tornqvist H, Belfrage P (1976) Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. J Biol Chem 251:813–819.
- Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.
- Tzavara ET, Li DL, Moutsimilli L, Bisogno T, Di Marzo V, Phebus LA, Nomikos GG, Giros B (2006) Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. Biol Psychiatry 59:508–515.
- Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007) Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27:3663–3676.
- Van Der Stelt M, Di Marzo V (2004) Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. Eur J Biochem 271:1827–1834.
- Van Sickle MD, Duncan M, Kingsley PJ, et al. (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 310:329–332.
- Villares J (2007) Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. Neuroscience 145:323–334.
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468–474.
- Walsh JP (1993) Depression of excitatory synaptic input in rat striatal neurons. Brain Res 1608(1):123–128.
- Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G (2003) Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. Proc Natl Acad Sci USA 100:1393–1398.
- Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, Tkatch T, Lovinger DM, Surmeier DJ (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50:443–452.
- Wartmann M, Campbell D, Subramanian A, Burstein SH, Davis RJ (1995) The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. FEBS Lett 359:133–136.
- Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588–592.
- Yin HH, Adermark L, Lovinger DM (2008a) Neurotensin reduces glutamatergic transmission in the dorsolateral striatum via retrograde endocannabinoid signaling. Neuropharmacology 54:79–86.
- Yin HH, Davis MI, Ronesi JA, Lovinger DM (2006a) The role of protein synthesis in striatal long-term depression. J Neurosci 26:11811–11820.

- Yin HH, Knowlton BJ (2004) Contributions of striatal subregions to place and response learning. Learn Mem 11:459–463.
- Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.
- Yin HH, Knowlton BJ, Balleine BW (2005a) Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. Eur J Neurosci 22:505–512.
- Yin HH, Knowlton BJ, Balleine BW (2006b) Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. Behav Brain Res 166:189–196.
- Yin HH, Lovinger DM (2006) Frequency-specific and D2 receptormediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 103:8251–8256.

- Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005b) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513–523.
- Yin HH, Prasad-Mulcare S, Hilario MRF, Clouse E, Davis MI, Hansson AC, Lovinger DM, Costa RM (2008b) Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nature Neuroscience 12(3):333–341.
- Zhu PJ, Lovinger DM (2005) Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurosci 25(26):6199–6207.
- Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci USA 96:5780–5785.

# Nitric Oxide Signaling in the Striatum

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# I. INTRODUCTION: THE NITRIC OXIDE SYSTEM

Nitric oxide (NO) is a gaseous neuromodulator which is a key player in the regulation of numerous physiological and pathophysiological processes in both the peripheral and central nervous system (Boehning and Snyder, 2003; Bredt, 2003; Garthwaite, 2008). NO was first identified as the "endothelial derived relaxation factor" (EDRF) in peripheral blood vessels (Palmer et al., 1987), and has since been implicated in numerous other critical neuronal processes. Perhaps most notably, NO has been shown to mediate various forms of synaptic plasticity, including short and long-term changes in the efficacy of excitatory and inhibitory synaptic transmission (Susswein et al., 2004). Three distinct isoforms of the NO-producing enzyme nitric oxide synthase (NOS; neuronal NOS, inducible NOS, endothelial NOS) have been described (Alderton et al., 2001; Garthwaite, 2008). Of these isoforms, type 1 (neuronal, nNOS) has been shown to be ubiquitously distributed

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. throughout the brain in an uneven manner, and is expressed in the nuclei of the basal ganglia in moderate levels (Bredt et al., 1990; Vincent, 1994).

#### A. Biosynthesis of NO

The initial purification of nNOS from the rat, pig, and human cerebellum revealed that this ~150kDa protein is activated by calcium/calmodulin and uses NADPH as an electron donor (Bredt and Snyder, 1990; Mayer et al., 1990; Schmidt and Murad, 1991). It is now known that nNOS exhibits a bidomain structure consisting of distinct oxygenase and reductase domains (Nathan and Xie, 1994a; Nathan and Xie, 1994b). Upon activation, nNOS monomers dimerize and together with two calmodulins, form a tetrameric complex (Griffith and Stuehr, 1995; Alderton et al., 2001; Stuehr et al., 2004). Electrons derived from NADPH are donated to the reductase domain of nNOS which via the redox carriers FAD and FMN, shuttle electrons to the oxygenase domain (Nathan and Xie, 1994a; Stuehr et al., 2004). Upon arrival in the oxygenase domain, the electrons interact with heme iron and tetrahydrobiopterin at the active site to catalyze the monooxygenase reaction which converts L-arginine into citrulline and NO co-products (Nathan and Xie, 1994a; Stuehr et al., 2004). In the first phase of this reaction, L-arginine is converted to N<sup>G</sup>-hydroxy-L-arginine and the hydroxylated nitrogen from this intermediate is processed further to yield NO (Stuehr et al., 1991). Thus, the conversion of L-arginine to NO and L-citrulline requires two successive monooxygenase reactions.

# B. nNOS-Expressing Interneurons and NO Effector Pathways

In the rat striatal complex, nNOS interneurons are readily revealed using NADPH-diaphorase histochemical or nNOS immunocytochemical staining techniques (Hope et al., 1991; Kawaguchi, 1993; Gracy and Pickel, 1997) (see also Chapter 8). Numerous studies using these techniques have shown that NOS interneurons are all relatively aspiny, and in addition to NO, synthesize and release GABA as well as neuropeptides such as somatostatin and neuropeptide Y (Kubota et al., 1993; Kawaguchi, 1997; Tepper et al., 2004). Neuroanatomical studies using unbiased stereological sampling techniques estimate that nNOS-containing interneurons make up less than 3 percent (~21,000 cells) of the total neuronal population of the striatum (West et al., 1996). However, the axons of nNOS interneurons are highly ramified and project to distal subregions of the striatum (up to 1 mm from soma), enabling these cells to exert a tremendous functional impact on both blood flow and neurotransmission in this highly integrative nucleus (Emson et al., 1993; Kawaguchi, 1997).

The most well studied signal transduction pathway for NO transmission in the brain involves the activation of the soluble guanylyl cyclase (sGC) signaling cascade and subsequent production of the second messenger cGMP (Murad, 2006; Garthwaite, 2008). Electrophysiological studies performed in intact systems and striatal slices from rats have demonstrated that NO potently modulates the activity of medium-sized spiny neurons (MSNs) (see also Chapter 5) via the activation of sGC and cGMP synthesis (Calabresi et al., 1999a; Calabresi et al., 1999b; West and Grace, 2004). MSNs contain very high levels of this NO effector enzyme as well as the other components of the sGC-cGMP second messenger system (Ariano, 1983; Ariano and Ufkes, 1983) and sGC activity is reported to be higher in the rat striatum than in any other region of the brain (Hofmann et al., 1977; Matsuoka et al., 1992). Ultrastructural studies showing that NOS immunoreactive processes form synaptic contacts on dendritic spine shafts of MSNs in the rat dorsal striatum and nucleus accumbens also indicate that the sGC signaling cascade is postsynaptic to presynaptic sources of NO (Calabresi et al., 1999b; Sancesario et al., 2000; Hidaka and Totterdell, 2001; French et al., 2005). This is supported further by studies using organotypic co-cultures which have demonstrated that DARPP-32-expressing striatal MSNs are innervated by a dense plexus of nitrergic terminals (Gomez-Urquijo et al., 1999). Interestingly, these NO producing inputs have been shown to be directly apposed to tyrosine hydroxylase expressing (i.e., dopaminergic) terminals (Hidaka and Totterdell, 2001). The interaction between these afferents will be discussed below.

Studies performed in brain slices from rats and mice have shown that the NO-sGC-cGMP mediated stimulation of cGMP-dependent protein kinase (PKG) activity results in increased DARPP-32 (Thr-34) phosphorylation in striatal MSNs (Tsou et al., 1993; Nishi et al., 2005). The PKGmediated increase in DARPP-32 (Thr-34) phosphorylation is rapid, transient and dependent on an increase in intracellular calcium levels induced following glutamatergic stimulation of NMDA, AMPA and metabotropic glutamate subtype 5 receptors (Nishi et al., 2005). Striatal DARPP-32 activation is thought to play a central role in mediating many of the physiological effects of dopamine (DA) and the interactions between DA and other neurotransmitters that modulate MSN activity and striatal output (Greengard, 2001). Together with DA receptor stimulation, this NOsGC-cGMP-PKG-DARPP-32 effector pathway has been shown to play a key role in regulating long-term changes in striatal synaptic efficacy (Calabresi et al., 2007), and as such, is likely to play an important role in the planning and execution of purposeful motor activity (Del Bel et al., 2005). These topics will be discussed in more detail below.

Other effects of NO-sGC-cGMP signaling are mediated via cGMP-dependent modulation of phosphodiesterase (PDE) activity and cyclic nucleotide metabolism (Murad, 2006; Garthwaite, 2008). At least seven different PDE subtypes are expressed in moderate to high levels in the striatum (Menniti et al., 2006) and inhibition of PDE (e.g., PDE10A) function has been shown to increase DARPP-32 activity (Nishi et al., 2008), membrane excitability, and responsiveness of MSNs to cortical inputs (West and Grace, 2004; Threlfell et al., 2009). Furthermore, intracellular application of a PDE inhibitor was shown to increase the duration of spontaneous up states known to be driven by glutamatergic inputs (West and Grace, 2004). PDE inhibition has also been shown to promote pharmacological long-term depression (LTD) at rat corticostriatal synapses (Calabresi et al., 1999b). Despite the above studies, the interactions between striatal PDEs and NO-cGMP signaling remain poorly understood.

In addition to activation of the sGC-cGMP-PKG cascades, NO has been implicated in regulating numerous, diverse membrane bound and soluble intracellular proteins via direct mechanisms involving protein modifications. For example, NO has been reported to activate ADP-ribosyl transferases, increase prostaglandin biosynthesis via stimulation of cyclo-oxygenases, and inhibit ribonucleotide reductase, cytochrome c oxidase activity, and monoaminergic transporter proteins (Brune and Lapetina, 1989; Lepoivre et al., 1991; Salvemini et al., 1993; Cleeter et al., 1994; Schuman et al., 1994; Kiss et al., 1999; Kiss et al., 2004). The facilitatory effect of NO on ADP-ribosylation was found to be associated with an increase in expression of the immediate early gene c-fos in striatal neurons (Morris, 1995). Multiple studies indicate that NO facilitates intracellular calcium release from mitochondrial pools in striatal neurons (Meini et al., 2000; Horn et al., 2002). NO may also modulate the activity of ion channels regulating neuronal membrane excitability directly via nitrosylation/nitrosation mechanisms or indirectly via PKG-dependent mechanisms (Ahern et al., 2002; Lipton, 2007). For example, NO has been demonstrated to alter NMDA and GABA-A receptor function, and potassium, calcium, chloride, and non-selective ion channels via both direct and indirect mechanisms (Ahern et al., 2002). However, it should be noted that the physiological significance of many of these non-cGMP dependent effects has been questioned (Garthwaite, 2008).

# II. AFFERENT REGULATION OF STRIATAL NO SYNTHESIS

# A. Role of Corticostriatal Afferents and Glutamate Receptors

As initially described by Garthwaite and colleagues in studies using cerebellar cells as a model system (Garthwaite et al., 1988), the primary signaling pathway of nNOS involves NMDA receptor-dependent stimulation of NO synthesis (Fig. 10.1). These studies used multiple experimental approaches to demonstrate that following NMDA receptor activation, a labile factor sensitive to hemoglobin is released from cerebellar cells. This factor was shown to be similar to EDRF in that it was able to relax vascular smooth muscle, had a short half life, and was protected by superoxide dismutase activity (Garthwaite et al., 1988). More recent studies have shown that low concentrations of glutamate induce a rapid and transient NMDA receptor-dependent phosphorylation of nNOS on ser 1412 which is mediated via Akt activation and essential for activation of nNOS (Rameau et al., 2007). Furthermore, nNOS is bound to postsynaptic density protein PSD-95 which anchors it in a functional complex with the NMDA receptor (Christopherson et al., 1999). As a result, NO is generated proximal to the plasma membrane and can diffuse freely out of the cell and modulate neurotransmission via its interactions with the downstream signaling molecules mention above.

In the rodent striatal complex, nNOS interneurons express NMDA, AMPA and metabotropic glutamate receptors (Kawaguchi et al., 1995; Gracy and Pickel, 1997; Kawaguchi, 1997; Nishi et al., 2005) and receive asymmetric glutamatergic synaptic contacts from the (frontal) cortex (Vuillet et al., 1989; Salin et al., 1990). Studies using electrophysiological and molecular techniques initially suggested that nNOS interneurons may be potently activated by stimulation of corticostriatal afferents (Kawaguchi, 1993; Berretta et al., 1997). Using an NO-selective microsensor, we recently demonstrated for the first time that striatal NO efflux is robustly increased in vivo by electrical stimulation of frontal cortical afferents in a frequency- and stimulus intensity-dependent manner via NMDA receptor- and nNOS-dependent mechanisms (Sammut et al., 2007b). These observations extend previous reports that intrastriatal infusion of NMDA potently activates NO efflux in a manner that can be blocked by NMDA receptor antagonists and nNOS inhibitors (Iravani et al., 1998; Crespi and Rossetti, 2004; Rossetti and Crespi, 2004).

# **B.** Regulation of Striatal NO Synthesis by Dopamine

In addition to glutamate receptors, *in situ* hybridization studies performed in rodents have shown that nNOS interneurons contain low levels of D1 DA receptor mRNA (Le et al., 1991) and express D5 receptor protein (Rivera et al., 2002; Centonze et al., 2003). To my knowledge, the co-localization of D2/3/4 receptors or mRNAs with markers of striatal nNOS interneurons has not been described. However, we have recently shown that both D1- and D2-like receptor activation strongly regulates striatal nNOS activity, albeit in opposing manners (Sammut et al., 2006; Sammut et al., 2007a). Accordingly, electrical and chemical stimulation of the substantia nigra



FIGURE 10.1 Model of striatal nNOS regulation and nitrergic modulation of MSN activity. Frontal corticostriatal inputs have been shown to target the dendrites of striatal nitrergic/GABAergic interneurons (Kawaguchi, 1997). Coherent activation of frontal cortical afferents exerts direct excitatory effects on nNOS positive interneurons via NMDA receptor activation (Crespi and Rossetti, 2004; Sammut et al., 2007b). Calcium influx resulting from NMDA receptor activation activates nNOS in a calmodulin-dependent manner and facilitates the conversion of L-arginine into L-citrulline and NO (Garthwaite, 2008). The nigrostriatal DA system facilitates nNOS activity via D1/5 receptor stimulation in a manner that is opposed by concurrent D2-like receptor activation (Sammut et al., 2006; Sammut et al., 2007a). In this model we propose that tonic NO signaling increases glutamatergic transmission across corticostriatal synapses via a sGC-cGMP-dependent mechanism (West and Grace, 2004; Sammut et al., 2007b). The impact of cGMP on MSN function is limited by numerous PDEs which metabolize cyclic nucleotides, some of which are activated (e.g., PDE2A) or inhibited (PDE3A) by cGMP (Menniti et al., 2006). While phasic NO-sGC-cGMP signaling can increase the responsiveness of MSNs to cortical input (West and Grace, 2004), the excitatory impact of NO is attenuated by DA-mediated D2 receptor activation which acts to dampen subsequent excitatory responses in MSNs (Ondracek et al., 2008). Thus, disruption of nNOS activity was shown to increase the magnitude of D2 receptor-mediated short-term depression of cortically-evoked spike activity induced *in vivo* during phasic stimulation of frontal cortical afferents (Ondracek et al., 2008). Under physiological conditions, it is likely that corticostriatal transmission controls feed-forward inhibitory and excitatory processes differentially to enable the coordination of purposeful motor behavior.

and systemic administration of the D1/5 receptor agonist SKF 81297 both robustly increased striatal NO efflux via nNOS and D1/5 receptor-dependent mechanisms (Liu et al., 2005b; Sammut et al., 2006; Sammut et al., 2007a). The facilitatory effects of electrical stimulation of the substantia nigra and SKF 81297 on striatal NO efflux were both attenuated by systemic administration of the D2-like receptor agonist quinpirole, whereas administration of the D2-like receptor antagonist eticlopride augmented evoked NO efflux (Sammut et al., 2007a). In agreement with our studies, D1/5 receptor activation has been shown to increase striatal tissue levels of cGMP (Altar et al., 1990; Di Stefano et al., 2005; Siuciak et al., 2006), whereas D1/5 antagonism produced the opposite effect (Altar et al., 1990; Di Stefano et al., 2005). Conversely, D2like receptor agonism decreased striatal tissue levels of cGMP (Di Stefano et al., 2005) and D2-like receptor antagonism increased these measures, potentially via activation of presynaptic heteroreceptors located on glutamatergic inputs to nNOS interneurons (Altar et al., 1990; Di Stefano et al., 2005). Additionally, studies using NADPH-diaphorase staining as an indirect measure of striatal nNOS activity have shown that administration of D1/5 receptor antagonists decreases enzyme activity, whereas D2-like receptor antagonists have the opposite effect (Morris et al., 1997). Taken together, these studies show that DA transmission regulates nNOS activity via both facilitatory (D1-like receptor activation) and inhibitory (D2-like receptor activation) mechanisms (see Fig. 10.1).

Pioneering work by Calabresi and colleagues demonstrated that D1-like receptor activation occurring following bath perfusion of SKF 38393 robustly stimulates the firing activity of electrophysiologically-identified nNOS interneurons in striatal brain slice preparations from both rats

(190

and mice (Centonze et al., 2002; Centonze et al., 2003). The excitatory effect of SKF 38393 on the membrane activity of nNOS interneurons was also observed in D1 receptor -/- mice suggesting that the D1-like receptor involved in the excitation of these cells is the D5 subtype (Centonze et al., 2003). Moreover, bath application of SKF 38393 strongly depolarized the membrane potential of nNOS cells recorded in rat striatal slices during tetrodotoxin co-perfusion, indicating that D5 receptor-dependent activation of striatal nNOS activity is likely mediated by a direct effect on striatal nNOS interneurons (Centonze et al., 2002). Similar studies performed in the presence of bath applied tetrodotoxin revealed that the D2-like receptor agonist quinpirole did not directly affect the membrane potential of nNOS interneurons (Centonze et al., 2002). Taken together, the above studies indicate that DA potently modulates NO synthesis and nNOS interneuron activity directly via stimulation of D1- and D2-like receptors. Given that D5 receptor agonism potently depolarizes the membrane potential of these interneurons and stimulates NO efflux (see above), it is likely that these events are related to each other such that membrane depolarization stimulates intracellular calcium influx and subsequently, facilitates nNOS activity. The inhibitory effect of D2-like receptor agonism observed in the neurochemical studies does not appear to be a direct effect, but may be mediated via D2-like receptor-dependent suppression of glutamatergic inputs (Fig. 10.1) or another indirect pathway.

# III. EFFECTS OF NO SIGNALING ON NEUROTRANSMITTER RELEASE

Considerable evidence has accumulated in the past 10–15 years indicating that endogenous (stimulated by local tissue infusion of the NO precursor L-arginine or N<sup>G</sup>-hydroxy-L-arginine) and exogenous (generated from NO-donors) NO facilitates the release of many neurotransmitters in the striatum including glutamate, DA, and acetylcholine (ACh). This work has been extensively reviewed (Kiss, 2000; Prast and Philippu, 2001; West et al., 2002). Thus, the following section is a brief summary and update on the recent progress towards understanding the mechanisms associated with NO signaling as it pertains to the modulation of major striatal afferent systems.

#### A. Regulation of Glutamate Release

It is generally accepted that NO signaling can act to amplify excitatory neurotransmission at glutamatergic synapses in numerous brain regions (Garthwaite, 2008). Studies using reduced striatal preparations have shown that NO can inhibit glutamate transporters leading to increases in extracellular glutamate concentrations (Lonart and Johnson, 1994; Pogun et al., 1994; Taskiran et al., 2003). In vivo microdialysis studies in rats have also shown that intrastriatal infusion of both NOS substrate and NO generators increased local glutamate efflux up to two-fold over basal levels (Guevara-Guzman et al., 1994; West and Galloway, 1997a). Likewise, studies using push-pull perfusion techniques have shown that local infusion of NO generators increased glutamate release in the dorsal striatum (Segovia et al., 1994) and nucleus accumbens (Kraus and Prast, 2002). Exogenous NO also facilitated glutamate release evoked in the nucleus accumbens via electrical stimulation of the hippocampal fimbria (Kraus and Prast, 2001; Kraus and Prast, 2002). NO produced following NMDA receptor activation may promote additional glutamate release (Montague et al., 1994; Bogdanov and Wurtman, 1997). Indeed, studies by Wurtman and colleagues showed that the generation of endogenous NO following local striatal NMDA infusion also increases extracellular glutamate levels in vivo in a NOS- and calciumdependent manner. Importantly, NMDA-induced glutamate release was shown to be attenuated in the cortex and striatum of nNOS knockout mice (Kano et al., 1998). Studies by Montague and colleagues showed that NMDA-mediated release of [<sup>3</sup>H]-glutamate and [<sup>3</sup>H]-norepinephrine from cortical synaptosomes is decreased following NOS inhibition or scavenging of NO by hemoglobin (Montague et al., 1994).

The above studies indicate that the facilitation of glutamate transmission by NO is complex. Indeed, the effects of NO on glutamate terminals may involve multiple modifications of transporter proteins and synaptic machinery as NO has been shown to: (1) inhibit glutamate uptake into terminals (Lonart and Johnson, 1994; Pogun et al., 1994; Taskiran et al., 2003) and synaptic vesicles via s-nitrosylation of transporter enzyme sulfyhydryl groups (Wolosker et al., 1996); and (2) facilitate the release of vesicular glutamate from hippocampal synaptosomes via a calcium-independent mechanism (Meffert et al., 1994). Additionally, intrastriatal cGMP infusion increases extracellular glutamate via an unknown mechanism (Guevara-Guzman et al., 1994; Kraus and Prast, 2002). Thus, these studies indicate that NO facilitation of glutamate transmission is a widespread and highly conserved mechanism involved in the nitrergic modulation of synaptic transmission. The effects of NO on electrophysiological measures of corticostriatal transmission are discussed below.

#### **B.** Regulation of Dopamine Release

Numerous studies performed in rats, mice and hamsters have demonstrated that endogenous NO produced via intrastriatal substrate (L-arginine or N<sup>G</sup>-hydroxy-L-arginine) infusion facilitates DA efflux in vitro (Zhu and Luo, 1992; Chaparro-Huerta et al., 1997; Liang and Kaufman, 1998) and in vivo (Strasser et al., 1994; Spatz et al., 1995; West and Galloway, 1997b; West and Galloway, 1997a). NO-induced DA efflux is dependent on activation of the nNOS isoform, dose of substrate utilized, and is potentiated following inhibition of glutamate reuptake (West and Galloway, 1997a; West and Galloway, 1997b). In the vast majority of cases, the effect of endogenous NO on DA release can be mimicked with multiple NO generating compounds (West et al., 2002). However, the facilitatory effects of exogenous NO on DA release are dependent on the NO generator used and the redox state and integrity of the experimental preparation (Buyukuysal, 1997; Trabace and Kendrick, 2000; Serra et al., 2001). Thus, in oxidatively stressed environments the reaction of the NO radical with superoxide anions promotes the formation of peroxynitrite (Lipton et al., 1994), which may act to inhibit DA release (Buyukuysal, 1997; Trabace and Kendrick, 2000; Serra et al., 2001). Conversely, under physiological conditions where the biological environment is not in a state of oxidative stress (i.e., in presence of adequate oxygen and antioxidants), NO donors (e.g., S-nitroso-N-acetylpenicillamine (SNAP), sodium nitroprusside) generally facilitate DA release (Nakahara et al., 1994; West and Galloway, 1996; West and Galloway, 1998; West et al., 2002). Most studies have found that DA release stimulated by NO generators is not mediated via sGC activation, but rather, is associated with the activation of an ionotropic glutamate receptor-dependent mechanism (Nakahara et al., 1994; West and Galloway, 1996; West and Galloway, 1997a; West et al., 2002; Rocchitta et al., 2004) or inhibition of the DA transporter (Pogun and Kuhar, 1994; Kiss et al., 2004), but see also work by Kendrick and colleagues (Trabace and Kendrick, 2000). Lastly, high output of NO production may result in the activation of negative feedback mechanisms regulating NMDA receptor function (West et al., 2002).

#### C. Regulation of Acetylcholine Release

Considerable work on NO-ACh interactions has been carried out in the ventral striatum by Prast and colleagues (Prast and Philippu, 2001). A series of reports performed in both awake and anesthetized rats demonstrated that low and moderate concentrations of various NO generators (diethylamine/ NO, SNAP, or 3-morpholinosydnonimine (SIN-1)) superfused into the nucleus accumbens facilitated the release of ACh (Prast et al., 1995; Prast et al., 1998; Kraus and Prast, 2001). At higher concentrations, local infusion of diethylamine/NO decreased the release rate of ACh via a GABA-A receptor-dependent mechanism (Prast et al., 1998). Further work showed that the sGC-cGMP signaling pathway is critically involved in the modulation of ACh efflux by NO (Guevara-Guzman et al., 1994; Prast et al., 1998; Kraus and Prast, 2002). This is supported by observations showing that local infusion of exogenous cGMP increases ACh efflux (Guevara-Guzman et al., 1994), whereas pharmacological disruption of endogenous cGMP synthesis following local infusions of sGC inhibitors decreased ACh efflux in the striatal complex (Prast et al., 1998). Furthermore, pharmacological disruption of endogenous cGMP metabolism using local PDE inhibitor infusions was shown to increase glutamate efflux in the NAc (Kraus and Prast, 2002). This increase in glutamate release was found to play an intermediate role in NO-mediated ACh release (Kraus and Prast, 2001). Therefore, similar to studies described above for DA release, the facilitatory effect of NO on ACh release was blocked by local infusions of ionotropic glutamate receptor antagonists (Prast et al., 1998). Studies by Calabresi and colleagues showing that NO increases the firing activity of electrophysiologically identified cholinergic interneurons recorded in striatal slices also strongly support the findings of the above neurochemical studies (Centonze et al., 2001).

## IV. REGULATION OF STRIATAL NEURON ACTIVITY AND OUTPUT BY NO SIGNALING

### A. Tonic NO Signaling

Several *in vivo* studies performed in rats have demonstrated that striatal extracellular nitrite (Ohta et al., 1994a), cGMP (Globus et al., 1995) and L-citrulline (Ohta et al., 1994a; Ohta et al., 1994b) levels are maintained in a steady-state which is sensitive to changes in NOS activity. Likewise, genetic disruption of nNOS activity decreases striatal cGMP levels by approximately 50% (Siuciak et al., 2006), indicating that steady-state nitrergic tone is the primary activator of sGC under basal conditions (see Fig. 10.1). Thus, as observed in the hippocampus (Hopper and Garthwaite, 2006), levels of "tonic" NO and cGMP are likely to play an important signaling role in the striatum. In support of this, we have recently

shown that systemic administration of the nNOS inhibitor 7-nitroindazole strongly inhibited the spontaneous firing activity of striatal neurons isolated during low frequency cortical stimulation (Ondracek et al., 2008). Moreover, in previous studies using intracellular recordings in vivo (West and Grace, 2004), we showed that disruption of local NO-sGC signaling using intrastriatal or intracellular application of selective enzyme inhibitors depressed glutamate-driven up states and evoked EPSPs, and decreased the responsiveness of identified MSNs to somatic injection of depolarizing current. Similar effects were observed following local infusion of the NO scavenger carboxy PT-IO (West and Grace, 2004). In most cases the inhibitory effects of these compounds on neuronal activity were reversed by co-application of cGMP analogues, indicating that tonic NO-cGMP signaling regulates the function of ion channels involved in controlling the basal membrane excitability of MSNs and their responsiveness to excitatory glutamatergic transmission (West and Grace, 2004).

#### **B.** Phasic NO Signaling

Recent studies performed in rats using extracellular recording techniques to investigate the impact of locally applied NO generating compounds on basal and evoked activity of striatal neurons have reported that phasic NO signaling can act to facilitate (West et al., 2002; Liu et al., 2005a) or inhibit (Di Giovanni et al., 2003; Galati et al., 2008) neuronal activity of putative MSNs. Studies by West and colleagues showed that reverse dialysis of the NO generating compound SNAP increased the firing rate and bursting of striatal neurons under non-stimulated conditions and during single-pulse electrical stimulation of the prefrontal cortex (West et al., 2002). Consistent with these findings, microiontophoresis of the NO generator sodium nitroprusside was shown to elevate the spontaneous firing rate of 51 out of 66 striatal neurons and to facilitate glutamate-evoked excitation in a subpopulation of putative MSNs (Liu et al., 2005a). This same study showed that microiontophoresis of the NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME) produced opposite effects on evoked activity (Liu et al., 2005a). In contrast to the above findings, microiontophoresis of the superoxide and NO generator SIN-1 was reported to decrease glutamate-induced excitations in 12 of 15 neurons, whereas L-NAME increased glutamate-evoked activity of putative MSNs (Di Giovanni et al., 2003). Similar outcomes were reported by Galati and colleagues (Galati et al., 2008).

Taken together, the above studies indicate that phasic NO signaling can have excitatory or inhibitory effects on striatal

neurons and that this may depend on the level of NO generated, the method of NO delivery, and the relative activity of the neurons under study. However, numerous methodological differences exist between each of these studies related to recording techniques, drug selection, drug delivery and data analysis which may have contributed to differences in outcomes. Moreover, a general criticism of all four studies is that each used only one NO generator and did not confirm the specificity of the purported NO effect. The exclusive use of SIN-1 as an NO generator in the studies by Di Giovanni and Galati is particularly problematic because SIN-1 produces superoxide along with NO, which together spontaneously react to form peroxynitrite (Hogg et al., 1992). Thus, it is possible that the inhibitory effects of SIN-1 on glutamate-evoked firing were not mediated by NO, but resulted from the actions of peroxynitrite. The specificity of the effects of the nonselective L-arginine analogues used in the above microiontophoretic studies is also unclear because only one inhibitor was used and no attempt was made to antagonize the effect with L-arginine. Lastly, and most importantly, none of the above reports identified the neuronal populations studied with neurochemical labeling techniques or antidromic activation.

# C. Regulation of Short- and Long-Term Synaptic Plasticity

The neurochemical studies discussed above indicate that nitrergic transmission is strongly activated by convergent and synchronous burst firing of corticostriatal neurons (Sammut et al., 2007b). To determine the impact of physiological concentrations of endogenous NO generated from local interneurons, we utilized a similar intermittent train stimulation protocol to activate frontal cortical afferents and NO synthesis (Ondracek et al., 2008). These studies examined the impact of train stimulation of the cortex on single-unit activity evoked via low frequency stimulation of the opposite frontal cortical hemisphere. We found that the most frequently observed response to train stimulation of the frontal cortex in rats treated with vehicle or a nNOS inhibitor was a short-term inhibition of cortically-evoked spike activity. This short-term inhibitory response was blocked by systemic administration of the D2 receptor antagonist eticlopride, suggesting that it was mediated by D2-like receptor stimulation (Ondracek et al., 2008). A subpopulation of neurons (~20%) recorded in vehicle-treated rats responded to train stimulation of the frontal cortex with a transient increase in cortically-evoked firing (Ondracek et al., 2008). Group comparisons of the frequency of these responses following systemic administration of the nNOS inhibitor revealed a significant increase in short-term inhibitory responses and an abolition of excitatory responses. These results demonstrate that burst stimulation of frontal cortical afferents in the intact animal activates a powerful feed-forward excitation mediated by phasic NO signaling which opposes concurrent D2-like receptor-dependent short-term inhibitory influences on striatal neuron activity (Ondracek et al., 2008).

We have also examined the role of tonic NO signaling in short-term plasticity observed across corticostriatal synapses during paired-pulse stimulation of the prefrontal cortex (West and Grace, 2004). In these studies, MSNs were recorded during intrastriatal infusion of either artificial cerebral spinal fluid (vehicle control) or the NO scavenger CPT-IO. We found that MSNs recorded in the presence of CPT-IO exhibited decreases in paired-pulse facilitation of EPSPs evoked via stimulation of corticostriatal pathways compared to control recordings (West and Grace, 2004). Taken together with the above studies using the intermittent train stimulation protocol (Ondracek et al., 2008), these observations demonstrate that under physiological conditions tonic and phasic NO signaling acts to promote short-term excitatory influences on corticostriatal synaptic activity.

The vast majority of neurochemical and electrophysiological studies discussed above indicate that in intact networks NO signaling primarily acts to facilitate glutamate transmission across corticostriatal synapses. In apparent contrast to these observations, studies by Calabresi and colleagues examining the role of the NO-sGC signaling pathway on long-term changes in corticostriatal plasticity in brain slice preparations from both rats and mice consistently find inhibitory effects (i.e., LTD) of NO on glutamate transmission (Calabresi et al., 2007). These studies showed that high-frequency stimulation of corticostriatal pathways or augmentation of NO levels using bath perfusion of NO generators (hydroxylamine or SNAP) induced LTD of corticostriatal transmission (Calabresi et al., 1999b). Inhibition of cGMP metabolism using local application of the PDE inhibitor zaprinast yielded similar results in rats (Calabresi et al., 1999b) and in wild-type, but not DARPP-32 -/-, mice (Calabresi et al., 2000). Pharmacological LTD was also reported to occlude further LTD elicited by high-frequency stimulation of corticostriatal pathways (Calabresi et al., 1999b). Pretreatment with the sGC inhibitor ODQ or the PKG inhibitor RP-8-Br-cGMPS blocked the LTD induced by electrical stimulation and pharmacological manipulations indicating that LTD is produced via a nNOS-sGC-cGMP-PKG-DARPP-32 signaling-dependent mechanism (Calabresi

et al., 1999a; Calabresi et al., 1999b; Calabresi et al., 2000). These studies provide clear and convincing evidence for a role of NO in mediating LTD of corticostriatal transmission in striatal brain slices. While the reasons for the apparent differences between outcomes observed in these in vitro studies and the above mentioned in vivo investigations are not known, differences in outcomes are often observed between intact and reduced preparations (Pare et al., 1998a; Pare et al., 1998b), particularly when different stimulation protocols and recording techniques are employed. For example, stimulation paradigms that elicit LTD in striatal slices have been shown to produce long-term potentiation (LTP) of corticostriatal neurotransmission in anesthetized rats (Charpier and Deniau, 1997), possibly resulting from the greater removal of the voltage-dependent magnesium block of NMDA receptors and higher levels of basal excitability observed in vivo (Pare et al., 1998a; Pare et al., 1998b). Alternatively, reports that LTP of corticostriatal transmission is attenuated in slices obtained from eNOS<sup>-/-</sup> mice or following treatment with the non-selective NOS inhibitor L-NAME (Doreulee et al., 2003), indicate that the role played by NO in striatal synaptic plasticity may also depend on the intensity and duration of corticostriatal pathway stimulation (stimulation paradigm) and the relative recruitment of different NOS isoforms. Additional studies are needed to resolve these issues.

# D. Regulation of Striatal Neuronal Synchrony and Output

We have examined the impact of phasic NO signaling, using the above mentioned train stimulation paradigm, on oscillatory activity recorded in the rat striatum. In these studies we performed dual NO microsensor and local field potential recordings concurrently in the contralateral and ipsilateral striatum, respectively (Sammut et al., 2007b). We found that systemic administration of the non-specific NOS and sGC inhibitor methylene blue simultaneously depressed evoked NO efflux and the peak oscillation frequency (within the delta band) of local striatal field potentials recorded immediately following termination of cortical train stimulation (Sammut et al., 2007b). As mentioned above, local application of NO-sGC inhibitors was observed to decrease the amplitude of spontaneous glutamate-driven up states in studies using in vivo intracellular recording techniques (West and Grace, 2004). Other investigators have shown that in addition to modifying activity in corticostriatal networks, inhibition of nNOS activity alters oscillatory activity within

striatopallidal circuits (Ferraro et al., 2002). Furthermore, O'Donnell and Grace have shown that train stimulation of corticostriatal pathways facilitates dye-coupling between MSNs in rat striatal slices in a manner which was blocked by NOS inhibitors and mimicked during bath perfusion of an NO generator (O'Donnell and Grace, 1997). These studies suggest that NO signaling may mediate electrotonic coupling between MSNs and act to synchronize oscillatory activity, and perhaps, spike discharge within a functional ensemble of MSNs. As shown in our recent studies, pharmacological disruption of this nitrergic neuromodulation is likely to compromise the integration of corticostriatal transmission and short-term plasticity in striatal MSNs (West and Grace, 2004; Sammut et al., 2007b; Ondracek et al., 2008). Together, these findings demonstrate that burst firing across corticostriatal synapses is detected and amplified by nNOS interneurons in a feed-forward manner which may facilitate the synchronization of striatal network activity with glutamate driven oscillations. Indeed, this kind of feed-forward facilitation of excitatory transmission by local interneurons has been reported to play an important role in integrating synaptic transmission in the spinal cord, hippocampus and basolateral amygdala (Silberberg et al., 2005; Woodruff et al., 2006).

Given the above, it is likely that perturbation of striatal NO signaling can lead to abnormal striatal output and disruption of neural integration in down-stream basal ganglia nuclei. In support of this, we have shown that local manipulations of striatal NO levels strongly modifies the activity of DA neurons in the substantia nigra indirectly via influences on striatonigral feedback pathways (West and Grace, 2000; West et al., 2002). These studies showed that intrastriatal infusion of nNOS substrate (NG-hydroxy-L-arginine) or NO generators (hydroxylamine or SNAP), concurrently with intermittent periods of striatal and cortical stimulation, increased the mean DA cell population firing rate as compared to vehicle treated controls. Local infusion of either the nNOS inhibitor 7-nitroindazole or the NO scavenger CPT-IO did not affect basal firing rate, however these compounds robustly increased the percentage of DA neurons responding to striatal stimulation with an initial inhibition followed by a rebound excitation (IE response). CPT-IO infusion also markedly decreased the current amplitude required to evoke an IE response during electrical stimulation of the striatum and prefrontal cortex (West and Grace, 2000). Thus, the above studies show that striatal NO signaling is likely to play an important role in modulating the excitability of striatal efferent pathways to both the globus pallidus and substantia nigra.

# V. ROLE OF STRIATAL NO-SGC SIGNALING IN MOTOR BEHAVIOR

A variety of psychopharmacological studies performed in behaving rodents indicate that striatal nNOS interneurons play a critical role in the generation of motor behavior (Del Bel et al., 2005). Thus, systemic and intrastriatal administration of NOS and sGC inhibitors has been reported to suppress basal locomotion and promote catalepsy (Stewart et al., 1994; Del Bel et al., 2004; Echeverry et al., 2007). Disruption of NOS function also potentiates catalepsy induced via D2-like receptor antagonist administration (Del Bel and Guimaraes, 2000; Cavas and Navarro, 2002). Additionally, locomotion stimulated by substance P (Mancuso et al., 1994), NMDA receptor antagonists (Deutsch et al., 1996), and D1/5 and D2-like receptor agonists (Starr and Starr, 1995) is decreased following systemic administration of NOS inhibitors. Elevations in striatal levels of cAMP and cGMP observed in PDE1B knock-out mice are associated with greater locomotor hyperactivity induced via administration of DA agonists (Reed et al., 2002). In summary, these studies demonstrate that in animals with intact DA innervation, striatal NO-sGC transmission promotes locomotor activity.

### VI. IMPACT OF DOPAMINE DEPLETION ON STRIATAL NO-SGC SIGNALING

Striatal NOS activity has been reported to be depressed in the striatal complex of post-mortem brains of patients with Parkinson's disease (PD) (Bockelmann et al., 1994; Eve et al., 1998) and DA-depleted animals (Sahach et al., 2000; de Vente et al., 2000; Barthwal et al., 2001; Sancesario et al., 2004). Although in the case of DA-depleted rodents, conflicting studies have been reported (Chalimoniuk and Langfort, 2007). Additionally, several studies have shown that striatal sGC mRNA and protein are upregulated in DA-depleted mice (Chalimoniuk et al., 2004; Chalimoniuk and Langfort, 2007). Studies measuring cGMP levels in striatal tissue from DA-depleted rodents have reported conflicting results (de Vente et al., 2000; Chalimoniuk et al., 2004; Sancesario et al., 2004; Chalimoniuk and Langfort, 2007; Giorgi et al., 2008). While the nature of these inconsistent findings is currently unclear, a likely explanation is suggested by work showing that mRNA, protein, and activity of striatal PDEs are elevated in DA-depleted rats (Sancesario et al., 2004; Giorgi et al., 2008). Given this, cGMP synthesis and catabolism may be abnormal in striatal MSNs, leading to irregular intracellular cGMP transients. Unfortunately, this has not been studied in the striatum of patients with PD. However, recent studies of PD patients showed that extracellular cGMP levels are elevated in the internal segment of the globus pallidus during clinically-effective deep brain stimulation (Stefani et al., 2005). Additionally, serum levels of cGMP are increased in patients with PD receiving L-DOPA therapy (Chalimoniuk and Stepien, 2004). This suggests that cGMP signaling cascades may be abnormal in PD and may reflect functional disturbances between DA and glutamate in the DA-depleted basal ganglia. Augmentation of corticostriatal transmission by NO-sGC-cGMP signaling may also contribute to the enduring changes in oscillatory activity and membrane excitability of MSNs observed in the DA-depleted striatum (Murer et al., 2002; Hammond et al., 2007). Most pathophysiological models and metabolic studies of PD predict that the net effect of these alterations is an imbalance in striatal output in which the indirect pathway becomes functionally hyperactive and the direct pathway is slightly hypoactive (Albin et al., 1989; Alexander and Crutcher, 1990; Wichmann and Delong, 2007). Therefore, our findings showing that NOsGC-cGMP signaling facilitates corticostriatal transmission and short-term changes in synaptic efficacy (West and Grace, 2004; Ondracek et al., 2008) suggest that increased postsynaptic responsiveness to NO and heightened cGMP production is likely to exacerbate the hyperactivity observed in the indirect (striatopallidal) output pathway in the dopamine-depleted striatum. Conversely, augmentation of corticostriatal transmission during NO signaling may act to partially alleviate the apparent hypofunction observed in the direct pathway in PD.

Given the above, it is likely that future studies examining the impact of DA-depletion on the regulation of striatal nNOS activity will further our understanding of sensorimotor integration within striatal networks involved in normal motor function and pathophysiological states. Moreover, a better understanding of the complexities of NO-sGC-cGMP signaling in identified MSNs and local interneurons in the intact and dopamine-depleted striatum holds considerable promise for the development of novel therapeutic strategies for restoring motor function in patients with PD.

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#### REFERENCES

- Ahern GP, Klyachko VA, Jackson MB (2002) cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO. Trends in Neurosciences 25:510–517.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. Biochem J 357:593–615.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Altar CA, Boyar WC, Kim HS (1990) Discriminatory roles for D1 and D2 dopamine receptor subtypes in the in vivo control of neostriatal cyclic GMP. Eur J Pharmacol 181:17–21.
- Ariano MA (1983) Distribution of components of the guanosine 3',5'phosphate system in rat caudate-putamen. Neuroscience 10:707–723.
- Ariano MA, Ufkes SK (1983) Cyclic nucleotide distribution within rat striatonigral neurons. Neuroscience 9:23–29.
- Barthwal MK, Srivastava N, Dikshit M (2001) Role of nitric oxide in a progressive neurodegeneration model of Parkinson's disease in the rat. Redox Rep 6:297–302.
- Berretta S, Parthasarathy HB, Graybiel AM (1997) Local release of GABAergic inhibition in the motor cortex induces immediate-early gene expression in indirect pathway neurons of the striatum. J Neurosci 17:4752–4763.
- Bockelmann R, Wolf G, Ransmayr G, Riederer P (1994) NADPHdiaphorase/nitric oxide synthase containing neurons in normal and Parkinson's disease putamen. J Neural Transm Park Dis Dement Sect 7:115–121.
- Boehning D, Snyder SH (2003) Novel neural modulators. Annu Rev Neurosci 26:105–131.
- Bogdanov MB, Wurtman RJ (1997) Possible involvement of nitric oxide in NMDA-induced glutamate release in the rat striatum: an *in vivo* microdialysis study. Neurosci Lett 221:197–201.
- Bredt DS (2003) Nitric oxide signaling in brain: potentiating the gain with YC-1. Mol Pharmacol 63:1206–1208.
- Bredt DS, Hwang PM, Snyder SH (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347:768–770.
- Bredt DS, Snyder SH (1990) Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 87:682–685.
- Brune B, Lapetina EG (1989) Activation of a cytosolic ADPribosyltransferase by nitric oxide-generating agents. J Biol Chem 264:8455–8458.
- Buyukuysal RL (1997) Effect of nitric oxide donors on endogenous dopamine release from rat striatal slices. I: Requirement to antioxidants in the medium. Fundam Clin Pharmacol 11:519–527.
- Calabresi P, Picconi B, Tozzi A, Di FM (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Calabresi P, Centonze D, Gubellini P, Marfia GA, Bernardi G (1999a) Glutamate-triggered events inducing corticostriatal long-term depression. J Neurosci 19:6102–6110.
- Calabresi P, Gubellini P, Centonze D, Picconi B, Bernardi G, Chergui K, Svenningsson P, Fienberg AA, Greengard P (2000) Dopamine and cAMP-Regulated Phosphoprotein 32 kDa Controls Both Striatal Long-Term Depression and Long-Term Potentiation, Opposing Forms of Synaptic Plasticity. J Neurosci 20:8443–8451.

- Calabresi P, Gubellini P, Centonze D, Sancesario G, Morello M, Giorgi M, Pisani A, Bernardi G (1999b) A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. J Neurosci 19:2489–2499.
- Cavas M, Navarro JF (2002) Coadministration of -NOARG and tiapride: Effects on catalepsy in male mice. Prog Neuropsychopharmacol Biol Psychiatry 26:69–73.
- Centonze D, Pisani A, Bonsi P, Giacomini P, Bernardi G, Calabresi P (2001) Stimulation of nitric oxide-cGMP pathway excites striatal cholinergic interneurons via protein kinase G activation. J Neurosci 21:1393–1400.
- Centonze D, Bracci E, Pisani A, Gubellini P, Bernardi G, Calabresi P (2002) Activation of dopamine D<sub>1</sub>-like receptors excites LTS interneurons of the striatum. Eur J Neurosci 15:2049–2052.
- Centonze D, Grande C, Saulle E, Martin AB, Gubellini P, Pavon N, Pisani A, Bernardi G, Moratalla R, Calabresi P (2003) Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 23:8506–8512.
- Chalimoniuk M, Langfort J (2007) The effect of subchronic, intermittent L-DOPA treatment on neuronal nitric oxide synthase and soluble guanylyl cyclase expression and activity in the striatum and midbrain of normal and MPTP-treated mice. Neurochem Int 50:821–833.
- Chalimoniuk M, Langfort J, Lukacova N, Marsala J (2004) Upregulation of guanylyl cyclase expression and activity in striatum of MPTPinduced parkinsonism in mice. Biochem Biophys Res Commun 324:118–126.
- Chalimoniuk M, Stepien A (2004) Influence of the therapy with pergolide mesylate plus L-DOPA and with L-DOPA alone on serum cGMP level in PD patients. Pol J Pharmacol 56:647–650.
- Chaparro-Huerta V, Beas-Zarate C, Guerrero MU, Feria-Velasco A (1997) Nitric oxide involvement in regulating the dopamine transport in the striatal region of rat brain. Neurochem Int 31:607–616.
- Christopherson KS, Hillier BJ, Lim WA, Bredt DS (1999) PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. J Biol Chem 274:27467–27473.
- Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH (1994) Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 345:50–54.
- Crespi F, Rossetti ZL (2004) Pulse of nitric oxide release in response to activation of N-methyl-D-aspartate receptors in the rat striatum: rapid desensitization, inhibition by receptor antagonists, and potentiation by glycine. J Pharmacol Exp Ther 309:462–468.
- de Vente J, van Ittersum MM, van Abeelen J, Emson PC, Axer H, Steinbusch HWM (2000) NO-mediated cGMP synthesis in cholinergic neurons in the rat forebrain: effects of lesioning dopaminergic or serotonergic pathways on nNOS and cGMP synthesis. Eur J Neurosci 12:507–519.
- Del Bel EA, da Silva CA, Guimaraes FS, Bermudez-Echeverry M (2004) Catalepsy induced by intra-striatal administration of nitric oxide synthase inhibitors in rats. Eur J Pharmacol 485:175–181.
- Del Bel EA, Guimaraes FS (2000) Sub-chronic inhibition of nitric-oxide synthesis modifies haloperidol-induced catalepsy and the number of NADPH-diaphorase neurons in mice. Psychopharmacology (Berl) 147:356–361.
- Del Bel EA, Guimarãúes FS, Bermúdez-Echeverry M, Gomes MZ, Schiaveto-de-Souza A, Padovan-Neto FE, Tumas V, Barion-Cavalcanti

AP, Lazzarini M, Nucci-da-Silva LP, de P (2005) Role of nitric oxide on motor behavior. Cell Mol Neurobiol 25:371–392.

- Deutsch SI, Rosse RB, Paul SM, Tomasino V, Koetzner L, Morn CB, Mastropaolo J (1996) 7-Nitroindazole and methylene blue, inhibitors of neuronal nitric oxide synthase and NO-stimulated guanylate cyclase, block MK-801-elicited behaviors in mice. Neuropsychopharmacology 15:37–43.
- Di Giovanni G, Ferraro G, Sardo P, Galati S, Esposito E, La Grutta V (2003) Nitric oxide modulates striatal neuronal activity via soluble guanylyl cyclase: an in vivo microiontophoretic study in rats. Synapse 48:100–107.
- Di Stefano A, Sozio P, Cacciatore I, Cocco A, Giorgioni G, Costa B, Montali M, Lucacchini A, Martini C, Spoto G, Di Pietrantonio F, Di Matteo E, Pinnen F (2005) Preparation and pharmacological characterization of trans-2-amino-5(6)-fluoro-6(5)-hydroxy-1-phenyl-2,3dihydro-1H-indenes as D2-like dopamine receptor agonists. J Med Chem 48:2646–2654.
- Doreulee N, Sergeeva OA, Yanovsky Y, Chepkova AN, Selbach O, Godecke A, Schrader J, Helmut L (2003) Cortico-striatal synaptic plasticity in endothelial nitric oxide synthase deficient mice. Brain Res 964:159–163.
- Echeverry MB, Salgado ML, Ferreira FR, da-Silva CA, Del Bel EA (2007) Intracerebroventricular administration of nitric oxide-sensitive guanylyl cyclase inhibitors induces catalepsy in mice. Psychopharmacology (Berl) 194:271–278.
- Emson PC, Augood SJ, Senaris R, Guerara GR, Kishimoto J, Kadowaki K, Norris PJ, Kendrick KM (1993) Chemical signalling and striatal interneurones. Prog Brain Res 99:155–165.
- Eve DJ, Nisbet AP, Kingsbury AE, Hewson EL, Daniel SE, Lees AJ, Marsden CD, Foster OJF (1998) Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. Brain Res Mol Brain Res 63:62–71.
- Ferraro G, Sardo P, Di Giovanni G, Galati S, La Grutta V (2002) Nitric oxide and cortico-striato-pallidal motor circuitry: Quantitative EEG analysis of surface and depth recordings. Neuroscience Research Communications 30:121–133.
- French SJ, Ritson GP, Hidaka S, Totterdell S (2005) Nucleus accumbens nitric oxide immunoreactive interneurons receive nitric oxide and ventral subicular afferents in rats. Neuroscience 135:121–131.
- Galati S, D'Angelo V, Scarnati E, Stanzione P, Martorana A, Procopio T, Sancesario G, Stefani A (2008) In vivo electrophysiology of dopamine-denervated striatum: focus on the nitric oxide/cGMP signaling pathway. Synapse 62:409–420.
- Garthwaite J (2008) Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci 27:2783–2802.
- Garthwaite J, Charles SL, Chess-Williams R (1988) Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336:385–388.
- Giorgi M, D'Angelo V, Esposito Z, Nuccetelli V, Sorge R, Martorana A, Stefani A, Bernardi G, Sancesario G (2008) Lowered cAMP and cGMP signalling in the brain during levodopa-induced dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. Eur J Neurosci 28:941–950.
- Globus MY, Prado R, Busto R (1995) Ischemia-induced changes in extracellular levels of striatal cyclic GMP: role of nitric oxide. Neuroreport 6:1909–1912.
- Gomez-Urquijo SM, Hokfelt T, Ubink R, Lubec G, Herrera-Marschitz M (1999) Neurocircuitries of the basal ganglia studied in organotypic

cultures: focus on tyrosine hydroxylase, nitric oxide synthase and neuropeptide immunocytochemistry. Neuroscience 94:1133–1151.

- Gracy KN, Pickel VM (1997) Ultrastructural localization and comparative distribution of nitric oxide synthase and N-methyl-D-aspartate receptors in the shell of the rat nucleus accumbens. Brain Res 747:259–272.
- Greengard P (2001) The neurobiology of slow synaptic transmission. Science 294:1024–1030.
- Griffith OW, Stuehr DJ (1995) Nitric oxide synthases: properties and catalytic mechanism. Annu Rev Physiol 57:707–734.
- Guevara-Guzman R, Emson PC, Kendrick KM (1994) Modulation of *in vivo* striatal transmitter release by nitric oxide and cyclic GMP. J Neurochem 62:807–810.
- Hammond C, Bergman H, Brown P (2007) Pathological synchronization in Parkinson's disease: networks, models and treatments. Trends Neurosci 30:357–364.
- Hidaka S, Totterdell S (2001) Ultrastructural features of the nitric oxide synthase-containing interneurons in the nucleus accumbens and their relationship with tyrosine hydroxylase-containing terminals. J Comp Neurol 431:139–154.
- Hofmann M, Spano PF, Trabucchi M, Kumakura K (1977) Guanylate cyclase activity in various rat brain areas. J Neurochem 29:395–396.
- Hogg N, rley-Usmar VM, Wilson MT, Moncada S (1992) Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. Biochem J 281:419–424.
- Hope BT, Michael GJ, Knigge KM, Vincent SR (1991) Neuronal NADPH diaphorase is a nitric oxide synthase. Proc Natl Acad Sci USA 88:2811–2814.
- Hopper RA, Garthwaite J (2006) Tonic and phasic nitric oxide signals in hippocampal long-term potentiation. J Neurosci 26:11513–11521.
- Horn TF, Wolf G, Duffy S, Weiss S, Keilhoff G, MacVicar BA (2002) Nitric oxide promotes intracellular calcium release from mitochondria in striatal neurons. FASEB J 16:1611–1622.
- Iravani MM, Millar J, Kruk ZL (1998) Differential release of dopamine by nitric oxide in subregions of rat caudate putamen slices. J Neurochem 71:1969–1977.
- Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA, Lo EH (1998) Effects of nitric oxide synthase gene knockout on neurotransmitter release *in vivo*. Neuroscience 86:695–699.
- Kawaguchi Y (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci 13:4908–4923.
- Kawaguchi Y (1997) Neostriatal cell subtypes and their functional roles. Neurosci Res 27:1–8.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends in Neurosciences 18:527–535.
- Kiss JP, Hennings ECP, Zsilla G, Vizi ES (1999) A possible role of nitric oxide in the regulation of dopamine transporter function in the striatum. Neurochem Int 34:345–350.
- Kiss JP, Zsilla G, Vizi ES (2004) Inhibitory effect of nitric oxide on dopamine transporters: interneuronal communication without receptors. Neurochem Int 45:485–489.
- Kiss JP (2000) Role of nitric oxide in the regulation of monoaminergic neurotransmission. Brain Res Bull 52:459–466.
- Kraus MM, Prast H (2002) Involvement of nitric oxide, cyclic GMP and phosphodiesterase 5 in excitatory amino acid and GABA release in the nucleus accumbens evoked by activation of the hippocampal fimbria. Neuroscience 112:331–343.

- Kraus MM, Prast H (2001) The nitric oxide system modulates the *in vivo* release of acetylcholine in the nucleus accumbens induced by stimulation of the hippocampal fornix/fimbria-projection. Eur J Neurosci 14:1105–1112.
- Kubota Y, Mikawa S, Kawaguchi Y (1993) Neostriatal GABAergic interneurones contain NOS, calretinin or parvalbumin. Neuroreport 5:205–208.
- Le MC, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.
- Lepoivre M, Fieschi F, Coves J, Thelander L, Fontecave M (1991) Inactivation of ribonucleotide reductase by nitric oxide. Biochem Biophys Res Commun 179:442–448.
- Liang LP, Kaufman S (1998) The regulation of dopamine release from striatum slices by tetrahydrobiopterin and L-arginine-derived nitric oxide. Brain Res 800:181–186.
- Lipton SA (2007) Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation. Curr Drug Targets 8:621–632.
- Lipton SA, Singel DJ, Stamler JS (1994) Neuroprotective and neurodestructive effects of nitric oxide and redox congeners. Ann NY Acad Sci 738:382–387.
- Liu CN, Liu X, Gao D, Li S (2005a) Effects of SNP, GLU and GABA on the neuronal activity of striatum nucleus in rats. Pharmacological Research 51:547–551.
- Liu D, Sammut S, West AR (2005b) Nitric oxide signaling modulates the responsiveness of striatal medium spiny neurons to electrical stimulation of the substantia nigra: Striatal nitrergic signaling. In: The Basal Ganglia VIII (Bolam JP, Ingham CA, Magill PJ, eds), pp. 503–512. New York: Springer Science and Business Media.
- Lonart G, Johnson KM (1994) Inhibitory effects of nitric oxide on the uptake of [3H]dopamine and [3H]glutamate by striatal synaptosomes. J Neurochem 63:2108–2117.
- Mancuso F, Calignano A, Sorrentino L (1994) Endogenous nitric oxide modulates behavioural effects elicited by substance P in rat. Eur J Pharmacol 271:329–333.
- Matsuoka I, Giuili G, Poyard M, Stengel D, Parma J, Guellaen G, Hanoune J (1992) Localization of adenylyl and guanylyl cyclase in rat brain by in situ hybridization: comparison with calmodulin mRNA distribution. J Neurosci 12:3350–3360.
- Mayer B, John M, Bohme E (1990) Purification of a Ca2 + /calmodulindependent nitric oxide synthase from porcine cerebellum. Cofactorrole of tetrahydrobiopterin. FEBS Lett 277:215–219.
- Meffert MK, Premack BA, Schulman H (1994) Nitric oxide stimulates Ca(2+)-independent synaptic vesicle release. Neuron 12:1235–1244.
- Meini A, Benocci A, Frosini M, Sgaragli G, Pessina G, Aldinucci C, Youmbi GT, Palmi M (2000) Nitric oxide modulation of interleukin-1[beta]-evoked intracellular Ca2+ release in human astrocytoma U-373 MG cells and brain striatal slices. J Neurosci 20:8980–8986.
- Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. Nat Rev Drug Discov 5:660–670.
- Montague PR, Gancayco CD, Winn MJ, Marchase RB, Friedlander MJ (1994) Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 263:973–977.
- Morris BJ, Simpson CS, Mundell S, Maceachern K, Johnston HM, Nolan AM (1997) Dynamic changes in NADPH-diaphorase staining reflect activity of nitric oxide synthase: evidence for a dopaminergic regulation of striatal nitric oxide release. Neuropharmacology 36:1589–1599.

- Morris BJ (1995) Stimulation of immediate early gene expression in striatal neurons by nitric oxide. J Biol Chem 270:24740–24744.
- Murad F (2006) Nitric Oxide and Cyclic GMP in Cell Signaling and Drug Development. N Engl J Med 355:2003–2011.
- Murer MG, Tseng KY, Kasanetz F, Belluscio M, Riquelme LA (2002) Brain Oscillations, Medium Spiny Neurons, and Dopamine. Cell Mol Neurobiol 22:611–632.
- Nakahara K, Yokoo H, Yoshida M, Tanaka M, Shigemori M (1994) [Effect of nitric oxide on central dopaminergic neurons]. No To Shinkei 46:1147–1153.
- Nathan C, Xie QW (1994a) Nitric oxide synthases: roles, tolls, and controls. Cell 78:915–918.
- Nathan C, Xie QW (1994b) Regulation of biosynthesis of nitric oxide. J Biol Chem 269:13725–13728.
- Nishi A, Kuroiwa M, Miller DB, O'Callaghan JP, Bateup HS, Shuto T, Sotogaku N, Fukuda T, Heintz N, Greengard P, Snyder GL (2008) Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. J Neurosci 28:10460–10471.
- Nishi A, Watanabe Y, Higashi H, Tanaka M, Nairn AC, Greengard P (2005) Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. Proc Natl Acad Sci USA 102:1199–1204.
- O'Donnell P, Grace AA (1997) Cortical afferents modulate striatal gap junction permeability via nitric oxide.. Neuroscience 76:1–5.
- Ohta K, Araki N, Shibata M, Hamada J, Komatsumoto S, Shimazu K, Fukuuchi Y (1994a) A novel *in vivo* assay system for consecutive measurement of brain nitric oxide production combined with the microdialysis technique. Neurosci Lett 176:165–168.
- Ohta K, Shimazu K, Komatsumoto S, Araki N, Shibata M, Fukuuchi Y (1994b) Modification of striatal arginine and citrulline metabolism by nitric oxide synthase inhibitors. Neuroreport 5:766–768.
- Ondracek JM, Dec A, Hoque KE, Lim SA, Rasouli G, Indorkar RP, Linardakis J, Klika B, Mukherji SJ, Burnazi M, Threlfell S, Sammut S, West AR (2008) Feed-forward excitation of striatal neuron activity by frontal cortical activation of nitric oxide signaling in vivo. Eur J Neurosci 27:1739–1754.
- Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526.
- Pare D, Lang EJ, Destexhe A (1998a) Inhibitory control of somatodendritic interactions underlying action potentials in neocortical pyramidal neurons in vivo: an intracellular and computational study. Neuroscience 84:377–402.
- Pare D, Shink E, Gaudreau H, Destexhe A, Lang EJ (1998b) Impact of spontaneous synaptic activity on the resting properties of cat neocortical pyramidal neurons *In vivo*. J Neurophysiol 79:1450–1460.
- Pogun S, Dawson V, Kuhar MJ (1994) Nitric oxide inhibits 3H-glutamate transport in synaptosomes. Synapse 18:21–26.
- Pogun S, Kuhar MJ (1994) Regulation of neurotransmitter reuptake by nitric oxide. Ann NY Acad Sci 738:305–315.
- Prast H, Fischer H, Werner E, Werner-Felmayer G, Philippu A (1995) Nitric oxide modulates the release of acetylcholine in the ventral striatum of the freely moving rat. Naunyn Schmiedebergs Arch Pharmacol 352:67–73.
- Prast H, Tran MH, Fischer H, Philippu A (1998) Nitric Oxide-Induced Release of Acetylcholine in the Nucleus Accumbens: Role of Cyclic GMP, Glutamate, and GABA. J Neurochem 71:266–273.
- Prast H, Philippu A (2001) Nitric oxide as modulator of neuronal function. Prog Neurobiol 64:51–68.

- Rameau GA, Tukey DS, Garcin-Hosfield ED, Titcombe RF, Misra C, Khatri L, Getzoff ED, Ziff EB (2007) Biphasic coupling of neuronal nitric oxide synthase phosphorylation to the NMDA receptor regulates AMPA receptor trafficking and neuronal cell death. J Neurosci 27:3445–3455.
- Reed TM, Repaske DR, Snyder GL, Greengard P, Vorhees CV (2002) Phosphodiesterase 1B Knock-Out Mice Exhibit Exaggerated Locomotor Hyperactivity and DARPP-32 Phosphorylation in Response to Dopamine Agonists and Display Impaired Spatial Learning. J Neurosci 22:5188–5197.
- Rivera A, Alberti I, Martin AB, Narvaez JA, de la CA, Moratalla R (2002) Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. Eur J Neurosci 16:2049–2058.
- Rocchitta G, Migheli R, Mura MP, Esposito G, Desole MS, Miele E, Miele M, Serra PA (2004) Signalling pathways in the nitric oxide donor-induced dopamine release in the striatum of freely moving rats: evidence that exogenous nitric oxide promotes Ca2+ entry through store-operated channels. Brain Res 1023:243–252.
- Rossetti ZL, Crespi F (2004) Inhibition of nitric oxide release *in vivo* by ethanol. Alcohol Clin Exp Res 28:1746–1751.
- Sahach VF, Baziliuk OV, Oleshko MM, Kotsiuruba OV, Bukhanevych OM, Appenzeller O (2000) [The nitric oxide system in a chronic deficiency of mesostriatal dopamine: the action of nitroglycerin]. Fiziol Zh 46:55–63.
- Salin P, Kerkerian-Le GL, Heidet V, Epelbaum J, Nieoullon A (1990) Somatostatin-immunoreactive neurons in the rat striatum: effects of corticostriatal and nigrostriatal dopaminergic lesions. Brain Res 521:23–32.
- Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P (1993) Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci USA 90:7240–7244.
- Sammut S, Bray KE, West AR (2007a) Dopamine D(2) receptor-dependent modulation of striatal NO synthase activity. Psychopharmacology (Berl) DOI: 10.1007/s00213-006-0681-z.
- Sammut S, Dec A, Mitchell D, Linardakis J, Ortiguela M, West AR (2006) Phasic dopaminergic transmission increases NO efflux in the rat dorsal striatum via a neuronal NOS and a dopamine D(1/5) receptor-dependent mechanism. Neuropsychopharmacology 31:493–505.
- Sammut S, Park DJ, West AR (2007b) Frontal cortical afferents facilitate striatal nitric oxide transmission *in vivo* via a NMDA receptor and neuronal NOS-dependent mechanism. J Neurochem 103:1145–1156.
- Sancesario G, Morello M, Reiner A, Giacomini P, Massa R, Schoen S, Bernardi G (2000) Nitrergic neurons make synapses on dual-input dendritic spines of neurons in the cerebral cortex and the striatum of the rat: implication for a postsynaptic action of nitric oxide. Neuroscience 99:627–642.
- Sancesario G, Giorgi M, D'Angelo V, Modica A, Martorana A, Morello M, Bengtson CP, Bernardi G (2004) Down-regulation of nitrergic transmission in the rat striatum after chronic nigrostriatal deafferentation. Eur J Neurosci 20:989–1000.
- Schmidt HH, Murad F (1991) Purification and characterization of a human NO synthase. Biochem Biophys Res Commun 181:1372–1377.
- Schuman EM, Meffert MK, Schulman H, Madison DV (1994) An ADPribosyltransferase as a potential target for nitric oxide action in hippocampal long-term potentiation. Proc Natl Acad Sci USA 91:11958–11962.
- Segovia G, Porras A, Mora F (1994) Effects of a nitric oxide donor on glutamate and GABA release in striatum and hippocampus of the conscious rat. Neuroreport 5:1937–1940.

- Serra PA, Esposito G, Delogu MR, Migheli R, Rocchitta G, Miele E, Desole MS, Miele M (2001) Analysis of S-nitroso-N-acetylpenicillamine effects on dopamine release in the striatum of freely moving rats: role of endogenous ascorbic acid and oxidative stress. Br J Pharmacol 132:941–949.
- Silberberg G, Grillner S, LeBeau FE, Maex R, Markram H (2005) Synaptic pathways in neural microcircuits. Trends Neurosci 28:541–551.
- Siuciak JA, McCarthy SA, Chapin DS, Fujiwara RA, James LC, Williams RD, Stock JL, McNeish JD, Strick CA, Menniti FS, Schmidt CJ (2006) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: Evidence for altered striatal function. Neuropharmacology 51:374–385.
- Spatz M, Yasuma Y, Strasser A, Kawai N, Stanimirovic D, McCarron R (1995) Modulation of striatal dopamine release in cerebral ischemia by L-arginine. Neurochem Res 20:491–496.
- Starr MS, Starr BS (1995) Do NMDA receptor-mediated changes in motor behaviour involve nitric oxide? Eur J Pharmacol 272:211–217.
- Stefani A, Fedele E, Galati S, Pepicelli O, Frasca S, Pierantozzi M, Peppe A, Brusa L, Orlacchio A, Hainsworth AH, Gattoni G, Stanzione P, Bernardi G, Raiteri M, Mazzone P (2005) Subthalamic stimulation activates internal pallidus: evidence from cGMP microdialysis in PD patients. Ann Neurol 57:448–452.
- Stewart J, Deschamps SE, Amir S (1994) Inhibition of nitric oxide synthase does not block the development of sensitization to the behavioral activating effects of amphetamine. Brain Res 641:141–144.
- Strasser A, McCarron RM, Ishii H, Stanimirovic D, Spatz M (1994) L-arginine induces dopamine release from the striatum in vivo. Neuroreport 5:2298–2300.
- Stuehr DJ, Kwon NS, Nathan CF, Griffith OW, Feldman PL, Wiseman J (1991) N omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine. J Biol Chem 266:6259–6263.
- Stuehr DJ, Wei CC, Santolini J, Wang Z, Aoyagi M, Getzoff ED (2004) Radical reactions of nitric oxide synthases. Biochem Soc Symp:39–49.
- Susswein AJ, Katzoff A, Miller N, Hurwitz I (2004) Nitric oxide and memory. Neuroscientist 10:153–162.
- Taskiran D, Kutay FZ, Pogun S (2003) Effect of carbon monoxide on dopamine and glutamate uptake and cGMP levels in rat brain. Neuropsychopharmacology 28:1176–1181.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR (2009) Inhibition of Phosphodiesterase 10A Increases the responsiveness of striatal projection neurons to cortical stimulation. J Pharmacol Exp Ther 328:785–795.
- Trabace L, Kendrick KM (2000) nitric oxide can differentially modulate striatal neurotransmitter concentrations via soluble guanylate cyclase and peroxynitrite formation. J Neurochem 75:1664–1674.

- Tsou K, Snyder GL, Greengard P (1993) Nitric oxide/cGMP pathway stimulates phosphorylation of DARPP-32, a dopamine- and cAMPregulated phosphoprotein, in the substantia nigra. Proc Natl Acad Sci USA 90:3462–3465.
- Vincent SR (1994) Nitric oxide: a radical neurotransmitter in the central nervous system. Prog Neurobiol 42:129–160.
- Vuillet J, Kerkerian L, Kachidian P, Bosler O, Nieoullon A (1989) Ultrastructural correlates of functional relationships between nigral dopaminergic or cortical afferent fibers and neuropeptide Y-containing neurons in the rat striatum. Neurosci Lett 100:99–104.
- West AR, Galloway MP (1998) Nitric oxide and potassium chloridefacilitated striatal dopamine efflux *in vivo*: role of calcium-dependent release mechanisms. Neurochem Int 33:493–501.
- West AR, Galloway MP (1997a) Endogenous nitric oxide facilitates striatal dopamine and glutamate efflux in vivo: Role of ionotropic glutamate receptor-dependent mechanisms. Neuropharmacology 36:1571–1581.
- West AR, Galloway MP, Grace AA (2002) Regulation of striatal dopamine neurotransmission by nitric oxide: effector pathways and signaling mechanisms. Synapse 44:227–245.
- West AR, Galloway MP (1996) Intrastriatal infusion of (±)-S-Nitroso-N-Acetylpenicillamine releases vesicular dopamine via an ionotropic glutamate receptor-mediated mechanism: an *in vivo* microdialysis study in chloral hydrate-anesthetized rats. J Neurochem 66:1971–1980.
- West AR, Galloway MP (1997b) Inhibition of glutamate reuptake potentiates endogenous nitric oxide-facilitated dopamine efflux in the rat striatum: an *in vivo* microdialysis study. Neurosci Lett 230:21–24.
- West AR, Grace AA (2000) Striatal nitric oxide signaling regulates the neuronal activity of midbrain dopamine neurons *in vivo*. J Neurophysiol 83:1796–1808.
- West AR, Grace AA (2004) The nitric oxide-guanylyl cyclase signaling pathway modulates membrane activity states and electrophysiological properties of striatal medium spiny neurons recorded *in vivo*. J Neurosci 24:1924–1935.
- West MJ, Ostergaard K, Andreassen OA, Finsen B (1996) Estimation of the number of somatostatin neurons in the striatum: an in situ hybridization study using the optical fractionator method. J Comp Neurol 370:11–22.
- Wichmann T, Delong MR (2007) Anatomy and physiology of the basal ganglia: relevance to Parkinson's disease and related disorders. Handb Clin Neurol 83:1–18.
- Wolosker H, Reis M, Assreuy J, de ML (1996) Inhibition of glutamate uptake and proton pumping in synaptic vesicles by S-nitrosylation. J Neurochem 66:1943–1948.
- Woodruff AR, Monyer H, Sah P (2006) GABAergic excitation in the basolateral amygdala. J Neurosci 26:11881–11887.
- Zhu XZ, Luo LG (1992) Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. J Neurochem 59:932–935.

# Role of Adenosine in the Basal Ganglia

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# I. INTRODUCTION: THE ADENOSINE SYSTEM

Adenosine is a nucleoside composed of the purine base adenine and ribose. Rather than a neurotransmitter, adenosine can be defined as a metabolite that also serves a signaling function. Physiological and balanced levels of adenosine are maintained by transporters that ensure equivalent extra- and intracellular adenosine concentrations. Adenosine can be phosphorylated to adenosine monophosphate (AMP) by adenosine kinase; conversely, AMP can be hydrolyzed to adenosine by 5'-nucleotidase. Concentrations of adenosine rise because of increased intracellular formation (e.g., increased cellular activity, hypoxia) or increased release of adenine nucleotides.

Adenosine acts on four G-protein-coupled receptors:  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ , and  $A_3$  (Fredholm, 1995).  $A_1$  and  $A_3$  receptors are

coupled to G<sub>i</sub>/G<sub>olf</sub> proteins, decrease cAMP levels, increase K<sup>+</sup> conductance, and decrease transient Ca<sup>2+</sup> conductance involved in transmitter release. A2A and A2B receptors are coupled to Gs/olf proteins and raise levels of cAMP (Fredholm, 1995; Ambrósio et al., 1996) (Fig. 11.1). Adenosine has similar potency at human A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> receptors (Fredholm et al., 2001), but shows lower potency at  $A_{2B}$  receptors. In areas where they are very abundant, adenosine stimulates A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub> receptors at physiological basal level (Svenningsson et al., 1999a; Fredholm et al., 2007), whereas in other locations adenosine receptors are activated only when the local formation of adenosine is increased (see Fig. 11.2 for details on synthesis and degradation of adenosine). In the following sections we will discuss A1 and A2A receptors, the adenosine receptor subtypes that play a role in basal ganglia (BG) function.



FIGURE 11.1 Functional interactions between D<sub>2</sub> dopamine, A<sub>2A</sub> adenosine and metabotropic glutamate 5 (mGluR5) receptors in striatopallidal neurons. At the intramembrane level, the A2A receptor and the D2 receptor interact antagonistically, whereas mGluR5 and A2A receptors act synergistically to counteract the D2-mediated signaling. The A<sub>2A</sub> receptor and the D<sub>2</sub> receptor also exert opposite effects on adenylyl cyclase (AC) levels and AC-regulated downstream molecules, such as PKA, DARPP-32, CREB-P and early genes. A synergistic interaction between A2A and mGluR5 receptors was demonstrated at the level of MAP kinases, DARPP-32 phosphorylation, early gene expression. Solid lines: stimulatory effect (+); broken lines: inhibitory effect (-); CaMK II/IV, calcium/calmodulin-dependent protein kinase type II/IV; cAMP, cyclic AMP; CREB, cAMP response element-binding protein; K+, potassium channel; DARPP-32-P (Thr75) and DARPP-32-P (Thr34), dopamine and cAMP-regulated phosphoprotein, phosphorylated at threonine residues 75 and 34, respectively; Gi, Go, inhibitory G-proteins, Gq, Gs, Golf, stimulatory G-proteins; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PP-1, PP-2, protein phosphatases 1 and 2. With permission of Dr Jadwiga Wardas [Prog Neurobiol (2007) 83:293-309].

# II. ADENOSINE RECEPTOR LOCALIZATION AND FUNCTION

#### A. A<sub>1</sub> Receptors

 $A_1$  receptors are widespread in the brain; however, their presence in the BG influences the functionality of the BG network. In the caudate-putamen (CPu),  $A_1$  receptors are located in both direct and indirect GABAergic efferent neurons, and in cholinergic interneurons, whereas presynaptic  $A_1$  receptors are found in corticostriatal afferents (Alexander and Reddington, 1989; Ferré et al., 1996). In addition,  $A_1$  receptors are localized on nerve terminals in globus pallidus (GP) and substantia migra (SN) (Fastbom et al., 1987).

In relation to presynaptic A1 receptors, a modulation of dopaminergic and glutamatergic neurotransmission has been reported. A1 receptors localized on corticostriatal nerve terminals indirectly inhibit dopamine release, by their ability to modulate glutamate release (Ambrósio et al., 1996; Borycz et al., 2007). In addition, a glutamateindependent modulation of dopamine transmission by the A<sub>1</sub> receptor has been reported, probably mediated by A<sub>1</sub> receptors directly located on dopaminergic nerve terminals (Borycz et al., 2007). These different mechanisms of modulation of dopamine neurotransmission seem to be region dependent, the first mechanism being prevalent in the nucleus accumbens shell, while the second being mostly active in the accumbens core and dorsal striatum (Borycz et al., 2007; O'Neill et al., 2007). Interestingly, since agonists of the A1 receptor can attenuate the evoked release of excitatory amino acids in the CPu, they may play a protective role against ischemic neuronal damage (Goda et al., 1998). The mechanism of this effect likely relies on the attenuation of Ca<sup>2+</sup> influx through voltage-dependent calcium channels, resulting in the decrease of glutamate release (Marchi et al., 2002).

#### **B.** A<sub>2A</sub> Receptors

In contrast to the widespread distribution of  $A_1$  receptors,  $A_{2A}$  receptors are preferentially and abundantly contained in GABAergic neurons of the indirect pathway, projecting from the CPu to the globus pallidus external segment (GPe), which also selectively express the D<sub>2</sub> dopamine receptor and the peptide enkephalin (Fink et al., 1992; Augood et al., 1994) (Fig. 11.3). Conversely, neurons of the direct pathway, projecting to the substantia nigra pars reticulata (SNr) or to the globus pallidus internal segment (GPi), which selectively express the D<sub>1</sub> dopamine receptor and the peptide dynorphin, do not contain appreciable levels of  $A_{2A}$ receptors (Schiffmann et al., 1991a) (Fig. 11.3). In addition, in the CPu  $A_{2A}$  receptors are present on cholinergic nerve terminals (Kurokawa et al., 1996) (Fig. 11.3).

Morphologically,  $A_{2A}$  receptors in the CPu are prevalent in dendrites and dendritic spines but less so in axons and axon terminals of recurrent collaterals projecting back to the CPu, or projecting from the cortical areas. Moreover,  $A_{2A}$  receptors are observed primarily at asymmetric synapses, suggesting that adenosine may be important in modulating excitatory input to striatal neurons. Similar to rodents, in primate and post-mortem human brain the





**FIGURE 11.2** Adenosine is formed both within cells and in the extracellular compartment, as a result of hydrolysis of AMP (adenosine monophosphate) through an action of 5'-nucleotidase. Hence adenosine formation depends on ATP breakdown and synthesis. Extracellular concentrations are maintained stable by the bidirectional transport through a specific transporter. Adenosine is then catabolized to inosine by the action of adenosine kinase (AKA) and adenosine deaminase (ADA). With permission of Dr Jadwiga Wardas.

expression of  $A_{2A}$  receptor and its mRNA is highly concentrated in both caudate and putamen (Schiffmann et al., 1991b; Hurley et al., 2000; Calon et al., 2004) (Fig. 11.4). Moreover, PET studies show that  $A_{2A}$  receptors are concentrated in the putamen > caudate nucleus > nucleus accumbens (Brooks et al., 2008).

### III. ADENOSINE RECEPTOR INTERACTIONS

Interactions between  $A_{2A}$  and  $D_2$  dopamine receptors at both post- and presynaptic levels play prominent roles in the control of BG functions. However, specific interactions between  $A_1$  and  $A_{2A}$  receptors and an antagonistic interaction between  $A_1$  and  $D_1$  receptors have also been shown to have an important role in BG functions and dysfunctions. Moreover, a positive cooperation between  $A_{2A}$  and glutamate mGlu<sub>5</sub> receptors has been revealed.

#### A. Biochemical Interactions: Postsynaptic Modulation of BG Neurotransmission

The indirect striatopallidal pathway plays a prominent role in mediating  $A_{2A}$  receptor function in motor responses in the CPu. In the intact CPu, adenosine via postsynaptic  $A_{2A}$  receptors excites striatopallidal neurons, opposing the inhibitory effect exerted by dopamine through  $D_2$  receptors. This mechanism likely relies on the coexpression of  $A_{2A}/D_2$  receptors in GABAergic striatopallidal neurons that interact with different modes to modulate neuronal activity. At the transcriptional level, the  $G_{s/olf}$ -coupled  $A_{2A}$  receptor activates the cAMP-PKA and MAPK signal, opposing  $G_i$ -coupled,  $D_2$  receptor-mediated inactivation of the same transduction signal (Fredholm, 1995; Schulte and Fredholm, 2003) (Fig. 11.1).

Moreover, a receptor-receptor interaction and a formation of functional heteromers between the two receptors has been reported (Hillion et al., 2002). Through a heteromerbased receptor-receptor interaction,  $A_{2A}$  receptor stimulation can modulate  $D_2$  binding affinity, by a direct molecular inhibition, or reducing  $D_2$  coupling to  $G_i$  protein. Similar to  $A_{2A}$ - $D_2$  receptor interaction, the existence of an antagonistic  $A_1$ - $D_1$  receptor interaction in striatonigral neurons has been observed. The  $A_1$  receptor, probably through a GTP-independent mechanism (Ferré et al., 1994; Sakiyama et al., 2007). The formation of  $A_1$ - $D_1$  receptor heteromers has been recently reported in the CPu, and it might sustain this direct



**FIGURE 11.3**  $A_{2A}$  adenosine receptor ( $A_{2A}$  R) antagonist activity in Parkinson's disease (PD). Mechanisms of both symptomatic effects and neuroprotection are illustrated. In PD an imbalance between direct and indirect striatal output pathway activity is generated by the lack of dopamine (indicated as a broken line).  $A_{2A}$  receptor antagonism in striatopallidal neurons and, likely, in the globus pallidus (GP), relieves their hyperactivity, restoring a balance between output pathways. Moreover,  $A_{2A}$  receptor blockade in acetylcholine interneurons restores acetylcholine tone, which might contribute to counteracting tremor. Thick lines: hyperactive neurons; dotted lines: hypoactive neurons. STN: subthalamic nucleus; SNr: substantia nigra pars reticulata; Ach: Acetylcholine; CPu: caudate-putamen. Th: thalamus. In the parkinsonian state, glial proliferation (ramified cells) is present both in the CPu and substantia nigra pars compacta (SNc). As neuroprotective agents,  $A_{2A}$  receptor antagonists attenuate dopaminergic cell degeneration and prevent glial response, through a mechanism which might involve  $A_{2A}$  receptors located either in neurons or in glial cells.

intramembrane interaction (Franco et al., 2007). Although  $A_1$  receptors are also localized on striatopallidal neurons, no evidence for an  $A_1$  receptor-mediated modulation of the striatal  $D_2$  receptor binding characteristics has been found.

Beside the intramembrane direct receptor-receptor interaction, an antagonistic  $A_1-D_1$  receptor interaction can take place at the transcriptional level (Ferré et al., 1999).  $A_1$ receptor agonists inhibit the  $D_1$  agonist-induced increase in the expression of *zif-268*, *c-fos* and *jun-B* mRNA levels in the dopamine-denervated striatum, suggesting a negative modulation by  $A_1$  receptors of the  $D_1$  receptor signaling at supersensitive  $D_1$  receptors, which might underlie the counteraction of  $D_1$ -mediated motor responses (Ferré et al., 1999).

The existence of a positive cooperation between  $A_1$  and  $A_{2A}$  receptors has been shown by the synergistic induction of *c-fos* expression in striatal neurons. In fact, while selective stimulation of each receptor subtype does not induce the expression of the early-gene, receptor coactivation increases striatal *c-fos* expression, probably due to an  $A_1$  enabling effect. As a mechanism,  $A_1$  receptor stimulation inhibits dopamine release, which in turn opposes  $A_{2A}$  receptor stimulating effects through an action on striatopallidal  $D_2$  receptors (Karcz-Kubicha et al., 2003, 2006). Recently, the formation

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#### **MONKEY A2A RECEPTOR**

**FIGURE 11.4** Representative examples of post-mortem autoradiography of 3H-SCH-58261 binding to  $A_{2A}$  receptors and  $A_{2A}$  receptor mRNA in the brain of non-human primate at three rostro-caudal stereotaxic coordinates of the basal ganglia including the caudate, putamen, nucleus accumbens, GPe and GPi, subthalamic nucleus and SN. With permission of Dr Therese DiPaolo [Prog in Neurobiol (2007) 83:293–309].

of  $A_1$ – $A_{2A}$  receptor heteromers has been suggested in striatal glutamatergic nerve terminals, where these receptors are colocalized (Ciruela et al., 2006). By modifying the binding characteristics of the  $A_1$  receptor for the agonist, heteromers would exert a fine-tuning modulation of glutamatergic neurotransmission (Ciruela et al., 2006).

More recent studies have shown that A2A receptors can form heteromers with non-dopaminergic G-protein coupled receptors such as the mGlu<sub>5</sub> receptor (Ferré et al., 2002).  $A_{2A}$ -mGlu<sub>5</sub> heteromers have been detected in glutamatergic striatal terminals in vivo, and in striatal neurons by *in vitro* studies, and have been suggested to play a role in striatal plasticity and to modulate the activity of striatopallidal neurons (Ferré et al., 2002). Co-stimulation of A2A and mGlu<sub>5</sub> receptors exerts a synergistic inhibitory effect on dopamine binding to  $D_2$  receptors on these neurons and a synergistic stimulatory action on striatal *c-fos* expression, ERK and DARPP-32 phosphorylation (Popoli et al., 2001; Ferré et al., 2002; Nishi et al., 2003). Accordingly, a synergy has been reported between A2A and mGlu5 antagonists in the modulation of motor responses (Coccurello et al., 2004).

A further interaction was reported for  $A_{2A}$  and  $CB_1$  cannabinoid receptors which may form heteromeric complexes as shown in co-transfected HEK-293T cells and rat CPu. In a human neuroblastoma cell line,  $CB_1$  receptor signaling was found to be completely dependent on  $A_{2A}$  receptor activation. Accordingly, blockade of  $A_{2A}$  receptors counteracted the motor depressant effects produced by intrastriatal administration of CB<sub>1</sub> receptor agonists (Carriba et al., 2007).

## **B.** Biochemical Interactions: Presynaptic Modulation of BG Neurotransmission

At the presynaptic level, the  $A_{2A}$  receptor modulates neuronal activity in both CPu and GP by regulating GABAergic, cholinergic and glutamatergic synaptic transmissions.

Ultrastructural localization studies suggest that  $A_{2A}$  receptors in the CPu can modulate GABAergic transmission at multiple cellular sites (Rosin et al., 1998, 2003; Hettinger et al., 2001). Presynaptic  $A_{2A}$  receptors, probably located on GABAergic collateral axons, exert an inhibitory modulation of GABA release from medium spiny projection neurons, likely relieving a GABA-mediated inhibition of these neurons (Mori et al., 1996). Moreover, striatal cholinergic nerve terminals express the  $A_{2A}$  receptor, which can modulate the acetylcholine (Ach) release (Brown et al., 1990; Kurokawa et al., 1996).  $A_{2A}$  receptor

agonists increase and  $A_{2A}$  receptor antagonists reduce Ach release in the CPu *in vivo* (Kurokawa et al., 1996), an effect modulated by the dopaminergic transmission, since dopamine depletion amplifies the  $A_{2A}$  receptor agonist effect (Kurokawa et al., 1996).

 $A_{2A}$  receptors located on corticostriatal terminals may modulate the striatal efflux of glutamate evoked by an excitotoxic insult (Popoli et al., 2002; Melani et al., 2003). The intrastriatal administration of an  $A_{2A}$  receptor antagonist reduces the glutamate efflux induced by the administration of the excitotoxin quinolinic acid (Gianfriddo et al., 2003), an effect which is of particular interest for the neuroprotective effect of these compounds in neurodegeneration induced by *in vivo* ischemia.

## IV. A<sub>2A</sub> RECEPTORS IN PARKINSON'S DISEASE: BIOCHEMICAL STUDIES

In order to discuss the role of  $A_{2A}$  receptors in BG function it is very important to consider pathologies such as Parkinson's disease (PD). Based on the unique cellular and regional distribution of these receptors and in line with growing evidence showing that  $A_{2A}$  receptor antagonists improve motor symptoms in animal models of PD and in clinical trials, the  $A_{2A}$  receptor has emerged as a major player in BG functions.

### A. Postsynaptic Interactions with the Dopamine System

The majority of studies on adenosine–dopamine interactions have been performed in dopamine-depleted or -denervated rodents and primates, and they form the basis for a proposed role of  $A_{2A}$  receptors in PD. Of particular relevance in this context are the increased levels of the  $A_{2A}$  receptor and its mRNA in the striatum of 6-hydroxydopamine (6-OHDA)-lesioned rats (Pinna et al., 2002) and in the putamen of post-mortem parkinsonian patients (Calon et al., 2004), which suggests a strict correlation between  $A_{2A}$  receptor modifications and dopamine depletion.

The expression of a number of proteins, including receptors, ion channels, phosphoproteins and transcription factors, can be indirectly regulated by  $A_{2A}$ - $D_2$  receptor antagonistic interactions both in normal and dopaminedenervated situations (Schulte and Fredholm, 2003; Sahin et al., 2007; Santini et al., 2007). Of particular interest is
the effect on the expression of DARPP-32 (dopamine and cAMP-regulated phosphoprotein), whose activation is strictly related to dopamine transmission (Fig. 11.1).  $A_{2A}$  receptor stimulation induces the phosphorylation of DARPP-32, which is antagonized by D<sub>2</sub> receptor agonists in models of PD (Santini et al., 2007). It is noteworthy that an aberrant activation of the PKA-MAPK pathway, as well as of DARPP-32, has been linked to the appearance of parkinsonian signs and to the motor response complications produced by dopaminomimetic therapy, suggesting that the  $A_{2A}$  receptor may have an important role in these responses (for details see Fig. 11.1).

Interestingly, A<sub>2A</sub> receptors and D<sub>2</sub> receptors exert an opposite effect on the CREB phosphorylation state, since A<sub>2A</sub> receptor agonists increase CREB phosphorylation, which is antagonized by  $D_2$  receptor agonists in co-transfected cells (Kull et al., 1999; Schulte and Fredholm, 2003) (Fig. 11.1). In PD models, an antagonistic interaction between A<sub>2A</sub> receptor and D<sub>2</sub> receptor at the transcriptional level is reflected by the synergistic induction of a variety of immediate early genes, such as c-fos, zif-268, NGFI-B, jun-B in the striatopallidal pathway, produced by L-DOPA, or D<sub>2</sub> receptor agonists and A<sub>2A</sub> receptor antagonists (Morelli et al., 1995; Boegman and Vincent, 1996; Le Moine et al., 1997; Svenningsson et al., 1999b) (Fig. 11.1). An intermembrane direct cross-talk between the A2A receptor and D<sub>2</sub> receptor has been described as an additional site of functional interaction in reserpinized rodents (Ferré et al., 1991). Stimulation of A2A receptors can decrease the ability of dopamine to bind the D<sub>2</sub> receptor. A<sub>2A</sub> receptor agonists cause a reduction in the affinity of dopamine D<sub>2</sub> agonist binding sites by a direct intramembrane, Gprotein independent, receptor-receptor interaction (Ferré et al., 1991). A possible explanation for this mode of interaction came from the discovery of functional heteromeric receptor complexes, comprising the A2A receptor and other G-protein coupled receptors, such as the D<sub>2</sub> receptor and  $D_3$  receptor. The antagonism of such a molecular interaction might contribute to the antiparkinsonian activity of A<sub>2A</sub> antagonists (Kull et al., 1999; Hillion et al., 2002). Recently, evidence for functional  $A_{2A}$ - $D_3$  heteromers has also been provided by in vitro studies, suggesting a further level of modulation of dopamine transmission by A2A receptors (Fuxe et al., 2007).

Interestingly, in PD experimental models the depletion of dopamine leads to a partial loss of spines and glutamatergic synapses in the indirect striatopallidal pathway (Day et al., 2006). Morphological changes are correlated with altered electrophysiological activity, underlining the importance of  $A_{2A}$  receptors in the control of neuronal activity associated with dopamine neuron degeneration in the indirect pathway (Day et al., 2006).

# **B.** Postsynaptic Interactions with the Glutamate System

Beneficial effects of  $A_{2A}$  receptor blockade on the regulation of the phosphorylation state of the  $\alpha$ -amino-3hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type of glutamate receptors by L-DOPA have been described. Hyperphosphorylation of the striatal AMPA receptor consequent to chronic administration of L-DOPA to 6-OHDA-lesioned rats, is in fact prevented by combined administration with the  $A_{2A}$  receptor antagonist istradefylline (Chase et al., 2003; Kachroo et al., 2005).

#### C. Presynaptic Interactions

Several pieces of evidence highlight the contribution of presynaptic  $A_{2A}$  receptor to the modulation of motor responses and indicate that GABA release from striatopallidal neurons is regulated through  $A_{2A}$  receptors contained in both the CPu and GP.  $A_{2A}$  receptors are highly expressed in the GP, mainly in the neuropil, where they can regulate pallidal extracellular GABA concentration and, thereafter, GP activity (Rosin et al., 2003). Stimulation of pallidal  $A_{2A}$  receptors by intrapallidal or intrastriatal infusion of  $A_{2A}$  receptor agonists enhances pallidal GABA outflow and produces hyperpolarization of striatopallidal neurons (Ferré et al.,1993; Ochi et al., 2000, 2004; Shindou et al., 2001; Mori and Shindou, 2003).

Following dopamine denervation, lack of dopamine generates an imbalance in the activity of striatonigral and striatopallidal pathways. Striatonigral neurons become hypoactive, whereas striatopallidal neurons, losing the inhibitory effect of dopamine while undergoing the stimulatory influence of adenosine, become hyperactive. In the GP, neuronal discharge rate and oscillatory activity are altered and extracellular GABA basal level is increased, suggesting that dopamine depletion, either directly or indirectly, disrupts the modulatory function of GP within the BG. Such imbalanced activity of striatal output pathways leads to a markedly increased inhibitory output from SNr/GPi to thalamocortical neurons, which produces hypokinetic symptoms as in PD (Fig. 11.3). An altered adenosinergic transmission might directly contribute to the imbalance in the activity of striatal neurons. In this regard, an interesting result mentioned above concerns the increase of A2A receptor mRNA reported in the striatum of 6-OHDA-lesioned rats and parkinsonian primates, as well as in PD patients chronically treated with L-DOPA (Pinna et al., 2002; Calon et al., 2004; Tomiyama et al., 2004). An abnormally increased A2A signaling would prevail over D2 signaling in striatopallidal neurons, contributing to the imbalance in the activity of the indirect versus the direct pathway and to the motor impairment in PD. Moreover, the abnormal A<sub>2A</sub> signaling would produce an altered responsiveness of striatopallidal neurons to L-DOPA, reducing its therapeutic efficacy at D<sub>2</sub> receptor site and contributing to the dysregulation of the striatal pathways which is associated with the development of L-DOPA-induced dyskinetic movements.

It is therefore hypothesized that the A2A receptor antagonist efficacy in PD firstly relies on the blockade of A2A receptors on striatopallidal neurons, which should dampen their excessive activity and restore some balance between striatonigral and striatopallidal neurons, consequently relieving thalamocortical activity. Moreover, blockade of A<sub>2A</sub> receptor prevailing tone could be one of the mechanisms underlying the absence of dyskinetic responses reported with A<sub>2A</sub> antagonists in PD animal models and in preliminary clinical trials (Morelli et al., 2007). In addition, A<sub>2A</sub> receptor antagonists acting at the GP level, cause a decrease of extracellular GABA in the GP and, as a consequence, decrease the outflow of glutamate in the SNr (Ochi et al., 2000; 2004). This mechanism offers a rationale for the use of A<sub>2A</sub> receptor antagonists as monotherapy in PD, as well as for the synergistic effect observed upon the concurrent administration of A2A receptor antagonists with L-DOPA or dopaminergic agonists (Schwarzschild et al., 2006).

In addition, since striatal Ach appears to play an important role in the genesis of parkinsonian tremor, an additional role of  $A_{2A}$  receptor antagonists in PD may be the modulation of Ach release which might underlie the antitremorigenic effect reported for these agents in the tremulous jaw movements rat model of PD (Salamone et al., 1998; Simola et al., 2004).

# D. A<sub>2A</sub> Receptor Control of Striatal GAD67 and Neuropeptides

Striatal peptides distinguish between the direct and indirect projection pathways (see Chapter 1). Moreover, peptide

modifications have been associated with changes in the activity of these neurons. In the dopamine-depleted striatum, dysregulation of striatal output pathways is reflected by altered levels of dynorphin, preprotachykinins (PPT) and enkephalin. A decrease in dynorphin and PPT mRNA levels reflects the hypoactivity of striatonigral neurons, whereas enhanced enkephalin levels reflect striatopallidal neuron hyperactivity (Gerfen et al., 1990). Moreover, prolonged administration of L-DOPA in rodent and primate models of PD results in adaptive changes in the expression of several genes, both in striatonigral and striatopallidal neurons (see Chapter 36). These changes have been associated with the development of L-DOPA-induced dyskinetic responses, reflecting an aberrant functionality of BG. For instance, increased expression of the neuropeptide dynorphin has been reported in striatonigral neurons, whereas increased expression of the enzyme GAD67 in these neurons suggests an abnormal overactivity of the direct pathway (Carta et al., 2002). Chronic treatment with A<sub>2A</sub> receptor antagonists as monotherapy selectively restores enkephalin levels, leaving unaffected neuropeptide expression in striatonigral neurons; this effect correlates with the selective activity of these drugs on striatopallidal neurons (Aoyama et al., 2002; Lundblad et al., 2003). Moreover, chronic administration of low doses of L-DOPA in association with  $A_{2A}$  receptor antagonists produces a full motor response not associated with adaptive modifications in the striatal levels of enkephalin, dynorphin and GAD67 mRNAs (Carta et al., 2002; Tronci et al., 2007). On the other hand, in contrast to a full dose of L-DOPA, this treatment regimen did not modify GAD67 mRNA expression in the GP and SNr (Carta et al., 2003). This latter effect might reflect the absence of dyskinetic responses associated with A2A receptor antagonists and L-DOPA treatments in rodent and primate models of PD (Grondin et al., 1999; Kanda et al., 2000).

### V. A<sub>2A</sub>-DOPAMINE INTERACTIONS IN PARKINSON'S DISEASE: BEHAVIORAL STUDIES

A pioneer study performed in the 1970s by Fuxe and Ungerstedt (1974) reported that the  $A_1/A_{2A}$  receptor antagonist caffeine induced contralateral turning behavior in unilaterally 6-OHDA-lesioned rats, the most widely used model of PD. That study provided the first evidence that antagonism of adenosine receptors may have important

implications in movement control by BG. Furthermore, that finding opened the search for more selective drugs with a high affinity for  $A_{2A}$  receptors which appear to be the most extensively involved in the control of motor behavior (Svenningsson et al., 1997). Indeed,  $A_{2A}$  receptor antagonists have emerged as an attractive non-dopaminergic target to improve the motor deficits that characterize PD (Morelli et al., 2007).

### A. Studies in Rodents

The first studies evaluating the actions of A2A receptors in neurologically intact rodents or in rodent models of PD showed that A<sub>2A</sub> receptor agonists induced pronounced depression of spontaneous and dopamine agonist-stimulated motor activity (Ferré et al., 1991; Vellucci et al., 1993) together with a reduction of the contralateral turning behavior induced by dopamine D<sub>1</sub> and D<sub>2</sub> agonists in unilaterally 6-OHDA-lesioned rats (Morelli et al., 1994). Conversely, in these lesioned rats, A2A receptor antagonists produced motor stimulant effects and markedly increased the number of contralateral turns induced by a threshold dose of L-DOPA, as well as by the stimulation of dopamine D<sub>1</sub> and D<sub>2</sub> receptors (Pinna et al., 1996; Pollack and Fink, 1996; Fenu et al., 1997; Le Moine et al., 1997). Interestingly,  $A_{2A}$  receptor antagonists have been shown to counteract specific motor deficits in rats even when administered without L-DOPA (Pinna et al., 2007). In line with these results, A<sub>2A</sub> receptor antagonists increase locomotor activity in MPTP-treated or reserpinized mice and reverse haloperidol-induced catalepsy in rats (Shiozaki et al., 1999; Hauber et al., 2001).

While acute studies reported that in unilaterally 6-OHDAlesioned rats A2A receptor antagonists render a low dose of a dopaminergic drug fully effective, in rats rendered dyskinetic by subchronic treatment with L-DOPA, A<sub>2A</sub> receptor antagonists produced an additive reduction in motor disability when administered with L-DOPA, without worsening dyskinesia (Jenner, 2003). These studies also showed that while a full dose of L-DOPA induced sensitization in turning behavior (indicative of dyskinetic potential), combination of the A<sub>2A</sub> receptor antagonist SCH 58261 plus a low dose of L-DOPA induced the same degree of contralateral turning on first administration (indicative of similar therapeutic activity) but did not produce sensitization in turning behavior (Pinna et al., 2001). Similar results were obtained in A2A KO mice (Fredduzzi et al., 2002). In line with these behavioral effects, the A<sub>2A</sub> receptor antagonist istradefylline reversed the increase in GABA levels in the GPe caused by 6-OHDA-induced denervation of the striatum in the rat (Ochi et al., 2000) and the denervation-induced increase in preproenkephalin A mRNA expression in the indirect output pathway (Lundblad et al., 2003). All together, these studies suggest that  $A_{2A}$  receptor antagonists might provide a beneficial effect in PD and might avoid worsening of L-DOPA initiated dyskinesia.

Promising effects of  $A_{2A}$  antagonists have also been observed on parkinsonian tremor, a symptom difficult to be antagonized by classical dopaminergic therapy. Blockade of  $A_{2A}$  receptors effectively counteracts tremulous jaw movements induced in rats by tacrine or haloperidol, suggesting a beneficial use of these drugs against tremor originated in the striatum (Correa et al., 2004; Simola et al., 2004). Interestingly, intrastriatal infusion of the  $A_{2A}$  receptor antagonist SCH BT2 has revealed a key role for the ventrolateral portion of the CPu (Simola et al., 2004), a striatal portion in which a specific increase in  $A_{2A}$  mRNA expression was detected following dopamine denervation in the 6-OHDA model of PD (Pinna et al., 2002).

In addition, blockade of  $A_{2A}$  receptors counteracted muscle rigidity induced by haloperidol and reserpine, as it decreased muscle resistance and reflex EMG activities (Wardas et al., 2001). Moreover, blockade of  $A_{2A}$  receptors potentiated the effect of L-DOPA on haloperidol-induced muscle rigidity, an effect which might be attributed to the synergistic action of  $A_{2A}$  receptor blockade with dopamine  $D_1$  and  $D_2$  receptors stimulation (Wardas et al., 2001).

### **B.** Studies in Primates

Preclinical data on primates rendered parkinsonian suggest that  $A_{2A}$  receptor antagonists may provide motor benefit as monotherapy, potentiate the benefit of dopamine agonists or low-dose of L-DOPA and do not exacerbate development of L-DOPA-induced dyskinesia.

In MPTP-treated common marmosets, oral administration of the  $A_{2A}$  receptor antagonist istradefylline dosedependently increased locomotor activity. In contrast, oral administration of selective  $A_1$  receptor antagonists had no effect on locomotor activity (Grondin et al., 1999; Kanda et al., 2000; Bibbiani et al., 2003). These observations indicate that the improvements in motor function, although modest, are mediated by selective antagonism of  $A_{2A}$ receptors.

When administered with  $D_1$  or  $D_2$  receptor agonists, an additive effect of  $A_{2A}$  receptor antagonists on locomotor

activity was observed. Moreover, when the  $D_2/D_3$  agonist quinpirole was administered 24 hours after istradefylline (a time at which istradefylline alone has no effect on motor function), a significant potentiation of its effects was observed compared with quinpirole alone, indicative of a synergistic interaction (Kanda et al., 2000). These observations suggest that istradefylline and a dopamine agonist might provide from additive to synergistic effects on motor functions.

Oral administration of a low, threshold dose of L-DOPA to MPTP-treated marmosets produced a non-significant increase in locomotor activity. Notably when  $A_{2A}$  receptor antagonists were given in association with a low dose of L-DOPA, they produced symptomatic relief comparable to that elicited by an optimal dose of L-DOPA alone but with less dyskinesia, although they did not suppress dyskinesia after it was established (Hauser and Schwarzschild, 2005). Therefore, similarly to what observed in rodents, a low dyskinetic potential of  $A_{2A}$  receptor antagonists was observed in MPTP-treated primates (Grondin et al., 1999; Bibbiani et al., 2003).

Administration of L-DOPA to MPTP-treated primates induces marked dyskinesia. In these L-DOPA-primed animals, subchronic administration of istradefylline alone improved locomotor function into the normal range and caused little or no dyskinesia (Kanda et al., 1996). This latter observation suggests that in PD patients with established dyskinesia, the addition of istradefylline might allow maintenance of the motor response with less dyskinesia using a lower L-DOPA dose.

## C. Clinical Trials with A<sub>2A</sub> Receptor Antagonists in PD Patients

Clinical trials with  $A_{2A}$  receptor antagonists are currently evaluating several compounds. The most advanced of these trials is the one on istradefylline. The results obtained so far in phase III, showed that istradefylline reduces OFF time in a late-stage patient population already receiving dopaminergic therapy, with no or minimal increase in non-troublesome dyskinesia (Hauser et al., 2003; Lewitt et al., 2008; Stacy et al., 2008). However, the effect on motor function was not statistically significant in any of these studies. Further evaluation of  $A_{2A}$  antagonists is also needed in conjunction with suboptimal doses of L-DOPA as suggested by the preclinical studies. Moreover, the potential for use of adenosine antagonists as monotherapy in non-dyskinetic PD patients has not been explored yet.

# VI. A<sub>2A</sub> RECEPTORS IN HUNTINGTON'S DISEASE

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by the expansion of a polymorphic CAG trinucleotide repeat encoding a polyglutamine tract within the huntingtin protein (The Huntington Disease Collaborative Research Group, 1993). GABAergic enkephalin neurons of the BG, which show the highest levels of expression of  $A_{2A}$  receptors, are the most vulnerable in HD. Such a selective neuronal vulnerability, which occurs despite ubiquitous expression of mutant and normal huntingtin, has suggested that A2A receptors might play a pathogenetic role in HD (Blum et al., 2003a). Furthermore, changes in  $A_{2A}$  receptor expression and signaling have been reported in various experimental models of HD, including one of the best-characterized transgenic models, the R6/2 mice (Mangiarini et al., 1996). In these animals, a transient increase in  $A_{2A}$  receptor density (Bmax) and  $A_{2\text{A}}$  receptor-dependent cAMP production was found at early presymptomatic ages (7-14 postnatal days) (Tarditi et al., 2006), while a highly significant decrease in A<sub>2A</sub> receptor binding was reported at later stages (12 weeks) (Cha et al., 1999). Furthermore, an aberrant amplification of A<sub>2A</sub> receptor-stimulated adenylyl cyclase was found in striatal-derived cells engineered to express mutant huntingtin (Varani et al., 2001). A similar alteration (namely increased A2A receptor-dependent adenylyl cyclase stimulation) was also present in the peripheral blood cells (platelets, lymphocytes and neutrophils) of patients carrying the mutant huntingtin gene with respect to healthy subjects (Varani et al., 2003). Interestingly, A2A receptor dysfunction seems to correlate with age at onset in HD patients (Maglione et al., 2005). Increased A2A receptor signaling (namely enhanced responsiveness of adenylyl cyclase to the selective A2A receptor agonist CGS 21680) was found also in sympthomatic R6/2 mice (Chou et al., 2005). Such an aberrant A<sub>2A</sub> receptor phenotype was accompanied by profound functional changes, since A2A receptor activation oppositely modulated NMDAinduced toxicity in the striatum of R6/2 vs wild type mice (Martire et al., 2007).

The interpretation of the functional significance of the aberrant  $A_{2A}$  receptor phenotype in HD mice is complicated by the conflicting data so far reported on the potential neuroprotective and neurodegenerative effects of these receptors in the brain. The same complex profile has emerged in experimental models of HD, in which both  $A_{2A}$ receptor agonists and antagonists have shown beneficial effects (see Popoli et al., 2008 for review). For instance,

pharmacological blockade or genetic inactivation of A2A receptors resulted in neuroprotection in pathogenetic models of HD (Popoli et al., 2002; Blum et al., 2003b, Fink et al., 2004). A partial protective effect was also observed in R6/2 mice after subchronic treatment, in early phases, with the A<sub>2A</sub> receptor antagonist SCH 58261 (Domenici et al., 2007). Unexpectedly, however, a chronic treatment with A2A receptor agonist CGS21680 (at later stages of the disease) was frankly beneficial in the same model (Chou et al., 2005). Since A<sub>2A</sub> receptors may mediate either "bad" responses (for example, stimulation of glutamate outflow and excessive glial activation), and "good" responses (such as trophic and antinflammatory effects), both their blockade and their activation can result neuroprotective according to the mechanisms involved in a given condition (or at a given stage of a disease).

Considering that HD is a chronically progressive illness, the multiple mechanisms involving  $A_{2A}$  receptors may play different relative roles along the degenerative process. Such different mechanisms can be influenced by  $A_{2A}$  receptor activation or blockade in different ways, even leading to opposite outcomes depending on the time of agonist/antagonist administration.

## VII. NEUROPROTECTIVE POTENTIAL OF A<sub>2A</sub> RECEPTOR ANTAGONISTS

Besides playing a role in BG-mediated motor functions and having beneficial effects on PD-associated motor deficits,  $A_{2A}$  receptor antagonists have been associated with neuroprotective effects in BG pathologies such as PD and HD.

A novel therapeutic potential of  $A_{2A}$  antagonists was offered by the epidemiological evidence of an inverse relationship between the consumption of caffeine, an  $A_1/A_{2A}$ receptor antagonist, and the risk of developing PD, which raised the exciting possibility of a neuroprotective potential for  $A_{2A}$  receptors antagonists (Ascherio et al., 2001; Schwarzschild et al., 2003).

In experimental rodent models of PD, blockade of  $A_{2A}$  receptors has been shown to substantially prevent the death of nigral dopaminergic neurons and the fall in striatal dopamine concentration (Chen et al., 2001; Ikeda et al., 2002; Pieri et al., 2005). Furthermore, a role for  $A_{2A}$  receptors in neurodegeneration was suggested by the absence of dopamine nigral degeneration consequent to MPTP administration in  $A_{2A}$  receptor genetically depleted mice (Carta et al., 2009; Chen et al., 2001). The mechanism underlying

neuroprotective activity of  $A_{2A}$  antagonists seems to differ from that mediating motor stimulating effects of these agents. Though several mechanisms have been hypothesized, involving either neuronal or glial  $A_{2A}$  receptors, no single mechanism has been shown to prevail.  $A_{2A}$  receptor stimulation on glutamatergic nerve terminals or astrocytes enhances glutamate release and might contribute to the excitotoxic component of neuronal death. Alternatively,  $A_{2A}$  receptors located on microglial cells might contribute to neuroinflammation, which has a prominent role in neuronal demise in PD (Hirsch et al., 2003; Chen and Pedata, 2008) (Fig. 11.3).

## VIII. ADENOSINE RECEPTORS AND COGNITIVE PROCESSES: ANY ROLE?

Several pieces of experimental evidence collected in the recent years have demonstrated that the BG are deeply involved in the modulation of cognitive processes. It is now well acknowledged that the striatum is critical to the generation of motor memories and motor habits (Packard and Knowlton, 2002; Willuhn and Steiner, 2008), as well as to the regulation of non-motor memory. Circuits connecting the striatum to the cerebral cortex (e.g., the fronto-striatal circuit), in fact, profoundly modulate functions such as attention, goal-directed behavior, motivation, organization and planning (Schmidtke et al., 2002; Grahn et al., 2008).

In light of this evidence, the previously described extensive regulation of BG functionality by adenosine might acquire particular importance to cognition-related processes. Notably, several investigations have indeed demonstrated that manipulation of adenosinergic transmission deeply impacts cognition. Thus, administration of the non-selective adenosine receptor antagonist caffeine to mice has been shown to improve the performance in the object recognition memory paradigm (Costa et al., 2008) and to attenuate the deficit in the spontaneous alternation task of cognitive function induced by the intracranial infusion of  $\beta$ -amyloid (a putative model of Alzheimer's disease) (Dall'Igna et al., 2007). Furthermore, in the rat, caffeine administration has been shown to enhance attention and to counteract the age-related decline in olfactionmediated memory (Prediger et al., 2005a; Higgins et al., 2007). Paralleling the beneficial effects observed in animals, independent investigations performed in humans have demonstrated the capability of caffeine in increasing attention and improving the performance in tests aimed at evaluating cognitive function (Lorist and Tops, 2003; Haskell et al., 2005). In addition to the latter findings, epidemiological studies have linked caffeine consumption to an inverse association with cognitive decline in elderly (Johnson-Kozlow et al., 2002; Ritchie et al., 2007). Results in line with those elicited by caffeine have been achieved following the selective manipulation of either A<sub>1</sub> or A2A receptors. A memory failure has been described in rats subsequently to either an intra-hippocampal infusion or a systemic administration of an A<sub>1</sub> receptor agonist (Ohno and Watanabe, 1996; Prediger and Takahashi, 2005). Conversely,  $A_1$  receptor antagonists, either infused intracranially or administered systemically, have been demonstrated to improve the performance in tests aimed at evaluating cognitive function (Pitsikas and Borsini, 1997; Pereira et al., 2002; Prediger and Takahashi, 2005). In agreement with these findings, memory deficits have been reported in rats overexpressing the human A<sub>2A</sub> receptor in the brain, whilst an amelioration of cognitive function has been described in mice bearing a genetic deletion of the A2A receptor as well as in both rats and mice administered with A2A receptor antagonists (Prediger and Takahashi, 2005; Prediger et al., 2005b; O'Neill and Brown 2006; Wang et al., 2006; Dall'Igna et al., 2007; Giménez-Llort et al., 2007; Higgins et al., 2007).

Up to now, only a few studies have employed a neuroanatomical approach aimed at determining the brain areas in which adenosine acts to modulate cognitive function. Such investigations are basically concerned with the A<sub>1</sub> receptors (Ohno and Watanabe, 1996; Pereira et al., 2002), whereas no studies on A2A receptors have been performed yet. Recent experiments have demonstrated that A2A receptors located in the nucleus accumbens regulate the execution of effort-related behavior (Font et al., 2008; Mingote et al., 2008), which is intimately linked to cognitive processes (Phillips et al., 2008). Therefore, the possibility that adenosine receptors located in the BG may participate in the modulation of cognitive function could exist. In particular, A<sub>2A</sub> receptors, which as previously mentioned are highly and almost selectively enriched on the striatopallidal neurons, might be involved in this process.

In light of the extensive adenosine–dopamine interactions existing in the BG, modulation of dopaminergic transmission might be a mechanism through which BG adenosine receptors would eventually influence cognitionrelated functions. However, controversial results on the relevance of such an interaction on cognitive processes have been obtained in experimental animals. On the one hand, it has been reported that the administration of an A2A receptor antagonist counteracts the impairment in social recognition memory induced in rats by the monoamine-depleting agent reserpine (Prediger et al., 2005b). This finding that could be envisioned as the consequence of a boosting effect on dopamine transmission exerted by the blockade of  $A_{2A}$ receptors. Notably, in line with this interpretation, a synergistic interaction between an A2A receptor antagonist and a  $D_2$  receptor agonist has also been described in the same experimental model (Prediger et al., 2005b). On the other hand, A2A receptor blockade has been demonstrated to be ineffective in counteracting the deficit in reversal learning displayed by rats bearing a 6-OHDA-induced dopaminergic denervation of the dorsomedial striatum (O'Neill and Brown, 2007). In this regards, it has to be highlighted that important methodological differences exist between these studies. In particular, such investigations employed a different kind of dopamine depletion (which was restricted to a specific striatal region in the 6-OHDA model being, conversely, spread to all the brain in the reserpine model) and analyzed different features of cognitive function (social recognition memory vs. reversal learning). Therefore, it is possible that adenosine-dopamine interactions may be relevant only to certain aspects of cognitive processes or, influence them in a fashion dependent on the BG region in which such interactions take place. Alternatively, adenosine receptors in the BG could regulate cognitive function by influencing either one or both glutamatergic and cholinergic neurotransmissions, which deeply modulate cognitive processes, and which extensively interact with adenosine transmission, although this hypothesis has been challenged (Cunha et al., 2008).

In recent years, a further interesting opportunity has been disclosed on a possible therapeutic use of  $A_{2A}$  receptor antagonists in helping to prevent craving and relapse in human heroin addicts.  $A_{2A}$  receptor antagonists inhibit morphine self-administration in rats, and eliminate reinstatement of the drug self-administration in heroin addicted rats (Sahraei et al., 1999; Yao et al., 2006).  $A_{2A}$  receptors in accumbal neurons are coexpressed with  $\mu$ -opioid receptors (MOR) and CB1 receptors. In these neurons, the  $A_{2A}$ receptor seems to play a permissive role in MOR-CB1 synergy to modulate cAMP/PKA signaling. Blockade of  $A_{2A}$  receptors could therefore disrupt the instatement of plastic changes cAMP/PKA-mediated, which contribute to the acquisition or maintenance of drug-seeking behaviour (Yao et al., 2006). Taken together, the data available thus far do not definitely clarify whether adenosine receptors located in the BG participate in the regulation of cognitive function mediated by adenosine. Nevertheless, considering the high enrichment of adenosine receptors in the BG and their intimate interaction with other receptors binding neurotransmitters critically involved in the modulation of cognitive processes, further and more detailed studies on this topic are necessary. In particular, it has to be ascertained which subtype(s) of adenosine receptors might be involved in such phenomena, the precise profile of cognition-related functions which could be modulated by adenosine receptors located in the BG and which BG region(s) might be eventually important to the adenosine-mediated modulation of cognitive function.

#### IX. CONCLUSIONS

How adenosine receptors can affect BG functions is explained by the role of adenosine as a signaling molecule in the nervous system. This type of modulation forms a new concept for how BG function is controlled in normal physiological circumstances and in pathological conditions. The selective localization of  $A_{2A}$  receptors to the BG and specifically to the indirect output pathway, offers a unique opportunity to modulate the dopamine output from the striatum that is believed critical to the occurrence of motor behavior. The ability of A2A receptor antagonists to modulate neurotransmission, first of all dopaminergic, GABAergic and glutamatergic transmission, places adenosine signaling in a prime position to influence motor function. Another field of potential utilisation of compounds binding adenosine receptors is neuroprotection. The current preclinical data strongly support a role for drugs binding A<sub>2A</sub> receptors in protecting neurons from the type of pathogenic processes believed to occur in PD and some stages of HD. Finally the action of adenosine receptors in brain areas directly connected to the BG might play important roles in cognitive functions opening new therapeutic possibilities for drugs acting at this level.

## REFERENCES

- Alexander SP, Reddington M (1989) The cellular localization of adenosine receptors in rat neostriatum. Neuroscience 28:645–651.
- Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM (1996) Modulation of Ca<sup>2+</sup> channels by activation of adenosine A1 receptors in rat striatal glutamatergic nerve terminals. Neurosci Lett 220:163–166.

- Aoyama S, Koga K, Mori A, et al. (2002) Distribution of adenosine A(2A) receptor antagonist KW-6002 and its effect on gene expression in the rat brain. Brain Res 953:119–125.
- Ascherio A, Zhang SM, Hernán MA, et al. (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neuro 50:56–63.
- Augood SJ, Emson PC (1994) Adenosine A2a receptor mRNA is expressed by enkephalin cells but not by somatostatin cells in rat striatum: a co-expression study. Mol Brain Res 22:204–210.
- Bibbiani F, Oh JD, Petzer JP, et al. (2003) A(2A) antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. Exp Neurol 184:285–294.
- Blum D, Galas MC, Pintor A, et al. (2003a) A dual role of adenosine A2A receptors in 3-nitropropionic acid-induced striatal lesions: implications for the neuroprotective potential of A2A antagonists. J Neurosci 23:5361–5369.
- Blum D, Hourez R, Galas MC, Popoli P, Schiffmann SN (2003b) Adenosine receptors and Huntington's disease: implications for pathogenesis and therapeutics. Lancet Neurol 2:366–374.
- Boegman RJ, Vincent SR (1996) Involvement of adenosine and glutamate receptors in the induction of c-fos in the striatum by haloperidol. Synapse 22:70–77.
- Borycz J, Pereira MF, Melani A, et al. (2007) Differential glutamate-dependent and glutamate-independent adenosine A1 receptor-mediated modulation of dopamine release in different striatal compartments. J Neurochem 101:355–363.
- Brooks DJ, Doder M, Osman S, et al. (2008) Positron emission tomography analysis of [11C]KW-6002 binding to human and rat adenosine A2A receptors in the brain. Synapse 62:671–681.
- Brown SJ, James S, Reddington M, Richardson PJ (1990) Both A1 and A2a purine receptors regulate striatal acetylcholine release. J Neurochem 55:31–38.
- Calon F, Dridi M, Hornykiewicz O, et al. (2004) Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. Brain 127:1075–1084.
- Carriba P, Ortiz O, Patkar K, et al. (2007) Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacol 32:2249–2259.
- Carta AR, Pinna A, Cauli O, Morelli M (2002) Differential regulation of GAD67, enkephalin and dynorphin mRNAs by chronic-intermittent l-dopa and A2A receptor blockade plus L-dopa in dopaminedenervated rats.. Synapse 44:166–174.
- Carta AR, Tabrizi MA, Baraldi PG, et al. (2003) Blockade of A2A receptors plus l-DOPA after nigrostriatal lesion results in GAD67 mRNA changes different from l-DOPA alone in the rat globus pallidus and substantia nigra reticulata. Exp Neurol 184:679–687.
- Carta AR, Kachroo A, Schintu N, et al. (2009) Inactivation of neuronal forebrain A(2A) receptors protects dopaminergic neurons in a mouse model of Parkinson's disease. J Neurochem Oct 8 [Epub ahead of print].
- Cha JH, Frey AS, Alsdorf SA, et al. (1999) Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354:981–989.
- Chase TN, Bibbiani F, Bara-Jimenez W, et al. (2003) Translating A2A antagonist KW6002 from animal models to parkinsonian patients. Neurology 61:S107–S111.

- Chen JF, Pedata F (2008) Modulation of ischemic brain injury and neuroinflammation by adenosine A2A receptors. Curr Pharm Des 14:1490–1499.
- Chen JF, Xu K, Petzer JP, et al. (2001) Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. J Neurosci 21:RC143.
- Chou SY, Lee YC, Chen HM, et al. (2005) CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. J Neurochem 93:310–320.
- Ciruela F, Casadó V, Rodrigues RJ, et al. (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080–2087.
- Coccurello R, Breysse N, Amalric M (2004) Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology 29:1451–1461.
- Correa M, Wisniecki A, Betz A, et al. (2004) The adenosine A2A antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to parkinsonism. Behav Brain Res 148:47–54.
- Costa MS, Botton PH, Mioranzza S, et al. (2008) Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus. Neurochem Int 53:89–94.
- Cunha GM, Canas PM, Melo CS, et al. (2008) Adenosine A2A receptor blockade prevents memory dysfunction caused by beta-amyloid peptides but not by scopolamine or MK-801. Exp Neurol 210:776–781.
- Dall'Igna OP, Fett P, Gomes MW, et al. (2007) Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25–35)-induced cognitive deficits in mice. Exp Neurol 203:241–245.
- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- Domenici MR, Scattoni ML, Martire A, et al. (2007) Behavioural and electrophysiological effects of the adenosine A2A receptor antagonist SCH58261 in R6/2 Huntington's disease mice. Neurobiol Dis 28:197–205.
- Fastbom J, Pazos A, Palacios JM (1987) The distribution of adenosine A1 receptors and 5'-nucleotidase in the brain of some commonly used experimental animals. Neuroscience 22:813–826.
- Fenu S, Pinna A, Ongini E, Morelli M (1997) Adenosine A2A receptor antagonism potentiates l-dopa-induced turning behaviour and c-fos expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 321:143–147.
- Ferré S, Herrera-Marschitz M, Grabowska-Andén M, Casas M, Ungerstedt U, Andén NE (1991) Postsynaptic dopamine/adenosine interaction: II. Postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. Eur J Pharmacol 192:31–37.
- Ferré S, Karcz-Kubicha M, Hope, et al. (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc Natl Acad Sci USA 99:11940–11945.
- Ferré S, O'Connor WT, Fuxe K, Ungerstedt U (1993) The striopallidal neuron: a main locus for adenosine–dopamine interactions in the brain. J Neurosci 13:5402–5406.
- Ferré S, O'Connor WT, Svenningsson P, et al. (1996) Dopamine D1 receptor-mediated facilitation of GABAergic neurotransmission in

the rat strioentopenduncular pathway and its modulation by adenosine A1 receptor-mediated mechanisms. Eur J Neurosci 8:1545–1553.

- Ferré S, Popoli P, Giménez-Llort L, et al. (1994) Postsynaptic antagonistic interaction between adenosine A1 and dopamine D1 receptors. Neuroreport 6:73–76.
- Ferré S, Rimondini R, Popoli P, et al. (1999) Stimulation of adenosine A1 receptors attenuates dopamine D1 receptor-mediated increase of NGFI-A, c-fos and jun-B mRNA levels in the dopamine-denervated striatum and dopamine D1 receptor-mediated turning behaviour. Eur J Neurosci 11:3884–3892.
- Fink JS, Kalda A, Ryu H, et al. (2004) Genetic and pharmacological inactivation of the adenosine A<sub>2A</sub> receptor attenuates 3-nitropropionic acid-induced striatal damage. J Neurochem 88:538–544.
- Fink JS, Weaver DR, Rivkees SA, et al. (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Mol Brain Res 14:186–195.
- Font L, Mingote S, Farrar AM, et al. (2008) Intra-accumbens injections of the adenosine A2A agonist CGS 21680 affect effort-related choice behavior in rats. Psychopharmacology 199:515–526.
- Franco R, Lluis C, Canela EI, et al. (2007) Receptor-receptor interactions involving adenosine A1 or dopamine D1 receptors and accessory proteins. J Neural Transm 114:93–104.
- Fredduzzi S, Moratalla R, Monopoli A, et al. (2002) Persistent behavioral sensitization to chronic I-DOPA requires A<sub>2A</sub> adenosine receptors. J Neurosci 22:1054–1062.
- Fredholm BB (1995) Purinoceptors in the nervous system. Pharmacol Toxicol 76:228–239.
- Fredholm BB, Chern Y, Franco R, Sitkovsky M (2007) Aspects of the general biology of adenosine A2A signaling. Prog Neurobiol 83:263–267.
- Fredholm BB, Irenius E, Kull B, Schulte G (2001) Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. Biochem Pharmacol 61:443–448.
- Fuxe K, Canals M, Torvinen M, et al. (2007) Intramembrane receptor-receptor interactions: a novel principle in molecular medicine. J Neural Transm 114:49–75.
- Fuxe K, Ungerstedt U (1974) Action of caffeine and theophyllamine on supersensitive dopamine receptors: considerable enhancement of receptor response to treatment with DOPA and dopamine receptor agonists. Med Biol 52:48–54.
- Gerfen CR, Engber TM, Mahan LC, et al. (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gianfriddo M, Corsi C, Melani A, et al. (2003) Adenosine A(2A) antagonism increases striatal glutamate outflow in the quinolinic acid rat model of Huntington's disease. Brain Res 979:225–229.
- Giménez-Llort L, Schiffmann SN, Shmidt T, et al. (2007) Working memory deficits in transgenic rats overexpressing human adenosine A2A receptors in the brain. Neurobiol Learn Mem 87:42–56.
- Goda H, Ooboshi H, Nakane H, Ibayashi S, Sadoshima S, Fujishima M (1998) Modulation of ischemia-evoked release of excitatory and inhibitory amino acids by adenosine A1 receptor agonist. Eur J Pharmacol 357:149–155.
- Grahn JA, Parkinson JA, Owen AM (2008) The cognitive functions of the caudate nucleus. Prog Neurobiol 86:141–155.
- Grondin R, Bédard PJ, Hadj Tahar A, Grégoire L, Mori A, Kase H (1999) Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology 52:1673–1677.

- Haskell CF, Kennedy DO, Wesnes KA, Scholey AB (2005) Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacology 179:813–825.
- Hauber W, Neuscheler P, Nagel J, Muller CE (2001) Catalepsy induced by a blockade of dopamine D1 or D2 receptors was reversed by a concomitant blockade of adenosine A(2A) receptors in the caudateputamen of rats. Eur J Neurosci 14:1287–1293.
- Hauser RA, Hubble JP, Truong DD (2003) Randomized trial of the adenosine A(2A) receptor antagonist istradefylline in advanced PD. Neurology 61:297–303.
- Hauser RA, Schwarzschild MA (2005) Adenosine A2A receptor antagonists for Parkinson's disease: rationale, therapeutic potential and clinical experience. Drugs Aging 22:471–482.
- Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 431:331–346.
- Higgins GA, Grzelak ME, Pond AJ, et al. (2007) The effect of caffeine to increase reaction time in the rat during a test of attention is mediated through antagonism of adenosine A2A receptors. Behav Brain Res 185:32–42.
- Hillion J, Canals M, Torvinen M, et al. (2002) Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 277:18091–18097.
- Hirsch EC, Breidert T, Rousselet E, et al. (2003) The role of glial reaction and inflammation in Parkinson's disease. Ann NY Acad Sci 991:214–228.
- Hurley MJ, Mash DC, Jenner P (2000) Adenosine A(2A) receptor mRNA expression in Parkinson's disease. Neurosci Lett 291:54–58.
- Ikeda K, Kurokawa M, Aoyama S, Kuwana Y (2002) Neuroprotection by adenosine A2A receptor blockade in experimental models of Parkinson's disease. J Neurochem 80:262–270.
- Jenner P (2003) A2A antagonists as novel non-dopaminergic therapy for motor dysfunction in PD. Neurology 61:S32–S38.
- Johnson-Kozlow M, Kritz-Silverstein D, Barrett-Connor E, Morton D (2002) Coffee consumption and cognitive function among older adults. Am J Epidemiol 156:842–850.
- Kachroo A, Orlando LR, Grandy DK, et al. (2005) Interactions between metabotropic glutamate 5 and adenosine A2A receptors in normal and parkinsonian mice. J Neurosci 25:10414–10419.
- Kanda T, Jackson MJ, Smith LA, et al. (2000) Combined use of the adenosine A(2A) antagonist KW-6002 with I-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol 162:321–327.
- Karcz-Kubicha M, Ferré S, Díaz-Ruiz O, et al. (2006) Stimulation of adenosine receptors selectively activates gene expression in striatal enkephalinergic neurons. Neuropsychopharmacology 31: 2173–2179.
- Karcz-Kubicha M, Quarta D, Hope BT, et al. (2003) Enabling role of adenosine A1 receptors in adenosine A2A receptor-mediated striatal expression of c-fos. Eur J Neurosci 18:296–302.
- Kull B, Ferré S, Arslan G, et al. (1999) Reciprocal interactions between adenosine A2A and dopamine D2 receptors in Chinese hamster ovary cells co-transfected with the two receptors. Biochem Pharmacol 58:1035–1045.
- Kurokawa M, Koga K, Kase H, Nakamura J, Kuwana Y (1996) Adenosine A2a receptor-mediated modulation of striatal acetylcholine release in vivo. J Neurochem 66:1882–1888.

- Le Moine C, Svenningsson P, Fredholm BB, Bloch B (1997) Dopamineadenosine interactions in the striatum and the globus pallidus: inhibition of striatopallidal neurons through either D2 or A2A receptors enhances D1 receptor-mediated effects on c-fos expression. J Neurosci 17:8038–8048.
- Lewitt PA, Guttman M, Tetrud JW, et al. (2008) Adenosine A(2A) receptor antagonist istradefylline (KW-6002) reduces "off" time in Parkinson's disease: A double-blind, randomized, multicenter clinical trial (6002-US-005). Ann Neurol 63:295–302.
- Lorist MM, Tops M (2003) Caffeine, fatigue, and cognition. Brain Cogn 53:82–94.
- Lundblad M, Vaudano E, Cenci MA (2003) Cellular and behavioural effects of the adenosine A2a receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. J Neurochem 84:1398–1410.
- Maglione V, Giallonardo P, Cannella M, et al. (2005) Adenosine A2A receptor dysfunction correlates with age at onset anticipation in blood platelets of subjects with Huntington's disease. Am J Genet B Neuropsychiatr Gen 139B:101–105.
- Mangiarini L, Sathasivam K, Seller M, et al. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506.
- Marchi M, Reiteri L, Risso F, et al. (2002) Effects of adenosine A1 and A2A receptor activation on the evoked release of glutamate from rat cerebrocortical synaptosomes. Br J Pharmacol 136:434–1440.
- Martire A, Calamandrei G, Felici F, et al. (2007) Opposite effects of the A<sub>2A</sub> receptor agonist CGS21680 in the striatum of Huntington's disease versus wild-type mice. Neurosci Lett 417:78–83.
- Melani A, Pantoni L, Bordoni F, et al. (2003) The selective A2A receptor antagonist SCH 58261 reduces striatal transmitter outflow, turning behavior and ischemic brain damage induced by permanent focal ischemia in the rat. Brain Res 959:243–250.
- Mingote S, Font L, Farrar AM, et al. (2008) Nucleus accumbens adenosine A2A receptors regulate exertion of effort by acting on the ventral striatopallidal pathway. J Neurosci 28:9037–9046.
- Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA (2007) Role of adenosine A2A receptors in parkinsonian motor impairment and I-DOPA-induced motor complications. Prog Neurobiol 83:293–309.
- Morelli M, Fenu S, Pinna A, Di Chiara G (1994) Adenosine A2 receptors interact negatively with dopamine D1 and D2 receptors in unilaterally 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 251:21–25.
- Morelli M, Pinna A, Wardas J, Di Chiara G (1995) Adenosine A2 receptors stimulate c-fos expression in striatal neurons of 6-hydroxydopamine-lesioned rats. Neuroscience 67:49–55.
- Mori A, Shindou T (2003) Modulation of GABAergic transmission in the striatopallidal system by adenosine A2A receptors: a potential mechanism for the antiparkinsonian effects of A2A antagonists. Neurology 61:S44–S48.
- Mori A, Shindou T, Ichimura M, Nonaka H, Kase H (1996) The role of adenosine A2a receptors in regulating GABAergic synaptic transmission in striatal medium spiny neurons. J Neurosci 16:605–611.
- Nishi A, Liu F, Matsuyama S, et al. (2003) Metabotropic mGlu5 receptors regulate adenosine A2A receptor signaling. Proc Natl Acad Sci USA 100:1322–1327.
- Ochi M, Koga K, Kurokawa M, Kase H, Nakamura J, Kuwana Y (2000) Systemic administration of adenosine A(2A) receptor antagonist reverses increased GABA release in the globus pallidus of unilateral 6-hydroxydopamine-lesioned rats: a microdialysis study. Neuroscience 100:53–62.

- Ochi M, Shiozaki S, Kase H (2004) Adenosine A(2A) receptor-mediated modulation of GABA and glutamate release in the output regions of the basal ganglia in a rodent model of Parkinson's disease. Neuroscience 127:223–231.
- Ohno M, Watanabe S (1996) Working memory failure by stimulation of hippocampal adenosine A1 receptors in rats. Neuroreport 7:3013–3016.
- O'Neill M, Brown VJ (2006) The effect of the adenosine A(2A) antagonist KW-6002 on motor and motivational processes in the rat. Psychopharmacology 184:46–55.
- O'Neill M, Brown VJ (2007) The effect of striatal dopamine depletion and the adenosine A2A antagonist KW-6002 on reversal learning in rats. Neurobiol Learn Mem 88:75–81.
- O'Neill C, Nolan BJ, Macari A, O'Boyle KM, O'Connor JJ (2007) Adenosine A1 receptor-mediated inhibition of dopamine release from rat striatal slices is modulated by D1 dopamine receptors. Eur J Neurosci 26:3421–3428.
- Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Annu Rev Neurosci 25:563–593.
- Pereira GS, Mello e Souza T, Vinadé ER, et al. (2002) Blockade of adenosine A1 receptors in the posterior cingulate cortex facilitates memory in rats. Eur J Pharmacol 437:151–154.
- Phillips AG, Vacca G, Ahn S (2008) A top-down perspective on dopamine, motivation and memory. Pharmacol Biochem Behav 90:236–249.
- Pierri M, Vaudano E, Sager T, Englund U (2005) KW-6002 protects from MPTP induced dopaminergic toxicity in the mouse. Neuropharmacology 48:517–524.
- Pinna A, Corsi C, Carta AR, Valentini V, Pedata F, Morelli M (2002) Modification of adenosine extracellular levels and adenosine A(2A) receptor mRNA by dopamine denervation. Eur J Pharmacol 20:75–82.
- Pinna A, di Chiara G, Wardas J, Morelli M (1996) Blockade of A2a adenosine receptors positively modulates turning behaviour and c-Fos expression induced by D1 agonists in dopamine-denervated rats. Eur J Neurosci 8:1176–1181.
- Pinna A, Fenu S, Morelli M (2001) Motor stimulant effect of the adenosine A<sub>2A</sub> receptor antagonist SCH 58261 do not develop tolerance after repeated treatment in 6-hydroxydopamine-lesioned rats. Synapse 39:233–238.
- Pinna A, Pontis S, Borsini F, Morelli M (2007) Adenosine A<sub>2A</sub> receptor antagonists improve deficits in initiation of movement and sensory motor integration in the unilateral 6-hydroxydopamine rat model of Parkinson's disease. Synapse 261:606–614.
- Pitsikas N, Borsini F (1997) The adenosine A1 receptor antagonist BIIP 20 counteracts scopolamine-induced behavioral deficits in the passive avoidance task in the rat. Eur J Pharmacol 328:19–22.
- Pollack AE, Fink JS (1996) Synergistic interaction between an adenosine antagonist and a D<sub>1</sub> dopamine agonist on rotational behavior and striatal c-Fos induction in 6-hydroxydopamine-lesioned rats. Brain Res 743:124–130.
- Popoli P, Blum D, Domenici MR, Burnouf S, Chern Y (2008) A critical evaluation of adenosine A2A receptors as potentially "druggable" targets in Huntington's disease. Curr Pharm Des 14:1500–1511.
- Popoli P, Pèzzola A, Torvinen M, et al. (2001) The selective mGlu(5) receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: interactions with adenosine A(2a) receptors. Neuropsychopharmacology 25:505–513.

- Popoli P, Pintor A, Domenici MR, et al. (2002) Blockade of striatal adenosine A2A receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. J Neurosci 22:1967–1975.
- Prediger RD, Batista LC, Takahashi RN (2005a) Caffeine reverses agerelated deficits in olfactory discrimination and social recognition memory in rats Involvement of adenosine A1 and A2A receptors. Neurobiol Aging 26:957–964.
- Prediger RD, Da Cunha C, Takahashi RN (2005b) Antagonistic interaction between adenosine A2A and dopamine D2 receptors modulates the social recognition memory in reserpine-treated rats. Behav Pharmacol 16:209–218.
- Prediger RD, Takahashi RN (2005) Modulation of short-term social memory in rats by adenosine A1 and A(2A) receptors. Neurosci Lett 376:160–165.
- Ritchie K, Carrière I, de Mendonça A, et al. (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). Neurology 69:536–545.
- Rosin DL, Hettinger BD, Lee A, Linden J (2003) Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 61:S12–S18.
- Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J (1998) Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. J Comp Neurol 401:163–186.
- Sahin B, Galdi S, Hendrick J, Greene RW, Snyder GL, Bibb JA (2007) Evaluation of neuronal phosphoproteins as effectors of caffeine and mediators of striatal adenosine A2A receptor signaling. Brain Res 1129:1–14.
- Sahraei H, Motamedi F, Khoshbaten A, Zarrindast MR (1999) Adenosine A(2) receptors inhibit morphine self-administration in rats. Eur J Pharmacol 383:107–113.
- Sakiyama Y, Hatano K, Kato T, Tajima T, Kawasumi Y, Ito K (2007) Stimulation of adenosine A1 receptors decreases in vivo dopamine D1 receptor binding of [11C]SCH23390 in the cat striatum revealed by positron emission tomography. Ann Nucl Med 21:447–453.
- Salamone JD, Mayorga AJ, Trevitt JT, Cousins MS, Conlan A, Nawab A (1998) Tremulous jaw movements in rats: a model of parkinsonian tremor. Prog Neurobiol 56:591–611.
- Santini E, Valjent E, Usiello A, et al. (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in I-DOPA-induced dyskinesia. J Neurosci 27:6995–7005.
- Schiffmann SN, Jacobs O, Vanderhaeghen JJ (1991a) Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an in situ hybridization histochemistry study. J Neurochem 57:1062–1067.
- Schiffmann SN, Libert F, Vassart G, Vanderhaeghen JJ (1991b) Distribution of adenosine A2 receptor mRNA in the human brain. Neurosci Lett 130:177–181.
- Schmidtke K, Manner H, Kaufmann R, Schmolck H (2002) Cognitive procedural learning in patients with fronto-striatal lesions. Learn Mem 9:419–429.
- Schulte G, Fredholm BB (2003) Signaling from adenosine receptors to mitogen-activated protein kinases. Cell Signal 15:813–827.
- Schwarzschild MA, Agnati L, Fuxe K, Chen JF, Morelli M (2006) Targeting adenosine A2A receptors in Parkinson's disease. Trends Neurosci 29:647–654.
- Schwarzschild MA, Xu K, Oztas E, et al. (2003) Neuroprotection by caffeine and more specific A2A receptor antagonists in animal models of Parkinson's disease. Neurology 61:S55–S61.

- Shindou T, Mori A, Kase H, Ichimura M (2001) Adenosine A(2A) receptor enhances GABA(A)-mediated IPSCs in the rat globus pallidus. J Physiol 532:423–434.
- Shiozaki S, Ichikawa S, Nakamura J, Kitamura S, Yamada K, Kuwana Y (1999) Actions of adenosine A2A receptor antagonist KW-6002 on drug-induced catalepsy and hypokinesia caused by reserpine or MPTP. Psychopharmacology 147:90–95.
- Simola N, Fenu S, Baraldi PG, Tabrizi MA, Morelli M (2004) Blockade of adenosine A2A receptors antagonizes parkinsonian tremor in the rat tacrine model by an action on specific striatal regions. Exp Neurol 189:182–188.
- Stacy M, Silver D, Mendis T, et al. (2008) A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson disease. Neurology 70:2233–2240.
- Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999a) Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 59:355–396.
- Svenningsson P, Fourreau L, Bloch B, Fredholm BB, Gonon F, Le Moine C (1999b) Opposite tonic modulation of dopamine and adenosine on c-fos gene expression in striatopallidal neurons. Neuroscience 89:827–837.
- Svenningsson P, Nomikos GG, Ongini E, Fredholm BB (1997) Antagonism of adenosine A<sub>2A</sub> receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate-putamen and nucleus accumbens. Neuroscience 79:753–764.
- Tarditi A, Camurri A, Varani K, et al. (2006) Early and transient alteration of adenosine A(2A) receptor signaling in a mouse model of Huntington disease. Neurobiol Dis 23:44–53.
- The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983.

- Tomiyama M, Kimura T, Maeda T, Tanaka H, Kannari K, Baba M (2004) Upregulation of striatal adenosine A2A receptor mRNA in 6-hydroxydopamine-lesioned rats intermittently treated with L-DOPA. Synapse 52:218–222.
- Tronci E, Simola N, Borsini F, et al. (2007) Characterization of the antiparkinsonian effects of the new adenosine A2A receptor antagonist ST1535: acute and subchronic studies in rats. Eur J Pharmacol 566:94–102.
- Varani K, Abbracchio MP, Cannella M, et al. (2003) Aberrant A<sub>2A</sub> receptor function in peripheral blood cells in Huntington's disease. FASEB J 17:2148–2150.
- Varani K, Rigamonti D, Sipione S, et al. (2001) Aberrant amplification of A<sub>2A</sub> receptor signaling in striatal cells expressing mutant Huntingtin. FASEB J 15:1245–1247.
- Vellucci SV, Sirinathsinghji DJS, Richardson PJ (1993) Adenosine A<sub>2</sub> receptor regulation of apomorphine-induced turning in rats with unilateral striatal dopamine denervation. Psychopharmacology 111:383–388.
- Wang JH, Ma YY, van den Buuse M (2006) Improved spatial recognition memory in mice lacking adenosine A2A receptors. Exp Neurol 199:438–445.
- Wardas J, Konieczny J, Lorenc-Koci E (2001) SCH 58261, an A(2A) adenosine receptor antagonist, counteracts parkinsonian-like muscle rigidity in rats. Synapse 41:160–171.
- Willuhn I, Steiner H (2008) Motor-skill learning in a novel runningwheel task is dependent on D1 dopamine receptors in the striatum. Neuroscience 153:249–258.
- Yao L, McFarland K, Fan P, Jiang Z, Ueda T, Diamond I (2006) Adenosine A2a blockade prevents synergy between mu-opiate and cannabinoid CB1 receptors and eliminates heroin-seeking behavior in addicted rats. Proc Natl Acad Sci USA 103:7877–7882.

# Regulation of Corticostriatal Synaptic Plasticity in Physiological and Pathological Conditions

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## I. INTRODUCTION

The striatum is the main input station of the basal ganglia; it receives excitatory afferents from the cortex and thalamus and a dense innervation from midbrain dopamine (DA) neurons (see Chapter 1). Striatal medium-sized spiny projection neurons (MSNs), which account for the large majority of striatal neurons (see Chapter 3), receive inputs from the whole cortical mantle and, in turn, project either directly to the output nuclei of the basal ganglia (in the socalled "direct pathway") or to the external segment of the globus pallidus (GPe) (in the so-called "indirect pathway") and thence to the output nuclei (Grillner et al., 2005).

MSNs are inhibitory GABAergic neurons that interact to some degree through local axon collaterals (see Chapter 5) and provide, via the direct pathway, powerful inhibitory control of the basal ganglia output nuclei neurons. By contrast, activity through the parallel indirect pathway results

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. in strong excitation of the basal ganglia output nuclei via increased activity of excitatory glutamatergic neurons in the subthalamic nucleus (STN) (Grillner et al., 2005) (see Chapter 1). Striatal MSN activity is modulated by DA arising from both the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) (see also Chapter 7). DA participates in information processing within the striatum, together with acetylcholine (ACh), GABA and other neurotransmitters released by striatal interneurons.

Physiological activity of the striatum is essential for the control of the whole basal ganglia and is thought to play a crucial role in the control of motor activity (Pisani et al., 2005) and other behavioral output. For example, parts of the striatum are involved in reward processing and in several forms of learning and memory, such as habit learning (see Chapter 32), goal-directed instrumental and reward-association learning and procedural and emotional learning (Hikosaka et al., 1999; Schultz et al., 2003; Yin and Knowlton, 2006). Accordingly,

altered activity of the striatum results in the onset of pathological conditions characterized by various motor and behavioral abnormalities including Parkinson's disease (PD) or Huntington's disease (HD) (see also Chapter 35).

The involvement of the striatum in numerous forms of learning is thought to be based on changes in neuronal activity occurring when specific behavioral and motor tasks are being learned and in the subsequent functional and morphological remodeling of the synapses linking cortical and striatal neurons. Studies in experimental models have demonstrated that striatal neurons are able to undergo almost all forms of Hebbian plasticity together with short-term forms of intrinsic plasticity, spike-timing dependent plasticity, depotentiation and other essential mechanisms aimed at preventing neural network destabilization (Calabresi et al., 2007; Berretta et al., 2008; Di Filippo et al., 2008a; Kreitzer and Malenka, 2008).

Synaptic plasticity at corticostriatal connections exerts a crucial role during physiological conditions in the regulation of reward, learning and memory processes (Kreitzer and Malenka, 2008). On the other hand, when a pathological insult alters the physiological activity of MSNs or causes the impairment of a specific neurotransmitter system, neurons can respond by undergoing non-physiological forms of synaptic plasticity that can be either beneficial or detrimental in nature (Calabresi et al., 2002; Picconi et al., 2003; Picconi et al., 2006a). In the following sections, we will describe the mechanisms underlying two prominent forms of striatal synaptic plasticity - long-term depression (LTD) and long-term potentiation (LTP) - during physiological conditions. Thereafter, we will summarize the downstream consequences on synaptic plasticity of different pathological insults such as those causing hypo- and hyperkinetic movement disorders and stroke.

## II. PHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF CORTICOSTRIATAL LONG-TERM DEPRESSION (LTD) AND LONG-TERM POTENTIATION (LTP)

LTD and LTP at corticostriatal synapses were described for the first time in 1992 (Calabresi et al., 1992b; Calabresi et al., 1992c) when pioneering studies of synaptic plasticity at glutamatergic corticostriatal synapses demonstrated that high-frequency stimulation (HFS, three trains of pulses at 100 Hz) of corticostriatal fibres (and possibly thalamostriatal fibres as well), in association with postsynaptic neuronal firing, was able to induce LTD of corticostriatal transmission onto striatal MSNs (Calabresi et al., 1992b). In particular, it was demonstrated that HFS was able to induce LTD (>2h) of both extracellularly recorded field potentials and intracellularly recorded excitatory postsynaptic potentials (EPSPs) and that subthreshold tetanic stimulation, which under control condition was not able to result in LTD, induced LTD when associated with membrane depolarization (Calabresi et al., 1992b).

## A. Role of Glutamate Receptors in LTD and LTP

Several neurotransmitters have been found to crucially influence the induction of both striatal LTD and LTP. Striatal LTD was found to be dependent on the activation of metabotropic glutamate receptors and independent from both NMDA receptor and GABA<sub>A</sub> receptor activation (Calabresi et al., 1992b). In particular, LTD induction was found to require the selective activation of mGluR1 (Gubellini et al., 2001; Gubellini et al., 2004) and Ca<sup>2+</sup> influx through voltage-dependent nifedipine-sensitive Ca<sup>2+</sup> channels, a sufficient intracellular free Ca<sup>2+</sup> concentration and the activation of Ca<sup>2+</sup>-dependent protein kinases (Calabresi et al., 1994) (Fig. 12.1).

In contrast to LTD, LTP was initially found to be expressed by corticostriatal synapses after the removal of magnesium from the extracellular medium, an experimental condition that results in deinactivation of NMDA glutamate receptors (Calabresi et al., 1992c) and thus to be dependent on the activation of these specific glutamate receptors (Calabresi et al., 1992c). According to this experimental evidence the initial hypothesis was that, in the striatum, LTD represented the physiological form of synaptic plasti city, while LTP was inducible only after a pharmacological manipulation of the experimental medium.

In 1997 the first demonstration of an *in vivo* form of synaptic plasticity was provided by Charpier and Deniau (1997). Interestingly, these authors showed that in their *in vivo* preparation the tetanus induced LTP of corticostriatal excitatory transmission (Charpier and Deniau, 1997). This latter evidence, together with the results of subsequent in vitro studies (Fino et al., 2005), demonstrated that LTP, as LTD, represents a physiological form of neuroplasticity expressed at corticostriatal synapses and that it is not the expression of a putative pathological process over-activating NMDA glutamate receptors.



**FIGURE 12.1** Modulation of corticostriatal LTD by glutamate, dopamine, endocannabinoids and acetylcholine. In experimental conditions, high-frequency stimulation (HFS) of corticostriatal fibers, in association with postsynaptic neuronal firing, is able to induce long-term depression (LTD) of corticostriatal transmission onto striatal medium spiny projection neurons. Activation of metabotropic glutamate receptors (mGluR1) by glutamate (Glu) is a required step for LTD induction. Activation of D2 dopamine (DA) receptors influences the phosphorylation state of DARPP-32 through modulation of the intracellular levels of cAMP. DARPP-32, in turn, functions as a potent inhibitor of protein phosphatase 1 (PP1) which regulates the functional activity of many physiological effectors, including AMPA glutamate receptors. Activation of D2 receptors on cholinergic interneurons results in the lowering of the M1 muscarinic receptor tone and further facilitates the induction of LTD by disinhibition of Cav1.3 Ca<sup>2+</sup> channels. Endocannabinoids (eCBs) are released by striatal MSNs following membrane depolarization ( $\Psi^+$ ), intracellular Ca<sup>2+</sup> elevation, D2 receptor stimulation and metabotropic glutamate receptor (mGluR) activation. After being released by the postsynaptic neurons, eCBs act as retrograde messengers activating presynaptic CB1 receptors and thus inducing a long-lasting depression of excitatory glutamatergic transmission. Other abbreviations: AC, adenylate cyclase; ACh, ace-tylcholine; AMPA, alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptors; Ca<sup>++</sup>, calcium ions; cAMP, cyclic AMP; CB1, cannabinoid receptor 1; DARPP-32, dopamine- and cAMP-regulated phosphoprotein 32 kDa; SNc, substantia nigra pars compacta;  $\Psi^+$ , membrane depolarization.

As far as the specific role played by NMDA receptors is concerned, it is worth remembering that striatal MSNs are known to show characteristic shifts of the membrane potential between two preferred levels, one more polarized, called the Down-state (varying from -94 to -61 mV), and one more depolarized, called the Up-state (varying from -71to -40 mV), triggered by increased activity of many convergent corticostriatal neurons (Wilson and Kawaguchi, 1996). During the Up-state the membrane potential of MSNs is closer to the threshold for NMDA receptor opening and action potentials are thought to be triggered by noisy fluctuations of the membrane potential. Fluctuation of the membrane potential leading to NMDA receptor "deinactivation" facilitates intracellular Ca<sup>2+</sup> rise which, in turn, might trigger the Ca<sup>++</sup>-dependent molecular mechanisms underlying LTP induction (Kerr and Plenz, 2002).

Therefore, based on current data (Calabresi et al., 2007), it is possible to speculate that repetitive activation of a corticostriatal synapse, such as that mimicked by the HFS protocol, occurring in the Up-state will probably favor LTP induction, according to the higher probability of

NMDA receptor opening at the Up-state membrane potential values. Conversely, the same tetanic stimulus occurring during the Down-state will probably lead to LTD of the efficacy of corticostriatal glutamatergic transmission.

However, although intracellular recordings from anesthetized animals have demonstrated that MSNs undergo spontaneous transitions between hyperpolarized and depolarized states, wakefulness is associated with a completely different pattern of temporally disorganized depolarizing synaptic events of variable amplitude (Mahon et al., 2006), suggesting that in the waking state the regulation of synaptic plasticity by state transitions might be more complex and not easily predictable.

#### **B.** Role of Dopamine Receptors

From the first studies on striatal LTD and LTP it has been evident that the induction of synaptic plasticity at corticostriatal synapses requires not only the neurotransmitter glutamate but also a series of other neurotransmitter and neuromodulators released from striatal interneurons and other neuronal structures (Calabresi et al., 1992b; Calabresi et al., 1992c). In particular, physiological DA signaling was found to critically modulate both LTD and LTP induction (see also Chapter 6). Indeed, the critical role of DA was evident in the first description of LTD. In particular, it was demonstrated that activation of both D1 and D2 DA receptors was required for LTD induction (Fig. 12.1) and that striatal LTP was critically dependent on the activation of D1/D5 receptors (Calabresi et al., 1992b; Calabresi et al., 1992a; Centonze et al., 1999; Kerr and Wickens, 2001; Schotanus and Chergui, 2008). A few years later, a critical role for D2 receptors in the regulation of the mechanisms underlying the direction of long-term changes in synaptic efficacy in the striatum was further demonstrated by utilizing recordings from D2 receptor-null mice (Calabresi et al., 1997).

In the striatum stimulation of D1 and D2 receptors is known to trigger opposite effects on the intracellular levels of cyclic AMP (cAMP), stimulating and inhibiting adenylyl cyclase activity, respectively (Greengard et al., 1999) (see also Chapter 26). cAMP levels, in turn, modulate the activity of PKA (cAMP-dependent protein kinase A), a major substrate of which is the DA and cAMP-regulated phosphoprotein 32 kDa (DARPP-32). DARPP-32 acts as a potent inhibitor of protein-phosphatase-1 (PP-1) that regulates the functional activity of many physiological effectors, including NMDA and AMPA glutamate receptors (Greengard et al., 1999). Interestingly, both LTP and LTD have been demonstrated to be fully abolished after the genetic disruption of DARPP-32 (Calabresi et al., 2000), highlighting the importance of the DA receptor/PKA/ DARPP-32/PP-1 pathway in the induction of bidirectional synaptic plasticity in the striatum.

The interpretation of the precise role played by DA in the regulation of corticostriatal plasticity is complicated by the lack of homogeneity in DA receptor expression in MSNs. In fact, the prevailing hypothesis is that D1 and D2 receptors are "segregated" between the two striatal projecting pathways, with D1 receptors being mainly expressed by MSNs of the "direct" striatonigral pathway and D2 receptors by MSNs of the "indirect" striatopallidal pathway (DeLong and Wichmann, 2007) (see also Chapter 1). This "segregation hypothesis" is partially in contrast with several experimental results suggesting that LTD (which requires D2 receptor activation) is expressed by the large majority of striatal MSNs rather than only by half of them (Calabresi et al., 1992b), and with the fact that, in BAC transgenic mice, D2 receptor antagonists block LTD induction in both D1- and D2 receptor-expressing MSNs, and not only in D2-expressing MSNs (Wang et al., 2006). Several

theories have been postulated to explain why both D1- and D2-expressing MSNs express D2 receptor-dependent LTD. For example, it has been demonstrated that activation of D2 receptors expressed by striatal cholinergic interneurons results in reduced ACh release and in subsequent lowering of M1 muscarinic receptor tone, an event that has been demonstrated to mediate LTD induction in D1-expressing striatonigral MSNs, through disinhibition of Cav1.3 Ca<sup>2+</sup> channels (Wang et al., 2006) (see also Chapter 6).

Another theory is that the D2 receptor-dependence of LTD in D1-expressing MSNs might represent a "spillover" artifact caused by the induction protocol. Indeed, it is possible that a high-intensity macrostimulation, when applied in the white matter separating the cortex from the striatum can give rise to a broad and diffuse activation of striatal neurons, resulting in the release of endocannabinoids (ECBs) from D2-expressing MSNs (Kreitzer and Malenka, 2008) and to the subsequent induction of an ECBs-dependent LTD also in D1-receptor expressing MSNs (see also below and Chapter 9).

## C. Role of Acetylcholine

Similar to glutamate and DA, endogenously produced ACh also seems to play a crucial role in modulating long-lasting striatal synaptic changes. Striatal large cholinergic interneurons, which are known to represent the main source of striatal ACh (Pisani et al., 2007), are autonomous pacemakers and are thus referred to as "tonically active neurons" (TANs) in behaving animals (Pisani et al., 2007) (see also Chapter 7). ACh from striatal cholinergic interneurons differentially modulates striatonigral and striatopallidal neurons and, through the interaction with DA, has an integrative and modulatory role in the basal ganglia circuit (Calabresi et al., 2006).

The ACh-dependent neuronal signaling is mediated by the activation of different subtypes of muscarinic and nicotinic receptors. ACh, acting at muscarinic receptors, has been demonstrated to influence both LTP and LTD induction. In particular, activation of M1-like muscarinic receptors has been demonstrated to be a required step for the induction of corticostriatal LTP (Calabresi et al., 1999) probably via a PKC (protein kinase C)-mediated mechanism while, as specified above, a lowered ACh concentration seems to facilitate LTD induction by lowering the M1 muscarinic receptor tone and thus disinhibiting Cav1.3  $Ca^{2+}$  channels (Wang et al., 2006) (Fig. 12.1). Different from M1 muscarinic receptors, the activation of M2-like muscarinic receptors seems to exert a negative influence on striatal LTP, probably via the reduction of glutamate release from corticostriatal fibres (Calabresi et al., 1998; Wang et al., 2006). Similar to muscarinic receptors, nicotinic ACh receptors seems to play an important role, as their blockade inhibits striatal LTD (Partridge et al., 2002).

#### D. Role of Endogenous Cannabinoids

In contrast to classical neurotransmitters, ECBs are released from postsynaptic neurons and travel backward across synapses, acting as retrograde synaptic messengers inhibiting neurotransmitter release by the activation of presynaptic CB1 receptors (Wilson and Nicoll, 2002) (see Chapter 9). ECBs, such as anandamide (AEA), have been demonstrated to be released by striatal MSNs following membrane depolarization, intracellular Ca<sup>2+</sup> elevation and D2 receptor stimulation (Di Marzo et al., 1994; Giuffrida et al., 1999), leading to the hypothesis that in the striatum AEA, released by the postsynaptic neurons after membrane depolarization, might act as a retrograde messenger activating presynaptic CB1 receptors and thus inducing LTD of excitatory glutamatergic transmission (Gerdeman et al., 2002; Ronesi et al., 2004; Kreitzer and Malenka, 2005; Di Filippo et al., 2008b) (Fig. 12.1).

It is worth noting that it has recently been proposed that striatal MSNs of the direct and indirect pathways might express different synaptic properties (Kreitzer and Malenka, 2007). In particular, it has been suggested that ECB release sufficient to trigger ECB-mediated LTD, is restricted to indirect pathway MSNs (Kreitzer and Malenka, 2007).

# E. Synaptic Plasticity Expressed by Striatal Interneurons

The physiological activity of striatal MSNs is known to be regulated by their interaction with various subpopulations of striatal interneurons, including three types of GABAergic cells (one coexpressing parvalbumin, one calretinin and one nitric oxide synthase) (see Chapter 8) and cholinergic interneurons (Kawaguchi et al., 1995; Pisani et al., 2007). Similar to striatal MSNs, striatal interneurons have also been demonstrated to express different forms of neuroplasticity. In particular, after tetanic stimulation of cortico/thalamostriatal fibers, striatal cholinergic interneurons express a form of LTP that has been demonstrated to require D5, but not D2 or NMDA receptor activation (Suzuki et al., 2001). The ability to express synaptic plasticity has also been investigated in striatal GABAergic interneurons and in particular in fast-spiking GABAergic interneurons, a specific subtype of striatal interneurons (Centonze et al., 2002). These cells have been demonstrated to express either LTP or LTD, depending on the pattern of synaptic stimulation, and to require NMDA receptor activation to express these forms of synaptic plasticity (Fino et al., 2008).

## III. SYNAPTIC DEPOTENTIATION AT CORTICOSTRIATAL SYNAPSES: A MECHANISM OF PHYSIOLOGICAL "FORGETTING"?

As introduced above, LTP probably represents the main accepted vertebrate model for learning and memory. Nevertheless, LTP operates by positive feedback rules that might potentially drive neuronal circuits toward maximal action potential firing frequency ranges, leading to synaptic saturation. For this reason, the ability to forget or "ignore" irrelevant synaptic signals is crucial to render neurons able to encode subsequent plastic changes. One of the key mechanisms that is thought to prevent neuronal network destabilization and to underlie synaptic "forgetting" is "depotentiation", which results from the reversal of an established LTP by a low-frequency stimulation protocol (LFS, 1–5 Hz) and seems to be distinct from *de novo* LTD (O'Dell and Kandel, 1994; Picconi et al., 2003).

It has been demonstrated that, in the striatum, synaptic depotentiation is prevented by the pretreatment of slices with PP-1 and protein phosphatase 2A (PP-2A) inhibitors, D1-like DA receptor agonists and adenylate cyclase activators (Picconi et al., 2003), suggesting an important role of the D1/PKA/DARPP-32 signaling pathway in the induction of this form of homeostatic plasticity. ACh also seems to play a role in the expression of striatal depotentiation. Indeed, depotentiation of MSNs is blocked by scopolamine, indicating that reversal of LTP in the striatum requires activation of muscarinic ACh receptors by endo genous ACh (Picconi et al., 2006a).

Depotentiation is not the only form of homeostatic plasticity in basal ganglia circuits. Other important mechanisms include global changes in synaptic strength, changes in neuronal excitability, spike-timing dependent plasticity (STDP) and the regulation of synapse number (Mahon et al., 2003; Stellwagen and Malenka, 2006).

In particular, recent studies have investigated the presence of STDP at striatal synapses (Fino et al., 2005; Pawlak and Kerr, 2008). It is now well-accepted that the temporal relationship between activity in the pre- and postsynaptic elements constitutes a determinant factor for the induction of synaptic plasticity, influencing both the magnitude and the direction of the induced synaptic change (Dan and Poo, 2004). On the basis of this hypothesis, striatal synaptic strength can be affected in different ways according to whether a presynaptic spike closely precedes, or follows, an EPSP (Fino et al., 2005; Pawlak and Kerr, 2008)

## IV. CORTICOSTRIATAL SYNAPTIC PLASTICITY IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

PD is a chronic and progressive neurodegenerative disorder of largely unknown etiology caused by a pathological cascade resulting is the degeneration of midbrain DA neurons of the SNc that project to the striatum (Lang and Lozano, 1998a; Lang and Lozano, 1998b) (see Chapter 34). The progressive loss of SNc neurons leads to an impairment in DA regulation of basal ganglia physiological activity and to subsequent alteration of the delicate balance of activity in the output nuclei that is thought to be essential for the normal regulation of motor function (Lang and Lozano, 1998a; Lang and Lozano, 1998b) (see also Chapters 25 and 38). Accordingly, in humans suffering from PD the loss of SNc neurons results in the onset of several motor symptoms such as tremor at rest, bradykinesia and rigidity (Lang and Lozano, 1998a;Lang and Lozano, 1998b).

In the last decades many advances have been made in the field of PD research and several theories have been postulated to explain the onset of neuronal network abnormalities that underlie symptom onset during PD (see Chapters 25 and 38). One of these theories postulates that the presence of an alteration of the main forms of synaptic plasticity in the striatum and in the motor cortex of PD patients might represent the synaptic basis underlying the onset of the disabling motor and cognitive symptoms of the disease (Pisani et al., 2005; Calabresi et al., 2006).

According to this theory, studies on experimental models of PD and in human subjects suffering from the disease have confirmed the link between SNc neuron degeneration and the loss of the main forms of synaptic plasticity. In neurotoxic models of PD, such as the 6-OHDA model, both LTD and LTP are lost (Calabresi et al., 1992b; Centonze et al., 1999;Kreitzer and Malenka, 2007). Nevertheless, in animals sustaining unilateral 6-OHDA lesions of the SNc, coricostriatal LTP can be restored by the chronic treatment with the DA precursor 1-3,4-dihydroxyphenylalanine (L-DOPA) (Picconi et al., 2003).

It has also been demonstrated that ECB-dependent LTD is selectively lost at indirect-pathway MSN synapses in

experimental models of PD and that it can be rescued either in the presence of a D2 receptor agonist or by the application of an inhibitor of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endo genous cannabinoid AEA (Kreitzer and Malenka, 2007). Interestingly, administration of the same drugs has been demonstrated to ameliorate symptoms in the same experimental PD models (Kreitzer and Malenka, 2007), providing support for the hypothesis of a correlation between synaptic plasticity abnormalities at corticostriatal synapses and PD symptoms.

An alteration of the main forms of synaptic plasticity, together with an impairment in DA neurotransmission, has been demonstrated to occur not only in neurotoxic models of PD but also in genetic models of the disease such as in the mice with genetic inactivation of the familial parkinsonism-linked gene DJ-1 (Goldberg et al., 2005) and in PINK1(-/-) mutant mice (Kitada et al., 2007).

The hypothesis of a link between synaptic alterations and PD has also been directly investigated in patients suffering from the disease. For example, Morgante et al., by utilizing a paired associative stimulation protocol, have been able to demonstrate the presence of aberrant motor cortex plasticity in PD (Morgante et al., 2006). Also, Prescott et al., by studying synaptic plasticity in PD patients undergoing therapeutic implantation of deep brain stimulating electrodes, have demonstrated the absence of potentiation in the substantia nigra pars reticulata of patients recorded in the absence of a pharmacological dopaminergic treatment (Calabresi et al., 2009).

In conclusion, the results of experimental and human studies indicate that, during PD, synaptic plasticity is altered both at corticostriatal synapses and in other brain structures whose function is thought to be crucial for normal motor control, suggesting a link between the onset of PD symptoms and the alteration of LTD and LTP.

## V. CORTICOSTRIATAL SYNAPTIC PLASTICITY IN EXPERIMENTAL MODELS OF HYPERKINETIC DISORDERS

### A. Huntington's Disease

HD is a neurodegenerative disorder that follows an autosomal-dominant pattern of inheritance (Bates, 2005). HD symptoms usually appear in mid-life and include psychiatric disturbances, involuntary movements and cognitive decline, progressing toward death about 10–20 years from onset. The HD-causing gene mutation consists of an expanded CAG trinucleotide repeat in the huntingtin (htt) gene and results in a neurodegenerative process that primarily affects striatal MSNs, with relative sparing of cholinergic interneurons (Ferrante et al., 1985).

The striatum thus represents the neuronal structure that is primarily involved in the neurodegenerative process that characterizes HD (see also Chapter 35). For this reasons it has been hypothesized that an impaired function of the intrastriatal neuronal network might represent the basis for the development of the cognitive and motor symptoms of the disease. According to the hypothesis of a pathogenetic role of synaptic plasticity abnormalities during HD, alterations in the induction and reversal of synaptic plasticity have been demonstrated in both neurotoxic (3-nitropropionic acid-treated rats) and genetic (R6/2 mice) models of the disease. Striatal MSNs recorded in both disease models have been demonstrated to express normal LTP, but to be unable to depotentiate their synapses after a low-frequency stimulation protocol (Picconi et al., 2006a; Di Filippo et al., 2007).

This inability of MSNs to reverse synaptic strength to pre-LTP levels might cause alterations in the homeostatic processes that are known to prevent neuronal circuit destabilization during information storage and probably accounts for the impaired behavioral flexibility described in HD patients at early clinical stages. Interestingly, cholinergic interneurons recorded from 3-NP-treated animals and HD R6/2 mice have been demonstrated to be unable to express physiological LTP (Picconi et al., 2006a; Di Filippo et al., 2007), suggesting that, since striatal synaptic depotentiation depends upon activation of muscarinic receptors, the lack of potentiation of cholinergic interneurons might be responsible for the absence of depotentiation in MSNs in HD experimental models. It has also been demonstrated, by recording spontaneous (sEPSCs) and miniature excitatory postsynaptic currents (mEPSCs) from striatal neurons of both toxic and genetic models of HD, that a significant down-regulation of glutamate transmission occurs during HD (Rossi et al., 2006). This latter evidence suggests that reduced synaptic excitation of the input structure of the basal ganglia might represents an electrophysiological correlate of HD and might potentially contribute to the impairment of synaptic plasticity that has been described in HD models.

#### B. L-DOPA-induced Dyskinesia

The discovery that DA deficiency due to SNc degeneration represents the central event leading to symptom onset in PD resulted in the subsequent introduction of a replacement therapy with the DA precursor L-DOPA (Mercuri and Bernardi, 2005) (see Chapter 36). This drug initially revolutionized the treatment of this disabling neurodegenerative disease. Unfortunately, in most patients (>90%), the treatment with L-DOPA, within 5–10 years of treatment initiation, is complicated by the onset of motor fluctuations and hyperkinetic involuntary movements, named dyskinesias (Mercuri and Bernardi, 2005).

It has been hypothesised that, after chronic treatment with L-DOPA, the development of dyskinesias might be associated with the presence of an impairment of the physiological forms of synaptic plasticity at corticostriatal synapses and in particular with the loss of synaptic depotentiation (Picconi et al., 2003; Calabresi et al., 2008). As described above, in the 6-OHDA model of PD LTP is lost but can be restored by chronic treatment with L-DOPA (Picconi et al., 2003). Interestingly, it has been demonstrated that depotentiation is selectively lost only in dyskinetic animals chronically treated with L-DOPA, whereas animals that do not develop involuntary movements maintain the physiological reversal of synaptic strength after LFS (Picconi et al., 2003), suggesting that the absence of this form of homeostatic synaptic plasticity might lead to the destabilization of neuronal circuits in the basal ganglia.

It is possible to hypothesize that the alteration in motor control and in the ability of synapses to undergo depotentiation during dyskinesias might be attributable to specific changes occurring along the D1/PKA/DARPP-32 signaling pathway leading to the inhibition of PP-1 activity (see also Chapter 36). Indeed, depotentiation at corticostriatal synapses involves protein phosphatase activity and, as introduced above, activation of D1 receptors results in PKA-catalyzed phosphorylation of DARPP-32 on Thr34, which, in turn, converts DARPP-32 into a potent inhibitor of PP-1. Interestingly, it has been demonstrated that dyskinetic animals express higher levels of Thr34phosphorylated DARPP-32 than nondyskinetic rats and drug-naïve controls (Picconi et al., 2003). It is also worth to remember that, in the striatum, synaptic depotentiation is prevented by pretreatment of slices with PP-1 and PP-2A inhibitors, D1-like DA-receptor agonists, and adenylyl cyclase activators (Picconi et al., 2003). All these factors point to a role of the D1/PKA/DARPP-32 signaling pathway in mediating the loss of synaptic depotentiation that accompanies the onset of involuntary movements in the 6-OHDA model of PD. In line with this hypothesis, it has been suggested that D1 receptor blockade might improve L-DOPA-induced dyskinesia (Grondin et al., 1999).

## VI. STRIATAL SYNAPTIC PLASTICITY AND NEURONAL ISCHEMIA

Stroke is the second most common cause of death and the principal cause of adult disability in the world (Feigin et al., 2003). Although in most cases stroke induces mild to severe permanent deficits (Varona et al., 2004), recovery is often dynamic, reflecting the ability of the injured neuronal net-work to adapt. It has repeatedly been documented that neuroplasticity occurs in the cerebral cortex and in subcortical structures after ischemic injuries. In particular, *in vitro* studies have demonstrated that oxygen and glucose deprivation (*in vitro* ischemia) is able to cause a potentiation of the glutamate-mediated excitatory synaptic transmission, named, post-ischemic LTP (i-LTP) (Di Filippo et al., 2008c).

After an ischemic insult, surrounding a core of severe injury named ischemic core, neuronal death evolves more slowly in a heterogeneous area called the ischemic penumbra. Neurons within the penumbra are functionally impaired but not yet dead and can sustain membrane potentials (Hossmann, 1994). i-LTP was described at corticostriatal synapses after transient oxygen and glucose deprivation in 2002 (Calabresi et al., 2002). Like hippocampal post-anoxic LTP (Crepel et al., 1993), striatal i-LTP is dependent on NMDA receptor activation and is prevented by the intraneuronal injection of Ca<sup>2+</sup> chelators (Calabresi et al., 2002).

Interestingly, i-LTP is expressed by striatal MSNs but not by striatal cholinergic interneurons that are known to be resistant to *in vivo* ischemia. This evidence might suggest that i-LTP represents a pathological form of neuroplasticity accounting for the cell type-specific vulnerability following ischemia and energy deprivation (Calabresi et al., 2002; Calabresi et al., 2003; Di Filippo et al., 2008c).

As described above, NMDA receptor activation is essential for the induction of i-LTP in the striatum. In particular, a critical role of NR2B subunit-containing NMDA receptors has been suggested (Picconi et al., 2006b). In particular, it has been demonstrated that NR2B subunitcontaining NMDA receptor antagonists selectively block i-LTP without affecting activity-dependent LTP (Picconi et al., 2006b) and that certain low-affinity and uncompetitive NMDA receptor antagonists, such as memantine, block i-LTP (Tozzi et al., 2007).

The importance of NMDA receptors for the induction of i-LTP might be explained by the fact that, after oxygen and glucose deprivation, neurons fail to generate sufficient ATP, ionic gradients are lost and glutamate is released, promoting over-activation of ionotropic NMDA glutamate receptors and excessive neuronal  $Ca^{2+}$  entry that, in turn, could represent an essential step for the induction of this form of synaptic plasticity.

As described above DA plays a crucial role in the regulation of physiological neuroplasticity in the striatum (Calabresi et al., 2007). Interestingly, endogenous DA, via D1 receptors, is also able to selectively facilitate the expression of i-LTP on the AMPA-mediated component of the EPSPs (Saulle et al., 2002) suggesting a critical role for this neurotransmitter also during striatal i-LTP. The precise functional role of striatal i-LTP is still debated. It has indeed been hypothesized that i-LTP could exert both a detrimental and a beneficial role after cerebral ischemia. In particular the potentially detrimental effects of i-LTP are suggested by the fact that it shares several key molecular pathways with the processes triggered by the ischemic insult (such as NMDA receptor activation and intracellular  $Ca^{2+}$  load) (Calabresi et al., 2002). According to this view, i-LTP could represent a factor enhancing the excitotoxic processes that are known to facilitate neuronal death after ischemia. On the other hand it is also generally accepted that neuroplasticity can mediate recovery after stroke. Thus, an alternative scenario is that the induction of i-LTP in the ischemic penumbra might represent an adaptive form of plasticity aimed at improving functional recovery after stroke (Di Filippo et al., 2008c) (Fig. 12.2).

### VII. CONCLUSIONS AND FUTURE PERSPECTIVES

The precise mechanisms underlying the induction of synaptic plasticity in the striatum are complex and still far from being unraveled. Nevertheless, many advances have been



FIGURE 12.2 The dual role played by post-ischemic LTP in the striatum.

made in the last two decades and the knowledge about learning mechanisms in basal ganglia neuronal circuits is progressively improving. Crucially, in the last years, it has been possible to observe the translation of the theoretical basis of synaptic plasticity into human studies performed utilizing transcranial magnetic stimulation techniques or direct neuronal stimulation by deep brain stimulation electrodes (see Chapter 39).

Interestingly, the possibility of modulating synaptic plasticity by means of pharmacological and neurophysiological manipulations has also been investigated.

The hope is that, in the future, it will be possible to directly influence the induction and the reversal of the main forms of synaptic plasticity in the basal ganglia neuronal circuits. The possibility of a direct modulation of the efficacy of synaptic transmission might indeed have a profound therapeutic impact with potential implications for the treatment of disabling neurological disease and for neurorehabilitation.

#### REFERENCES

- Bates GP (2005) History of genetic disease: the molecular genetics of Huntington disease a history. Nat Rev Genet 6:766–773.
- Berretta N, Nistico R, Bernardi G, Mercuri NB (2008) Synaptic plasticity in the basal ganglia: a similar code for physiological and pathological conditions. Prog Neurobiol 84:343–362.
- Calabresi P, Centonze D, Gubellini P, Bernardi G (1999) Activation of M1-like muscarinic receptors is required for the induction of corticostriatal LTP. Neuropharmacology 38:323–326.
- Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998) Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. Eur J Neurosci 10:3020–3023.
- Calabresi P, Centonze D, Pisani A, Cupini L, Bernardi G (2003) Synaptic plasticity in the ischaemic brain. Lancet Neurol 2:622–629.
- Calabresi P, Di Filippo M, Ghiglieri V, Picconi B (2008) Molecular mechanisms underlying levodopa-induced dyskinesia. Mov Disord 23(Suppl 3):S570–S579.
- Calabresi P, Gubellini P, Centonze D, et al. (2000) Dopamine and cAMPregulated phosphoprotein 32kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. J Neurosci 20:8443–8451.
- Calabresi P, Maj R, Mercuri NB, Bernardi G (1992a) Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. Neurosci Lett 142:95–99.
- Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992b) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:4224–4233.
- Calabresi P, Mercuri NB, Di Filippo M (2009) Synaptic plasticity, dopamine and Parkinson's disease: one step ahead. Brain 132:285–287.
- Calabresi P, Picconi B, Parnetti L, Di Filippo M (2006) A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholine synaptic balance. Lancet Neurol 5:974–983.

- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopaminemediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1994) Post-receptor mechanisms underlying striatal long-term depression. J Neurosci 14:4871–4881.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1992c) Long-term potentiation in the striatum is unmasked by removing the voltagedependent magnesium block of NMDA receptor channels. Eur J Neurosci 4:929–935.
- Calabresi P, Saiardi A, Pisani A, Baik JH, Centonze D, Mercuri NB, Bernardi G, Borrelli E (1997) Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. J Neurosci 17:4536–4544.
- Calabresi P, Saulle E, Centonze D, Pisani A, Marfia GA, Bernardi G (2002) Post-ischaemic long-term synaptic potentiation in the striatum: a putative mechanism for cell type-specific vulnerability. Brain 125:844–860.
- Centonze D, Bracci E, Pisani A, Gubellini P, Bernardi G, Calabresi P (2002) Activation of dopamine D1-like receptors excites LTS interneurons of the striatum. Eur J Neurosci 15:2049–2052.
- Centonze D, Gubellini P, Picconi B, Calabresi P, Giacomini P, Bernardi G (1999) Unilateral dopamine denervation blocks corticostriatal LTP. J Neurophysiol 82:3575–3579.
- Charpier S, Deniau JM (1997) In vivo activity-dependent plasticity at cortico-striatal connections: evidence for physiological long-term potentiation. Proc Natl Acad Sci USA 94:7036–7040.
- Crepel V, Hammond C, Krnjevic K, Chinestra P, Ben-Ari Y (1993) Anoxia-induced LTP of isolated NMDA receptor-mediated synaptic responses. J Neurophysiol 69:1774–1778.
- Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23–30.
- Delong MR, Wichmann T (2007) Circuits and circuit disorders of the basal ganglia. Arch Neurol 64:20–24.
- Di Filippo M, Picconi B, Tantucci M, Ghiglieri V, Bagetta V, Sgobio C, Tozzi A, Parnetti L, Calabresi P (2008a) Short-term and long-term plasticity at corticostriatal synapses: Implications for learning and memory. Behav Brain Res 199:108–118.
- Di Filippo M, Picconi B, Tozzi A, Ghiglieri V, Rossi A, Calabresi P (2008b) The endocannabinoid system in Parkinson's disease. Curr Pharm Des 14:2337–2347.
- Di Filippo M, Tozzi A, Costa C, Belcastro V, Tantucci M, Picconi B, Calabresi P (2008c) Plasticity and repair in the post-ischemic brain.. Neuropharmacology 55:353–362.
- Di Filippo M, Tozzi A, Picconi B, Ghiglieri V, Calabresi P (2007) Plastic abnormalities in experimental Huntington's disease. Curr Opin Pharmacol 7:106–111.
- Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691.
- Feigin VL, Lawes CM, Bennett DA, Anderson CS (2003) Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet Neurol 2:43–53.
- Ferrante RJ, Kowall NW, Beal MF, Richardson EP Jr., Bird ED, Martin JB (1985) Selective sparing of a class of striatal neurons in Huntington's disease. Science 230:561–563.
- Fino E, Deniau JM, Venance L (2008) Cell-specific spike-timingdependent plasticity in GABAergic and cholinergic interneurons in corticostriatal rat brain slices. J Physiol 586:265–282.

- Fino E, Glowinski J, Venance L (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 25:11279–11287.
- Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451.
- Giuffrida A, Parsons LH, Kerr TM, Rodriguez de FF, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358–363.
- Goldberg MS, Pisani A, Haburcak M, et al. (2005) Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45:489–496.
- Greengard P, Allen PB, Nairn AC (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 23:435–447.
- Grillner S, Hellgren J, Menard A, Saitoh K, Wikstrom MA (2005) Mechanisms for selection of basic motor programs – roles for the striatum and pallidum. Trends Neurosci 28:364–370.
- Grondin R, Doan VD, Gregoire L, Bedard PJ (1999) D1 receptor blockade improves L-dopa-induced dyskinesia but worsens parkinsonism in MPTP monkeys. Neurology 52:771–776.
- Gubellini P, Pisani A, Centonze D, Bernardi G, Calabresi P (2004) Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases. Prog Neurobiol 74:271–300.
- Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, Conquet F, Calabresi P (2001) Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology 40:839–846.
- Hikosaka O, Nakahara H, Rand MK, Sakai K, Lu X, Nakamura K, Miyachi S, Doya K (1999) Parallel neural networks for learning sequential procedures. Trends Neurosci 22:464–471.
- Hossmann KA (1994) Viability thresholds and the penumbra of focal ischemia. Ann Neurol 36:557–565.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.
- Kerr JN, Plenz D (2002) Dendritic calcium encodes striatal neuron output during up-states. J Neurosci 22:1499–1512.
- Kerr JN, Wickens JR (2001) Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum *in vitro*. J Neurophysiol 85:117–124.
- Kitada T, Pisani A, Porter DR, et al. (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc Natl Acad Sci USA 104:11441–11446.
- Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543–554.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Kreitzer AC, Malenka RC (2005) Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25:10537–10545.
- Lang AE, Lozano AM (1998a) Parkinson's disease. First of two parts. N Engl J Med 339:1044–1053.
- Lang AE, Lozano AM (1998b) Parkinson's disease. Second of two parts. N Engl J Med 339:1130–1143.
- Mahon S, Casassus G, Mulle C, Charpier S (2003) Spike-dependent intrinsic plasticity increases firing probability in rat striatal neurons in vivo. J Physiol 550:947–959.
- Mahon S, Vautrelle N, Pezard L, Slaght SJ, Deniau JM, Chouvet G, Charpier S (2006) Distinct patterns of striatal medium spiny neuron

activity during the natural sleep-wake cycle. J Neurosci 26: 12587–12595.

- Mercuri NB, Bernardi G (2005) The 'magic' of L-dopa: why is it the gold standard Parkinson's disease therapy? Trends Pharmacol Sci 26:341–344.
- Morgante F, Espay AJ, Gunraj C, Lang AE, Chen R (2006) Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. Brain 129:1059–1069.
- O'Dell TJ, Kandel ER (1994) Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. Learn Mem 1:129–139.
- Partridge JG, Apparsundaram S, Gerhardt GA, Ronesi J, Lovinger DM (2002) Nicotinic acetylcholine receptors interact with dopamine in induction of striatal long-term depression. J Neurosci 22:2541–2549.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 28:2435–2446.
- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci 6:501–506.
- Picconi B, Passino E, Sgobio C, et al. (2006a) Plastic and behavioral abnormalities in experimental Huntington's disease: a crucial role for cholinergic interneurons. Neurobiol Dis 22:143–152.
- Picconi B, Tortiglione A, Barone I, et al. (2006b) NR2B subunit exerts a critical role in postischemic synaptic plasticity. Stroke 37:1895–1901.
- Pisani A, Bernardi G, Ding J, Surmeier DJ (2007) Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30:545–553.
- Pisani A, Centonze D, Bernardi G, Calabresi P (2005) Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. Mov Disord 20:395–402.
- Prescott IA, Dostrovsky JO, Moro E, Hodaie M, Lozano AM, Hutchison WD (2009) Levodopa enhances synaptic plasticity in the substantia nigra pars reticulata of Parkinson's disease patients. Brain 132:309–318.
- Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679.
- Rossi S, Prosperetti C, Picconi B, De Chiara V, Mataluni G, Bernardi G, Calabresi P, Centonze D (2006) Deficits of glutamate transmission in the striatum of toxic and genetic models of Huntington's disease. Neurosci Lett 410:6–10.
- Saulle E, Centonze D, Martin AB, Moratalla R, Bernardi G, Calabresi P (2002) Endogenous dopamine amplifies ischemic long-term potentiation via D1 receptors. Stroke 33:2978–2984.
- Schotanus SM, Chergui K (2008) Dopamine D1 receptors and group I metabotropic glutamate receptors contribute to the induction of longterm potentiation in the nucleus accumbens. Neuropharmacology 54:837–844.
- Schultz W, Tremblay L, Hollerman JR (2003) Changes in behavior-related neuronal activity in the striatum during learning. Trends Neurosci 26:321–328.
- Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-alpha. Nature 440:1054–1059.
- Suzuki T, Miura M, Nishimura K, Aosaki T (2001) Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. J Neurosci 21:6492–6501.

- Tozzi A, Costa C, Di Filippo M, et al. (2007) Memantine reduces neuronal dysfunctions triggered by in vitro ischemia and 3-nitropropionic acid. Exp Neurol 207:218–226.
- Varona JF, Bermejo F, Guerra JM, Molina JA (2004) Long-term prognosis of ischemic stroke in young adults. Study of 272 cases. J Neurol 251:1507–1514.
- Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, Tkatch T, Lovinger DM, Surmeier DJ (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50:443–452.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 16:2397–2410.
- Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science 296:678–682.
- Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci 7:464–476.

# **Organization of the Globus Pallidus**

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## I. INTRODUCTION: THE GLOBUS PALLIDUS IN THE BASAL GANGLIA CIRCUITRY

The pallidum consists of the globus pallidus that lies dorsal to the anterior commissure and the ventral pallidum that lies ventral to the anterior commissure. The globus pallidus is a sensorimotor and associative function-related structure. while the ventral pallidum is a prefrontal and limbic function-related structure. This chapter focuses mainly on the globus pallidus. Figure 13.1 shows the major neuronal connections between the basal ganglia and related nuclei (see also Chapter 1). The major input nuclei of the basal ganglia, the striatum and the subthalamic nucleus (STN), receive inputs from the cerebral cortex, intralaminar thalamic nuclei, and brainstem nuclei including the substantia nigra (SN) and the pedunculopontine tegmental nucleus (PPN). Dopamine neurons in the substantia nigra pars compacta (SNc) project heavily to the striatum, but send moderate projections also to other basal ganglia nuclei, including the globus pallidus and STN. Overall, the afferent organization implies that the basal ganglia receive inputs from various stages of information processing, from the brainstem to the cerebral cortex.

The GPe and GPi have distinct functional roles, although the two nuclei share some morphological and physiological similarities as described below. The GPe receives major inputs from the input nuclei of the basal ganglia, the striatum and STN, and sends outputs to most of the basal ganglia nuclei, including the striatum, GPi, STN, and SN. These connections suggest that the main role of GPe is to integrate information from the two input nuclei and send processed output back to the basal ganglia nuclei. The GPi and the substantia nigra pars reticulata (SNr) receive inputs from the basal ganglia input nuclei, GPe, and brainstem nuclei, including PPN, and send their outputs out of the basal ganglia, including to thalamic nuclei, and to the brainstem. Thus, the GPi and SNr are output nuclei of the basal ganglia.

# II. ANATOMY OF THE STRIATUM AND THE GLOBUS PALLIDUS

Figures 13.2A and 13.2C are, respectively, a tilted-horizontal section of a rat brain and a coronal section of a monkey brain immunostained for the calcium-binding protein calbindind28K (CaBP). The striatum is a telencephalic derivative. The striatum of the rodent is a single large nucleus, whereas the striatum of the primate is divided by the anterior limb of the internal capsule into the caudate nucleus and the putamen (Fig. 13.2A,C). The striatum consists of the patch compartment (striosome) and the matrix compartment. The



FIGURE 13.1 Major neuronal connections between basal ganglia and related nuclei. The basal ganglia consist of the striatum (Str), the subthalamic nucleus (STN), the external segment of the globus pallidus (GPe), the internal segment of the globus pallidus (GPi), the substantia nigra pars reticulata (SNr), and the substantia nigra pars compacta (SNc). The Str and STN are input nuclei of the basal ganglia and receive inputs from the cerebral cortex, the centromedian/parafascicular thalamic complex (CM-PF), and the pedunculopontine tegmental nucleus (PPN). The GPe receives major inputs from the input nuclei and sends its outputs to most of the basal ganglia output nuclei, including the Str, GPi, STN, SNr and SNc. Basal ganglia output nuclei, GPi and SNr, receive major inputs from various basal ganglia nuclei including PPN and then send their outputs to the ventral anterior thalamic nucleus (VA), CM-PF, and the brainstem, including PPN. The SNc projects heavily to Str. The SNc also sends moderate projections to all other basal ganglia nuclei.

projection neurons in the matrix compartment receive inputs mainly from pyramidal neurons in the layer III and superficial layer V of the cortex and project to the pallidum and the SNr, while neurons in the patch compartment receive inputs mainly from the deep layer V and layer VI neurons of the cortex and project to the SNc (Gerfen, 1992) (see Chapter 1). The projection neurons in the matrix compartment of the rostromedial region of the striatum contain CaBP. Thus, these striatal regions and their projection sites in the globus pallidus, which contain CaBP-containing striatal axons, are darkly stained. The projection neurons in the patch compartment and the dorsolateral region of the dorsal striatum are CaBP-free.

The globus pallidus is a diencephalic derivative, which is composed of the GPe and GPi. In the primate, the GPe is located medially to the putamen and is separated by a thin lateral medullary lamina. The medial medullary lamina separates the GPe and the GPi. The primate GPi is farther divided into the lateral and the medial portions by the accessory medullary lamina (Fig. 13.2C). The internal capsule is located at medial to the medial portion of GPi. The rodent GPe is located medial to the striatum as it is in primates. However, the rodent GPi consists of a small group of neurons and is encapsulated in the internal capsule, hence it is also called the entopeduncular nucleus. There are other differences between the GPi of primates and rodents as described below.

#### A. Functional Territories of the GPe and GPi

All areas of the cerebral cortex send topographically organized projections and form various functional territories in the striatum, including the sensorimotor, association, and limbic territories (Selemon and Goldman-Rakic, 1985; Donoghue and Herkenham, 1986; McGeorge and Faull, 1989) (see Chapter 1). Among these, the motor territory in the striatum is the largest and most extensively studied. The motor territories in the cortex and their striatal projection sites can be divided into sub-territories including the higher order motor, primary motor, and motor association territories. In monkeys, afferents from the primary motor cortex (M1) form a large territory in the putamen (referred to as the M1 territory herein). The M1 territory roughly corresponds to the CaBP-poor region in Figure 13.2C. A portion of the M1 territory also receives afferents from the primary somatosensory cortex (Flaherty and Graybiel, 1993). Afferents from the supplementary motor area (SMA) and the premotor area of monkeys converge and form another large motor association territory (referred to as the SMA territory herein) in the putamen. These two territories are mostly segregated with small overlap and occupy most of the caudolateral aspect of the putamen. Each territory presents a rough body representation with the face at ventral and the leg at dorsal regions (Parthasarathy and Graybiel 1997; Takada et al. 1998; Kaneda et al., 2002). The rodent M1 (i.e., the lateral agranular cortex) terminal area also roughly corresponds to the CaBP-poor region in the striatum (Fig. 13.2A). The M2 (the medial agranular cortex) of the rodent is considered the motor association area, which is underdeveloped and cannot be separated into higher order motor areas. The M2 terminal area in the striatum is located medially to the M1 terminal area in the CaBP-rich area (Fig. 13.2A,B).

Anterograde tracing studies in rats and monkeys revealed that striatal axons form two disk shaped terminal fields in both the GPe and GPi (Wilson and Phelan, 1982; Kita and Kita, 2001; Kaneda et al., 2002). Single axon tracing studies in rats and monkeys further revealed that these terminal areas are created by each striatal axon forming multiple narrow



**FIGURE 13.2** The striato-pallidal projections and functional territories of the GPe and GPi. A and C: a tilted-horizontal (tilted rostrodorsal to caudoventral direction to include the dorsal part of striatum (Str), GPe, and GPi in a same plane) section of a rat (A) and a coronal section of a monkey brain immunostained for the calcium binding protein calbindin-d28K (CaBP) (B). A: The rat GPe is located medial to the striatum, and rat GPi is encapsulated in the internal capsule (ic). C: In the monkey, the GPe is located medially to the putamen (Put) and separated by the thin lateral medullary lamina (lml). The medial medullary lamina (mml) separates the GPe and GPi. The accessory medullary lamina (aml) further separates the monkey GPi into lateral (GPi-l) and medial portion (GPi-m). At ventral to the GPi, some of GPi efferent axons form a thin white matter called the ansa lenticularis (al). CaBP immunohistochemistry poorly stained the patch compartment of the striatum (p in A and C mark some of patches). The CaBP-poor regions correspond to the primary motor territory. CaBP staining is rich in the matrix compartment of the rostromedial region of the striatum and its projection sites in the globus pallidus (white stars in A and C). The CaBP-rich regions roughly correspond to the association and limbic territories of the striatum and pallidum. B: Striatal neurons located in the CaBP-poor matrix region of rat striatum form two terminal areas in the middle of the GPe. D: The primary motor (M1) and the supplementary motor area (SMA) territories in monkey Put are mostly segregated with partial overlap, and each territory have a corresponding body representation. Projections from each territory of Put form two territories in GPe and GPi.

disk-shaped terminal fields (Chang et al., 1981; Kawaguchi et al., 1990; Parent et al., 1995; Kita 1996). In a global view, the topographical organization in the cortico-striatal projections is maintained in the striato-pallidal projection. Thus, neurons

in the two motor related territories in primate putamen form multiple motor territories in the GPe and GPi (Fig. 13.2B,D). Like in the putamen, the M1 and SMA representing territories in the GPe and GPi are mostly segregated though they both have partly overlapped regions. A rough body representation, with the head at the ventral and the foot at the dorsal regions, can be found in each territory (Fig. 13.2D) (Yoshida et al., 1993; Kaneda et al., 2002).

The GPe and GPi receive major excitatory inputs from the STN, which receives major excitatory inputs only from the frontal lobe and mainly from the motor cortices. Like the putamen, the monkey STN contains multiple motor territories, including one receiving inputs mainly from M1, SMA, and higher order motor cortices, and each territory shows somatotopical representation (Nambu et al., 1996; Inase et al., 1999). The motor related territories in the STN are mostly segregated with small overlap. Anterograde tracing studies showed that STN-pallidal projections form multiple disk shaped terminal fields in the GPe and GPi, similar to striato-pallidal projections (Smith et al., 1990). A single axon tracing study in monkeys revealed that approximately 70% of STN neurons form multiple terminal fields in both GPe and GPi, some of which also project to SNr (Sato et al., 2000b). In comparison, most rodent STN neurons project to the GPe, GPi and SNr and form multiple terminal fields in each nucleus (Kita and Kitai, 1987).

In vivo recording studies suggest that the somatotopically organized M1-striato-pallidal projections converge to somatotopically organized M1-STN-pallidal projections. Figure 13.3 shows responses of basal ganglia neurons evoked after electrical stimulation of a small area of M1. These response patterns and response latencies are very similar in rats and monkeys. The responses of GPe and GPi neurons consist of an early excitation, an inhibition, and a late excitation. The early excitation is AMPA receptor-mediated and evoked through the cortico-STN-GPe and -GPi pathways. The conduction times of these disynaptic pathways are 8-11 ms. The early excitation is followed by a GABA<sub>A</sub> receptor-mediated inhibition evoked by the Cx-striato-GPe and -GPi pathways (Nambu et al., 2000; Kita et al., 2004; Tachibana et al., 2008). The conduction times of these disynaptic inhibitory pathways is much longer, generally on the order of 16-20 ms. The late excitation consists of multiple components (Kita et al., 2004; Tachibana et al., 2008). Many of the GPe and GPi neurons that evoked early excitation also showed inhibition, and neurons evoking the early excitation alone or evoking the inhibition alone were rare. These observations suggest that the somatotopically organized M1-striato-pallidal projection overlaps very precisely with the M1-STN-pallidal projection.



**FIGURE 13.3** Responses of basal ganglia neurons evoked after electrical stimulation of a small area of the M1. Peri-stimulus time histograms of unit discharge present typical spontaneous firing rates (spike rate prior to stimulus onset) and response sequences in each nucleus. Response patterns and response latencies are very similar in the rat and monkey. The responses of GPe and GPi neurons consist of an early excitation, an inhibition, and a late excitation. The early excitation is evoked by fast conducting cortico-STN-GPe or -GPi disynaptic connections. The inhibition is evoked by slow conducting Cx-Str-GPe or -GPi disynaptic connections. The late excitation consists of multiple components. Most of the GPe and GPi neurons that evoked early excitation also showed inhibition, and neurons evoking only early excitation or inhibition are rarely observed, suggesting that the disynaptic connections via the Str and STN converge in a very precise manner in the GPe and GPi. Open and closed circles represent glutamatergic and GABAergic synapses, respectively.

# **B.** Morphological Characteristics of GPe Neurons

The majority of GPe neurons are GABAergic projection neurons (Mugnaini and Oertel, 1985; Smith et al., 1987; Kita, 1994; Kita and Kitai, 1994) (see also Chapter 14). In rats and monkeys, approximately {2/3} of the GPe neurons contain the calcium-binding protein parvalbumin (PV), and immunostaining for PV can be used to observe somatodendritic and axonal processes of these neurons (Fig. 13.4C,D) (Kita and Kitai, 1994; Kita 1996; Kita and Kita, 2001). These neurons have large aspiny primary, varicose secondary, and tertiary dendrites with occasional complex endings with many appendages of various types (Fig. 13.5A). Dendrites of GPe neurons form a disk-like dendritic field with the plane of the disc parallel to the lateral medullary lamina (Yelnik et al., 1984; Kita, 1996). The orientations of disk-shaped terminal fields formed by striatal and STN axons mentioned above are in the same orientation as that of the discoidal dendritic field. Thus, neurons with a discoidal dendritic field and discoidal afferent axons form a disk shaped functional territory. Approximately {1/3} of rat GPe neurons have sparsely spinous dendrites that form a radiating dendritic field (Fig. 13.5B). These neurons are often immunonegative for PV but express preproenkephalin mRNA (Hoover and Marshall, 2002). The GPe contains a small number of small neurons, which are suggested to be interneurons (DiFiglia et al., 1982; Falls et al., 1983; Cooper and Stanford, 2002). Some of these small neurons contain GABA and calretinin (Cooper and Stanford, 2002).

## C. Projection Sites of GPe Axons

The GABA/PV-containing GPe neurons with discoidal dendritic field project to the GPi, STN, and SNr (Kita



**FIGURE 13.4** Parvalbumin (PV) immunoreactive neurons in monkey globus pallidus and trajectories of GPi efferent axons. A: A tilted-horizontal section of a monkey brain immunostained for the calcium binding protein PV. Immunohistochemistry for PV stains a class of interneurons in the striatum and most of neurons in the GPe and GPi. In a global view, strongly PV-immunopositive areas in the putamen and caudoventral globus pallidus correspond to motor-related areas. PV-positive bundles of GPi axons cross the internal capsule (ic) medially and form the comb system. B: A schematic presentation of GPi efferent axons. Ventrally exiting axons from GPi-l or GPi-m form ansa lenticularis (al, also see Fig. 13.2C), and medially exiting axons cross the ic, with some forming the lenticular fasciculus (lf). These axons merge and become thalamic fasciculus (tf) when they are dorsal to the zona incerta (ZI). Each GPi axon emits several branches and projects to the densicellular (VAdc) and parvicellular (VApc) parts of the ventral anterior thalamic nucleus, CM, PF, and PPN. C and D: High magnification view of GPe (C) and GPi (D) show that many fusiform shaped somata and large dendrites are oriented perpendicular to the fiber bundles.



**FIGURE 13.5** Drawing of rat GPe neurons and an axon. A: A neuron with large aspiny primary, varicose secondary, and tertiary dendrites with occasional complex endings (arrow heads) with many appendages. The scale in B also applies to A. B: A neuron with sparsely spineous dendrites. C: An axon of a GPe neuron that has extensive local axon collaterals in GPe and multiple small terminal fields in GPi.

and Kitai, 1994; Bevan et al., 1998; Sato et al., 2000a, Baufreton et al., 2009). In rats, the GABA/enkephalin-containing GPe neurons with a radiating dendritic field project to the striatum in addition to GPi, GPe, and STN (Staines and Fibiger, 1984; Kita and Kitai, 1994; Kita and Kita, 2001; Sadek et al., 2007) (see also Chapter 14). Like in the rat, approximately 30% of monkey GPe neurons project to the striatum (Kita et al, 1999). However, in monkeys, GPe neurons projecting to the striatum do not project to the GPi, STN, or the SNr (Sato et al., 2000a). Axons from both types of GPe neurons form large boutons that contain small round or elongated vesicles and multiple mitochondria, and the boutons form symmetric synapses on the somata and proximal dendrites of GPi and STN neurons (Chang et al., 1983; Shink and Smith 1995; Baufreton et al., 2009). Recent single cell studies in rats estimated that each GPe neuron forms approximately 275 synapses on 2% of total STN neurons, that single STN neurons maximally receive input from 2% of GPe neurons, and that small fractions of GPe-STN input are sufficiently powerful to inhibit and synchronize the autonomous activity of STN neurons (Bevan, et al., 2007; Baufreton et al., 2009). In the striatum, approximately 50% of GPe boutons terminate on aspiny GABAergic interneurons (Bevan et al., 1998) and the other 50% on the dendritic shafts of spiny projection neurons. The interneurons targeted by the GPe axons receive several boutons, suggesting a powerful inhibitory control by the GPe (Bevan et al., 1998 and 2002). The topographic arrangements of the GPe-STN and GPe-striatal projections are in register with that of the STN-GPe and striato-GPe projections, suggesting precise reciprocal loops.

All GPe projection neurons in the monkey and the rodent emit local collateral axons (Kita and Kitai, 1994; Bevan et al., 1998; Sato et al., 2000a; Sadek et al., 2007). Single neuron studies revealed that each GPe axon forms terminal fields within or near the dendritic field of the parent neuron and other terminal fields some distance away from the parent dendritic field (Fig. 13.5C) (Kita and Kitai, 1994; Sato et al., 2000; Sadek et al., 2007). These local axons terminate on the somata and proximal dendrites and can evoke powerful inhibition to GPe neurons.

## D. Morphological Characteristics of GPi Neurons

Most GPi neurons are GABAergic and project to the thalamus and the brainstem, as mentioned above. Additionally, the GPi appears to have no interneurons. Like the GPe neurons, most of the GPi neurons are immunoreactive for PV (Fig. 13.4D). Golgi studies report a single class of GPi neurons having medium-sized somata with thick and long aspiny dendrites, which form a discoidal dendritic field with the plain parallel to the medial medullary lamina (Yelnik et al, 1984). Intracellular labeling in rats showed that some GPi neurons have dendrites with occasional complex endings (Nakanishi et al., 1991). Thus, the somato-dendritic morphology of GPe and GPi neurons are thought to be very similar. However, unlike the GPe, GPi neurons do not emit extensive local axon collaterals (Nakanishi et al., 1991; Parent et al., 2001).

#### E. Projection Sites of GPi Axons

The motor-related territories occupy a large area of monkey GPi. Some efferent axons from monkey GPi exit ventrally and travel through the ansa lenticularis (Figs 13.2C, 13.4B). Other efferent axons exit medially and cross the internal capsule to form the lenticular fasciculus (Fig. 13.4A,B). Some other axons run through the area in between the two fasciculi. These efferent axons are seen as single wide fiber bundle when they cross the internal capsule and become the thalamic fasciculus dorsal to the zona incerta. Studies with neuro-tracing and ablative lesion in monkeys suggest that afferent axons in these fasciculi can originate from either the lateral or medial portion of GPi and that the caudoventral motor territory of GPi sends axons mainly through the lenticular fasciculus, while the rostral non-motor territory of GPi sends axons mainly through the ansa lenticularis (Sidibe et al., 1997; Baron et al., 2001; Parent and Parent, 2004).

GPi neurons project in a topographically organized manner to the zona incerta, the densicellular (VAdc) and parvicellular (VApc) parts of the ventral anterior thalamic nucleus, the centromedian thalamic nucleus (CM), the parafascicular thalamic nucleus (Pf), and the PPN (Ilinsky and Kultas-Iinsky, 1987; Parent et al, 2001). Single axon tracing studies revealed that each axon terminates in many of these targets through collateral axons (Fig. 13.4B) (Parent et al., 2001). The GPi projection to the PPN is mainly to the pars dissipata of the PPN, in the area that contains glutamatergic, GABAergic, and sparse cholinergic neurons, and the pars dissipata is located medially to the cholinergic cell rich pars compacta of PPN (Lee et al., 1988; Shink et al., 1997). A small number of GPi neurons project to the lateral habenular nucleus, and thus, they are considered limbic-related (Parent et al., 2001). Axons of GPi neurons form large terminals that contain pleomorphic vesicles and many mitochondria, and these terminals form symmetric synapses predominantly with proximal dendrites (Sidibe et al., 1997; Shink et al., 1997; Ilinsky et al., 1997).

The GPi of the primate, an output nucleus of the basal ganglia, contains multiple motor representation areas as mentioned above, suggesting that the GPi has multiple output channels. For instance, the GPi-thalamo-cortical projections are organized such that the outputs from different territories of the GPi are largely segregated and innervate different motor areas including M1, SMA, and premotor areas. It has also been shown that outputs from the two terminal areas formed by striatal axons in the lateral and the medial portion of the GPi converge at a motor cortex. For instance, M1, SMA, and premotor areas receive afferents from both lateral and medial portions of GPi (Hoover and Strick, 1993).

There are some differences in the efferent projections of rat and monkey GPi. In the monkey, GPi and SNr efferent axons form largely segregated terminal fields in the thalamus, while in rats, they form overlapped terminal fields. A large number of neurons in the rostroventral part of rat GPi projects to the lateral habenular nucleus (Van der Kooy and Carter, 1981; Rajakumar et al., 1994), while GPihabenular projection in the monkey is small. Conversely, the GPi projection to PPN in the rat appears less numerous compared to those in monkeys (Steininger et al., 1992).

# III. PHYSIOLOGY OF THE GLOBUS PALLIDUS

# A. Physiological Properties of GPe Neurons

Unit recording in awake monkeys and rodents distinguished two types of firing patterns in GPe neurons. The majority of GPe neurons show 20-100 Hz irregular firing intermingled with long pauses of approximately 100-600 ms (DeLong, 1971; Gardiner and Kitai, 1992; Magill et al., 2000; Ni et al., 2000). GPe neurons pause more often when animals are in low-arousal state (Elias et al., 2007). A small number of GPe neurons fire with low frequency and in bursts. It is not certain whether these two firing patterns represent two distinct cellular groups or a single group with different states. Results of in vivo and in vitro intracellular recordings suggest that most GPe projection neurons, both with aspiny discoidal or sparsely spinous radiating dendrites, have autonomous firing and can generate sustained high frequency firings with no prominent spike accommodation upon stimulation. The autonomous firing is supported by membrane properties such as strong hyperpolarization-activated inward current (HCN2) and Nav1.6 currents (Chan et al., 2004; Mercer et al., 2007). GPe neurons also have membrane properties that can support irregular firing and pauses, and the appearance of regular or irregular firing is dependent on the membrane potential level (Hashimoto and Kita, 2006). GPe neurons in vitro do not show spontaneous long pauses. However, long pauses could be induced when the dendrites of GPe neurons were depolarized while the soma was hyperpolarized (Hashimoto and Kita, 2006). Long pauses of some monkey GPe neurons were resistant to local application of GABA<sub>A</sub> receptor antagonist, though the application blocked M1 or putamen-induced inhibition. These observations suggest involvement of novel ionic channels in the generation of long pauses. These observations also suggest a possibility that the two firing patterns observed in vivo studies present two different states of the same type of GPe neuron. In vitro studies revealed that some of these high frequency firing neurons have a prominent hyperpolarization-activated inward current, low-threshold Ca<sup>++</sup>-spikes, or an early K-current. However, the differences in the membrane properties are often not distinctive enough to unequivocally classify them into different subclasses (Gunay et al., 2008). A small number of GPe neurons recorded in vitro lacked spontaneous firing and had a prominent spike accommodation and long-duration spikes (Kita and Kitai, 1991; Cooper and Stanford, 2000). In sum, GPe neurons have been classified based on their morphology, projection sites, chemical markers, and in vivo and vitro physiological

properties. However, clear relationships between the different types have not been established.

#### **B.** Physiological Properties of GPi Neurons

Unit recordings from monkey GPi report a single class of neurons with irregular, high frequency firing without long pauses (DeLong, 1971; Ni et al., 2000). *In vitro* studies on GPi neurons are scarce. Intracellular recording studies in slice preparations suggest GPi neurons have similar physiological properties to PV immunoreactive GPe neurons. A majority of GPi neurons have autonomous firing and are capable of maintaining sustained high frequency firing. These neurons also have a prominent hyperpolarization-activated inward current, low-threshold Ca<sup>++</sup>-spikes, or an early K-current (Nakanishi et al., 1990 and 1991; Kita, 2001).

#### C. Synaptic Inputs to GPe and GPi Neurons

The high frequency autonomous firing of GPe and GPi neurons provides a condition in which both excitatory and inhibitory synaptic inputs, even small ones, can effectively alter activity of postsynaptic neurons. Indeed, local application of GABA<sub>A</sub> receptor or AMPA receptor antagonists increase regularity of firing in the awake monkey pallidum; this demonstrates that GABAergic inputs from striatal and GPe neurons and glutamatergic inputs from STN modulate the autonomous firing of GPe and GPi neurons (Kita et al., 2004; Tachibana et al., 2008). Below are more detailed descriptions of major synaptic inputs to GPe and GPi neurons.



**FIGURE 13.6** Synaptic connections within the basal ganglia. Approximately half of the striatal medium spiny GABAergic neurons project only to the GPe and the other half to the GPe, SNc, and SNr. Some of the nigra projecting striatal axons also innervate to the GPi. Striatal axons form small boutons and synapse mainly on dendrites of target neurons. Approximately  $\{2/3\}$  of GPe neurons have aspiny dendrites and contain GABA and the calcium binding protein parvalbumin (PV). These neurons project to the GPi, STN, and SNr, form large boutons, and synapse mainly on the somata and proximal dendrites of target neurons. The remaining  $\{1/3\}$  of GPe neurons have sparsely spinous dendrites. These neurons project to the striatum and STN. The GPi and SNr are basal ganglia output nuclei and project to the thalamic nuclei and the brainstem. STN neurons are glutamatergic. STN axons form medium sized boutons and form synapses mainly on the somata and dendritic shafts of both GP<sub>e</sub> and GPi neurons.

#### 1. Striatal Inputs

Approximately 65% of boutons in the GPe and GPi are of striatal origin and contain GABA. In GPe, approximately 2/3 of striatal boutons are formed by axons terminating only in GPe (Fig. 13.6). The remaining boutons are formed by collaterals of axons projecting to GPi and/or SNr (Kawaguchi et al., 1990; Parent et al., 1995; Wu et al., 2000). Although a massive number of striatal neurons project to the GPe (the ratio of the number of neurons in the striatum:GPe is approximately 60:1 in rats, Oorschot, 1996), the number of boutons belonging to each axon is relatively small (between 100 and 250) and these boutons are sparsely distributed (Kawaguchi et al., 1990; Yelnik et al., 1996).

In GPi, Str boutons are formed by collaterals of some of axons projecting to SNr. Some Str-SNr axons do not emit collaterals in GPi. Therefore it appears that there are no axons that project only to GPi (Kawaguchi et al., 1990) (Fig. 13.6). In monkeys, all striato-SNr axons emit collaterals in GPe and GPi, and a small number of striatal neurons project to the GPe and GPi but not to SNr (Parent et al., 1995). Striatal boutons are small or medium in size (less than 1  $\mu$ m), contain large pleomorphic vesicles, and form symmetric synapses mainly on the dendrites of GPe and GPi neurons. Stimulation of a striatal axon evokes a long latency (2–12 ms) and very small (less than 3 mV or 10 pA) GABA<sub>A</sub> receptor-mediated IPSPs/IPSCs in GPe and GPi neuron in vitro (Ogura and Kita, 2000; Kita 2007). Because each striato-GPe/GPi axon evokes only a small response, the simultaneous activation of a group of striatal neurons is required to produce strong inhibition in GPe and GPi.

#### 2. GPe Inputs

Approximately 15% of the boutons in the GPe and GPi are formed by collateral axons of GPe neurons and contain GABA (Fig. 13.5C). In contrast to striatal axons, GPe axons form relatively large boutons (over 1  $\mu$ m) containing mitochondria



**FIGURE 13.7** Electron micrographs of rat GPe tissues immunostained for PV. A: A soma of PV-positive neuron containing an indented nucleus. B: A high magnification photomicrograph of the area marked in A. Small PV-negative boutons forming symmetric synapses cover the soma. A PV-negative bouton (marked by an arrowhead) forms asymmetric synapse with a PV-negative and a PV-positive dendrite. C: A PV-positive dendrite forms a symmetric synapse with PV-positive bouton (a double arrowhead). D: Large PV-positive boutons form symmetric synapses with a PV-negative soma. Two PV-positive myelinated axons and a PV-negative bouton forming an asymmetric synapse (marked by an arrow) are also observed.



**FIGURE 13.8** Recording of a continuously firing GPi neuron in a slice preparation shows spontaneous IPSPs (some are marked by arrows). A: The spontaneous IPSPs were very effective in delaying the next spike generation. B: IPSPs recorded from a GPi neuron to GPe stimulation. The amplitude of the IPSPs was small when it was evoked at the early part of the spike after hyperpolarization (e.g., a trace marked by a small arrowhead). In contrast, the amplitude was large when it was evoked in the later recovery phase of the after the hyperpolarization (e.g., marked by a large arrowhead); thus, the IPSPs effectively delayed the next spike generation. This observation suggests that synchronized activation of GPe resets the firing of a large number of GPi neurons.

and small round or elongated vesicles, and they form symmetrical synapses on the somata and proximal dendrites (Chang et al., 1983; Hazrati et al., 1990; DiFiglia et al., 1982; Falls et al., 1983; Okoyama et al., 1987; Kita 1994; Shink and Smith, 1995; Sadek et al., 2007) (Fig. 13.7). Large spontaneous IPSPs observed in GPe neurons in vitro are attributed to these synapses (Fig. 13.6). The topographic arrangements of the STN-GPe and striato-GPe projections overlap with that of the GPe-STN and GPe- striatal projections, suggesting precise reciprocal loops. Activation of GPe axons evokes short latency and large IPSCs ranging from 10 to over 100 pA in target neurons (Ogura and Kita, 2000; Kita, 2001; Matsui and Kita, 2003; Baufreton et al., 2009). The large intra-GPe collateral and GPe-GPi inputs play a significant role in controlling the firing rate, firing pattern, and synchronization of GPe and GPi neurons. For instance, when a large spontaneous IPSP occurred in the recovery phase of the spike after hyperpolarization of a GPi neuron, the next spike was effectively delayed (Fig. 13.8A). Figure 13.8B shows that GPe stimulation-evoked IPSPs effectively reset the firing of a GPi neuron. These observations suggest that large IPSPs can synchronize firing of GPe and GPi neurons postsynaptic to a GPe axon. One of the most remarkable observations made in experimental parkinsonian monkeys is the synchronization of firing of a large number of GPe and GPi neurons (Raz et al., 2000). It is possible that the synchronized activity is due to an increase of the GABAergic inhibition of GPe synapses after the dopamine loss.

GABA<sub>B</sub> receptors are expressed mainly on extrasynaptic membranes, and some are also expressed on presynaptic membranes of GABAergic and glutamatergic boutons (Chen et al., 2004; Charara et al., 2005). Repetitive activation of GPe axons induces a slow GABA<sub>B</sub> receptormediated IPSP/inhibition in both GPe and GPi neurons (Kaneda and Kita, 2005; Kita et al., 2006), while there has been no convincing report so far that striatal synapses can evoke GABA<sub>B</sub> receptor-mediated responses in pallidal neurons. Studies in monkeys suggest that GABA released from local collateral axon terminals maintains the ambient level of GABA at a relatively high approximately 0.5 µM in GPe, which is enough to activate extrasynaptic GABA<sub>B</sub> receptors in GPe in vivo (Galvan et al., 2005; Kita et al., 2006). The activation of presynaptic GABA<sub>B</sub> receptors on the boutons of GPe axons suppresses GABA release (Kaneda and Kita, 2005). Thus, it is likely that both preand postsynaptic GABA<sub>B</sub> receptors play important roles in the control of GPe neuronal activity.

#### 3. STN Synapses

Approximately 20% of boutons in the GPe and GPi form asymmetric synapses (DiFiglia et al., 1982; Falls et al., 1983; Okoyama et al., 1987; Shink and Smith, 1995). Many of these boutons are thought to belong to the axons from the STN (see also Chapter 15), although the precise number is unknown. Other possible origins of asymmetric synapses include the frontal cortex, the intralaminar thalamic nuclei, and the pars dissipata of the PPN. The STN boutons are medium in size, contain small pleomorphic vesicles, and form synapses mainly on the somata and dendritic shafts of both GPe and GPi neurons (Fig. 13.7). Stimulation of the STN evokes short latency AMPA receptor-mediated excitatory responses in GPe and GPi neurons (Kita and Kitai, 1991; Nakanishi et al., 1991; Tachibana et al., 2008). Large EPSPs evoked in pallidal neurons can trigger slow potentials, probably low threshold calcium spikes, with a burst of action potentials (Robledo and Feger, 1990; Naksnishi et al., 1991). Pallidal neurons also express metabotropic glutamate receptors (mGluRs). The group I mGluRs, mGluR1 and mGluR5, are highly localized on the postsynaptic membrane of the rodent and primate GPe neurons (Hanson and Smith 1999; Poisik et al. 2003). An in vitro study revealed that repetitive activation, such as bursts, of STN axons can evoke very slow mGluR1 mediated excitations in GPe neurons (Kaneda et al., 2007).

#### 4. Presynaptic Modulations

The synaptic release of GABA and glutamate in GPe is modulated by several presynaptic receptors, including GABA<sub>B</sub> autoreceptors as mentioned above (Chen and Yung., 2003; Kaneda and Kita, 2005), group III mGluR (Marino et al., 2003; Matsui and Kita, 2003), mu, delta and kappa opioid (Stanford and Cooper, 1999; Ogura and Kita, 2000), adenosine A2A (Shindou et al., 2002), serotonin (Kita et al., 2007; Hashimoto and Kita, 2008; Rav-Acha et al., 2008), and dopamine (Cooper and Stanford, 2001; Watanabe et al., 2009). Amongst these, only the activation of adenosine A2A receptors increases while others decrease the release of GABA or glutamate. Recent studies found that dopamine and serotonin exert very strong presynaptic suppression of GABA release in the globus pallidus (Cooper and Stanford, 2001; Kita et al., 2007; Hashimoto and Kita, 2008; Rav-Acha et al., 2008; Watanabe et al., 2009). Another interest-ing presynaptic modulator is cannabinoid receptormediated depolarization-induced suppression of synaptic transmissions. The striato-GPe axons express abundant cannabinoid CB1 receptors (Sanudo-Pena et al., 1998; Matyas et al., 2006). Strong depolarization of postsynaptic sites of GPe neurons by glutamatergic inputs releases cannabinoid, which activates presynaptic CB1 receptors and suppresses GABA or glutamate release (Freiman and Szabo, 2005; Engler et al., 2006).

### **IV. FUNCTIONAL CONSIDERATIONS**

The basal ganglia input nuclei, the striatum and STN, receive various stages of inputs from the brainstem to the cerebral cortex and send their outputs to the GPe and GPi/SNr. The studies on the organizations of these pathways and on the cellular morphology revealed existence of

multiple functional territories in the striatum, globus pallidus, and STN. Furthermore, the sensorimotor territories in each nucleus have somatotopic organizations. In vivo recording studies suggest that information originating from the cortex travels through two pathways, one through the striatum and another through the STN, and converge in a very precise manner on GPe and GPi neurons belonging to the same functional territory. These findings suggest the existence of well-organized multiple parallel information processing systems in the basal ganglia. The activity of multiple channels in the parallel system may be coordinated by intrinsic connections made by the interneurons in the striatum and by intrinsic collateral axons in the GPe and STN.

The GPe receives its main inputs from basal ganglia input nuclei and projects to most of the nuclei in the basal ganglia. Thus, the GPe manages the information processing within the basal ganglia. GPe neurons have various ionic channels that can support high frequency autonomous firing. GPe neurons also have various ionic channels that can modulate the rate of the autonomous firing. Ionic channels also modulate the synaptic inputs from the striatal, intrinsic collateral inputs and the STN, and in return, these inhibitory and excitatory inputs activate or deactivate ionic channels. Because of these interactions, even small inhibitory or excitatory inputs might effectively alter the firing pattern of GPe neurons. Under normal conditions, characteristic high frequency irregular firing with pauses or low frequency firing with bursts of GPe neurons in vivo is generated by an ensemble of synaptic actions and ionic currents of the somata and dendritic membrane. The efferent axons of GPe neurons terminating on the somata and proximal dendrites of striatal, GPe, GPi, and STN neurons may exert powerful controls on the neurons through their own individual functional channels of the parallel pathway system. On the other hand, the intrinsic collateral axons of GPe neurons control neurons in other functionally related channels that may be necessary for a sequential activation of various body parts in a controlled voluntary movement.

The GPi is a basal ganglia output nucleus and projects to the thalamus and the brainstem. The GPi receives main inputs from basal ganglia input nuclei and from GPe. GPi neurons also have membrane properties to support autonomous firing and the firing is modulated by striatal, GPe, and STN inputs, similarly to GPe neurons. Under normal conditions, the activity of each GPi neurons in vivo is irregular without long pauses. The differences in the firing pattern between GPe and GPi neurons suggest possible differences in some of the membrane properties.

Physiological observations made in patients undergoing surgical treatments and in animal models of Parkinson's disease showed a number of changes in neuronal activities in the basal ganglia related to dopamine deficiency (see also Chapters 25 and 38). These observations suggest that the striato-GPe GABAergic input is increased and striato-GPi input is decreased (Nambu et al., 2005) and that the burst activity of GPe, GPi, and STN neurons is greatly increased after loss of nigrostriatal dopaminergic projections (Filion and Tremblay, 1991; Hutchison et al., 1994; Boraud et al., 1998; Magill et al., 2001). Responses of GPe neurons to joint stimulation became less selective, responding to single joint movement under normal conditions but responding to multiple joints after being rendered parkinsonian (Filion et al., 1988; Rothblat and Schneider, 1995). Responses of GPe neurons to striatal stimulation increased not only the response amplitude but also the number of responsive areas such that many GPe neurons showed convergent responses to both the caudate nucleus and the putamen (Tremblay et al., 1989).

Abnormal firing patterns such as oscillation and synchronization of firing in a large number of GPe and GPi neurons have also been observed in subjects with hypokinetic (e.g., Parkinson's disease) or hyperkinetic (e.g., hemiballismus and dystonia) movement disorders (Nini et al., 1995; Bergman et al., 1998; Raz et al., 2001; Vitek, 2002; Wichmann and DeLong, 2003) (see also Chapter 39). In dopamine-depleted parkinsonian monkeys, correlated firing was observed not only between nearby pallidal neurons but also between neurons in different functional territories (Nini et al., 1995; Bergman et al., 1998; Raz et al., 2001), although it is debatable whether the overt neuronal synchronization is common in the basal ganglia of Parkinson's disease patients (Levy et al., 2002). The findings cited above suggest a breakdown of channel-specific information processing and a breakdown of channel-independent information processing performed in specific functional territories and somatotopic organization. Abnormal oscillation and synchronization of firing could be due to changes in the membrane properties, changes in synaptic output of GPe axons, and changes in STN afferent inputs. For instance, the level of ambient GABA in the GPe increases after a lesion of nigral dopaminergic neurons (Robertson et al., 1991; Galvan et al., 2005), suggesting an increase in the GABA release from the GPe axons, and the resulting large IPSPs promote synchronization of pallidal and STN neurons postsynaptic to the GPe neuron, as mentioned above. Abnormal oscillation and synchronization could be also due to changes in the membrane

properties that alter the properties of the autonomous firing of pallidal or STN neurons. Future studies will clarify the functional significance of the multiple functional channels in the basal ganglia and how basal ganglia disorders relate to the malfunction of the basal ganglia.

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#### REFERENCES

- Baron MS, Sidibe M, DeLong MR, Smith Y (2001) Course of motor and associative pallidothalamic projections in monkeys. J Comp Neurol 429:490–501.
- Baufreton J, Kirkham E, Atherton JF, Menard A, Magill PJ, Bolam JP, Bevan MD (2009) Sparse but selective and potent synaptic transmission from the globus pallidus to the subthalamic nucleus. J Neurophysiol 102:532–545.
- Bergman H, Feingold A, Nini A, Raz A, Slovin H, Abeles M, Vaadia E (1998) Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. Trends Neurosci 21:32–38.
- Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bevan MD, Hallworth NE, Baufreton J (2007) GABAergic control of the subthalamic nucleus. Prog Brain Res 160:173–188.
- Bevan MD, Magill PJ, Terman D, Bolam JP, Wilson CJ (2002) Move to the rhythm: oscillations in the subthalamic nucleus-external globus pallidus network. Trends Neurosci 25:525–531.
- Boraud T, Bezard E, Guehl D, Bioulac B, Gross C (1998) Effects of L-DOPA on neuronal activity of the globus pallidus externalis (GPe) and globus pallidus internalis (GPi) in the MPTP-treated monkey. Brain Res 787:157–160.
- Chan CS, Shigemoto R, Mercer JN, Surmeier DJ (2004) HCN2 and HCN1 channels govern the regularity of autonomous pacemaking and synaptic resetting in globus pallidus neurons. J Neurosci 24:9921–9932.
- Chang HT, Kita H, Kitai ST (1983) The fine structure of the rat subthalamic nucleus: an electron microscopic study. J Comp Neurol 221:113–123.
- Chang HT, Wilson CJ, Kitai ST (1981) Single neostriatal efferent axons in the globus pallidus: a light and electron microscopic study. Science 213:915–918.
- Charara A, Pare JF, Levey AI, Smith Y (2005) Synaptic and extrasynaptic GABA-A and GABA-B receptors in the globus pallidus: an electron microscopic immunogold analysis in monkeys. Neuroscience 131:917–933.
- Chen L, Boyes J, Yung WH, Bolam JP (2004) Subcellular localization of GABAB receptor subunits in rat globus pallidus. J Comp Neurol 474:340–352.

- Chen L, Yung WH (2003) Effects of the GABA-uptake inhibitor tiagabine in rat globus pallidus. Exp Brain Res 152:263–269.
- Cooper AJ, Stanford IM (2000) Electrophysiological and morphological characteristics of three subtypes of rat globus pallidus neurone *in vitro*. J Physiol 527(2):291–304.
- Cooper AJ, Stanford IM (2001) Dopamine D2 receptor mediated presynaptic inhibition of striatopallidal GABA(A) IPSCs in vitro. Neuropharmacology 41:62–71.
- Cooper AJ, Stanford IM (2002) Calbindin D-28k positive projection neurones and calretinin positive interneurones of the rat globus pallidus. Brain Res 929:243–251.
- DeLong MR (1971) Activity of pallidal neurons during movement. J Neurophysiol 34:414–427.
- Difiglia M, Pasik P, Pasik T (1982) A Golgi and ultrastructural study of the monkey globus pallidus. J Comp Neurol 212:53–75.
- Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397–403.
- Elias S, Joshua M, Goldberg JA, Heimer G, Arkadir D, Morris G, Bergman H (2007) Statistical properties of pauses of the highfrequency discharge neurons in the external segment of the globus pallidus. J Neurosci 27:2525–2538.
- Engler B, Freiman I, Urbanski M, Szabo B (2006) Effects of exogenous and endogenous cannabinoids on GABAergic neurotransmission between the caudate-putamen and the globus pallidus in the mouse. J Pharmacol Exp Ther 316:608–617.
- Falls WM, Park MR, Kitai ST (1983) An intracellular HRP study of the rat globus pallidus. II. Fine structural characteristics and synaptic connnections of medially located large GP neurons. J Comp Neurol 220:229–245.
- Filion M, Tremblay L (1991) Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res 547:142–151.
- Filion M, Tremblay L, Bedard PJ (1988) Abnormal influences of passive limb movement on the activity of globus pallidus neurons in parkinsonian monkeys. Brain Res 444:165–176.
- Flaherty AW, Graybiel AM (1993) Two input systems for body representations in the primate striatal matrix: experimental evidence in the squirrel monkey. J Neurosci 13:1120–1137.
- Freiman I, Szabo B (2005) Cannabinoids depress excitatory neurotransmission between the subthalamic nucleus and the globus pallidus. Neuroscience 133:305–313.
- Galvan A, Villalba RM, West SM, Maidment NT, Ackerson LC, Smith Y, Wichmann T (2005) GABAergic modulation of the activity of globus pallidus neurons in primates: *in vivo* analysis of the functions of GABA receptors and GABA transporters. J Neurophysiol 94:990–1000.
- Gardiner TW, Kitai ST (1992) Single-unit activity in the globus pallidus and neostriatum of the rat during performance of a trained head movement. Exp Brain Res 88:517–530.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 15:133–139.
- Gunay C, Edgerton JR, Jaeger D (2008) Channel density distributions explain spiking variability in the globus pallidus: a combined physiology and computer simulation database approach. J Neurosci 28:7476–7491.
- Hanson JE, Smith Y (1999) Group I metabotropic glutamate receptors at GABAergic synapses in monkeys. J Neurosci 19:6488–6496.
- Hashimoto K, Kita H (2006) Slow oscillatory activity of rat globus pallidus neurons in vitro. Eur J Neurosci 23:443–453.

- Hashimoto K, Kita H (2008) Serotonin activates presynaptic and postsynaptic receptors in rat globus pallidus. J Neurophysiol 99:1723–1732.
- Hazrati LN, Parent A, Mitchell S, Haber SN (1990) Evidence for interconnections between the two segments of the globus pallidus in primates: a PHA-L anterograde tracing study. Brain Res 533:171–175.
- Hoover BR, Marshall JF (2002) Further characterization of preproenkephalin mRNA-containing cells in the rodent globus pallidus. Neuroscience 111:111–125.
- Hoover JE, Strick PL (1993) Multiple output channels in the basal ganglia. Science 259:819–821.
- Hutchison WD, Lozano AM, Davis KD, Saint-Cyr JA, Lang AE, Dostrovsky JO (1994) Differential neuronal activity in segments of globus pallidus in Parkinson's disease patients. Neuroreport 5:1533–1537.
- Ilinsky IA, Kultas-Ilinsky K (1987) Sagittal cytoarchitectonic maps of the Macaca mulatta thalamus with a revised nomenclature of the motorrelated nuclei validated by observations on their connectivity. J Comp Neurol 262:331–364.
- Ilinsky IA, Yi H, Kultas-Ilinsky K (1997) Mode of termination of pallidal afferents to the thalamus: a light and electron microscopic study with anterograde tracers and immunocytochemistry in *Macaca mulatta*. J Comp Neurol 386:601–612.
- Inase M, Tokuno H, Nambu A, Akazawa T, Takada M (1999) Corticostriatal and corticosubthalamic input zones from the presupplementary motor area in the macaque monkey: comparison with the input zones from the supplementary motor area. Brain Res 833:191–201.
- Kaneda K, Kita H (2005) Synaptically released GABA activates both pre- and postsynaptic GABA(B) receptors in the rat globus pallidus. J Neurophysiol 94:1104–1114.
- Kaneda K, Kita T, Kita H (2007) Repetitive activation of glutamatergic inputs evokes a long-lasting excitation in rat globus pallidus neurons in vitro. J Neurophysiol 97:121–133.
- Kaneda K, Nambu A, Tokuno H, Takada M (2002) Differential processing patterns of motor information via striatopallidal and striatonigral projections. J Neurophysiol 88:1420–1432.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 10:3421–3438.
- Kita H (1994) Parvalbumin-immunopositive neurons in rat globus pallidus: a light and electron microscopic study. Brain Res 657:31–41.
- Kita H (1996) Glutamatergic and GABAergic postsynaptic responses of striatal spiny neurons to intrastriatal and cortical stimulation recorded in slice preparations. Neuroscience 70:925–940.
- Kita H (2001) Neostriatal and globus pallidus stimulation induced inhibitory postsynaptic potentials in entopeduncular neurons in rat brain slice preparations. Neuroscience 105:871–879.
- Kita H (2007) Globus pallidus external segment. Prog Brain Res 160:111–133.
- Kita H, Chiken S, Tachibana Y, Nambu A (2006) Origins of GABA(A) and GABA(B) receptor-mediated responses of globus pallidus induced after stimulation of the putamen in the monkey. J Neurosci 26:6554–6562.
- Kita H, Kita T (2001) Number, origins, and chemical types of rat pallidostriatal projection neurons. J Comp Neurol 437:438–448.
- Kita H, Kitai ST (1987) Efferent projections of the subthalamic nucleus in the rat: Light and electron microscopic analysis with the PHA-L Method. J Comp Neurol 260:435–452.
- Kita H, Kitai ST (1991) Intracellular study of rat globus pallidus neurons: membrane properties and responses to neostriatal, subthalamic and nigral stimulation. Brain Res 564:296–305.

- Kita H, Kitai ST (1994) The morphology of globus pallidus projection neurons in the rat: an intracellular staining study. Brain Res 636:308–319.
- Kita H, Nambu A, Kaneda K, Tachibana Y, Takada M (2004) Role of ionotropic glutamatergic and GABAergic inputs on the firing activity of neurons in the external pallidum in awake monkeys. J Neurophysiol 92:3069–3084.
- Kita H, Tokuno H, Nambu A (1999) Monkey globus pallidus external segment neurons projecting to the neostriatum. Neuroreport 10:1467–1472.
- Lee HJ, Rye DB, Hallanger AE, Levey AI, Wainer BH (1988) Cholinergic vs. noncholinergic efferents from the mesopontine tegmentum to the extrapyramidal motor system nuclei. J Comp Neurol 275:469–492.
- Levy R, Hutchison WD, Lozano AM, Dostrovsky JO (2002) Synchronized neuronal discharge in the basal ganglia of parkinsonian patients is limited to oscillatory activity. J Neurosci 22:2855–2861.
- Magill PJ, Bolam JP, Bevan MD (2000) Relationship of activity in the subthalamic nucleus-globus pallidus network to cortical electroencephalogram. J Neurosci 20:820–833.
- Magill PJ, Bolam JP, Bevan MD (2001) Dopamine regulates the impact of the cerebral cortex on the subthalamic nucleus-globus pallidus network. Neuroscience 106:313–330.
- Marino MJ, Valenti O, O'Brien JA, Williams DL Jr., Conn PJ (2003) Modulation of inhibitory transmission in the rat globus pallidus by activation of mGluR4. Ann NY Acad Sci 1003:435–437.
- Matsui T, Kita H (2003) Activation of group III metabotropic glutamate receptors presynaptically reduces both GABAergic and glutamatergic transmission in the rat globus pallidus. Neuroscience 122:727–737.
- Matyas F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF (2006) Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. Neuroscience 137:337–361.
- McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- Mercer JN, Chan CS, Tkatch T, Held J, Surmeier DJ (2007) Nav1.6 sodium channels are critical to pacemaking and fast spiking in globus pallidus neurons. J Neurosci 27:13552–13566.
- Mugnaini E, Oertel WH (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. Amsterdam: Elsevier.
- Nakanishi H, Kita H, Kitai ST (1990) Intracellular study of rat entopeduncular nucleus neurons in an *in vitro* slice preparation: electrical membrane properties. Brain Res 527:81–88.
- Nakanishi H, Kita H, Kitai ST (1991) Intracellular study of rat entopeduncular nucleus neurons in an *in vitro* slice preparation: response to subthalamic stimulation. Brain Res 549:285–291.
- Nambu A, Takada M, Inase M, Tokuno H (1996) Dual somatotopical representations in the primate subthalamic nucleus: evidence for ordered but reversed body-map transformations from the primary motor cortex and the supplementary motor area. J Neurosci 16:2671–2683.
- Nambu A, Tachibana Y, Kaneda K, Tokuno H, Takada M (2005) Dynamic model of basal ganglia functions and Parkinson's disease. In: The Basal Ganglia VIII (Bolam JP, Ingham CA, Magill PJ Eds), pp. 307– 312. New York: Springer.
- Nambu A, Tokuno H, Hamada I, Kita H, Imanishi M, Akazawa T, Ikeuchi Y, Hasegawa N (2000) Excitatory cortical inputs to pallidal neurons via the subthalamic nucleus in the monkey. J Neurophysiol 84:289–300.
- Ni Z, Bouali-Benazzouz R, Gao D, Benabid AL, Benazzouz A (2000) Changes in the firing pattern of globus pallidus neurons after the

degeneration of nigrostriatal pathway are mediated by the subthalamic nucleus in the rat. Eur J Neurosci 12:4338–4344.

- Nini A, Feingold A, Slovin H, Bergman H (1995) Neurons in the globus pallidus do not show correlated activity in the normal monkey, but phase-locked oscillations appear in the MPTP model of parkinsonism. J Neurophysiol 74:1800–1805.
- Ogura M, Kita H (2000) Dynorphin exerts both postsynaptic and presynaptic effects in the Globus pallidus of the rat. J Neurophysiol 83:3366–3376.
- Okoyama S, Nakamura Y, Moriizumi T, Kitao Y (1987) Electron microscopic analysis of the synaptic organization of the globus pallidus in the cat. J Comp Neurol 265:323–331.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a stereological study using the cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Parent A, Charara A, Pinault D (1995) Single striatofugal axons arborizing in both pallidal segments and in the substantia nigra in primates. Brain Res 698:280–284.
- Parent M, Levesque M, Parent A (2001) Two types of projection neurons in the internal pallidum of primates: single-axon tracing and threedimensional reconstruction. J Comp Neurol 439:162–175.
- Parent M, Parent A (2004) The pallidofugal motor fiber system in primates. Parkinsonism Relat Disord 10:203–211.
- Parthasarathy HB, Graybiel AM (1997) Cortically driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. J Neurosci 17:2477–2491.
- Poisik OV, Mannaioni G, Traynelis S, Smith Y, Conn PJ (2003) Distinct functional roles of the metabotropic glutamate receptors 1 and 5 in the rat globus pallidus. J Neurosci 23:122–130.
- Rajakumar N, Rushlow W, Naus CC, Elisevich K, Flumerfelt BA (1994) Neurochemical compartmentalization of the globus pallidus in the rat: an immunocytochemical study of calcium-binding proteins. J Comp Neurol 346:337–348.
- Rav-Acha M, Bergman H, Yarom Y (2008) Pre- and postsynaptic serotoninergic excitation of globus pallidus neurons. J Neurophysiol 100:1053–1066.
- Raz A, Vaadia E, Bergman H (2000) Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine vervet model of parkinsonism [In Process Citation]. J Neurosci 20:8559–8571.
- Robertson RG, Graham WC, Sambrook MA, Crossman AR (1991) Further investigations into the pathophysiology of MPTP-induced parkinsonism in the primate: an intracerebral microdialysis study of gamma-aminobutyric acid in the lateral segment of the globus pallidus. Brain Res 563:278–280.
- Robledo P, Feger J (1990) Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res 518:47–54.
- Rothblat DS, Schneider JS (1995) Alterations in pallidal neuronal responses to peripheral sensory and striatal stimulation in symptomatic and recovered parkinsonian cats. Brain Res 705:1–14.
- Rye DB, Saper CB, Lee HJ, Wainer BH (1987) Pedunculopontine tegmental nucleus of the rat: cytoarchitecture, cytochemistry, and some extrapyramidal connections of the mesopontine tegmentum. J Comp Neurol 259:483–528.
- Sadek AR, Magill PJ, Bolam JP (2007) A single-cell analysis of intrinsic connectivity in the rat globus pallidus. J Neurosci 27: 6352–6362.
- Sanudo-Pena MC, Patrick SL, Khen S, Patrick RL, Tsou K, Walker JM (1998) Cannabinoid effects in basal ganglia in a rat model of Parkinson's disease. Neurosci Lett 248:171–174.
- Sato F, Lavallee P, Levesque M, Parent A (2000a) Single-axon tracing study of neurons of the external segment of the globus pallidus in primate. J Comp Neurol 417:17–31.
- Sato F, Parent M, Levesque M, Parent A (2000b) Axonal branching pattern of neurons of the subthalamic nucleus in primates. J Comp Neurol 424:142–152.
- Selemon LD, Goldman-Rakic PS (1985) Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. J Neurosci 5:776–794.
- Shindou T, Nonaka H, Richardson PJ, Mori A, Kase H, Ichimura M (2002) Presynaptic adenosine A2A receptors enhance GABAergic synaptic transmission via a cyclic AMP dependent mechanism in the rat globus pallidus. Br J Pharmacol 136:296–302.
- Shink E, Sidibe M, Smith Y (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: II. Topography and synaptic organization of pallidal efferents to the pedunculopontine nucleus. J Comp Neurol 382:348–363.
- Shink E, Smith Y (1995) Differential synaptic innervation of neurons in the internal and external segments of the globus pallidus by the GABA- and glutamate-containing terminals in the squirrel monkey. J Comp Neurol 358:119–141.
- Sidibe M, Bevan MD, Bolam JP, Smith Y (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: I. Topography and synaptic organization of the pallidothalamic projection. J Comp Neurol 382:323–347.
- Smith Y, Hazrati LN, Parent A (1990) Efferent projections of the subthalamic nucleus in the squirrel monkey as studied by the PHA-L anterograde tracing method. J Comp Neurol 294:306–323.
- Smith Y, Parent A, Seguela P, Descarries L (1987) Distribution of GABAimmunoreactive neurons in the basal ganglia of the squirrel monkey (*Saimiri sciureus*). J Comp Neurol 259:50–64.
- Staines WA, Fibiger HC (1984) Collateral projections of neurons of the rat globus pallidus to the striatum and substantia nigra. Exp Brain Res 56:217–220.
- Stanford IM, Cooper AJ (1999) Presynaptic mu and delta opioid receptor modulation of GABAA IPSCs in the rat globus pallidus in vitro. J Neurosci 19:4796–4803.

- Steininger TL, Rye DB, Wainer BH (1992) Afferent projections to the cholinergic pedunculopontine tegmental nucleus and adjacent midbrain extrapyramidal area in the albino rat. I. Retrograde tracing studies. J Comp Neurol 321:515–543.
- Tachibana Y, Kita H, Chiken S, Takada M, Nambu A (2008) Motor cortical control of internal pallidal activity through glutamatergic and GABAergic inputs in awake monkeys. Eur J Neurosci 27:238–253.
- Takada M, Tokuno H, Nambu A, Inase M (1998) Corticostriatal projections from the somatic motor areas of the frontal cortex in the macaque monkey: segregation versus overlap of input zones from the primary motor cortex, the supplementary motor area, and the premotor cortex. Exp Brain Res 120:114–128.
- Tremblay L, Filion M, Bedard PJ (1989) Responses of pallidal neurons to striatal stimulation in monkeys with MPTP-induced parkinsonism. Brain Res 498:17–33.
- van der Kooy D, Carter DA (1981) The organization of the efferent projections and striatal afferents of the entopeduncular nucleus and adjacent areas in the rat. Brain Res 211:15–36.
- Vitek JL (2002) Pathophysiology of dystonia: a neuronal model. Mov Disord 17(Suppl. 3):S49–S62.
- Watanabe K, Kita T, Kita H (2009) Presynaptic actions of d2-like receptors in the rat cortico-striato-globus pallidus disynaptic connection in vitro. J Neurophysiol 101:665–671.
- Wichmann T, DeLong MR (2003) Pathophysiology of Parkinson's disease: the MPTP primate model of the human disorder. Ann NY Acad Sci 991:199–213.
- Wilson CJ, Phelan KD (1982) Dual topographic representation of neostriatum in the globus pallidus. BRES 243:354–359.
- Wu Y, Richard S, Parent A (2000) The organization of the striatal output system: a single-cell juxtacellular labeling study in the rat. Neurosci Res 38:49–62.
- Yelnik J, Francois C, Percheron G, Tande D (1996) A spatial and quantitative study of the striatopallidal connection in the monkey. Neuroreport 7:985–988.
- Yelnik J, Percheron G, Francois C (1984) A Golgi analysis of the primate globus pallidus. II. Quantitative morphology and spatial orientation of dendritic arborizations. J Comp Neurol 227:200–213.
- Yoshida S, Nambu A, Jinnai K (1993) The distribution of the globus pallidus neurons with input from various cortical areas in the monkeys. Brain Res 611:170–174.

# **Projections from Pallidum to Striatum**

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#### I. INTRODUCTION

The functional anatomical concepts of the basal ganglia that have been put forward over the past two decades by Alexander and Crutcher, DeLong and Albin et al. (Albin et al., 1989; DeLong, 1990; Alexander and Crutcher, 1990) have tremendously advanced our thinking of basal ganglia function and dysfunction. In these concepts the globus pallidus plays a crucial role as a center processing cortical signals that it receives via the striatum and relaying this information to other basal ganglia components and to the thalamus (see also Chapter 13). Globus pallidus in primates consists of an internal and external part, whereas in rodents and carnivores the homologues of the two constituent nuclei are entopeduncular nucleus and globus pallidus, respectively. For rodent globus pallidus and primate globus pallidus pars externa (GPe), it is its efferent connection with the subthalamic nucleus (STN) that figures most prominently in basal ganglia models, and it is impressive how robust and powerful these models have proven to be, whilst taking into account a rather limited number of GPe axonal connections. Recent years have witnessed a gradual incorporation of more of the GPe's manifold inputs and outputs into our models (for a review see Chapter 1). Much attention has focused on the reciprocal relationship between the GPe and the STN, and the output of the GPe to the internal segment of globus pallidus (GPi) (Bevan et al., 2002; Obeso et al., 2006). As the details start to unravel it is becoming clear that the GPe can no longer be considered a relay station along the axis running from the cortex via the striatum to the thalamus, but that it occupies a central position receiving cortical information via disynaptic and monosynaptic pathways and that it can profoundly influence basal ganglia processing and output at all levels (Kita, 1992; Naito and Kita, 1994; Bevan et al., 1998; Kita, 2007). This turns the spotlights to other GPe efferent connections that may offer deeper understanding of GPe function. One of these connections, that has hitherto not received a great deal of attention, is the axonal projection from the GPe to the striatum. Early indications that such a projection might exist come from the work of Kinnier Wilson (1913) and Mettler (1943). Yet how substantial is this pallidal efferent and what are its characteristics? This chapter will present an overview of the neuroanatomical tracing studies on the pallidostriatal connection, its neurochemical features and its regulation.

# II. GENERAL ANATOMY OF PALLIDOSTRIATAL PROJECTIONS

The striatal inputs from the pallidum in rodent, carnivore and primate appear to arise most if not only from the GPe and the ventral pallidum (see also Chapter 13). A few retrogradely labeled cells have been seen in the GPi (Smith and Parent, 1986), but other tract-tracing studies examining the GPe in which the GPi or the entopeduncular nucleus were co-examined state that no pallidostriatal cells were found in the latter nuclei (e.g. Beckstead, 1983; Parent et al., 1983; Walker et al., 1989). Other tracing studies aimed at establishing the GPi/entopeduncular nucleus efferents have, to our knowledge, never reported pallidal fibers in the striatum (for reviews, see Smith et al., 1998; Bolam et al., 2000). The population of pallidal neurons from which efferents to the striatum originate is quite large. In a key paper on the pallidostriatal neurons in the rat, Kita and Kita (2001) calculated the number of immunocytochemically identified GPe neurons labeled with a retrograde neuroanatomical tracer that was injected in the caudate-putamen and concluded that about a third of all GPe neurons issues fibers to the striatum. The same approach was used in two monkeys (Kita et al., 1999), in which 30% and 35% of GPe neurons was observed to be labeled after application of the retrograde tracer WGA-HRP (wheat germ agglutinin-horseradish peroxidase) in the caudate nucleus or putamen. In single cell labeling studies, six out of 23 (~25%) intra- or juxtacellularly labeled GPe neurons were found to project to the striatum (Kita and Kitai, 1994; Bevan et al., 1998).

Neuroanatomical labeling of pallidostriatal fibers was first demonstrated using anterograde tracing with tritiated amino-acids in the cat (Nauta, 1979). The conclusions from this study were carefully worded, for technical limitations prohibited the labeling from being unequivocally identified as originating specifically from globus pallidus. Subsequently, a number of studies employing retrograde or anterograde tracers at high resolution have confirmed the initial observations in cat (Jayaraman, 1983; Beckstead, 1983; Fisher et al., 1985; Spooren et al., 1991), monkey (Beckstead, 1983; Parent et al., 1983; Smith and Parent, 1986; Spooren et al., 1996; Kita et al., 1999) and rat (Staines et al., 1981; Staines and Fibiger, 1984; Takada et al., 1986; Shu and Peterson, 1988; Walker et al., 1989; Kuo and Chang, 1992; Groenewegen et al., 1993; Brog et al., 1993; Rajakumar et al., 1994; Shammah-Lagnado et al., 1996; Kita and Kita, 2001; Miwa et al., 2001). Labeling of passing fibers, the most likely source of error, could be excluded (for instance, see Staines and Fibiger, 1984).

All sectors of the ventral striatum – comprising ventral parts of the caudate-putamen (or, in non-rodents, caudate nucleus and putamen), nucleus accumbens and olfactory tubercle – have been successfully targeted with retrograde injections, except for the olfactory tubercle (Phillipson and Griffiths, 1985; Spooren et al., 1991; Kuo and Chang, 1992; Groenewegen et al., 1993; Churchill and Kalivas, 1994; Spooren et al., 1996). Both nucleus accumbens' core and shell throughout their rostrocaudal extent and along the medial to lateral axis were found to be reached by inputs arising from the ventral pallidum and, in some cases, the GPe (Phillipson and Griffiths, 1985; Kuo and Chang, 1992; Churchill and Kalivas, 1994). Injections of retrograde tracers in the caudate-putamen have all been placed at rostrocaudal levels anterior to the decussation of the anterior commissure. At these levels, dorsal, central and lateral parts of the caudate-putamen have been injected and shown to receive pallidal fibers. This means that sensorimotor, associative as well as limbic-associated territories of the caudate-putamen receive pallidal information (Staines et al., 1981; Staines and Fibiger, 1984; Takada et al., 1986; Walker et al., 1989; Kita and Kita, 2001; Miwa et al., 2001).

Anterograde studies in rat confirm the widespread projections from pallidum to caudate-putamen and nucleus accumbens (Shu and Peterson, 1988; Kuo and Chang, 1992; Groenewegen et al., 1993; Rajakumar et al., 1994; Shammah-Lagnado et al., 1996). In an elaborate study, with systematically placed deposits of anterograde tracers along the GPe's anteroposterior and mediolateral axes, Shammah-Lagnado et al. (1996) describe that both rostral and caudal GPe send axonal projections to the caudate-putamen, in a topographic fashion. Caudal parts of the caudate-putamen, where no retrograde injections have yet been reported, receive a slightly less dense pallidal innervation compared to rostral parts. With respect to the ventral striatum, Groenewegen et al. (1993) and Kuo and Chang (1992) show extensive, widespread projections from both medial and lateral ventral pallidum to nucleus accumbens, ventral caudate-putamen and olfactory tubercle. Both the dorsal and ventral pallidostriatal connections have been confirmed with electrophysiological methods in rat and guinea pig (Walker et al., 1989; Hakan et al., 1992; Nambu and Llinas, 1997; Bevan et al., 1998). It may be concluded that, in the rat, all pallidal regions emit fibers to the striatum and that all striatal regions are reached by these fibers although fiber densities may vary (see below).

Retrograde tracing in monkeys has produced partly conflicting data. Although studies agree that tracer deposits in putamen result in pallidal cellular labeling (Beckstead, 1983; Parent et al., 1983; Smith and Parent, 1986; Kita et al., 1999), this is not the case for the caudate nucleus. Whereas in the squirrel monkey few or no pallidal cells were labeled after injections in the caudate nucleus (Parent et al., 1983; Smith and Parent, 1986), Kita et al. (1999) did find evidence

for a pallidal projection to the body of the caudate nucleus in the macaque. This may point to a possible difference between old and new world monkeys as to how extensive the GPe to caudate nucleus connections are. However, at present, data from anterograde tracing experiments that could validate these results are lacking, so that technical limitations cannot be ruled out. Anterograde tracing from ventral pallidum and central GPe in the macaque shows strong projections to the caudate nucleus, whilst tracer deposits in other parts of the GPe result in selective fiber labeling in putamen (Spooren et al., 1996). In addition, the latter study convin cingly demonstrates widespread projections from ventral pallidum and GPe to nucleus accumbens, olfactory tubercle, caudate nucleus and putamen using both anterograde and retrograde tracing. GPe projections to caudate nucleus and putamen have been confirmed in the macaque with electrophysiology (Tremblay and Filion, 1989).

In the cat, retrograde labeling of pallidal neurons has been reported after tracer deposits in the head and body of the caudate nucleus and in rostral and "middle part" of putamen (Jayaraman, 1983; Beckstead, 1983; Fisher et al., 1985). The early anterograde study of Nauta (1979) in the cat agrees with these data in showing widespread radioactive labeling in head and body of caudate nucleus and in putamen. As regards the ventral striatum and ventral pallidum, only data from anterograde experiments are available, showing a topographically organized projection from ventral pallidum to nucleus accumbens, olfactory tubercle and caudate nucleus (Spooren et al., 1991).

Taken together, the tracing data in monkey and cat present a very similar, albeit less detailed picture compared to the rat: the GPe and the ventral pallidum emit a substantial fiber contingent to the putamen, caudate nucleus and ventral striatum.

#### **III. TOPOGRAPHY**

The pallidal inputs of the striatum obey a precise topographical arrangement. Two close but non-contiguous applications of different retrograde tracers along the mediolateral extent of the rat caudate-putamen give rise to cellular labeling in medial and lateral parts of the GPe, respectively (Staines and Fibiger, 1984). No co-localization of the two tracers was seen in the GPe, revealing a purely parallel type of pallidostriatal organization that is similar to the striatopallidal organization. The study further shows a strict relationship with the substantia nigra in which labeled GPe territories reach only those sectors of the striatum and substantia nigra that are interconnected. The comparison with the striatopallidal system is also made in other publications. By using immunostaining for calbindin, Kita and Kita (2001) were able to identify the injection site of retrograde tracer in either sensorimotor or associative sectors of the caudate-putamen and in doing so visualized projections to these areas from functionally corresponding regions in the GPe. Beckstead (1983) noticed in the cat that "the pallidostriatal projection reciprocates rather faithfully the topography of the striatopallidal projection". The same appears to hold true for the ventral pallidum (Phillipson and Griffiths, 1985; Spooren et al., 1991; Groenewegen et al., 1993; Spooren et al., 1996).

A clear, fine-grained rostrocaudal and dorsoventral topography has been revealed in the pallidofugal system in an extensive survey of anterograde labeling from all GPe districts (Shammah-Lagnado et al., 1996). For instance, from the caudal half of the GPe anteriorly placed anterograde tracer deposits result in fiber labeling in (more) rostrally located regions of the caudate-putamen compared to the caudal parts of the caudate-putamen that are reached by tracers coming from posteriorly placed deposits. In addition, tracer injections in dorsal or ventral territories of the GPe result in varicose axonal fiber labeling in dorsal and ventral parts of the caudate-putamen, respectively. As mentioned previously, the GPe projections to the caudate-putamen are less dense coming from the caudomedial part of the GPe compared to rostrolateral parts.

An important question is whether the pallidostriatal connection is truly a mirror image of the striatopallidal projection (Gerfen, 2004). On a gross scale such recipro city has been found, as outlined above, but detailed analysis of tracing data brings to light some discrepancies. In an anterograde study using PHA-1 (Phaseolus vulgaris leucoagglutinin) in the rat GPe, Rajakumar (1994) noticed that pallidostriatal axons arrive in striatal regions that contain no retrogradely labeled striatopallidal cell bodies, suggesting that pallidostriatal projections target a wider field than the striatal area from which the pallidal inputs originate (Fig. 14.1). However, retrograde transport of PHA-1 should be interpreted with great caution. Retrograde axonal uptake of the tracer is highly variable (Groenewegen and Wouterlood, 1990), precluding firm conclusions. Also, other experimental findings in the rat, as presented in the already mentioned report of Staines and Fibiger (1984) do not provide evidence for (strongly) overlapping projection patterns from pallidal subregions. On the other hand, our own experimental data do support Rajakumar's suggestions of a non-reciprocal pallidostriatal projection. Using Biotin Dextran as a simultaneous retrograde and anterograde



GP/GPe

**FIGURE 14.1** Schematic representation of pallidal efferents (dark brown arrows) which arrive in a region of the striatum (light green) that is larger than the striatal district from which emanate the striatopallidal projections (dark green patch and dark green arrows). Conversely, striatal sectors (dark green patch) receive information (light brown arrows) from pallidal sectors (light brown patch) that are larger than the pallidal field (dark brown patch) to which the striatal district (dark green patch) projects fibers (Rajakumar et al., 1994; Spooren et al., 1996; Kita et al., 1999). To view a color version of this image please visit http://www.elsevierdirect .com/companion/9780123747679

tracer in the ventral pallidostriatal pathway, we observed overlap of nucleus accumbens regions with retrogradely labeled neurons and those with anterogradely labeled fibers, but the latter regions were much more extensive than the zones holding labeled cell bodies (Fig. 14.2).

The problem of strictly reciprocal connectivity between the striatum and the GPe in the rat may at present remain unresolved. In the monkey, however, there is evidence to suggest that the topographies of striatopallidal and pallidostriatal systems are different. Kita et al. (1999) observe the pallidal areas that contain neuronal cell bodies retrogradely labeled with WGA-HRP after tracer injection in the striatum to be much larger than those displaying dense anterogradely labeled axons (Fig. 14.1). Furthermore, in contrast to the findings of Staines et al. (1981) in the rat, overlap of projection patterns arising from different locations in the GPe and the ventral pallidum indeed were seen in the monkey (Spooren et al., 1996). This leads to the conclusion that pallidal territories also emit fibers to striatal regions from which they do not receive afferents (Fig. 14.1). Clearly, this organization consisting of both open and closed loops would not only enable feedback to the striatum but also large scale communication between otherwise segregated loops that connect the cortex with the basal ganglia and thalamus. Whether species differences indeed exist remains to be established in more detailed neuroanatomical experiments, particularly in rat.

# IV. CHARACTERISTICS OF PALLIDOSTRIATAL NEURONS

The main finding in the above reviewed retro- and anterograde anatomical tracer studies is that pallidal efferents to



FIGURE 14.2 Mapping of regions in rat nucleus accumbens that show labeling of retrogradely labeled cell bodies and anterogradely labeled axons after injection with Biotin Dextran in ventral pallidum. In A, micrograph inset of frontal section through rat brain shows outline of area that is charted in main figure. Red territory delineates region with high density of retrogradely labeled cells, blue indicates region with retrogradely labeled cells and anterogradely labeled fibers, green territory represents the area in which only fibers but no cells are seen. Note that green region with fiber labeling is larger than blue region with cell soma labeling. B. Photomicrograph showing cell bodies and varicose axons in nucleus accumbens in the small boxed area in A on the border between red and blue regions. Retrogradely filled cells (an individual cell is indicated with an arrow) and anterogradely filled fibers (double arrow points to cluster of varicose fibers) are black, having been immunostained using nickel-enhanced DAB. The purple-blue stained cells in the section do not contain any retrograde tracer (arrow heads) and have been (counter)stained using Cresyl-violet. (From data kindly provided by Henk J. Groenewegen; rat # 98061). Bar in B represents 50 µm. CA, anterior commissure; CP, caudate-putamen; LV, lateral ventricle; NAc, nucleus accumbens; OT, olfactory tubercle. To view a color version of this image please visit http://www.elsevierdirect .com/companion/9780123747679

the striatum are widespread. This conclusion is corroborated by the observations on individually labeled pallidostriatal neurons showing pallidal axons that can be traced for the length of several millimeters in both rat and monkey (Bevan et al., 1998; Sato et al., 2000). However, more restricted axonal arborizations are also found. The population of pallidostriatal neurons is quite heterogeneous. Differences have been reported in cell size, morphology, striatal projection patterns and collateralization.

Cell size averages  $215 \pm 63.8 \mu m^2$  in primate (Sato et al., 2000), while in rat mean sizes are  $219 \pm 27.9$  and  $152 \pm 21.9 \mu m^2$  depending on whether cells were immunopositive or immunonegative for parvalbumin, respectively (Kita and Kita, 2001). In ventral pallidum, cell sizes range from 75 to  $525 \mu m^2$  (Kuo and Chang, 1992).

Practically all cell shapes have been reported: round, oval, polygonal, triangular (Beckstead, 1983; Kuo and Chang, 1992; Kita and Kitai, 1994; Sato et al., 2000). There is some consensus on the dendritic morphology of the pallidostriatal neurons. In both monkey and rat, the majority of neurons have thick, smooth dendrites that fan out in a discoid shape (Sato et al., 2000). A minority of pallidostriatal cells has a radial-shaped dendritic pattern with aspiny or moderately

spined dendrites (Kita and Kitai, 1994). These cell types probably belong to the type I and type II classes of neurons distinguished by Nambu and Llinas (1994) (Nambu and Llinas, 1994; Bevan et al., 1998). The location of the pallidostriatal subtypes may differ. The cells with radial dendritic patterns have been found in the central region of the GPe and along its medial border, whereas the (majority) type with discoid dendritic pattern is found scattered throughout the nucleus (Sato et al., 2000). This organization may be related to the complicated intrinsic pattern of connectivity that was recently described in the GPe (Sadek et al., 2007).

The axonal arborizations of the pallidostriatal neurons are complex. Within the GPe, axons branch profusely and collaterals either remain within the boundaries of the dendritic tree or grow well beyond its limits (Bevan et al., 1998; Sato et al., 2000). In coursing to the striatum, axons ramify into 1-5 main collaterals that enter the caudateputamen, caudate nucleus or putamen in rat and monkey, respectively (Bevan et al., 1998; Sato et al., 2000). From these main branches arborizations with different densities and differently sized territories develop. Bevan et al. (1998) in the rat show examples of a neuron with extensive axonal arborizations that cover a distance of several millimeters, and, in contrast, a neuron with much more restricted, i.e. several hundreds of microns, axonal ramifications. This difference was reflected in the number of boutons being about a third lower in the neuron with the restricted axonal projection pattern. Apparently, pallidostriatal neurons target either restricted or (very) large striatal territories.

Although somatodendritic morphology and axonal arborizations in rat and monkey pallidostriatal neurons are quite similar, there is a major species difference as regards axonal collaterals to other basal ganglia nuclei. Whereas in monkey the pallidostriatal neurons have been shown to belong to a separate population of pallidal projection neurons that does not project to other major GPe output targets (Sato et al., 2000), in rat it has been found that the pallidostriatal neurons may send axonal branches to the thalamus, STN, entopeduncular nucleus and substantia nigra pars reticulata and pars compacta (Staines and Fibiger, 1984; Takada et al., 1986; Kita and Kitai, 1994; Bevan et al., 1998). In cat, collaterals have been observed in the cortex (Fisher et al., 1985). The complexity of the collateralization is summarized in Figure 14.3. The implication of the species difference between rat and monkey is that in rat single GPe neurons can simultaneously control any number of basal ganglia components. In monkey, the same (pallidal) information may be distributed to



**FIGURE 14.3** Summary diagram showing the pallidostriatal projections and collaterals to other basal ganglia components, thalamus and cortex in different species. See text for references. GPe, globus pallidus pars externa and rodent globus pallidus; GPi, globus pallidus pars interna; SN, substantia nigra; STN, subthalamic nucleus; Str, striatum; Thal, thalamus; VP, ventral pallidum; VS, ventral striatum. Brain diagram inspired by Nauta and Feirtag (1986).

several basal ganglia components, but it is conveyed by separate cells. Finally, given the difference in projection targets between rostral and caudal parts of the GPe in the rat – for instance, caudal GPe does not project to the STN (Shammah-Lagnado et al., 1996)-it is likely that the organization of multinuclear collaterals will be found to change along the rostrocaudal axis of GPe.

### V. STRIATAL TARGETS OF PALLIDOSTRIATAL NEURONS

The pallidostriatal neurons establish synaptic contacts with medium spiny projection neurons and with aspiny interneurons (Bevan et al., 1998; Kita, 2007). The latter neurons belong to two populations, one expressing parvalbumin, the other containing nitric oxide (Bevan et al., 1998). The parvalbumin-expressing interneurons make up the largest population of GABAergic striatal interneurons. They are strongly interconnected through gap junctions and by way of their synaptic contacts with striatal projection neurons, they are able to command full control over striatal output (see Bolam et al., 2000, for review). Consequently, the pallidostriatal neurons are in a position to mono- or disynaptically regulate activity of the (two) striatal output pathways.

# VI. CHEMICAL NEUROANATOMY AND REGULATION OF PALLIDOSTRIATAL NEURONS

Like all pallidal projection neurons, the pallidostriatal neurons are GABAergic (Kita, 2007). The pallidostriatal neurons have been shown to be immunopositive or -negative for the calcium-binding protein parvalbumin (Kita and Kita, 2001). The majority of neurons are immunonegative for parvalbumin (Kita and Kita, 2001). The latter population also expresses enkephalin (Hoover and Marshall, 1999). However, not all pallidostriatal neurons are enkephalinergic (Fig. 14.4); estimates range from 26% (Hoover and Marshall, 1999) to 54% and 56% (Voorn et al., 1999; Hoover and Marshall, 2004). Hoover and Marshall (1999) state that the pallidostriatal neurons are "more frequently preproenkephalinergic" than the pallido-subthalamic neurons. A small minority of pallidonigral cells (6% of 537 pallidonigral cells) was found to contain preproenkephalin mRNA; no double-labeling was observed after retrograde injection in the cortex or entopeduncular nucleus (Voorn et al., 1999). Together, these findings provide evidence for a sizable population of enkephalinergic pallidal neurons that issues fibers to only one of the major pallidal output targets.

Administration of dopamine D2 receptor antagonists has been shown to result in the induction of the immediate early gene c-fos (Ruskin and Marshall, 1997; Miwa et al., 1998; Miwa et al., 2001; Billings and Marshall, 2003). In addition, such drug treatment or lesioning of the dopaminergic system with 6-hydroxydopamine causes upregulation of pallidal GAD and enkephalin, which, as stated above, are expressed in pallidostriatal neurons (Schuller et al., 1999; Voorn et al., 1999). Other neuropeptides that



**FIGURE 14.4** Presence and absence of preproenkephalin mRNA in retrogradely labeled pallidostriatal neurons in the rat. A. Fluorescent retrogradely labeled cells (with Fluorogold) in GPe after tracer injection in anterior caudate-putamen. B. Outlines of retrogradely labeled neurons is superimposed on radioactive in situ hybridization visualization of preproenkephalin mRNA. Enkephalinergic cell bodies are identified by aggregates of silver grains. Arrows point to retrogradely labeled neurons that do not contain labeling for preproenkephalin mRNA. ×10 magnification.

are affected by dopamine depletion are substance P and neurotensin (Martorana et al., 2003). These effects may be brought about via presynaptic mechanisms, via the striatopallidal route, and/or via postsynaptic processes involving the STN (Walker et al., 1989; Ruskin and Marshall, 1997; Miwa et al., 1998; Miwa et al., 2001). However, the fact that dopamine D2 receptor synthesis has been demonstrated in enkephalinergic pallidostriatal neurons (Marshall et al., 2001) makes a direct effect of dopaminergic manipulation on pallidostriatal neurons very likely. Moreover, the above data imply that under conditions of altered dopamine neurotransmission, as in Parkinson's disease, the pallidostriatal connection will be severely affected. No stereological analyses have been carried out on the various populations of pallidostriatal neurons thus far. On the basis of the available data reviewed above, we may estimate that about 30% of pallidal neurons projects to the striatum. Approximately 50% of the pallidostriatal neurons are enkephalinergic (and parvalbumin immunonegative) and ~40% of the enkephalinergic neurons express the dopamine D2 receptor so that they are under direct (postsynaptic) dopaminergic control (Hoover and Marshall, 2004). So far only one other monoamine receptor, 5HT-2A receptor, has been observed in the pallidostriatal neurons (Bubser et al., 2001).

#### VII. FUNCTIONAL CONSIDERATIONS

As our understanding of the GPe function advances it becomes clear that this nucleus is more than a relay station in basal ganglia circuitry (see Chapter 13). However, to include the pallidostriatal connection in upgraded basal ganglia diagrams as a (mere) feedback system would be to overlook its importance. Two aspects are of interest in this respect: the role of the pallidostriatal projection in circuitry engaging the cortex and the STN and its integrative characteristics.

In recent years, much attention has focused on the direct cortical inputs to the STN and the partly reciprocal connections of the nucleus with the GPe and the GPi/ entopeduncular nucleus (Obeso et al., 2006). This implies that cortical information can reach the GPe via two pathways and it essentially puts the GPe in control over (the excitability of) basal ganglia output stations to the thalamus and cortex (Fig. 14.5). Upon cortical stimulation, the GPe neurons show facilitation via the excitatory output from the STN followed by inhibition via the inhibitory inputs from the striatum (see (Kita and Kita, 2001; Obeso et al., 2006). Evidently, these signals will be transmitted to the striatum via the pallidostriatal neurons, thus providing



**FIGURE 14.5** Diagram showing two pathways along which cortical information can reach GPe. Excitatory signals reach GPe via the "fast excitation" pathway from the cortex to the STN. Via the "slow inhibition" route, inhibitory signals reach GPe from the striatum, which, in turn, has been activated by cortical inputs. GPe, globus pallidus pars externa and rodent globus pallidus; GPi, globus pallidus pars interna; SN, substantia nigra; STN, subthalamic nucleus; Str, striatum. Adapted from Kita (2007).

not only feed-back but also feed-forward signals (Fig. 14.5). The pallidal feed-forward input that arrives in the striatum at short latency will be capable of influencing corticostriatal inputs. It will affect, as reviewed above, both striatal projection neurons and interneurons, and set conditions for striatal processing of cortical signals. These conditions will depend on the nature of the pallidostriatal input, which, on basis of the somatodendritic and axonal morphology of the pallidostriatal neurons, may be argued to have strong integrative characteristics.

The morphology of the pallidostriatal neurons suggests important integrative capacities. Dendritic arborizations are elaborate and local axon collaterals may reach spectacular proportions (Bevan et al., 1998; Sato et al., 2000). The shape of the dendritic field and the stream-like organization of the striatopallidal projections have led Percheron et al. (Percheron et al., 1984) to propose a strong convergence of striatal information on pallidal neurons and, hence, a major integrative role for the GPe. Another morphological aspect concerning integration may be the degree of precision in the reciprocity of the striatopallidal and pallidostriatal projections. As illustrated in Figure 14.1, there is evidence to suggest much wider projection fields from the pallidum to the striatum than the striatal field from which input to the pallidal subregions emanates. Finally, the fact that the pallidostriatal neurons reach the syncytium of parvalbumin-containing striatal interneurons allows for strong coordination and/or synchronization of activity of striatal projection neurons over widely spaced striatal regions. The pallidostriatal neurons may thus play a role in the increased lateral connectivity in the striatum (and the pallidum) that is seen after cortical stimulation, that is, during global activation (Magill et al., 2006). Synchronization of neuronal firing in and among basal ganglia components and the cortex is an important process, that is disturbed in Parkinson 's disease (Brown, 2003; Stoffers et al., 2007) (see also Chapters 25 and 38). The GPe-STN-GPi circuit and the connection between the GPe and the striatum may play key roles in this process (Bevan et al., 2002; Obeso et al., 2006). The fact that manipulation of the dopaminergic neurotransmission in the pathway connecting this circuit with the striatum (as mentioned above) induces clear cut (patho)physiological changes warrants further functional neuroanatomical investigations of the pallidostriatal connection.

#### REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Beckstead RM (1983) A pallidostriatal projection in the cat and monkey. Brain Res Bull 11:629–632.
- Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bevan MD, Magill PJ, Terman D, Bolam JP, Wilson CJ (2002) Move to the rhythm: oscillations in the subthalamic nucleus-external globus pallidus network. Trends Neurosci 25:525–531.
- Billings LM, Marshall JF (2003) D2 antagonist-induced c-fos in an identified subpopulation of globus pallidus neurons by a direct intrapallidal action. Brain Res 964:237–243.
- Bolam JP, Hanley JJ, Booth PA, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196(Pt 4):527–542.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol 338:255–278.
- Brown P (2003) Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson's disease. Mov Disord 18:357–363.
- Bubser M, Backstrom JR, Sanders-Bush E, Roth BL, Deutch AY (2001) Distribution of serotonin 5-HT(2A) receptors in afferents of the rat striatum. Synapse 39:297–304.
- Churchill L, Kalivas PW (1994) A topographically organized gammaaminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. J Comp Neurol 345:579–595.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Fisher RS, Boylan MK, Hull CD, Buchwald NA, Levine MS (1985) Branched projections of pallidal and peripallidal neurons to neocortex

and neostriatum: a double-labeling study in the cat. Brain Res 326:156–159.

- Gerfen CR (2004) Basal Ganglia. In: The Rat Nervous System (Paxinos G Ed),. San Diego: Elsevier Academic Press.
- Groenewegen HJ, Berendse HW, Haber SN (1993) Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. Neuroscience 57:113–142.
- Groenewegen HJ, Wouterlood FG (1990) Light and electron microscopic tracing of neuronal connections with *Phaeolus vulgaris*-leucoagglutinin (PHA-L), and combinations with other neuroanatomical techniques. In: Analysis of Neuronal Microcircuits and Synaptic Interactions (Björklund A, Hökfelt T, Wouterlood FG, Van den Pol AN Eds.), pp. 47–124. Amsterdam-New York-Oxford: Elsevier.
- Hakan RL, Berg GI, Henriksen SJ (1992) Electrophysiological evidence for reciprocal connectivity between the nucleus accumbens septi and ventral pallidal region. Brain Res 581:344–350.
- Hoover BR, Marshall JF (2004) Molecular, chemical, and anatomical characterization of globus pallidus dopamine D2 receptor mRNAcontaining neurons. Synapse 52:100–113.
- Hoover BR, Marshall JF (1999) Population characteristics of preproenkephalin mRNA-containing neurons in the globus pallidus of the rat. Neurosci Lett 265:199–202.
- Jayaraman A (1983) Topographic organization and morphology of peripallidal and pallidal cells projecting to the striatum in cats. Brain Res 275:279–286.
- Kinnier Wilson SA (1913) An experimental research into the anatomy and physiology of the corpus striatum.. Brain 36:427–492.
- Kita H (1992) Responses of globus pallidus neurons to cortical stimulation: intracellular study in the rat. Brain Res 589:84–90.
- Kita H (2007) Globus pallidus external segment. Prog Brain Res 160:111–133.
- Kita H, Kita T (2001) Number, origins, and chemical types of rat pallidostriatal projection neurons. J Comp Neurol 437:438–448.
- Kita H, Kitai ST (1994) The morphology of globus pallidus projection neurons in the rat: an intracellular staining study. Brain Res 636:308–319.
- Kita H, Tokuno H, Nambu A (1999) Monkey globus pallidus external segment neurons projecting to the neostriatum. Neuroreport 10:1467–1472.
- Kuo H, Chang HT (1992) Ventral pallido-striatal pathway in the rat brain: a light and electron microscopic study. J Comp Neurol 321:626–636.
- Magill PJ, Pogosyan A, Sharott A, Csicsvari J, Bolam JP, Brown P (2006) Changes in functional connectivity within the rat striatopallidal axis during global brain activation *in vivo*. J Neurosci 26:6318–6329.
- Marshall JF, Henry BL, Billings LM, Hoover BR (2001) The role of the globus pallidus D2 subfamily of dopamine receptors in pallidal immediate early gene expression. Neuroscience 105:365–378.
- Martorana A, Fusco FR, D'Angelo V, Sancesario G, Bernardi G (2003) Enkephalin, neurotensin, and substance P immunoreactivity neurones of the rat GP following 6-hydroxydopamine lesion of the substantia nigra. Exp Neurol 183:311–319.
- Mettler FA (1943) Extensive unilateral cerebral removals in the primate: Physiologic effects and resultant degeneration. J Comp Neurol 80:69–148.
- Miwa H, Fuwa T, Nishi K, Kondo T (2001) Subthalamo-pallido-striatal axis: a feedback system in the basal ganglia. Neuroreport 12:3795–3798.
- Miwa H, Fuwa T, Nishi K, Mizuno Y (1998) Effects of the globus pallidus lesion on the induction of c-Fos by dopaminergic drugs in the striatum possibly via pallidostriatal feedback loops. Neurosci Lett 240:167–170.

- Naito A, Kita H (1994) The cortico-pallidal projection in the rat: an anterograde tracing study with biotinylated dextran amine. Brain Res 653:251–257.
- Nambu A, Llinas R (1997) Morphology of globus pallidus neurons: its correlation with electrophysiology in guinea pig brain slices. J Comp Neurol 377:85–94.
- Nambu A, Llinas R (1994) Electrophysiology of globus pallidus neurons in vitro. J Neurophysiol 72:1127–1139.
- Nauta HJ (1979) Projections of the pallidal complex: an autoradiographic study in the cat. Neuroscience 4:1853–1873.
- Nauta WJH, Feirtag M (1986) Fundamental Neuroanatomy. New York: W.H. Freeman.
- Obeso JA, Rodriguez-Oroz MC, Javier BF, Guridi J (2006) The globus pallidus pars externa and Parkinson's disease. Ready for prime time? Exp Neurol 202:1–7.
- Parent A, Mackey A, De Bellefeuille L (1983) The subcortical afferents to caudate nucleus and putamen in primate: a fluorescence retrograde double labeling study. Neuroscience 10:1137–1150.
- Percheron G, Yelnik J, Francois C (1984) A Golgi analysis of the primate globus pallidus. III. Spatial organization of the striato-pallidal complex. J Comp Neurol 227:214–227.
- Phillipson OT, Griffiths AC (1985) The topographic order of inputs to nucleus accumbens in the rat. Neuroscience 16:275–296.
- Rajakumar N, Elisevich K, Flumerfelt BA (1994) The pallidostriatal projection in the rat: a recurrent inhibitory loop? Brain Res 651:332–336.
- Ruskin DN, Marshall JF (1997) Differing influences of dopamine agonists and antagonists on Fos expression in identified populations of globus pallidus neurons. Neuroscience 81:79–92.
- Sadek AR, Magill PJ, Bolam JP (2007) A single-cell analysis of intrinsic connectivity in the rat globus pallidus. J Neurosci 27:6352–6362.
- Sato F, Lavallee P, Levesque M, Parent A (2000) Single-axon tracing study of neurons of the external segment of the globus pallidus in primate. J Comp Neurol 417:17–31.
- Schuller JJ, Billings LM, Marshall JF (1999) Dopaminergic modulation of pallidal preproenkephalin mRNA. Brain Res Mol Brain Res 69:149–153.
- Shammah-Lagnado SJ, Alheid GF, Heimer L (1996) Efferent connections of the caudal part of the globus pallidus in the rat. J Comp Neurol 376:489–507.
- Shu SY, Peterson GM (1988) Anterograde and retrograde axonal transport of Phaseolus vulgaris leucoagglutinin (PHA-L) from the globus pallidus to the striatum of the rat. J Neurosci Methods 25:175–180.
- Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86:353–387.
- Smith Y, Parent A (1986) Differential connections of caudate nucleus and putamen in the squirrel monkey (Saimiri sciureus). Neuroscience 18:347–371.
- Spooren WP, Lynd-Balta E, Mitchell S, Haber SN (1996) Ventral pallidostriatal pathway in the monkey: evidence for modulation of basal ganglia circuits. J Comp Neurol 370:295–312.
- Spooren WP, Veening JG, Groenewegen HJ, Cools AR (1991) Efferent connections of the striatopallidal and amygdaloid components of the substantia innominata in the cat: projections to the nucleus accumbens and caudate nucleus. Neuroscience 44:431–447.
- Staines WA, Atmadja S, Fibiger HC (1981) Demonstration of a pallidostriatal pathway by retrograde transport of HRP-labeled lectin. Brain Res 206:446–450.

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- Staines WA, Fibiger HC (1984) Collateral projections of neurons of the rat globus pallidus to the striatum and substantia nigra. Exp Brain Res 56:217–220.
- Stoffers D, Bosboom JL, Deijen JB, Wolters EC, Berendse HW, Stam CJ (2007) Slowing of oscillatory brain activity is a stable characteristic of Parkinson's disease without dementia. Brain 130:1847–1860.
- Takada M, Ng G, Hattori T (1986) Single pallidal neurons project both to the striatum and thalamus in the rat. Neurosci Lett 69:217–220.
- Tremblay L, Filion M (1989) Responses of pallidal neurons to striatal stimulation in intact waking monkeys. Brain Res 498:1–16.
- Voorn P, van de Witte S, Tjon G, Jonker AJ (1999) Expression of enkephalin in pallido-striatal neurons. Ann NY Acad Sci 877:671–675.
- Walker RH, Arbuthnott GW, Wright AK (1989) Electrophysiological and anatomical observations concerning the pallidostriatal pathway in the rat. Exp Brain Res 74:303–310.

# The Subthalamic Nucleus: From *In Vitro* to *In Vivo* Mechanisms

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# I. INTRODUCTION

The purpose of this chapter is to give an overview of the functional properties of the subthalamic nucleus (STN) and to describe recent insights in its role in normal and pathological brain processes, with emphasis on Parkinson's disease (PD) and epileptic disorders. Due to its strategic position in the organization of the basal ganglia (Fig. 15.1A) (see Chapter 1), and its specific electrophysiological and synaptic properties, the STN has a cardinal and leading situation in basal ganglia functions. First, the STN provides,

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. together with the striatum, the input stage of the basal ganglia, integrating and conveying the cortical and thalamic information to the basal ganglia output nuclei (Smith et al., 1998). Second, whereas 98.9% of neurons in the basal ganglia are GABAergic (Oorschot, 1996), the STN is composed of glutamatergic projection neurons, and thus it has long been considered as "the driving force" of the basal ganglia. Finally, at the single-cell level, STN neurons possess a distinctive set of non-synaptic ion channels that drive various autonomous activities and set the context in which the afferent synaptic inputs are integrated and converted into



FIGURE 15.1 Functional anatomy of the STN. (A) Afferent and efferent circuit anatomy of the STN. The STN can be subdivided into three main morphofunctional territories: a large dorsolateral motor region, a ventromedial associative territory, and a medial limbic sector. The dorsolateral sector receives excitatory inputs from various motor cortical areas, including the primary motor cortex (M1), premotor cortex (PM), supplementary motor area (SMA) and the cingulate motor area (CMA). The same STN region integrates monosynaptic excitatory afferents from the centromedian (CM) and the parafascicular (Pf) thalamic nuclei. Its conveys these cortical and thalamic information to the main output nuclei of the basal ganglia (SNr, substantia nigra pars reticulata; GPi, internal segment of the globus pallidus) and to the external segment of the globus pallidus (GPe), which sends back inhibitory projections to STN neurons. (B) Three-dimensional reconstruction of a STN neuron, which was juxtacellularly injected with neurobiotin. The cell body was located in the region of the STN receiving inputs from motor cortex. Note the numerous dendritic ramifications (in yellow) and the axon (in orange) that divides, close to the cell body, in anterior (Ant) and posterior collaterals directed to the GPe and SNr, respectively. (C) Distribution of synaptic contacts on STN neurons. The GABAergic synapses (GABA), arising from the GPe, are widely distributed on the soma, proximal and distal dendrites, with the indicated percentage. In contrast, the glutamatergic connections (Glu) from thalamus and cortex are restricted to distal dendrites. The output activity of STN neurons is sculpted by subtle interactions between these synaptic inputs and various, non-synaptic, ion channels (voltage-gated Na<sup>+</sup> channels, Na<sub>y</sub>; voltage-gated Ca<sup>2+</sup> channels, Ca<sup>2+</sup> v and small conductance Ca<sup>2+</sup>-dependent K<sup>+</sup> (SK) channels). (D) Typical responses of STN cells to cortical stimulation. Two excitatory peaks, with short- and long-latency onsets, are interrupted by a brief period of inhibition. (B) Modified from (Paz et al., 2007); (D), modified from (Maurice et al., 1998). To view a color version of this image please visit http://www.elsevierdirect .com/companion/9780123747679

firing patterns (Beurrier et al., 1999; Bevan and Wilson, 1999; Beurrier et al., 2000; Song et al., 2000; Do and Bean, 2003; Hallworth et al., 2003; Do and Bean, 2004).

Although the STN is a relatively small component of the basal ganglia, in terms of size and total number of neurons (Oorschot, 1996), its participation in large-scale normal brain oscillations is now recognized (Magill et al., 2000, 2001; Bevan et al., 2006) and causal links between aberrant activities in STN neurons and movement disorders, such as PD, are widely assumed (Bevan et al., 2002, 2006) (see Chapters 25, 38 and 39). More recently, it has been demonstrated that the STN is also involved in generalized epilepsy (Deransart et al., 1998; Vercueil et al., 1998), by propagating paroxysmal oscillations to basal ganglia output structures and, subsequently, controlling thalamo-cortical excitability (Paz et al., 2005a, 2005b, 2006, 2007).

After a brief description of the input-output synaptic organization of the STN, we will detail the active membrane properties of STN neurons, accurately described from in vitro experiments, which, after a complex interplay with synaptic inputs, subtly tune their firing patterns. We will then review the recent findings concerning the various types of normal spontaneous activities of STN neurons in vivo, and their relationships to cortical synchronization and vigilance states. The role of the dopaminergic system, and of its alteration, in the modulation of activity in the STN and related networks will be addressed, with a special emphasis on the abnormal oscillatory neuronal pattern associated with PD. Finally, the functional implication of the STN in non-convulsive generalized epilepsy will be highlighted, based on the recent in vivo electrophysiological investigations performed in a genetic model of absence seizures.

# II. SYNAPTIC ORGANIZATION OF THE SUBTHALAMIC NUCLEUS AND RESPONSES TO CORTICAL STIMULATION

The STN is a lens-shaped structure resting on the internal capsule (see Fig. 15.1B). It is principally composed of glutamatergic output neurons, having a soma of  $20\mu$ m of diameter that gives rise to four to five primary dendrites with spiny long-distance arborizations (Beurrier et al., 1999; Bevan and Wilson, 1999; Paz et al., 2005a) (Fig. 15.1B).

#### A. Inputs

Like most other components of the basal ganglia, the STN is subdivided into different functional regions, including motor, associative and limbic territories, each with specific input-output connections (Parent and Hazrati, 1995). The large dorsolateral portion of the STN corresponds to the motor territory; the ventromedial portion to the associative territory and the medial part to the limbic sector (Fig. 15.1A). Because abnormal activity of the STN is associated with profound movement disorders, the motor input-output circuit of the STN is the most investigated, and thus the best characterized (Bevan et al., 2006).

The motor sector of the STN receives somatotopically organized monosynaptic glutamatergic inputs from the primary motor cortex, pre- and supplementary motor areas and cingulate motor cortex (Afsharpour, 1985) (Fig. 15.1A). The motor STN is also connected by glutamatergic afferents arising from the centromedian and parafascicular nuclei of the thalamus (Bevan and Bolam, 1995), which also project to the striatum and the cerebral cortex. Cortical and thalamic terminals make asymmetrical synaptic contacts with the dendrites and spines of STN neurons (Bevan et al., 1995). The thalamic terminals contact larger postsynaptic targets, and therefore presumably more proximal regions of dendrites, than do the cortical terminals (Bevan et al., 1995) (Fig. 15.1C).

The major GABAergic input to the STN is derived from the external segment of the globus pallidus (GPe) (see Chapter 13 by Kita). The pallidal projections are ipsilateral, topographic (Smith et al., 1990; Shink et al., 1996; Bevan et al., 1997) and largely reciprocated by an excitatory synaptic feedback from the STN (Shink et al., 1996). In rats, all GPe neurons that spread within the basal ganglia innervate the STN but also project to other basal ganglia nuclei *via* axon collaterals (Bevan et al., 1998). The GABAergic pallidal projections are widely distributed on the STN neurons membrane. These inhibitory synapses, which are symmetric in nature (Smith et al., 1990), contact the somata (31%), the proximal (39%) and distal (30%)dendrites of STN neurons (Smith et al., 1990) (Fig. 15.1C). It is likely that the distal dendritic GABAergic projections have a particular functional impact on the integrative properties of STN neurons since they are co-aligned with cortical and thalamic inputs (Smith et al., 1990; Bevan et al., 1995). Minor GABAergic projections to the STN arise from the mesopontine tegmentum, forming asymmetric synapses (Bevan and Bolam, 1995). GABAergic type-A receptors are concentrated at GPe-STN synapses (Galvan et al., 2004) and are responsible for a phasic Cl<sup>-</sup>-dependent current at GPe-STN synaptic connections (Hallworth and Bevan, 2005). However, synchronous high-frequency firing in GPe neurons leads to the activation of peri- and extrasynaptic GABAergic type-B receptors (Galvan et al., 2004), resulting in a long-lasting K<sup>+</sup>-dependent postsynaptic hyperpolarization and subsequent burst firing in STN neurons (Hallworth and Bevan, 2005).

Histochemical, morphological, pharmacological and electrophysiological studies have converged to provide definitive evidence for a direct dopaminergic pathway from the substantia nigra to the STN (Hassani et al., 1997; Francois et al., 2000; Cragg et al., 2004). Dopaminergic terminals form conventional symmetrical synaptic contacts with all parts of STN neurons (Cragg et al., 2004).

#### **B.** Outputs

Most STN neurons are glutamatergic projection neurons and provide a powerful excitatory input to the GPe and to the two output structures of the basal ganglia, the internal segment of the GP (GPi), and the substantia nigra pars reticulata (SNr) (see Chapter 13). These output nuclei exert a tonic GABAergic inhibitory influence on thalamic relay neurons and brainstem targets (Parent and Hazrati, 1995). The firing of STN neurons is precisely regulated by powerful feedback inhibition from the reciprocally connected GABAergic pallidal projections (Shink et al., 1996; Bevan et al., 2002, 2006), which interfere with complex postsynaptic intrinsic membrane properties (see Fig. 15.1C). Recent findings indicate that STN can be also reciprocally connected with the cerebral cortex, with dense subthalamo-cortical projections terminating in superficial cortical layers (Degos et al., 2008). The functional implication of these subthalamo-cortical projections remains unclear but they might amplify paroxysmal synchronized activities in the cortico-basal ganglia loop (see below).

#### C. Responses to Cortical Stimulation

Sequential activation of excitatory and inhibitory synapses on STN neurons has been unveiled in vivo by the extracellularly recorded responses of STN units to electrical stimulations of the related cerebral cortex. Stimulation of ipsilateral frontal and prefrontal cortices typically leads to a short-latency triphasic response in STN neurons (Maurice et al., 1998) (Fig. 15.1D), which is initiated by an early monosynaptic excitation likely caused by the activation of glutamatergic AMPA and NMDA receptors at cortico-subthalamic synaptic connections (Nakanishi et al., 1988). This initial excitation is shortly followed by feedback, probably Cl<sup>-</sup>-dependent, inhibition from the GPe, which in turn is followed by a second phase of excitation resulting, at least in part, from inhibition of the GPe by striatum and subsequent disinhibition of the STN (Maurice et al., 1998; Magill et al., 2004). The post-inhibitory rebound of firing found in STN neurons may also result from interplay between their intrinsic electrical properties and inhibitory synaptic inputs. Indeed, the membrane hyperpolarization induced by GPe

inhibitory synaptic inputs could lead to a de-inactivation of low-threshold  $Ca^{2+}$  channels and a transient boost of voltage-gated Na<sup>+</sup> current (Baufreton et al., 2005) in the postsynaptic membrane, so that upon repolarization a rebound firing would be generated (Bevan et al., 2006). A similar sequence of synaptic and intrinsic events in STN neurons has also been described from in vivo intracellular recordings during spontaneous paroxysmal cortical synchronization (Paz et al., 2005a) (see below).

# III. CELLULAR BASIS OF SINGLE-SPIKE AND BURST FIRING IN SUBTHALAMIC NUCLEUS NEURONS *IN VITRO*

#### A. Burst Firing

Approximately half of STN neurons recorded in brain slices exhibits two states of activity: a switch from a tonic singlespike discharge to a burst firing mode depending on their membrane potential (Fig. 15.2A) (Beurrier et al., 1999;



**FIGURE 15.2** Ionic currents underlying the burst firing mode of STN neurons in vitro. (A) Switch of firing mode according to membrane potential in a STN neuron recorded in current-clamp mode. When hyperpolarized to -62 mV, by intracellular injection of current, the neuron displays the burst firing mode and switches to the single-spike mode when depolarized to -52 mV. (B) The cascade of ionic currents involved in the different phases of the burst firing mode (see text for explanations). (C) Voltage dependency of the activation and deinactivation of the rebound discharge. The rebound discharge is recorded at the break of a hyperpolarizing current pulse (left). Its amplitude and rise time depend on the value of the membrane potential at the end of the negative current pulse: at -78 mV (bottom trace), the rebound discharge is not evoked; at -72 mV (middle trace), it triggers a spike; at around -55 mV (top trace) the amplitude of the rebound increases and the spike delay decreases. Deinactivation of  $I_T$  is also voltage-dependent (right): the rebound discharge is not evoked when the membrane is held for 300 ms at -60 mV; at -74 mV, a small rebound appears and at -78 mV, the amplitude of the rebound is sufficient to trigger a burst of spikes. Modified from (Beurrier et al., 1999).

Baufreton et al., 2003). Burst firing is observed when the membrane potential is hyperpolarized, whereas tonic single-spike activity is triggered upon depolarization. Pharmacological investigations performed in whole-cell recordings in brain slices allowed identifying the ionic conductances involved in the different phases of the bursting activity of STN neurons (Fig. 15.2B) (Beurrier et al., 1999).

The burst plateau (Fig. 15.2B, c-d). The burst plateau is partly due to the activation of L-type  $Ca^{2+}$  channels ( $I_{I}$ ). The relative long duration of the bursts and their blockade by a chelator of intracellular Ca<sup>2+</sup> suggest also the participation of Ca<sup>2+</sup>-dependent inward currents such as the nonspecific cationic current (I<sub>CAN</sub>). Voltage-dependent Na<sup>+</sup> channels enable STN neurons to fire action potentials during the plateau but are not crucial for the burst firing mode as the rhythmic membrane depolarizations underlying bursts are still observed in the presence of tetrodotoxin (TTX), a blocker of voltage-dependent Na<sup>+</sup> channels. This also demonstrates that burst activity in STN neurons is an autonomous property generated in the absence of synaptic input. However, action potentials discharge amplifies Ca<sup>2+</sup> entry during the plateau depolarization by activating more  $I_{\rm L}$  and, possibly, other types of high-threshold Ca<sup>2+</sup> currents present in STN neurons (Song et al., 2000).

The burst repolarization (Fig. 15.2B, d–a). The increase in intracellular Ca<sup>2+</sup> concentration during the plateau leads to the activation of Ca<sup>2+</sup>-dependent K<sup>+</sup> currents ( $I_{K,Ca}$ ). Blockade of  $I_{K,Ca}$  by application of low concentration of tetraethylammonium and apamin prevents burst repolarization and confirms a role of these channels in this phase. The balance between depolarizing ( $I_L$ ,  $I_{CAN}$ ) and hyperpolarizing ( $I_{K,Ca}$ ) currents, in favor of the latter, explains the repolarization of the membrane to the peak of the afterhyperpolarization.

The interburst (Fig. 15.2B, a–b) and burst depolarizations (Fig. 15.2B, b–c). The membrane of STN neurons then spontaneously depolarizes as  $I_{K,Ca}$  decays because of  $Ca^{2+}$  clearance mechanisms (interburst depolarization). STN neurons also display a hyperpolarization-activated cationic current ( $I_h$ ), which is an inward current activated by hyperpolarization (Beurrier et al., 1999; Bevan and Wilson, 1999; Do and Bean, 2003). The hyperpolarization of the membrane between two bursts may activate  $I_h$ . The decay of  $I_{K,Ca}$ , combined to the possible activation of  $I_h$ , leads to membrane depolarization sufficient to activate a low-threshold  $Ca^{2+}$  current known as the transient  $Ca^{2+}$ current ( $I_T$ ).  $I_T$  activation depolarizes the membrane to the threshold potential of  $I_L$  so that the cycle repeats.

In addition to its role in burst firing mode,  $I_{\rm T}$  enables STN neurons to generate a rebound discharge following hyperpolarization: this discharge can be triggered by the intracellular injection of negative current (Fig. 15.2C) or by the natural occurrence of inhibitory postsynaptic potentials. Activation and inactivation of  $I_{\rm T}$  are voltagedependent:  $I_{\rm T}$  activates at membrane potentials positive to approximately -65 mV (Fig. 15.2C, left) and inactivates during a maintained depolarization. Deinactivation occurs only if the membrane is hyperpolarized for a sufficient amount of time (Fig. 15.2C, right). This latter property has a significant functional impact on STN neurons firing: it implies that  $I_{\rm T}$  can be activated only when STN neurons are depolarized from a relatively hyperpolarized membrane potential allowing its deinactivation. Therefore, tonic depolarization of the membrane potential results in the inactivation of  $I_{\rm T}$  and may explain in part the switch from burst discharge to tonic single-spike activity when STN neurons are depolarized.

#### **B.** Single-Spike Activity

While burst firing mode is observed only in a subset of STN neurons, all of them are able to fire repetitively in single-spike mode at around 5-17Hz.Similarly to the burst firing mode, the single-spike mode is an intrinsic property of STN neurons that is not affected by the application of glutamatergic and GABAergic receptor antagonists (Bevan and Wilson, 1999; Beurrier et al., 2000). In these neurons, the persistent  $Na^+$  current ( $I_{NaP}$ ), which activates below spike threshold, is the principal current responsible for the pacemaker depolarization that brings the membrane from the peak of the afterspike hyperpolarization to the spike threshold (Bevan and Wilson, 1999; Beurrier et al., 2000). The role of  $I_{\text{NaP}}$  in single-spike activity was mainly deduced from its voltage-dependence since drugs that block it are also blockers of the fast Na<sup>+</sup> current underlying action potentials (Fig. 15.3A). Voltage-clamp experiments using a slow depolarizing ramp demonstrated that  $I_{NaP}$  is activated from approximately  $-55 \,\mathrm{mV}$ , in a potential range crossed by the membrane during the interspike interval, and is completely blocked by application of TTX (Fig. 15.3B).

Although activation of  $I_{\text{NaP}}$  is likely sufficient to depolarize the membrane of STN neurons to reach action potential threshold during the interspike interval, another type of Na<sup>+</sup> current, initially described in Purkinje cells, also promotes pacemaking firing of STN neurons (Do and Bean, 2003, 2004). This current, called resurgent Na<sup>+</sup> current,



**FIGURE 15.3** Na<sup>+</sup> currents are essential for the single-spike mode of STN neurons in vitro. (A) Tonic activity is completely abolished in the presence of TTX. (B) Persistent Na<sup>+</sup> current ( $I_{NaP}$ ) recorded in voltage-clamp mode in response to a depolarizing ramp applied at 5 mV.s<sup>-1</sup> in the absence (Control) and presence of TTX. Adapted from (Beurrier et al., 2000). (C) Application of a SK<sub>Ca</sub> blocker, apamin (100 nM), disrupts the rhythmic spontaneous single-spike activity of STN neurons by abolishing the slow single-spike afterhyperpolarization. Modified from (Bevan and Wilson, 1999). (D) Ionic currents critical for single-spike activity of STN neurons.

is TTX-sensitive and displays unusual gating behavior: at voltages around -50 to -60 mV, where classical Na<sup>+</sup> channels are closed due to inactivation, a transient Na<sup>+</sup> current can be detected in STN neurons. The unique properties of the resurgent Na<sup>+</sup> current (slow inactivation combined to activation immediately after a spike) may explain the ability of STN neurons to fire rhythmically at very high frequency.

While  $I_h$  is not essential for the single-spike activity (Bevan and Wilson, 1999; Beurrier et al., 2000), it can favor this mode of discharge in some cells by maintaining the membrane potential at depolarized values where tonic activity is present (Beurrier et al., 2000).

 $Ca^{2+}$  currents and  $Ca^{2+}$ -dependent currents do not play a significant role in the pacemaker depolarization during single-spike firing in contrast to their involvement in the burst firing mode. Their voltage ranges of activation and recovery from inactivation are not traversed by the membrane during the tonic activity. However, high-threshold  $Ca^{2+}$  currents

activated during action potentials are responsible for a  $Ca^{2+}$  entry in STN neurons, which in turn activates a small-conductance  $Ca^{2+}$ -dependent potassium channel (SK<sub>Ca</sub>). SK<sub>Ca</sub> currents underlie the medium duration of action potential afterhyperpolarization and are essential for the regularity of tonic activity. Specific blockade of SK<sub>Ca</sub> by apamin does not prevent the spontaneous firing of STN neurons but greatly reduces afterhyperpolarization and disrupts the rhythmicity of spontaneous single-spike firing (Hallworth et al., 2003) (Fig. 15.3C). The ionic currents participating in the single-spike mode are summarized in Figure 15.3D.

The properties of STN neurons we described above were all observed and characterized in brain slices. However, most of them, such as the rebound discharge and the burst firing mode, are also found *in vivo* (Paz et al., 2005a), demonstrating that both normal and pathological activities of STN neurons are shaped by a complex interplay between intrinsic properties and network activity.



**FIGURE 15.4** In vivo spontaneous activity of STN neurons and its relationship to cortical synchronization. (A) Multiunit recording during ketamine anesthesia demonstrating that firing of STN neurons in close proximity is tightly correlated during rhythmic synchronizations in the ipsilateral electrocorticogram (ECoG). The bottom two traces represent the unit activities as indicated by the corresponding numbers in the raw data. (B) Urethane anesthesia. Whereas neurons in the STN exhibit low-frequency rhythmic firing ( $\sim$ 1 Hz) during periods of slow-wave activity in the ipsilateral cortex (Intact cortex), ipsilateral cortical ablation results in a temporal disorganization of STN activity (cortical ablation). Same representation as in (A) except in the lower panel where the top trace corresponds to the ECoG contralateral to the STN recording. (C) Simultaneous recordings of a STN neuron, a GPe neuron and the corresponding ECoG during ketamine anesthesia. Whereas both cells exhibit correlated bursts during recurrent cortical synchrony, brief periods of reduction of cortical slow-wave amplitude and rhythmicity (under white bar) lead to a loss of correlated activity in the same neurons. (D) Polygraphic recordings (ECoG and EMG) and unit activity in the STN across the sleep-wave cycle. The activity of the STN neuron clearly shifts from a bursting pattern during slow-wave sleep to a more regular tonic-like firing during active waking. (A) and (C), modified from (Magill et al., 2000) (B), modified from (Magill et al., 2001). (D), modified from (Urbain et al., 2000).

# C. *In Vivo* Activities of STN Neurons and their Relation to Cortical Patterns

Recent in vivo electrophysiological recordings of rat STN neurons and related cortical field potentials demonstrated that the cerebral cortex can pattern activity in the STN, which thus provides a potent device to propagate cortical information within basal ganglia networks (Magill et al., 2000, 2001; Bevan et al., 2002, 2006).

#### **D.** Anesthesia-Dependent Slow Oscillations

Under ketamine and/or urethane anesthesia, generating slow cortical oscillations and rhythmic discharge in corticofugal neurons (Cowan and Wilson, 1994; Contreras and Steriade, 1995; Mahon et al., 2001), action potential discharge properties of STN neurons are strictly related to the coincidental cortical activity (Magill et al., 2000, 2001) (Fig. 15.4A–C). During cortical slow oscillations (~1Hz), neighboring STN neurons fire correlated large bursts of action potentials (of several hundred milliseconds duration) with a periodicity that closely matches the cortical rhythm (Magill et al., 2000)

(Fig. 15.4A), suggesting a powerful phasic excitation of basal ganglia output nuclei during repetitive cortical synchronizations. Consistent with the massive glutamatergic innervation of STN neurons by cortical projections (Bevan et al., 1995), the in vivo removal of cortical synaptic inputs by ablation results in a slower, tonic, firing of STN neurons, which was similar to the discharge pattern found in brain slice preparations (Beurrier et al., 1999; Bevan and Wilson, 1999) (see Fig. 15.2A). Slow oscillatory cortical activities can be also reflected by trains of action potentials in GPe neurons with frequencies very similar to that observed in STN neurons (Magill et al., 2000; Bevan et al., 2006). As suggested by simultaneous recordings of GPe and STN units, this correlated activity in the two nuclei is likely due to the periodic firing of the STN and subsequent feedforward excitation of the GPe (Bevan et al., 2000) (Fig. 15.4C). This is also supported by the loss of correlated activity in STN and GPe neurons during brief periods of spontaneous reduction of cortical slow-wave amplitude and rhythmicity (Magill et al., 2000) (Fig. 15.4C). However, in the rat anesthetized with ketamine, paired recordings also show complex and variable firing patterns within the GPe, including synchronized activity, inversely correlated burst-firing and even uncorrelated patterns (Magill et al., 2000, 2001). Under urethane anesthesia, resulting in slower cortical oscillations, GPe neurons can show regular tonic discharge, irrespective of slow-wave activity in the cortex, whereas coincident rhythmic patterns in cortex and STN are simultaneously recorded (Magill et al., 2000, 2001; Bevan et al., 2002).

### E. Natural Patterns

The complex relationships between cortical patterns and the correlated activity in the STN-GPe network have been highlighted from in vivo extracellular recordings during the natural sleep-wake cycle (Urbain et al., 2000). In nonanesthetized head-restrained rats, STN neurons activity shifts from a random pattern during wakefulness to recurrent bursting discharges in slow-wave sleep (Urbain et al., 2000) (Fig. 15.4D). In contrast, GPe neurons recorded in the course of this study exhibit a relative regular firing whatever the state of vigilance. However, the activity of STN and GPe neurons displays, during voluntary or passive movement, spatiotemporal changes that are correlated to motor activity in a complex manner (reviewed in Bevan et al., 2002). During active movement, the activity in both nuclei is rarely correlated but it is highly structured with a precise somatotopic specificity (reviewed in Bevan et al., 2002).

# IV. SUBTHALAMIC NUCLEUS, DOPAMINE AND PARKINSONISM

As pointed out above, a substantial body of work demonstrated that oscillatory activities are an important feature of the normal operation of STN and connected cerebral regions. Recently, it has been shown that dopaminergic denervation of the basal ganglia profoundly affects the integration of excitatory cortical and GABAergic pallidal inputs to STN neurons, leading to abnormal neuronal patterns that resonate within the basal ganglia networks and propagate to other motor systems (see Chapter 25). A major challenge for future work will be to precisely determine how dopamine modulates the oscillatory activities generated in the STN and related nuclei and how the pathological oscillations of basal ganglia, induced by the alteration of the dopaminergic system, are causally linked with movement disorders.

#### A. Dopaminergic Control of STN Activity

Multiple studies suggest that the mesencephalic dopaminergic system regulates directly and indirectly the excitability and firing patterns of STN neurons. The presynaptic activation of D2-like receptors is responsible for a diminution of the release of both glutamate and GABA on STN neurons (Shen and Johnson, 2000). Consistently, chronic depletion of dopamine increases GABAergic and NMDA-dependent currents in STN cells following application of GABA and glutamate (Shen and Johnson, 2005). The dopaminergic system is also efficient for a robust control of intrinsic excitability of STN cells, leading to a modulation of firing rates and patterns. For instance, activation of type-5 dopaminergic receptors (D5) potentiates the Ca2+-dependent component of the bursting discharges (Baufreton et al., 2003), whereas activation of D1 and D2 receptors depolarizes and amplifies autonomous firing in STN neurons, partly due to the occlusion of a K<sup>+</sup> current (Baufreton et al., 2003; Bevan et al., 2006).

The striatal-GPe synaptic transmission is also controlled by dopamine and, consequently, provides potent indirect system influencing STN neurons activity. The excitability of striato-pallidal neurons is decreased by the activation of the somatodendritic D2 receptors (Hernandez-Lopez et al., 2000). In addition, the initial probability of release at GABAergic striato-pallidal synapses is reduced by the stimulation of the same type of presynaptic receptors (Cooper and Stanford, 2001) and, as a synergistic inhibitory effect, the activation of postsynaptic D4 receptors reduces GABA-mediated current in pallidal neurons (Shin et al., 2003). As a consequence of this complex dopaminergic control of striatal-GPe transmission, a deficit in dopamine will increase the excitability of striato-pallidal cells and enhances the GABAergic inhibition of pallidal cells, which would lead to a reduced pallidal inhibition of STN neurons (Bevan et al., 2006).

# B. Aberrant Oscillations in the GPe-STN Network in Parkinsonism

The motor disability in PD consists of poverty and slowness of movement (akinesia and bradykinesia), tremor at rest, muscle stiffness (rigidity), as well as gait and balance abnormalities (see also Chapter 38). Some or all of these symptoms are likely due to a loss of dopamine in the basal ganglia resulting from degeneration of dopaminergic neurons of the substantia nigra pars compacta. Whereas normal information processing in the STN and GPe is characterized by brain state-dependent spatiotemporal patterns of firing (see above), STN and GPe neurons display more correlated, synchronous and rhythmic patterns of activity in parkinsonian humans and animals (Bevan et al., 2002; Gatev et al., 2006). This transformation in the pattern of activity in the STN and GPe in PD, which is mirrored by consistent changes in the activity of the basal ganglia output nuclei, is likely to result in a profound alteration in the coding of information by the basal ganglia and might, therefore, contribute to the pathogenesis of the PD and the related motor symptoms.

Extracellular recordings of local field potentials and single-cell activities from parkinsonian patients undergoing functional neurosurgery revealed an elevated rhythmic activity in the GPe, STN and cortex with various frequency ranges including, the tremor frequency (4–10Hz), the alpha (8-13 Hz) and beta activities (14-30 Hz) (Bevan et al., 2002; Gatev et al., 2006). When dopaminergic agonists are administrated, the rhythmic activities below 30 Hz are relatively reduced in both cortex and basal ganglia and are subsequently replaced by higher frequency rhythms in the gamma range (30-70 Hz) (Bevan et al., 2002). The abnormal rhythmicity in cortex and basal ganglia associated with PD seems to be an ubiquitous electrophysiological modification consecutive to dopamine depletion since it is encountered in monkeys treated with MPTP (Gatev et al., 2006) and rats with altered dopaminergic transmission (Degos et al., 2009).

Although the mechanisms of pathological rhythmic activities in the STN-GPe network in patients and experimental models of PD remain unclear (see also Chapters 25 and 38), two main and non-exclusive processes could be involved. Indeed, abnormal synchronized oscillations could emerge from endogenous properties of the STN-GPe circuitry and/or be driven by afferent rhythmic activity from the cortex. Hence, it has been demonstrated that the STN-GPe network can support in vitro correlated rhythmic discharges when isolated from the cortex and the striatum (Plenz and Kitai, 1999). This activity would be initiated from synchronous bursting activity in GABAergic GPe neurons, generating sufficient hyperpolarization in STN neurons for a post-inhibitory rebound burst activity which, in turn, drives bursting discharges in GPe neurons and leads to the maintenance of the rhythm (Plenz and Kitai, 1999). Alternatively, the cortex is a potent external source that can pattern rhythmic activity in the STN and GPe. The principal in vivo effect of dopamine depletion in the anesthetized rat is the expression by the GPe neurons of low-frequency oscillatory activity and a more intense oscillatory activity in the STN, both correlated with the cortical rhythm (Magill et al., 2001). Since the temporal and amplitude properties of cortical slow-wave activity are not modified, it is likely that the STN and GPe under dopaminergic depletion are more sensitive to cortical synchronized oscillations (Magill et al., 2001).

# V. THE SUBTHALAMIC NUCLEUS AS A REMOTE CONTROL SYSTEM FOR CORTICAL SEIZURES

As stated above, motor symptoms of PD are associated with abnormal rhythmic activities in the STN and connected structures. Recent pharmacological and electrophysiological investigations in genetic models of absence epilepsy (Deransart et al., 1998; Vercueil et al., 1998; Deransart and Depaulis, 2002; Paz et al., 2005a, 2005b, 2006, 2007) demonstrated that the STN is also critically involved in the modulation of cortical seizure activity, expanding the impact of this deep and central nucleus in the triggering and modulation of pathological brain oscillations.

The Genetic Absence Epilepsy Rats from Strasbourg, or GAERS, exhibit spontaneous behavioral arrests with apparent loss of consciousness coincident with widespread spike-and-wave discharges (SWDs) in cortical and thalamic electroencephalograms, which closely resemble the electroclinical symptoms of typical absence epilepsy (Danober et al., 1998). Moreover, seizures are specifically abolished in GAERS by the anti-absence drugs used in patients, making this rats strain the more reliable animal model of the human absence epilepsy (Depaulis and van Luitjelaar, 2005; Polack and Charpier, 2009). We will examine the consistent pharmacological, deep-brain stimulation and electrophysiological studies that strongly support the hypothesis that the STN actively propagates cortical paroxysms in the basal ganglia and could control the cortical ictogenesis via the subthalamo-nigro-thalamo-cortical circuit.

### A. Pharmacological and Deep-Brain Stimulation Studies in Generalized Epilepsy

Pharmacological alteration of synaptic transmission at different levels of the basal ganglia circuits, as well as high frequency electrical stimulations of the STN, demonstrated that the subthalamo-nigral pathway provides a subcortical control system for seizure generation and/or generalization. Specifically, the occurrence of absence seizures is significantly decreased in GAERS (Deransart et al., 1996, 1998; Vercueil et al., 1998; Deransart and Depaulis, 2002) (Fig. 15.5A) by: (1) an alteration of the excitatory synaptic influence of the STN on the SNr; (2) a bilateral injection of a GABA agonist in the STN; (3) a blockade of subthalamic



FIGURE 15.5 Propagation of cortical epileptic discharges in basal ganglia networks. (A) Simplified representation of the changes in activity of the different structures of basal ganglia during pharmacological manipulations having antiepileptic effects in GAERS (adapted from Deransart et al., 1998; Vercueil et al., 1998). The agonists of different receptors are indicated, the stricken words represent the corresponding receptor antagonists (see text for details). In (A) and (B), the red and black arrows represent glutamatergic (Glu) excitatory (+) and GABAergic (GABA) inhibitory (-) projections, respectively. (B) Simplified diagram positioning the subthalamic nucleus (STN) in the processes of propagation of cortical paroxysms in the corticobasal ganglia loop. Cortical spike-and-wave discharges (SWDs) (top trace) propagate to the striatum and the STN with an imbalance (relative thickness of corresponding arrows) between the activity of corticostriatal (CStr) and corticosubthalamic (CSth) neurons. Striatal output neurons (Output) are silenced (black cross) via a feedforward inhibition, because of the activation of GABAergic striatal interneurons (Intern), whereas subthalamo-nigral neurons produce a rhythmic excitation of substantia nigra pars reticulata (SNr) neurons. In turn, nigral neurons inhibit ventromedial (VM) thalamocortical neurons, which also receive excitatory inputs from cortico-thalamic (CThal) neurons. The red thick arrows represent the pathway by which the SWDs propagate actively from cortex to basal ganglia and their feedback to the cerebral cortex that could underlie the control of absence seizures by the basal ganglia. The scheme also indicates the in vivo simultaneous recordings of cortical EEG and intra- (intra) and/or extracellular (extra) activities of basal ganglia neurons, as illustrated in this and the following figure. (C,D) Rhythmic bursting in the corticosubthalamopallidal network during SWDs. (C1) Spontaneous intracellular activity of a CSth neuron (bottom trace) simultaneously recorded with the EEG (top trace). (C2) Spontaneous intracellular activity of a STN neuron (bottom trace) during a SWD (top trace). (C3) Simultaneous extracellular recordings of a GPe (gray) and of a STN (black) neuron during interictal and ictal periods. (D) Chronological scenario of propagation of SWDs in the cortico-subthalamo-pallidal networks. Examples of two successive EEG spike-wave complexes (top traces) and the corresponding intracellular activity of a CSth neuron, extracellular firing of an STN neuron (STNe), intracellular recording from an STN neuron (STNi), and extracellular firing of a GPe neuron. (1) The early discharge of the CSth neurons promotes the early discharge in the STN neurons that (2) excite the GPe neurons, which in turn inhibit STN neurons (3). (4) The late firing of CSth neurons participates in maintaining the STN firing. (B-D) modified from (Paz et al., 2005a). Abbreviations: CSth, corticosubthalamic; CThal, corticothalamic; EEG, electroencephalogram; Extra, extracellular; Intern, interneuron; Intra, intracellular; Glu, glutamate; GP, globus pallidus; SNr, substantia nigra pars reticulate; STN, subthalamic nucleus; VM, ventromedial thalamic nucleus. To view a color version of this image please visit http://www .elsevierdirect.com/companion/9780123747679

or nigral glutamatergic NMDA receptors; (4) bilateral excitotoxic lesions of the STN; and (5) high-frequency stimulations (130 Hz) of the STN. Consistently, the cortical paroxysms are also suppressed by injection of GABAergic type-A receptor agonists into the SNr or by activation of GABAergic striatonigral neurons following intrastriatal injection of NMDA or dopaminergic D1 receptor agonists. Conversely, the pharmacological blockade of GABAergic type-A receptors in the SNr or the blockade of striatal D1 receptors aggravates absence seizures (Deransart et al., 2000, 2001) (Fig. 15.5A).

Taken together, these findings suggested that an alteration in the balance between synaptic excitation (from the STN) and inhibition (from the striatum) of SNr neurons controls the occurrence of cortical seizures, a relative increase in inhibition or excitation having anti- or pro-epileptic effects, respectively.

# B. Propagation of SWDs in Basal Ganglia Networks: Functional Imbalance Between Cortico-Subthalamo-Nigral and Cortico-Striato-Nigral Pathways

In theory, the differential modulation of SWDs by the basal ganglia, which can aggravate or interrupt the seizure activity, could originate from specific patterns in the activity of the cortico-striato-nigral and cortico-subthalamo-nigral pathways, affecting the firing of nigral output cells and, consequently, the activity of thalamo-cortical neurons. A series of recent studies have been specifically designed to elucidate the electrical activity occurring in the different neuronal elements of the basal ganglia during absence seizures in the GAERS and to unmask the mechanisms by which these subcortical nuclei can affect the cortical ictogenesis (Fig. 15.5B).

The occurrence of cortical SWDs is associated in the corticostriatal (CStr) neurons with rhythmic, sub- or suprathreshold, depolarizations phase-locked with the paroxysmal cortical activity. The GABAergic striatal output neurons, which receive a large number of converging CStr inputs (Kincaid et al., 1998; Mahon et al., 2001), exhibit simultaneously large-amplitude rhythmic depolarizing synaptic potentials, which however remain mostly subthreshold (Slaght et al., 2004). This inability of striato-nigral cells to generate action potential during the seizure is due to a feed-forward inhibition originating from the intense cortical activation of the intrastriatal GABAergic interneurons (Slaght et al., 2004), which produce a powerful inhibitory shunting effect on synaptic depolarizations in striatal output cells (Slaght et al., 2004) (Fig. 15.5B).

In contrast with the silencing of the cortico-striato-nigral pathway during seizures, the excitatory cortico-subthalamonigral pathway shows an intense activity, which wipes out the residual synaptic inhibition in the SNr. Cortical SWDs are concomitant with a sudden and drastic modification in the activity of cortico-subthalamic (CSth) neurons. Their interictal irregular firing is converted, during seizures, into a step-like behavior with rhythmic depolarizations generating repetitive discharges (Paz et al., 2005a) (Fig. 15.5C1). Interestingly, the firing rate of CSth neurons during SWDs is higher than that of CStr neurons (Fig. 15.5B), likely resulting from the expression in CSth neurons of a specific set of voltage-gated inward currents favoring bursting activity (Paz et al., 2005a). Since the axonal conduction velocity in CSth neurons (7 m/s) is higher than that of CStr neurons (1.5 m/s)(Mahon et al., 2001; Slaght et al., 2004; Paz et al., 2005a), the propagation of cortical influx in the cortico-subthalamic circuit appears more efficient and faster. This discrepancy between the two segregated corticofugal pathways projecting to the two basal ganglia entrances has likely a functional impact that remains to be elucidated in other, normal and pathological, brain processes.

# C. Rhythmic Bursting in STN and GPe Neurons During Seizures and its Repercussion on SNr Cells

The STN neurons recorded intracellularly in vivo in the GAERS (Paz et al., 2005a) display passive and active membrane properties similar to those described in vitro from normal rats (see above), strongly suggesting that the excitability of STN neurons in the epileptic rats is not altered. However, contrasting with that observed in striatal cells, the spontaneous activity of STN neurons doubles during SWDs and the corresponding firing profile switches from an irregular interictal pattern to high-frequency bursts of action potentials time-locked with the spike component of the SWD (Fig. 15.5C2, C3 and D). The rhythmic bursting in the STN is often composed of an early action potential, usually preceding the corresponding surface spike, followed by a short electrical silence (25 ms) and then a high-frequency cluster of action potentials (Fig. 15.5C3). This pattern of electrical events in the STN is very similar to that observed after electrical stimulation of the frontal cortex (see Fig. 15.1D) (Maurice et al., 1998). In vivo intracellular recordings unveiled that the intra-burst



**FIGURE 15.6** Blockade of the subthalamo-nigral transmission has an antiepileptic effect: cellular and network mechanisms. (A) Experimental design to investigate the role of the subthalamo-nigro-thalamo-cortical pathway in the control of cortical SWDs. Intranigral injection of kynurenate (kynu) blocks the subthalamo-nigral excitatory synaptic transmission. Pharmacological manipulations are made simultaneously with EEG, extracellular recordings of SNr and VM thalamic neurons and intracellular recordings of cortical neurons. (B) Histogram representing the mean firing frequency (freq.) of a VM thalamic neuron (bin, 20s) at the indicated times before (t1) or after (t2, t3) injection of kynurenate in the SNr. Each vertical bar below the histogram indicates the occurrence of an SWD in the corresponding EEG. Note that the injection of kynurenate in the SNr blocks cortical paroxysms and concomitantly increases the VM thalamic cell firing rate. The top traces depict EEG records at the indicated times. (C) Simultaneous recordings of the cortical EEG (EEG cx) (top traces) and of the extracellular activity (bottom traces) of an SNr neuron (extra SNr), a VM neuron (extra VM) and the intracellular activity of a cortical neuron (intra Cx) before (control) and after kynurenate (kynu) injection in SNr. Intra-SNr injection of kynurenate suppresses cortical SWDs together with, a deactivation of SNr neurons resulting in a dramatic reduction in the firing rate, a disinhibition of VM thalamic neurons and an inhibition of cortical neurons. Adapted from (Paz et al., 2007). Abbreviations: CSth, corticosubthalamic; CThal, corticothalamic; cx, cerebral cortex; EEG, electroencephalogram; Extra, extracellular; Glu, glutamate; Intra, intracellular; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VM, ventromedial thalamic nucleus.

pattern in STN neurons is sculpted by a sequence of synaptic and intrinsic events (Fig. 15.5D) including, an early depolarizing synaptic potential, followed by a short synaptic hyperpolarization and a rebound of excitation, which is likely due to both the activation of  $I_{\rm T}$  (see Fig. 15.2C) concomitant with late discharge of CSth neurons (Paz et al., 2005a) (Fig. 15.5D). The transient hyperpolarization reverses in polarity at around  $-73 \,\text{mV}$ , suggesting the involvement of Cl<sup>-</sup>-dependent conductance due to the activation of GABAergic type-A receptors. This hypothesis is supported by simultaneous extracellular recordings from STN and GPe neurons showing that GABAergic pallidal neurons exhibit, during SWDs, rhythmic clusters of action potentials (Fig. 15.5C3, D), which are correlated with the inhibitory synaptic potentials in STN neurons (Fig. 15.5D). Altogether, these findings suggest that cortical seizures impose rhythmic activity in STN neurons via a complex interplay between active membrane properties, CSth inputs and reciprocal synaptic connections with GPe cells.

The rhythmic bursting in the subthalamo-nigral neurons (Paz et al., 2005a), together with the lack of action potential firing in striato-nigral neurons during ictal activity (Slaght et al., 2004), induces a change in the balance between excitation and inhibition in the basal ganglia output nuclei that leads to a sudden reinforcement of the synaptic excitation originating from the STN. This is responsible for a synchronized bursting in the SNr neurons during cortical SWDs (Deransart et al., 2003; Paz et al., 2007) (see Fig. 15.6C, Control, extra SNr).

### D. Control of Ictogenesis by the Subthalamo-Nigro-Thalamo-Cortical Pathway

The striatal output neurons being silenced during seizures (Slaght et al., 2004) (Fig. 15.5B), the SWDs will propagate to the output nuclei of basal ganglia through a rhythmic bursting in the glutamatergic STN neurons, recurring with the frequency of cortical epileptic discharges (Paz et al., 2005a). Thus, the subthalamo-nigral pathway provides a potent network by which the basal ganglia can interfere with the seizure activity via the subsequent feed-back nigro-thalamo-cortical pathway. Such a complex network mechanism has been revealed by in vivo injection of kynurenate, a non-specific blocker of glutamatergic transmission, in the SNr (Paz et al., 2007) (Fig. 15.6A). This interruption of the transmission between STN and nigro-thalamic neurons produces a powerful antiepileptic effect together with a remarkable change in the activity of both SNr neurons and their targets in the ventromedial (VM) thalamic nucleus (Fig. 15.6B,C).

Desynchronized and random firing patterns of VM thalamic neurons in the absence of paroxysms become highly correlated and time-locked to the cortical spikes during seizures (Paz et al., 2007) (Fig. 15.6C, Control, extra VM). Injection of kynurenate in the SNr suppresses cortical seizures and concomitantly reduces dramatically the firing rate of SNr neurons (Fig. 15.6C, Kynu, extra SNr) and, consequently, increases the spontaneous activity of VM thalamic cells (Paz et al., 2007) (Fig. 15.6C, Kynu, extra VM), via a disinhibitory process (Chevalier and Deniau, 1990). The subsequent temporal disorganization of the thalamic activity, resulting in a sustained arrhythmic firing pattern leads to a restoration of a desynchronized cortical activity. This is consistent with the thalamic tonic firing recorded during active waking, whereas rhythmic bursting in the thalamus, as occurring during SWDs (Charpier et al., 1999; Polack and Charpier, 2006; Paz et al., 2007), is mainly correlated with cortical slow-waves associated with unconsciousness (Glenn and Steriade, 1982; Llinas and Steriade, 2006).

The cortical mechanisms underlying the control of seizures by the STN were revealed by the impact of the blockade of the subthalamo-nigral transmission on the intracellular activities of cortical neurons (Paz et al., 2007) (Fig. 15.6(A, C intra Cx). The antiepileptic effect of intra-SNr injection of kynurenate results from a disappearance of oscillations in cortical neurons together with a membrane hyperpolarization ( $\sim -7 \text{ mV}$ ), leading to a dramatic reduction in the firing rate and a diminution of membrane input resistance and time constant (Fig. 15.6C, Kynu, intra Cx). These synergistic changes in cortical neurons activity and excitability may originate from an activation of cortical GABAergic interneurons by the sustained arrhythmic firing pattern of the non-specific VM thalamo-cortical cells (Swadlow, 2003; Thomson and Bannister, 2003).

# E. Is There an On-line Control of Cortical Seizures by the STN?

Can the subthalamo-nigral pathway control on-line the cortical epileptic activities? The synchronized bursting of STN neurons, at once the start of seizure, induces repetitive discharges in SNr cells (Deransart et al., 2003; Paz et al., 2007) and subsequent rhythmic synaptic inhibition in thalamic cells (Paz et al., 2007). The iterative synaptic hyperpolarizations in thalamic cells will favor intrinsic bursting in the thalamo-cortical projections (Llinas and Steriade, 2006), which could amplify, via a resonance mechanism, the cortico-thalamic abnormal oscillations (Paz et al., 2007). Moreover, the rhythmic bursting of STN neurons could be rapidly transmitted back to the cortex *via* subthalamo-cortical projections (Degos et al., 2008), which might reinforce the paroxysmal synchronized activity in the cortex.

As a potent mechanism for seizure termination by the cortico-basal ganglia loop, the firing of STN and SNr neurons is often reduced and becomes arrhythmic around 500 ms prior to the end of the seizure (Deransart et al., 2003; Paz et al., 2007). As a consequence, the attenuation of the activity of nigro-thalamic cells is coincident with a desynchronized activity in thalamic neurons, which leads to the decrease in cortical neurons excitability (Paz et al., 2007) and, consequently, to the resolution of the cortical epileptic discharge.

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#### REFERENCES

- Afsharpour S (1985) Topographical projections of the cerebral cortex to the subthalamic nucleus. J Comp Neurol 236:14–28.
- Baufreton J, Atherton JF, Surmeier DJ, Bevan MD (2005) Enhancement of excitatory synaptic integration by GABAergic inhibition in the subthalamic nucleus. J Neurosci 25:8505–8517.
- Baufreton J, Garret M, Rivera A, de la Calle A, Gonon F, Dufy B, Bioulac B, Taupignon A (2003) D5 (not D1) dopamine receptors potentiate burst-firing in neurons of the subthalamic nucleus by modulating an L-type calcium conductance. J Neurosci 23:816–825.

- Beurrier C, Bioulac B, Hammond C (2000) Slowly inactivating sodium current (I(NaP)) underlies single-spike activity in rat subthalamic neurons. J Neurophysiol 83:1951–1957.
- Beurrier C, Congar P, Bioulac B, Hammond C (1999) Subthalamic nucleus neurons switch from single-spike activity to burst-firing mode. J Neurosci 19:599–609.
- Bevan MD, Bolam JP (1995) Cholinergic, GABAergic, and glutamateenriched inputs from the mesopontine tegmentum to the subthalamic nucleus in the rat. J Neurosci 15:7105–7120.
- Bevan MD, Wilson CJ (1999) Mechanisms underlying spontaneous oscillation and rhythmic firing in rat subthalamic neurons. J Neurosci 19:7617–7628.
- Bevan MD, Francis CM, Bolam JP (1995) The glutamate-enriched cortical and thalamic input to neurons in the subthalamic nucleus of the rat: convergence with GABA-positive terminals. J Comp Neurol 361:491–511.
- Bevan MD, Clarke NP, Bolam JP (1997) Synaptic integration of functionally diverse pallidal information in the entopeduncular nucleus and subthalamic nucleus in the rat. J Neurosci 17:308–324.
- Bevan MD, Atherton JF, Baufreton J (2006) Cellular principles underlying normal and pathological activity in the subthalamic nucleus. Curr Opin Neurobiol 16:621–628.
- Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Bevan MD, Wilson CJ, Bolam JP, Magill PJ (2000) Equilibrium potential of GABA(A) current and implications for rebound burst firing in rat subthalamic neurons in vitro. J Neurophysiol 83:3169–3172.
- Bevan MD, Magill PJ, Terman D, Bolam JP, Wilson CJ (2002) Move to the rhythm: oscillations in the subthalamic nucleus-external globus pallidus network. Trends Neurosci 25:525–531.
- Charpier S, Leresche N, Deniau JM, Mahon S, Hughes SW, Crunelli V (1999) On the putative contribution of GABA(B) receptors to the electrical events occurring during spontaneous spike and wave discharges. Neuropharmacology 38:1699–1706.
- Chevalier G, Deniau JM (1990) Disinhibition as a basic process in the expression of striatal functions. Trends Neurosci 13:277–280.
- Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15:604–622.
- Cooper AJ, Stanford IM (2001) Dopamine D2 receptor mediated presynaptic inhibition of striatopallidal GABA(A) IPSCs in vitro. Neuropharmacology 41:62–71.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- Cragg SJ, Baufreton J, Xue Y, Bolam JP, Bevan MD (2004) Synaptic release of dopamine in the subthalamic nucleus. Eur J Neurosci 20:1788–1802.
- Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C (1998) Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol 55:27–57.
- Degos B, Deniau JM, Chavez M, Maurice N (2009) Chronic but not acute dopaminergic transmission interruption promotes a progressive increase in cortical beta frequency synchronization: relationships to vigilance state and akinesia. Cereb Cortex 19:1616–1630.
- Degos B, Deniau JM, Le Cam J, Mailly P, Maurice N (2008) Evidence for a direct subthalamo-cortical loop circuit in the rat. Eur J Neurosci 27:2599–2610.

- Depaulis A, van Luitjelaar G (2005) Genetic models of absence epilepsy. In: The Rat Models of Seizures and Epilepsy (Pitkanen A, Schwartzkroin P, Moshe S, eds), pp. 233–248. San Diego (California): Elsevier.
- Deransart C, Depaulis A (2002) The control of seizures by the basal ganglia? A review of experimental data. Epileptic Disord 4(Suppl. 3):S61–S72.
- Deransart C, Marescaux C, Depaulis A (1996) Involvement of nigral glutamatergic inputs in the control of seizures in a genetic model of absence epilepsy in the rat. Neuroscience 71:721–728.
- Deransart C, Vercueil L, Marescaux C, Depaulis A (1998) The role of basal ganglia in the control of generalized absence seizures. Epilepsy Res 32:213–223.
- Deransart C, Riban V, Le B, Marescaux C, Depaulis A (2000) Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat. Neuroscience 100:335–344.
- Deransart C, Le-Pham BT, Hirsch E, Marescaux C, Depaulis A (2001) Inhibition of the substantia nigra suppresses absences and clonic seizures in audiogenic rats, but not tonic seizures: evidence for seizure specificity of the nigral control. Neuroscience 105:203–211.
- Deransart C, Hellwig B, Heupel-Reuter M, Leger JF, Heck D, Lucking CH (2003) Single-unit analysis of substantia nigra pars reticulata neurons in freely behaving rats with genetic absence epilepsy. Epilepsia 44:1513–1520.
- Do MT, Bean BP (2003) Subthreshold sodium currents and pacemaking of subthalamic neurons: modulation by slow inactivation. Neuron 39:109–120.
- Do MT, Bean BP (2004) Sodium currents in subthalamic nucleus neurons from Nav1.6-null mice. J Neurophysiol 92:726–733.
- Francois C, Savy C, Jan C, Tande D, Hirsch EC, Yelnik J (2000) Dopaminergic innervation of the subthalamic nucleus in the normal state, in MPTP-treated monkeys, and in Parkinson's disease patients. J Comp Neurol 425:121–129.
- Galvan A, Charara A, Pare JF, Levey AI, Smith Y (2004) Differential subcellular and subsynaptic distribution of GABA(A) and GABA(B) receptors in the monkey subthalamic nucleus. Neuroscience 127:709–721.
- Gatev P, Darbin O, Wichmann T (2006) Oscillations in the basal ganglia under normal conditions and in movement disorders. Mov Disord 21:1566–1577.
- Glenn LL, Steriade M (1982) Discharge rate and excitability of cortically projecting intralaminar thalamic neurons during waking and sleep states. J Neurosci 2:1387–1404.
- Hallworth NE, Bevan MD (2005) Globus pallidus neurons dynamically regulate the activity pattern of subthalamic nucleus neurons through the frequency-dependent activation of postsynaptic GABAA and GABAB receptors. J Neurosci 25:6304–6315.
- Hallworth NE, Wilson CJ, Bevan MD (2003) Apamin-sensitive small conductance calcium-activated potassium channels, through their selective coupling to voltage-gated calcium channels, are critical determinants of the precision, pace, and pattern of action potential generation in rat subthalamic nucleus neurons in vitro. J Neurosci 23:7525–7542.
- Hassani OK, Francois C, Yelnik J, Feger J (1997) Evidence for a dopaminergic innervation of the subthalamic nucleus in the rat. Brain Res 749:88–94.
- Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal

medium spiny neurons reduce L-type Ca<sup>2+</sup> currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. J Neurosci 20:8987–8995.

- Kincaid AE, Zheng T, Wilson CJ (1998) Connectivity and convergence of single corticostriatal axons. J Neurosci 18:4722–4731.
- Llinas RR, Steriade M (2006) Bursting of thalamic neurons and states of vigilance. J Neurophysiol 95:3297–3308.
- Magill PJ, Bolam JP, Bevan MD (2000) Relationship of activity in the subthalamic nucleus-globus pallidus network to cortical electroencephalogram. J Neurosci 20:820–833.
- Magill PJ, Bolam JP, Bevan MD (2001) Dopamine regulates the impact of the cerebral cortex on the subthalamic nucleus-globus pallidus network. Neuroscience 106:313–330.
- Magill PJ, Sharott A, Bevan MD, Brown P, Bolam JP (2004) Synchronous unit activity and local field potentials evoked in the subthalamic nucleus by cortical stimulation. J Neurophysiol 92:700–714.
- Mahon S, Deniau JM, Charpier S (2001) Relationship between EEG potentials and intracellular activity of striatal and cortico-striatal neurons: an in vivo study under different anesthetics. Cereb Cortex 11:360–373.
- Maurice N, Deniau JM, Glowinski J, Thierry AM (1998) Relationships between the prefrontal cortex and the basal ganglia in the rat: physiology of the corticosubthalamic circuits. J Neurosci 18:9539–9546.
- Nakanishi H, Kita H, Kitai ST (1988) An N-methyl-D-aspartate receptor mediated excitatory postsynaptic potential evoked in subthalamic neurons in an in vitro slice preparation of the rat. Neurosci Lett 95:130–136.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a stereological study using the cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. Brain Res Brain Res Rev 20:128–154.
- Paz JT, Deniau JM, Charpier S (2005a) Rhythmic bursting in the corticosubthalamo-pallidal network during spontaneous genetically determined spike and wave discharges. J Neurosci 25:2092–2101.
- Paz JT, Polack PO, Slaght SJ, Deniau JM, Charpier S (2005b) Propagation of cortical paroxysms in basal ganglia circuits during absence seizures. In: The Basal Ganglia VIII (Bolam JP, Ingham CA, Magill PJ, eds), pp. 55–65. New York: Springer.
- Paz JT, Chavez M, Saillet S, Deniau JM, Charpier S (2007) Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigrothalamic pathway. J Neurosci 27:929–941.
- Paz JT, Polack PO, Slaght SJ, Deniau JM, Mahon S, Charpier S (2006) Propagation and dynamic processing of cortical paroxysms in the basal ganglia networks during absence seizures. In: Generalized

seizures: From Clinical Phenomenology to Underlying Systems and Networks (Hirsch E, Andermann F, Chauvel P, Engel J, Lopes da Silva F, Luders H, eds), pp 75-91.

- Plenz D, Kitai ST (1999) A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. Nature 400:677–682.
- Polack PO, Charpier S (2006) Intracellular activity of cortical and thalamic neurones during high-voltage rhythmic spike discharge in Long-Evans rats in vivo. J Physiol 571:461–476.
- Polack PO, Charpier S (2009) Ethosuximide converts ictogenic neurons initiating absence seizures into normal neurons in a genetic model: Epilepsia.
- Shen KZ, Johnson SW (2000) Presynaptic dopamine D2 and muscarine M3 receptors inhibit excitatory and inhibitory transmission to rat subthalamic neurones in vitro. J Physiol 525(Pt 2):331–341.
- Shen KZ, Johnson SW (2005) Dopamine depletion alters responses to glutamate and GABA in the rat subthalamic nucleus. Neuroreport 16:171–174.
- Shin RM, Masuda M, Miura M, Sano H, Shirasawa T, Song WJ, Kobayashi K, Aosaki T (2003) Dopamine D4 receptor-induced postsynaptic inhibition of GABAergic currents in mouse globus pallidus neurons. J Neurosci 23:11662–11672.
- Shink E, Bevan MD, Bolam JP, Smith Y (1996) The subthalamic nucleus and the external pallidum: two tightly interconnected structures that control the output of the basal ganglia in the monkey. Neuroscience 73:335–357.
- Slaght SJ, Paz T, Chavez M, Deniau JM, Mahon S, Charpier S (2004) On the activity of the corticostriatal networks during spike-and-wave discharges in a genetic model of absence epilepsy. J Neurosci 24:6816–6825.
- Smith Y, Bolam JP, Von Krosigk M (1990) Topographical and Synaptic Organization of the GABA-Containing Pallidosubthalamic Projection in the Rat. Eur J Neurosci 2:500–511.
- Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86:353–387.
- Song WJ, Baba Y, Otsuka T, Murakami F (2000) Characterization of Ca(2+) channels in rat subthalamic nucleus neurons. J Neurophysiol 84:2630–2637.
- Swadlow HA (2003) Fast-spike interneurons and feedforward inhibition in awake sensory neocortex. Cereb Cortex 13:25–32.
- Thomson AM, Bannister AP (2003) Interlaminar connections in the neocortex. Cereb Cortex 13:5–14.
- Urbain N, Gervasoni D, Souliere F, et al. (2000) Unrelated course of subthalamic nucleus and globus pallidus neuronal activities across vigilance states in the rat. Eur J Neurosci 12:3361–3374.
- Vercueil L, Benazzouz A, Deransart C, Bressand K, Marescaux C, Depaulis A, Benabid AL (1998) High-frequency stimulation of the subthalamic nucleus suppresses absence seizures in the rat: comparison with neurotoxic lesions. Epilepsy Res 31:39–46.

# Neurophysiology of Substantia Nigra Dopamine Neurons: Modulation by GABA

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#### I. INTRODUCTION

It has been more than three decades now since B.S. Bunney, G.K. Aghajanian and their colleagues at Yale published the first electrophysiological studies of midbrain dopamine (DA) neurons in vivo (Bunney et al., 1973a,b). These extracellular single unit recordings revealed that DA neurons in the substantia nigra (SN) pars compacta (SNc) and ventral tegmental area (VTA) of chloral hydrate-anesthetized rats fired spontaneously at low rates with occasional slow bursts. In addition, these investigators showed that DA neurons were powerfully inhibited by intravenous administration of amphetamine or apomorphine, effects that were completely reversed by administration of antipsychotic drugs. Moreover, the firing of DA neurons was also suppressed by iontophoretic application of DA directly to DA neurons themselves, suggesting the presence of "presynaptic" receptors for dopamine, now known as somatodendritic autoreceptors, on the DA neurons themselves. Subsequent studies revealed that the source of the DA that activated these autoreceptors was the dendrites of the DA neurons themselves (Groves et al., 1975; Geffen et al., 1976; Paden et al., 1976), and that these dendrites made synapses with other DA dendrites (Wilson et al., 1977a) thereby creating a self-inhibitory network of DA neurons. These pioneering studies were prompted, in large part, by a desire to understand the sites and mechanisms of action of antipsychotic drugs and provided the impetus for a myriad of subsequent electrophysiological and anatomical experiments by laboratories all over the world.

Over the succeeding 30 years, it has become clear that mesencephalic DA neurons and the DA innervation of the forebrain play crucial roles not only in the execution of voluntary movement and as sites of action for antipsychotic drugs and stimulant drugs of abuse, but also as core components of neural systems regulating reward, reinforcement and addiction, as well as several types of higher cognitive function including various forms of associative learning (Schultz, 1997, 2007). The number of papers published on DA neurons exceeds 25,000 (PubMed, February 2009), far too many for any one review to even attempt to cover. This chapter will limit itself to a review of the basic anatomy of the SN and the morphological and electrophysiological characteristics of SN DA neurons, and the control of these neurons by GABAergic inputs. Other aspects of the afferent control of DA neurons have been reviewed elsewhere (Kitai et al., 1999; Diana and Tepper, 2002; Misgeld, 2004; Tepper and Lee, 2007; Lee and Tepper, 2009) (see also Chapters 23 and 31).

# II. NEUROCYTOLOGY OF NIGROSTRIATAL DOPAMINE NEURONS

Most of the cell bodies of origin of the *nigrostriatal* DA system are located in a densely packed, relatively thin shell,  $300-500 \mu$ m thick, the SNc (A9 in the terminology of Dahlstrom and Fuxe, 1964), dorsal to the larger and more diffuse substantia nigra pars reticulata (SNr) that comprises predominantly GABA projection neurons (Lee and Tepper, 2007). There are approximately 25,000 DA neurons bilaterally in the rat SN (Oorschot, 1996; Nair-Roberts et al., 2008). Smaller numbers of striatally projecting neurons are also found in the adjacent retrorubral field (A8) as well as in isolated patches in the SNr (Deutch et al., 1986). It is worth pointing out that many "nigrostriatal" DA neurons have been shown by retrograde labeling to collateralize to multiple regions including the cingulate and the prefrontal cortices (Fallon, 1981; Takada and Hattori, 1986).

Nigral DA neurons have been divided into dorsal and ventral tier groups (Fallon and Moore, 1978) (see also Chapter 1). The dorsal tier neurons express calbindin whereas the ventral tier neurons do not (Gerfen et al., 1987a,b; but see also Neuhoff et al., 2002). It has been argued on the basis of retrograde tracing that the dorsal tier neurons preferentially innervate the striatal matrix compartment whereas the ventral tier neurons innervate the striosome/patch compartment (Gerfen et al., 1987a,b). However, a more recent study using a novel anterograde tracing technique shows quite clearly that single nigrostriatal neurons innervate both patch and matrix compartments (Matsuda et al., 2009). Similarly, it has been claimed that the dorsal tier neurons have dendrites oriented principally mediolaterally in pars compacta whereas the ventral tier neurons extend dendrites ventrally into the pars reticulata (Fallon et al.,

1978). However, subsequent intracellular labeling studies suggest that many or most nigrostriatal neurons have dendrites that arborize within pars compacta as well as one or two ventrally directed dendrites (Kita et al., 1986; Tepper et al., 1987; Grace and Onn, 1989; cf Fig. 16.1).

In rats, nigral DA neurons are medium to large sized, 12-25 µm in diameter and exhibit multipolar, fusiform or polygonal somata that emit three to five thick, smooth dendrites that taper rapidly to about 1 µm or less in diameter. Dendrites are aspiny, but occasionally emit sparse thorn- or spine-like appendages. There are usually one or two ventrally directed dendrites that course through SNr perpendicular to the surface of the SNc. These are often the largest and longest dendrites issued by the neuron, can exceed 1 mm in length and extend throughout the entire dorsoventral extent of the SNr where they often form dendritic fascicles (Juraska et al., 1977; Kita et al., 1986; Tepper et al., 1994; 1987; cf Fig. 16.1). Most of the dorsal dendrites are shorter than the ventrally directed dendrites but are similar in other respects, and arborize in, and sometime extend beyond SNc, in all directions.

The earliest morphological descriptions of DA neurons from histofluorescence material stressed the varicose nature of their dendrites and the possible implications of the varicosities for dendritic DA release (e.g., Bjorklund and Lindvall, 1975). However, material from intracellular labeling with HRP, biocytin or Lucifer Yellow (Kita et al., 1986; Tepper et al., 1987, Grace and Onn 1989; Yung et al., 1991), or immunocytochemistry (Tepper et al., 1994) shows that most of the dendrites from DA neurons in mature animals are smooth, with some varicosities in the finer higher order dendrites. The previously observed varicosities were probably attributable to areas of aggregation of histofluorescent material rather than changes in dendritic caliber (Tepper et al., 1987).

It is sometimes claimed that the axon most commonly emerges from a dendrite at a relatively great distance from the soma (up to 240  $\mu$ m; Hausser et al., 1995), but this observation may have arisen from a selection artifact for the largest neurons in vitro where dendritic recording is easiest, since observations from several other studies indicate that the axon typically emerges from the soma or a proximal dendrite, usually within 30  $\mu$ m of the soma (Grace and Bunney, 1983a; Tepper et al., 1987; Grace and Onn, 1989; Matsuda et al., 2009; cf Fig. 16.1).

In marked contrast to the projection neurons of virtually all other basal ganglia nuclei, DA neurons of the SN do not emit local axon collaterals (Juraska et al., 1977;



FIGURE 16.1 Neuroanatomical organization of substantia nigra and neurocytology of nigral DA neurons. A. Coronal section through rat midbrain immunostained for tyrosine hydroxylase, illustrating the densely packed DA neurons of the substantia nigra pars compacta (SNc) and the adjacent ventral tegmental area (VTA). Note the numerous DA fibers that penetrate deep into the pars reticulata (SNr). Substantia nigra is bordered ventrally by the cerebral peduncles or crus cerebri (cc). B. Higher magnification micrograph of the area within the box in A illustrating the fasciculation of some of the ventral dendrites, coursing perpendicular to the surface of the SNc. Note that the dendrites are for the most part non-varicose. C. Photomontage of an electrophysiologically identified DA neuron in the pars compacta (SNc) of the substantia nigra filled with biocytin after whole cell recording in vitro. The dorsal dendrites arborize mostly within pars compacta and the neuron extends one thick, smooth and unbranched dendrite several hundred microns ventral through pars reticulata (SNr). D. A photomicrograph of an electrophysiologically identified DA neuron in substantia nigra in a 350 µm coronal section stained with biocytin following in vitro whole cell recording. The axon (large arrow) could be followed (small arrows) as it coursed medially for several hundred microns in this single optical plane. Inset shows the axon emerging from a short, thick proximal dendrite approximately 25 µm from the center of the soma. Source: 16.1C modified from Iribe et al. (1999); used with permission.

Tepper et al., 1987; Matsuda et al., 2009). After emerging from the cell and exhibiting an often initially tortuous and recurving trajectory, the axons course medially and rostral to SN, coalesce into a tract often referred to as the *medial forebrain bundle* that traverses the fields of Forel and projects into the forebrain. As they ascend, the axons arborize sparsely in the subthalamic nucleus (STN; Cragg et al., 2004), and then continue rostral and anterior, fanning out laterally through the globus pallidus (GP) where they form a small arborization (Lindvall and Bjorklund, 1979; Matsuda et al., 2009) before reaching their principal target, the striatum. In the striatum the axons from single cells branch profusely and form large, dense arborizations of varicose processes that occupy an average volume of approximately 0.5 mm<sup>3</sup> (Matsuda et al., 2009). Nigrostriatal axons form Gray's Type II symmetrical synapses, mainly on the dendrites or the necks of the dendritic spines of the striatal spiny projection neurons. Interestingly, although some of the DA synapses are made by terminal boutons or en passant varicosities, many of the synapses very small and are formed by thinner intervaricose segments of the axons (Pickel et al., 1981; Freund et al., 1984; Groves et al., 1994). These are easy to miss in single electron microscopic thin sections, especially if one is concentrating on varicosities, and almost certainly have contributed to the confusion about whether DA terminals actually form typical morphologically defined synapses in the striatum or not (see, e.g., Groves et al., 1994; Descarries et al., 1996 for discussion).

### III. ELECTROPHYSIOLOGICAL PROPERTIES OF NIGROSTRIATAL DOPAMINE NEURONS

### A. Extracellular Recordings

In in vivo extracellular recordings from anesthetized adult rats or mice, nigral DA neurons fire spontaneously between approximately 2–8 spikes/sec, with a mean firing rate around 4 spikes/sec (Bunney et al., 1973a,b). Estimates of the proportion of mesencephalic DA neurons that are spontaneously active in vivo in anesthetized rats vary widely, with some authors claiming that up to 50% of the neurons are normally silent (e.g., Chiodo, 1988; Floresco et al., 2003) to others who claim that the large majority of the neurons are spontaneously active under normal conditions (e.g., Dai and Tepper, 1998). Spontaneous action potentials are unusually wide, between 2.5 and 4 ms long in duration depending on filter settings and electrode characteristics (Bunney et al., 1973a,b).

Nigrostriatal DA neurons are readily identified by antidromic activation following stimulation of the striatum, GP or medial forebrain bundle. Like other monoaminergic neurons nigrostriatal DA neurons exhibit slow conduction velocities in the range of 0.4–0.5 m/sec. Antidromic responses of nigrostriatal DA neurons usually consist of a small spike, termed the initial segment (IS) spike, even when antidromically activated at low rates. Very often when the antidromic response is a "full" spike consisting of both the IS and the somatodendritic (SD) spike, there is a marked delay between the IS and SD components causing a notch in the initial positive part of the extracellularly recorded waveform. The same IS-SD break is often observed in spontaneous action potentials as well (Bunney et al., 1973a,b; Guyenet and Aghajanian, 1978; Deniau et al., 1978; Tepper et al., 1984a,b, 1984a,b; Trent and Tepper, 1991; cf Fig. 16.2).

DA neurons recorded in vivo in anesthetized rodents exhibit three distinct modes or patterns of firing that are clearly distinguishable from inspection of their autocorrelation histograms (Tepper et al., 1995). The most common pattern of activity is a random, or irregular mode of firing, characterized by an initial prolonged trough in the autocorrelation function representing a long post-firing inhibition. The next most common firing pattern is a regular, pacemaker-like firing, characterized by constant interspike



FIGURE 16.2 Basic electrophysiological characteristics of rodent nigral DA neurons. A. In vivo intracellular recording of an antidromically identified nigrostriatal neuron in a urethane-anesthetized rat. Note long, slow spike afterhyperpolarization and regular spontaneous firing pattern. The 4th spike in the train misses, revealing the underlying LVA  $Ca^{2+}$  membrane potential oscillation that drives spontaneous pacemaker spiking. B. Extracellularly recorded antidromic responses of a nigrostriatal neuron in a urethane-anesthetized rat. The asterisk marks a collision extinction in the 3rd sweep. Antidromic responses consist almost exclusively of the IS spike except for the red trace in the 4th sweep that is a full IS-SD spike. The arrow points to the IS-SD break. C. Extracellularly recorded action potentials from two different nigrostriatal neurons in a chloral hydrate-anesthetized mouse illustrate the typical long duration spike and IS-SD break (arrow). Each spike is the overlay of five spikes averaged from 10 consecutive spontaneous action potentials. D. Distribution of spontaneous firing rates of nigral DA neurons in a chloral hydrate-anesthetized rat. E. Extracellularly recoded spontaneous spike trains recorded from three different antidromically identified nigrostriatal neurons in chloral hydrate-anesthetized mice illustrating the three different patterns of spontaneous activity seen in vivo. F. Autocorrelograms generated from spike trains (insets) of three different nigrostriatal neurons illustrating the distinct histogram shapes that characterize the three firing patterns in urethane-anesthetized rats. G. In vitro whole cell recordings from SNc DA neurons in mice. G1. Hyperpolarizing current injections result in a slowly developing sag in the voltage response due to activation of I<sub>h</sub>, that results in a time dependent inward rectification that reduces the input resistance of the neuron by about 67% (G2). G3. Depolarizing current injection in a DA neuron hyperpolarized to  $-75 \,\text{mV}$  results in an LTS and subsequent HVA Ca<sup>2+</sup> spike as well as a rebound LTS. G4. Relaxation following hyperpolarizing current injections of varying durations show that the rebound spike is all-or-none. G5. Addition of a low concentration of Ni<sup>2+</sup> blocks the rebound slow spike identifying it as an LTS. Source C: redrawn from Brazhnik et al. (2008). Copyright 2008 by the Society for Neuroscience; D: reprinted from Dai and Tepper (1998), used with permission; F: redrawn from Tepper et al. (1995). Copyright 1995 by the Society for Neuroscience. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

intervals, a low coefficient of variation, and a lack of bursting. The third and least common mode (but the one that has generated the most interest) is burst firing, characterized by stereotyped bursts of 2–8 action potentials in which the first intraburst interspike interval is typically around 60 ms, followed by progressively increasing interspike intervals and progressively decreasing spike amplitudes (Grace and Bunney, 1984a,b; Tepper et al., 1995; Paladini and Tepper, 1999; Brazhnik et al., 2008; cf Fig. 16.2). The maximal instantaneous intraburst firing rate in anesthetized rodents is typically in the range of 12–15 Hz (Grace and Bunney, 1984b; Tepper et al., 1990) although significantly higher maximal intraburst firing rates have been observed in unanesthetized behaving rats (e.g., Kiyatkin and Rebec, 1998; Hyland et al., 2002).

The same three patterns of activity are observed in unanesthetized and immobilized (Wilson et al., 1977b) and/or freely moving rats (Freeman et al., 1985; Diana et al., 1989; Hyland et al., 2002) although in general a higher proportion of nigrostriatal neurons in vivo exhibit burst firing whereas a lower proportion exhibit pacemakerlike firing in unanesthetized preparations. Single DA neurons can spontaneously change firing patterns, or be induced to change by various experimental manipulations and SN DA neuron firing patterns can best be thought of as a existing along a continuum, with the pacemaker-like firing on one end and bursty firing on the other (Tepper et al., 1995; Paladini and Tepper, 1999; Celada et al., 1999; Lee et al., 2004).

The mechanisms controlling the firing patterns are of great interest to basal ganglia researchers for many reasons. Different firing patterns could lead to qualitatively different effects with respect to dendritic release of DA in SN (Bjorklund and Lindvall, 1975; Groves et al., 1975; Cheramy et al., 1981) and/or release of DA in striatum. Experimentally induced burst firing (Suaud-Chagny et al., 1992; Lee et al., 2004) or electrical stimulation of the medial forebrain bundle that mimics burst firing (e.g., Gonon and Buda, 1985, Gonon, 1988, Bean and Roth, 1991, Manley et al., 1992; Chergui et al., 1994) leads to increased extracellular DA levels in striatum and/or cortex compared to pacemaker-like firing. This results from saturation of the DA transporter that is responsible for regulating extracellular DA levels (Chergui et al., 1994; Miller and Abercrombie, 1999; but see also Rice and Cragg, 2008) rather than from increased release per pulse. Higher extracellular levels of DA could lead to qualitatively different effects than lower levels if, for example,

a significant fraction of striatal  $D_1$  receptors were located predominantly extra- or perisynaptically (Caille et al., 1996; Gonon, 1997). Under most conditions, firing rate and pattern appear to regulate somatodendritic and axon terminal DA release in parallel. Under some conditions however, IS and/or axonal and SD activity become dissociated (e.g., Grace, 1990, Trent and Tepper, 1991) leading to independent regulation of DA release in SN and axon terminal regions (Cobb and Abercrombie, 2003). Perhaps most importantly, a number of studies have shown that DA neurons respond to reward, or stimuli that predict reward by firing a short burst (e.g., Schultz, 1997, 2007).

#### **B. Intracellular Recordings**

There have only been a handful of in vivo intracellular recording studies of nigral DA neurons due to substantial technical challenges including the depth of the substantia nigra, the need to traverse several heavily myelinated regions, the anatomical organization of the SNc, and the responses of the neurons to intracellular penetration. As such, these recordings, mostly obtained by Grace and Bunney in the early to mid 1980s, represented a technical tour de force and were extremely valuable. The results from their earlier recordings confirm those from the earliest extracellular recordings described above. In addition they revealed that nigral DA neurons exhibit a prolonged spike after hyperpolarization and a slowly developing depolarizing sag in the membrane potential in response to strong hyperpolarizing current injections, identified the presumed IS spike seen in extracellular recordings, and showed the first intracellularly recorded synaptic responses in DA neurons (Grace and Bunney, 1983a,b; 1984a,b; 1985; Tepper et al., 1987).

Virtually all subsequent intracellular recordings of DA neurons have been obtained in vitro, first with sharp electrodes (Kita et al., 1986; Grace, 1990; 1991) and more recently with whole cell recordings. These have confirmed and extended the in vivo recordings and showed that the sag in nigrostriatal neurons is due to activation of a hyperpolarization activated cation channel (HCN) that mediates the depolarizing current,  $I_h$ . The prolonged spike afterhyperpolarization is due to activation of the apamin-sensitive calcium-activated K<sup>+</sup> channel, SK (Shepard and Bunney, 1991). Finally these neurons express a variety of calcium channels enabling both low and high threshold Ca<sup>2+</sup> spikes as well as a slow oscillatory potential that drives rhythmic single spiking in vitro and probably in vivo as well (Grace, 1991; Kang and Kitai, 1993a,b; Nedergaard et al., 1993;

Galarraga and Bargas, 1995; Wilson and Callaway, 2000). Nigrostriatal neurons were also shown to possess somatodendritic DA  $D_2$  autoreceptors that hyperpolarize the neuron by opening an inwardly rectifying K<sup>+</sup> (GIRK) channel (Lacey et al., 1987, 1989).

### IV. NEUROANATOMY OF GABA AFFERENTS TO NIGRAL DOPAMINE NEURONS

Most of the afferents to the SN are GABAergic and at least 70% of the synapses formed on nigral DA neurons are GABAergic (Rinvik and Grofova, 1970; Gulley and Smithberg, 1971; Ribak et al., 1976; Bolam and Smith, 1990). The SN is rich in both  $GABA_A$  and  $GABA_B$ receptors. These display regional segregation, with in situ hybridization and immunostaining showing that the GABAB<sub>R1</sub> and GABAB<sub>R2</sub> subunits (Charara et al., 2000) are expressed at significantly greater abundance in nigral DA neurons than in SNr GABA neurons, or in any other basal ganglia nucleus. Conversely, mRNA levels (Lu et al., 1999) and immunostaining for virtually all GABA<sub>A</sub> receptor subunits, particularly  $\alpha_1$  and  $\alpha_2$  subunits, are greater in the SNr than in the SNc. Most or all of the GABAA subunit immunostaining is constrained to postsynaptic specializations of symmetric synapses (Fujiyama et al., 2000), whereas GABA<sub>B</sub> subunits label both presynaptic terminals, where they serve as inhibitory GABA autoreceptors, as well as dendrites (for review, see Boyes and Bolam, 2007). Interestingly, a large proportion of the postsynaptic GABA<sub>B</sub> subunits appear to be located extrasynaptically (Boyes and Bolam, 2003). Although nigrostriatal DA and SNr GABA neurons express both GABA<sub>A</sub> and GABA<sub>B</sub> receptors, the responses of nigral neurons to GABA released from afferents is complex and varies depending on the nature of the afferent stimulation (Celada et al., 1999; Lee et al., 2004; cf Fig. 16.4).

The densest and best-characterized GABAergic inputs arise from the spiny projection neurons of the striatum (Grofová and Rinvik, 1970; Grofová, 1975; Hattori et al., 1973a,b; Somogyi et al., 1981; Bolam and Smith, 1990) and the GP (Hattori et al., 1975; Smith and Bolam, 1990). Striatonigral efferents colocalize substance P and dynorphin and arise from both the patch and matrix compartments (Gerfen and Young, 1988), which preferentially or selectively innervate the SNc and SNr respectively (Gerfen, 1984). However, DA and GABA dendrites overlap extensively in SNr, and the striatal inputs to DA neurons terminate mostly on distal dendrites (Bolam and Smith, 1990), so the degree of segregation of patch inputs to nigrostriatal neurons and matrix inputs to GABA neurons is probably not as clear-cut as is often assumed.

Striatal inputs to SN form symmetric Gray's Type II synapses (Grofova and Rinvik, 1970) and, as mentioned above, target the more distal dendritic regions of nigrostriatal and SNr neurons making only a relatively small proportion of synapses onto DA cell bodies (Bolam and Smith, 1990). This anatomical arrangement suggests that individual striatonigral neurons probably do not exert powerful inhibitory effects on nigrostriatal neurons and therefore simultaneous activation of a number of striatonigral neurons would be required to produce a substantial effect on a postsynaptic nigrostriatal neuron. Given the phasic and episodic nature of the spontaneous activity of striatal spiny projection neurons, and their very low overall mean firing rate (Wilson, 1993), it is unlikely that the striatum provides a significant GABA afferent tone to the nigrostriatal neurons.

Pallidal inputs to SN are also dense, with single GP GABA projection neurons often forming terminal arborizations in both SNc and SNr and innervating both DA and GABA neurons (Grofová 1975; Hattori et al., 1975; Totterdell et al., 1984; Smith and Bolam, 1989, 1990; Bevan et al., 1998). Pallidal boutons tend to be larger than striatal boutons, and form symmetric synapses on the cell bodies and proximal dendrites of nigrostriatal neurons, sometimes forming multiple repeated contacts and/or pericellular baskets around somata (Smith and Bolam, 1990). GP neurons typically fire spontaneously at 50-80 spikes/second in vivo in anesthetized rats (Celada et al., 1999) and can exceed 200 spikes/second (Kita, 2007). In contrast to the striatal inputs, given the electrotonically favored location of their synaptic inputs and high tonic firing rate, GP is likely a main contributor to the significant GABA tone that exists in SN in vivo (see below).

A third source of GABAergic inputs to nigrostriatal neurons arises locally (Grace and Bunney, 1979, 1985; Nitsch and Riesenberg, 1988) and is comprised of the local axon collaterals of the SNr GABA projection neurons (Tepper et al., 1995). These neurons emit a large diameter axon that issues several locally arborizing collaterals that exhibit varicosities en passant as well as terminal varicosities in SNr and SNc (Deniau et al., 1982; Grofova et al., 1982; Mailly et al., 2003; Lee and Tepper, 2009) before ascending to their principal sites of termination in the thalamus and tectum. Electron microscopic analyses reveal that

the varicosities are large boutons that form symmetric synapses with the somata and proximal dendrites of DA neurons in SNc and SNr. Individual collateral branches often form proximal multiple en passant synapses or pericellular baskets (Tepper et al., 2003; Lee and Tepper, 2007), similar to those formed by pallidal terminals (Smith and Bolam, 1990). SNr projection neurons typically fire spontaneously around 15–30 spikes/second in vivo and can exceed 100 spikes/second (Deniau et al., 2007). The SNr collateral input provides a crucial source of GABA to nigrostriatal neurons and plays a particularly important role in the modulation of their firing pattern and response to excitatory and inhibitory afferent inputs as described below.

#### V. NEUROPHYSIOLOGY OF GABA AFFERENTS

#### A. Responses to Striatal Stimulation

Both DA and GABA SN neurons in vivo respond to ipsilateral striatal stimulation with monosynaptic IPSPs (Yoshida and Precht, 1971; Grace and Bunney, 1983a, 1985) that lead to inhibition of spontaneous activity (Collingridge and Davies, 1981; Tepper et al., 1990). The latency to the onset of striatal-evoked inhibition is relatively long, (in rats and mice exceeding 10 ms; Tepper et al., 1990; Paladini et al., 1999a; Brazhnik et al., 2008), consistent with the relatively slow conduction velocity and long latency antidromic responses of striatonigral neurons ( $\sim$ 10 ms) following nigral stimulation in rats (Ryan et al., 1986).

Interestingly, some nigrostriatal neurons respond to weak striatal stimulation with an increase in firing rather than a decrease (Collingridge and Davies 1981; Grace and Bunney, 1985). This is due to a preferential inhibition of the GABA SNr projection neurons by the weaker striatal stimuli. This occurs because DA neurons are considerably less sensitive to GABAA receptor activation than the SNr GABA neurons (Grace and Bunney, 1979, 1985; Waszczak et al., 1980, 1981; Collingridge and Davies 1981; Tepper et al., 1986; Gulacsi et al., 2003) and thus there is little or no monosynaptic inhibition in the nigrostriatal neurons to the weaker stimulation whereas the SNr neurons are potently inhibited. Because of this difference in sensitivity to GABA<sub>A</sub> receptor activation (see below) coupled with the fact that the SNr GABA projection neurons are tonically active at a high rate and innervate the nigrostriatal neurons at proximal locations, the end result of weak striatal stimulation is a disinhibition of the nigrostriatal neuron from the tonic SNr input. This disinhibition is a key factor in the functioning of nigrostriatal neurons and their response to many afferent inputs, discussed at greater length below.

#### **B.** Responses to Pallidal Stimulation

Nigrostriatal neurons respond to stimulation of ipsilateral GP with a short latency monosynaptic IPSPs (Tepper et al., 1986) and inhibition of spontaneous activity (Paladini et al., 1999), consistent with the anatomical findings. However, if the GP is stimulated chemically by local infusion of bicuculline (which increases the mean firing rate of GP neurons by 55%), nigrostriatal neurons respond with a modest but statistically significant *increase* in firing rate but a dramatic increase in burst firing (Celada et al, 1999; Lee et al., 2004; cf Fig. 16.4). The opposite occurs if muscimol is infused into GP. Under these conditions GP activity is almost completely suppressed and the nigrostriatal neurons respond with a modest decrease in firing rate and a shift away from bursty or random firing to a pacemaker-like firing pattern (Celada et al., 1999).

These effects are clearly opposite to what one expects from excitation or inhibition of a monosynaptic inhibitory input. The explanation for these seemingly incompatible results is the same as that for striatal-induced excitation of nigrostriatal neurons and depends again on the different sensitivities of DA and SNr GABA neurons to stimulation of GABA<sub>A</sub> receptors. When GP neurons are activated by an electrical stimulus, all the neurons within the field of the stimulating electrode are depolarized and induced to fire simultaneously. This causes a massive and nearly synchronous release of GABA in the SN a few ms later. The synchronous nature of electrically stimulated release probably leads to greater extracellular levels of GABA than those that result from the chemical stimulation of the GP which, although exciting large numbers of GP neurons, does so in an asynchronous manner. Thus, the extracellular levels of GABA that are controlled almost exclusively by diffusion and uptake into presynaptic terminals and glia (Schousboe and Waagepetersen, 2007; Kirmse et al., 2008) are likely to be lower following chemical stimulation of GP where the asynchronous release allows the uptake mechanisms to clear the released GABA more efficiently than after electrical stimulation when all of the GABA is released simultaneously putting a much greater load on the uptake system. This leads to the situation where following chemical stimulation, there is a much greater inhibitory

response from the SNr projection neurons than from the less sensitive DA neurons, resulting in a selective inhibition of the SNr GABA neurons and a consequent disinhibition of the DA neurons. Conversely, following electrical stimulation of GP, the synchronous release of GABA is sufficient to inhibit both the nigrostriatal neurons as well as the SNr GABA neurons and so the monosynaptic inhibition of the DA neurons becomes apparent, although it is no doubt opposed by simultaneous disinhibition from the SNr neurons.

Evidence in support of this hypothesis comes from in vivo recordings of SNr GABA projection neurons following infusions of muscimol or bicuculline into GP. Whereas such infusions cause modest disinhibition and inhibition of nigrostriatal neuron firing rates, respectively, in antidromically identified nigrothalamic neurons such infusions lead to greater than a doubling or a complete cessation of spontaneous activity, respectively (Celada et al., 1999; Lee et al., 2004).

This is almost certainly the same mechanism that is responsible for the paradoxical excitatory effects of locally or systemically administered muscimol on nigrostriatal neuron activity (MacNeil et al., 1978; Walters and Lakoski, 1978; Grace and Bunney, 1979) and striatal DA release (Martin and Haubrich, 1978; Santiago and Westerink, 1992), or the excitatory effects of  $\mu$  opioids on DA neurons that lack  $\mu$  receptors (Lacey et al., 1989) and illustrates the crucial role that the SNr neurons play in the responses of nigrostriatal DA neurons to many different drugs and afferent inputs.

A similar sort of "paradoxical" response occurs in nigral DA neurons to activation of an extra-basal ganglia afferent, the lateral habenula. Stimulation of the lateral habenula produces potent inhibition in nigral DA neurons in rodent and monkey whereas lesions of this area lead to increased forebrain DA release (Ji and Shepard, 2007; Matsumoto and Hikosaka, 2007; Hikosaka et al., 2008). The paradox arises from the fact that the output of the lateral habenula appears to be glutamatergic and would be expected to produce excitation of nigral DA neurons. There are a number of possible explanations for this, but a disynaptic loop involving lateral habenular projections to SNr projection neurons and feed forward inhibition of nigrostriatal neurons remains a distinct possibility.

As one final example of the complexity of the interactions of nigral afferents with intranigral microcircuitry, it has been reported that chemical or electrical stimulation of the STN produces excitation and burst firing in nigrostriatal DA neurons in vivo (Smith and Grace, 1992; Chergui et al., 1994). This should not be surprising since the STN is a glutamatergic nucleus and sends a monosynaptic projection to the SN (Hammond et al., 1978). Remarkably, however, in both of the experiments just referred to, the predominant *initial* response to chemical or electrical stimulation of STN was either no effect or inhibition of nigral neurons. Excitation and/or burst firing were only seen in a minority of the neurons. Once again, this was almost certainly due to preferential activation of pars reticulata GABA projection neurons by the subthalamic input, since 90% of the synapses made by subthalamic afferents synapse onto GABA dendrites in SNr and only about 10% synapse directly onto DA dendrites (Lee and Tepper, 2009). Subsequent in vitro intracellular recordings showed that nigral DA neurons respond to STN stimulation with a depolarizing synaptic potential (DPSP) that exhibits a reversal potential around  $-38 \,\mathrm{mV}$ , a value very close to spike threshold (Iribe et al., 1999). Pharmacological dissection of the DPSP revealed it to be the result of near-simultaneous activation of a monosynaptic glutamatergic input with a reversal potential near 0mV, presumably originating from STN, and a GABA<sub>A</sub> IPSP. The IPSP resulting from electrical stimulation of STN could have come from inadvertent activation of descending GABA fibers from striatum or GP, but this was ruled out when the IPSP survived knife cuts just anterior to STN several days before the recordings, and by the demonstration that blocking the glutamatergic input pharmacologically completely eliminated the IPSP (Iribe et al., 1999). These data showed that chemical or electrical stimulation of STN simultaneous activates a monosynaptic EPSP and a disynaptic GABA<sub>A</sub>-mediated IPSP from the SNr projection neurons that together produce mixed, but initially inhibitory effects on DA neurons, as first reported by Robledo and colleagues (Robledo et al., 1988; Robledo and Feger, 1990). It also cannot be ruled out that the STN-elicited burst firing of nigrostriatal neurons is meditated in part through a monosynaptic excitation of GP neurons that then preferentially inhibit SNr neurons and produce bursting in nigrostriatal neurons via disinhibition just as chemical stimulation of the GP does (Celada et al., 1999; Lee et al., 2004).

Thus, under many conditions, the responses of nigrostriatal neurons to inhibitory and excitatory afferents are filtered by parallel innervation of the SNr GABA projection neurons, whose powerful inhibitory or disinhibitory effects on nigrostriatal neurons are a major factor in the response of the nigrostriatal neurons to afferent input.



FIGURE 16.3 Inhibitory responses of nigral DA neurons following stimulation of GABAergic afferents. A. Raw spike trains recorded extracellularly illustrating responses to single pulse electrical stimulation of A1, dorsolateral striatum, A2, GP, and A3, ventromedial thalamus (which antidromically activates SNr GABA projection neurons) in mice. Each trace consists of the overlay of 25-37 consecutive sweeps at 0.67 Hz. Note the slow onset and long duration of the striatal-evoked inhibition compared to the rapid onset and shorter duration following activation of GP and SNr afferents. B. Typical PSTHs showing thalamic (SNr) elicited inhibition (B1) is eliminated when the recording pipette contains the GABA<sub>A</sub> selective antagonist, bicuculline (BIC) (B2). C. Inhibition elicited by single pulse GP stimulation (C1) is completely blocked by pressure application of BIC using multibarrel pipettes (C2). After recovery from BIC (C3), subsequent pressure application of the GABA<sub>B</sub>-selective antagonist, CGP55845A (CGP) not only fails to block inhibition, but actually produces a slight augmentation and prolongation of the inhibition as well as a slight reduction in spontaneous firing rate (see text for explanation). D. PSTHs showing pharmacologically distinguishable early and late components of the inhibitory response of a nigral DA neuron to single pulse striatal stimulation in mice. D1. Control stimulation (660 µA pulse delivered at 0 msec) elicits a long duration (>200 ms) inhibition with a delayed onset. D2. Pressure application of CGP eliminates the late component of the inhibition but not the early component that is augmented by blockade of presynaptic GABA<sub>B</sub> autoreceptors. D3. Subsequent simultaneous application of CGP plus the GABA<sub>A</sub> antagonist, picrotoxin (PTX) eliminates both components of the evoked inhibition except for the small post-excitation inhibition following the PTX-induced increased firing. E. Same as D for a different cell but the order of drug application is reversed. E1. Striatal train stimulation elicits stronger inhibition than the single pulse stimulation in D1. E2. Application of PTX eliminates the early part of the inhibition but has no effect on the later portion. E3. Subsequent simultaneous application of PTX and CGP eliminates all inhibition. Note presence of similar excitatory response to stimulation in the presence of PTX as seen in D3. (See Paladini et al. (1999a) for explanation.) F. Simultaneous pre- and postsynaptic GABA<sub>B</sub> effects. F1. Striatal train stimulation elicits only a minimal early inhibition but a significant late inhibition (blue line). F2. Following CGP application, the same stimuli now evoke a clear early inhibition (red arrows) and the late inhibition is substantially attenuated. G. Facilitation of GABAergic inhibition is blocked by GABA<sub>A</sub> antagonists in mice. G1. GP train stimulation evokes inhibition with a weak early and strong late component. G2. Local CGP application greatly strengthens the early inhibition while completely eliminating the late inhibition. G3. Subsequent application of CGP and PTX eliminates all inhibitory responses. H. Presynaptic effects of GABA<sub>B</sub> antagonists. H1. Control PSTH with single pulse thalamic stimulation set to subthreshold current elicits no response. H2. Local application of CGP unmasks a clear inhibitory response to the identical stimulus. Note that there is a slight decrease background firing rate H3. Subsequent simultaneous application of the GABA<sub>B</sub> antagonist and the GABA<sub>A</sub> antagonist, bicuculline, eliminates the unmasked inhibition. I. Blocking GABA uptake greatly enhances the late component of the evoked inhibition. II. Single pulse striatal stimulation elicits a strong early inhibition and a weaker late inhibition. I2. Local application of the selective GABA uptake inhibitor, nipecotic acid (UPT), selectively augments the late inhibition. I3. Subsequent application of CGP and UPT completely blocks the late component of the inhibition but not the early component. Data in A. D. G and I taken from extracellular recordings chloral-hydrate anesthetized mice. Data in B. C and H taken from extracellular recordings in urethane-anesthetized rats. Source B: redrawn from Tepper et al. (1995), Copyright 1995 by the Society for Neuroscience, C, H: redrawn from Paladini et al. (1999a), used with permission; D-G, I: redrawn from Brazhnik et al. (2008). Copyright 2008 by the Society for Neuroscience. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

#### C. Responses to SNr Stimulation

A reciprocal relation between the spontaneous activity of SNc DA neurons and an unidentified population of non-DA SNr neurons was first described by Grace and colleagues (Grace and Bunney, 1979, Grace et al., 1980) on the basis of simultaneous extracellular recordings. This was a landmark observation. Although Grace and Bunney (1979) were careful to consider several possibilities for the identity of the non-DA neuron, including the idea that it might be a SNr projection neuron, subsequent reports identified the SNr neuron as a unique "zona reticulata interneuron" located just ventral to the SNc on the basis of electrophysiological characteristics including lack of antidromic responding from thalamus, superior colliculus or striatum, an excitatory response to noxious stimuli and great sensitivity to GABA (Grace and Bunney, 1985; Bunney et al., 1991; Smith and Grace, 1992). From then on, the idea that there is a SNr GABA interneuron that engages in a feedforward inhibitory circuit with nigrostriatal DA neurons became firmly entrenched in the literature (e.g., Mereu and Gessa, 1985; Johnson and North, 1992; Santiago and Westerink, 1992; Zhang et al., 1992, 1993; Cameron and Williams, 1993, Yung and Hausser, 1993; Bontempi and Sharp, 1997).

There had been reports of presumed local circuit or interneurons in SNr based principally on Golgi studies (Gulley and Wood, 1971; Juraska et al., 1977; Schwyn and Fox, 1974; Francois et al., 1979), but for the most part these appeared to be smaller versions of the projection neurons whose size and dendritic orientation are known to vary with location in the SNr (Juraska et al., 1977). These neurons were not reported to be present in great abundance in SNr and there is little known about their afferent or efferent connectivities or even whether they truly represent a cell type distinct from the SNr projection neurons. There have also been reports of a small proportion of GABA neurons recorded in rostral SNc in vitro that were physiologically similar but not identical to SNr projection neurons (Yung et al., 1991), and a small population of GABA neurons in a restricted rostro-caudal region of the SNc has been shown to increase c-fos expression in response to DAergic stimulation (Hebb and Robertson, 2000). There are little or no data concerning the efferent connectivities, or function of these SNc neurons, but it is important to not overlook the potential importance of a neuronal population simply because it makes up a small proportion of the total cell number in a nucleus (Tepper et al., 2004, 2008; Tepper and Bolam, 2004).

However, it had been recognized for some time that SNr projection neurons emit axon collaterals, both in SNr as well as in SNc (Deniau et al., 1982; Grofova et al., 1982). Stimulation of the SNr in vitro produces IPSP/Cs in nigrostriatal neurons (Yung et al., 1991; Johnson and North, 1992; Hajós and Greenfield, 1993, 1994; Hausser and Yung, 1994) but it was impossible to determine if these arise from activation of a population of interneurons or by activation of SNr projection neurons that innervate DA neurons via their axon collaterals.

One approach to address this question in vivo was to record from nigrostriatal DA neurons while stimulating the ipsilateral ventral thalamus or superior colliculus (Tepper et al., 1995). Although there were no known monosynaptic projections from these nuclei to the nigrostriatal neurons, such stimuli would be expected to antidromically activate a population of SNr GABA projection neurons selectively without driving any putative GABA interneurons. Such stimuli would produce inhibition in nigrostriatal neurons if they were directly innervated by SNr collaterals, excitation if the nigrostriatal neurons were innervated by GABA interneurons that were themselves innervated by the SNr projection neurons, and no effect if there were no connection at all between the SNr projection neurons and the nigrostriatal neurons. The results were clear-cut, with thalamic or tectal stimulation producing strong inhibition of the nigrostriatal neurons at latencies only a little longer than the SNr neuron antidromic conduction times (Tepper et al., 1995; Paladini et al., 1999a; Brazhnik et al., 2008).

The SNr GABA axon collaterals were subsequently carefully mapped (Mailly et al, 2003), and electron microscopic evidence was provided for the existence of synapses formed by collaterals of SNr projection neurons onto nigrostriatal neurons (Tepper et al., 2002; Lee and Tepper, 2009). Results from a subsequent experiment where non-DA SNr neurons were recorded in vitro, stained with biocytin and reconstructed in order to compare their somatodendritic and axonal morphology with their electrophysiological characteristics revealed a morphologically and electrophysiologically homogenous population of neurons in SNr, differing only in co-expression of either parvalbumin or calretinin (Lee and Tepper, 2007). Thus the SNr GABAergic input to nigrostriatal DA neurons arises predominantly or exclusively from the axon collaterals of the SNr principal cells, which play a dual role as both projection neurons and the source of intranigral GABAergic inhibition (Deniau et al., 2007).

# D. Why are SNr Neurons so Much More Sensitive to GABA than Nigrostriatal Neurons?

As mentioned earlier, SNr GABA neurons exhibit apparently greater sensitivity to inhibition by GABA or  $GABA_A$ agonists than nigral DA neurons. There are several possible reasons. First, there is a differential distribution of GABA<sub>A</sub> subunits on DA and SNr GABA neurons (Boyes and Bolam, 2007), and different subunit combinations generate receptors with markedly different biophysical properties (for recent review see Goetz et al., 2007).

Second, there are markedly different chloride regulatory mechanisms in nigrostriatal and SNr neurons. SNr projection neurons express KCC2, the typical  $K^+$ -Cl<sup>-</sup> co-transporter found in most mature CNS neurons (Rivera et al., 1999) that is responsible for maintaining the  $[Cl^-]_i$  low enough so that GABA<sub>A</sub> IPSPs are hyperpolarizing (Farrant and Kaila, 2007). These IPSPs exhibit a reversal potential around - 71 mV measured with gramicidin perforated patch recordings in vitro. In contrast, DA neurons lack KCC2 and exhibit a significantly more depolarized GABA<sub>A</sub> IPSP reversal potential around - 63 mV (Gulácsi et al., 2003). Furthermore, the reversal potential in DA neurons, but not in SNr GABA neurons, is highly dependent on the presence of bicarbonate indicating that DA neurons depend on a less efficient Na<sup>+</sup>- dependent Cl<sup>-</sup>/bicarbonate exchanger (NDAE; see Farrant and Kaila, 2007 for review) in order to extrude  $[Cl^-]_i$  and be capable of generating a hyperpolarizing IPSP. Thus, GABA<sub>A</sub> receptor activation produces a significantly smaller hyperpolarization in DA neurons than in SNr GABA neurons, and this is likely to account, at least in part, for their relative insensitivity to GABA compared to SNr output neurons (Gulácsi et al., 2003).

The difference in sensitivity to GABA of the two nigral neuron types is at the heart of much of the circuit-level phenomena that occur in SN including the increase in burst firing in nigrostriatal neurons triggered by disinhibition from the SNr neurons following chemical stimulation of the GP described above.

### E. Pharmacology of GABAergic Synaptic Responses in Nigrostriatal Neurons In Vivo

In in vitro experiments, local stimulation in SN elicits biphasic IPSP/Cs in nigral DA neurons. The early component shows a rapid onset, relatively brief duration, exhibits a reversal potential near the Cl<sup>-</sup> equilibrium potential, and is blocked by selective  $GABA_A$  receptor antagonists, bicuculline or picrotoxin. The later component has a slower onset, a greatly increased duration, and is unaffected by  $GABA_A$  antagonists but is blocked by selective  $GABA_B$ receptor antagonists (Johnson and North, 1992; Cameron and Williams, 1993; Hajós and Greenfield, 1993, 1994; Hausser and Yung, 1994). The GABA<sub>B</sub> component is most consistently observed following stimulation with brief high frequency train stimuli (e.g., Hausser and Yung, 1991; Johnson and North, 1992; Saitoh et al., 2004).

However in vivo in rats, single pulse stimulation of striatum, GP or antidromic activation of SNr projection neurons with stimuli up to 1 mA produces inhibition of nigral DA neurons that is completely blocked by local application of GABA<sub>A</sub> antagonists (Tepper et al., 1995; Paladini et al., 1999a). The GABA<sub>B</sub> antagonists CGP35348 or CGP55845A not only fail to block the inhibition, but in many cases lead to an augmented inhibitory response (Fig. 16.3). Train stimuli similar to those used in the in vitro experiments elicited longer duration, more powerful inhibition from striatum or GP. In some of these cases bicuculline or picrotoxin completely blocked the augmented inhibition. In other cases only the early part of the inhibition was blocked and the later portion remained. However, as with single pulse stimulation the GABA<sub>B</sub> antagonists, saclofen or CG-55845A, did not block any of inhibition resulting from train stimulation nor did the SK channel blocker, apamin or the D2 receptor antagonist, eticlopride (Paladini et al., 1999a).

If the stimulus intensity is adjusted to be just below threshold for evoking inhibition, application of GABA<sub>B</sub> antagonists unmasks an inhibitory response that can then be blocked by application of picrotoxin or bicuculline (Paladini et al., 1999a; cf Fig. 16.3. Thus in vivo in rats, inhibition in nigral DA neurons evoked from all three principal GABA afferents appears to be mediated predominantly or exclusively through stimulation of postsynaptic GABA<sub>A</sub> receptors. The potentiation of the inhibition by application of GABA<sub>B</sub> antagonists arises from blockade of presynaptic inhibitory GABA<sub>B</sub> autoreceptors located on the terminals of striatal, pallidal and SNr neurons that synapse onto DA neurons (Giralt et al., 1990; Hausser and Yung, 1994; see Misgeld et al., 2007 for review). This blockade results in increased stimulus-evoked GABA release that produces larger GABAA receptor-mediated inhibition that can be completely blocked by GABA<sub>A</sub> antagonists (Paladini et al., 1999a).

It is rather puzzling that it is so difficult to elicit GABA<sub>B</sub> inhibition in DA neurons in vivo when the neurons
clearly have abundant expression of GABA<sub>B</sub> receptors and exogenous application of GABA<sub>B</sub> agonists both in vivo (Engberg et al., 1993; Ehrhardt et al., 1998, 2002) and in vitro (Lacey et al., 1988) result in strong hyperpolarization and inhibition. One explanation is that a significant fraction of postsynaptic GABA<sub>B</sub> receptors on SNc neurons is located at extrasynaptic sites some distance from the site of GABA release (e.g., Boyes and Bolam, 2003). Activation of these receptors would require particularly intense stimuli in order to evoke enough GABA release to overcome the uptake mechanisms and allow diffusion away from the synapse to reach the extrasynaptic GABA<sub>B</sub> receptors. This is precisely the case in hippocampus where interneuronal inhibition of pyramidal cells is mediated exclusively by GABA<sub>A</sub> receptors except when the interneuron is stimulated by high frequency trains or when a large population of interneurons is firing synchronously (Scanziani, 2000). Therefore it may simply be that most previous in vivo experiments with intact uptake mechanisms have been unable to stimulate striatum, GP or SNr neurons strongly enough for this synaptic overflow to occur.

Indeed this seems to be the case. Recent experiments in vivo in mice reveal that stimulation of striatum, GP or antidromic activation of SNr projection neurons with single pulses delivered at amplitudes similar to those used in previous experiments in rats evoked inhibitory responses of significantly greater duration than those observed in rats (Brazhnik et al., 2008). Furthermore, the inhibitory responses could be seen to be composed of two discrete components, an early inhibition that could be selectively blocked by GABAA receptor antagonists and a later component, not usually seen in rats, that was unaffected by bicuculline or picrotoxin but that was blocked by the selective GABA<sub>B</sub> antagonist, CGP55845A. Although the late component could be blocked by CGP55845A, as in rats, there was no evidence for a tonic GABA<sub>B</sub> mediated inhibitory tone (Brazhnik et al., 2008; cf Fig. 16.3).

Local application of CGP55845A not only potentiated the early  $GABA_A$ -mediated inhibition following activation of striatal, pallidal or SNr inputs as in rats, but also resulted in a significant *decrease* in spontaneous firing rate, a trend that was evident but not statistically significant in the previous studies in rats. Both of these effects were attributable to action at inhibitory presynaptic GABA<sub>B</sub> terminal autoreceptors, as in rats.

GABA uptake blockers had relatively little effect on the early part of the evoked inhibition but greatly augmented the late component, an effect that was selectively antagonized by  $GABA_B$  antagonists (Brazhnik et al., 2008; cf Fig. 16.3). Masked postsynaptic  $GABA_B$  inhibitory effects that can be revealed by the application of GABA uptake blockade have also been shown to occur in striatum (Kirmse et al., 2008). In all other respects, the responses to GABA afferent inputs were identical in rats and mice.

## F. Why are Postsynaptic GABA<sub>B</sub> Responses Seen in Response to Stimulation of GABA Afferents in Mice In Vivo, but not in Rats?

There are several possible explanations for the appearance of GABA<sub>B</sub> postsynaptic effects in vivo in mice when previous experiments failed to see them in rats. Anesthetic differences is one possibility - most of the rat experiments were done under urethane anesthesia whereas the mice were anesthetized with chloral hydrate. Another possibility is that there is significant species difference in GABAergic signaling in SN between rats and mice, but that seems highly unlikely especially given the ready elicitation of GABA<sub>B</sub> IPSP/Cs in vitro in rat slices. Rather, the most likely explanation is simply the difference in size between rat and mouse brains. Neuronal packing density varies inversely with brain volume (Tower, 1954). This causes identical stimuli delivered in rat and mouse brain to stimulate a much larger number of neurons in the mouse. Further, since the mouse brain is smaller, a given volume of brain tissue corresponds to a greater *proportion* of all the cell in a given nucleus so stimulating equal volumes in rat and mouse would not only activate a larger number of neurons in the mouse but also a larger fraction of the total population of efferents, a variable that seems likely to be related to the maximum total receptor binding, uptake capacity and strength of synaptic response. Stimulation in a smaller brain is thus likely to result in greater extracellular levels of GABA some of which escapes the synapse and is available to diffuse to extrasynaptic GABA<sub>B</sub> receptors.

There are observations that support this hypothesis. A study of the postnatal changes in nigrostriatal neurons in rats showed that striatal evoked inhibition was especially potent in neonatal rat pups, lasting for several hundred ms, but by the time the rats had reached 21 days of age, the average duration of inhibition did not differ from that in adults. (Tepper et al., 1990). Three weeks of age is well before the rat striatum has fully matured anatomically or physiologically (Tepper and Trent, 1993; Tepper et al., 1998) but is a period when the size of the brain is close to that of adult rats. Additional support comes from in vitro

release studies where field stimulation of cortical or striatal slices released 80% more norepinephrine and 300% more DA in mouse slices than in rat slices using the same stimuli, indicating that identical stimuli released far more transmitter in mouse than in rat. Finally, in the in vivo mouse studies just described, electrical stimulation of striatum, GP or thalamus evoked much longer duration inhibition than identical stimulation in rats (Tepper et al., 1995, Paladini et al., 1999a; Brazhnik et al., 2008).

## G. Effects of GABA Receptor Antagonists on Spontaneous Activity in Nigrostriatal Neurons

In addition to blocking striatal, pallidal or SNr-evoked inhibition of nigrostriatal neurons, GABA receptor antagonists also affected both the firing rate and firing pattern of the spontaneous activity of nigrostriatal neurons. Local application of bicuculline methiodide produced a modest but statistically significant 25% increase in firing rate (Tepper et al., 1995, Paladini and Tepper, 1999). Two other GABA<sub>A</sub> antagonists that do not block the SK channel as does bicuculline methiodide (Johnson and Seutin, 1997), picrotoxin and gabazine, exerted smaller, less consistent excitatory effects on firing rate (Paladini and Tepper, 1999). Local application of the selective GABA<sub>B</sub> receptor antagonists, 2-OH-saclofen or CGP55845A, exerted even more modest, but opposite effects on firing rate, producing small decreases in spontaneous activity (Tepper et al., 1995; Paladini and Tepper, 1999; cf Figure 16.3 C).

In marked contrast to the relatively modest effects on nigrostriatal neuron firing rate, GABA<sub>A</sub> antagonists, exerted consistent and dramatic effects on the firing patterns of SNc DA neurons. Local application of bicuculline methiodide produced a dramatic increase in the CV, the proportion of neurons firing in bursts and the percentage of spikes fired in bursts (Tepper et al., 1995; Paladini and Tepper, 1999). Other GABAA antagonists that lack the SK channel blocking ability of bicuculline methiodide (Johnson and Seutin, 1997), picrotoxin and gabazine, mimicked the potent effects of bicuculline at switching neurons from pacemaker or random firing to burst firing. (Paladini and Tepper, 1999; Brazhnik et al., 2008; cf Fig. 16.4). Regardless of the initial firing pattern, all the  $GABA_A$ antagonists caused the majority of nigrostriatal neurons to shift to a bursty firing pattern. There was no correlation between the effects of GABAA antagonists on firing pattern and baseline firing rate or drug-induced changes in firing rate (Paladini and Tepper, 1999). This suggests that the mechanisms that modulate firing pattern and firing rate are different, and at least partially independent, and further, that altering GABAergic input to DA neurons has a greater effect on firing pattern than on firing rate.

In contrast,  $GABA_B$  antagonists produced opposite effects on firing pattern. Although these effects were usually less dramatic then those of the GABA antagonists, local application of CGP35348 or CGP 55845A led to a shift along the firing pattern continuum away from burst firing towards the pacemaker-like pattern. The regularization in firing pattern was evident in a number of indices including a decreased CV, a decrease in the percentage of spikes fired in bursts, and increase in the mean number of peaks in the autocorrelation histogram, and decrease in the numbers of neurons firing in the bursty mode and an increase in the proportion of neurons firing in the pacemaker pattern (Tepper et al., 1995; Paladini and Tepper, 1999; Brazhnik et al., 2008).

These results indicated that in vivo there exists a GABA tone on nigrostriatal neurons that produces a tonic activation of  $GABA_A$  receptors. The level of activation of nigrostriatal  $GABA_A$  receptors seems able to modulate the firing pattern in a very effective and rapid way. One could imagine that momentary decreases in GABAergic input resulting from brief pauses in the high tonic firing rate of the SNr neurons would reduce the GABA<sub>A</sub> tone, and, like brief applications of GABA<sub>A</sub> antagonists, produce a burst. On the other hand increases in GABAergic input resulting from increased SNr activity would increase the level of GABA<sub>A</sub> receptor stimulation and suppress burst firing.

In contrast, there is no tonic stimulation of the postsynaptic GABA<sub>B</sub> receptors on nigrostriatal neurons, consistent with findings in other brain regions including striatum and hippocampus (Scanziani, 2000; Kirmse et al., 2008, but see Erhardt et al., 1999). The effects of the GABA<sub>B</sub> antagonists result from action at presynaptic GABA<sub>B</sub> autoreceptors on the terminals of the GABA afferents that lead to increased GABA release and increased GABA<sub>A</sub> receptor stimulation. The modest inhibitory effects of GABA<sub>B</sub> antagonists on spontaneous activity and the facilitation of GABA<sub>A</sub>-mediated afferent induced inhibition suggest that unlike the postsynaptic GABA<sub>B</sub> receptors, the presynaptic GABA<sub>B</sub> autoreceptors, that are located on GABA afferents from striatum, GP and SNr, *are* tonically stimulated in vivo, albeit to a relatively small degree.

For reasons discussed above, the  $GABA_A$  tone is unlikely to originate from the striatum, but could arise



FIGURE 16.4 Blocking GABA<sub>A</sub> inputs to nigrostriatal neurons evokes bursty firing in vivo. A1. Pre-drug control neuron exhibits pacemaker firing. A2. Local application of the GABAA antagonist, bicuculline, produces a dramatic shift to a bursty firing pattern along with an increase in firing rate within a few seconds. A3. The effects of bicuculline wear off in about 7 minutes and the subsequent application of the GABA<sub>B</sub> antagonist, CGP55845A, fails to elicit burst firing, and in fact contributes to increased regularity of firing as indicated by an increase in the number of peaks in the autocorrelogram (9) compared to that in the pre-drug control (7). A4. Distribution of firing patterns of rat nigrostriatal neurons in vivo under control conditions, after local application of bicuculline or the GABA<sub>B</sub> antagonist, 2-OH saclofen, through the recording pipette. B. Manipulation of GP firing rate by infusion of drugs exerts paradoxical effects on SNc activity. B1. Control SNc neuron firing in the random mode. B2. Following infusion of muscimol into GP that produced almost complete inhibition of GP firing, the nigrostriatal neuron shifts to pacemaker firing accompanied by a small decrease in firing rate. B3. GP infusion of bicuculline produced a 58% increase in GP firing rate and caused the DA neuron to switch to busty firing, accompanied by a slight increase in firing rate. B4. Distribution of firing patterns for all SNc neurons following pallidal drug infusions. B5. Ratemeter record of the neuron shown in B1-3 shows that the dramatic changes in firing pattern are accompanied by very modest changes in firing rate. Note that the changes in firing rate and pattern are opposite to what would result from monosynaptic pallidal input to SNc neurons. B6. In contrast to the SNc neurons, the firing rate of one representative SNr projection neuron (B7) more than doubles following GP muscimol infusion and its spontaneous activity is completely suppressed following GP infusion of bicuculline, consistent with a powerful monosynaptic inhibitory pallidal input. C. Simultaneous recording of nigrostriatal neuron activity and striatal DA levels measured by microdialysis. C1. Control neuron firing randomly. C2. GP infusion of bicuculline causes neuron to shift to a bursty firing pattern with a modest increase in firing rate, the typical response as shown in C3. Simultaneous microdialysis in striatum reveals that the switch to the bursty firing pattern causes a 44% increase in extracellular DA levels. Source A1-3: redrawn from Paladini and Tepper (1999); A4: redrawn from Tepper et al. (1995), copyright 1995 by the Society for Neuroscience; B1-6: redrawn from Celada et al. (1999), used with permission; C: redrawn from Lee et al. (2004).

from either GP or SNr or both. However if we recall that pallidal-evoked inhibition of nigrostriatal neurons is only seen following the relatively strong, synchronous GABA release that occurs with electrical stimulation whereas the GABA release that accompanies pharmacological stimulation that causes asynchronous activation of GP produces disinhibition by preferentially inhibiting the SNr neurons, it seems most likely that the major source of the GABA<sub>A</sub> tone that modulates the firing pattern of the nigrostriatal neurons is the local axon collateral system of the SNr projection neurons.

The mechanism of the GABA<sub>A</sub>-mediated burst suppression is not completely understood, but NMDA-induced burst firing of nigral DA neurons in vitro can be blocked by the selective GABA<sub>A</sub> agonist, isoguvacine. The blockade is independent of membrane polarization but is associated with a large conductance increase (Paladini et al., 1999b). This effect has been simulated in compartmental models of DA neurons by a few groups where it has been explained by alterations in the interactions of membrane potential, conductance and dendritic coupling (Canavier, 1999; Komendatov et al., 2004; Kusnetsov et al., 2006).

## H. Afferent Regulation of Burst Firing in Nigrostriatal Neurons

The burst firing of DA neurons only occurs spontaneously in vivo, suggesting that in addition to intrinsic conductances, intact afferents are required for burst initiation and/or maintenance (Kita et al., 1986; Lacey et al., 1989, Grace and Onn, 1989). Bursts cannot be elicited by simple intracellular current injection but can be evoked by local electrical stimulation under appropriate conditions in vitro and/or pressure application of glutamate or NMDA (Morikawa et al., 2003; Blythe et al., 2007). Bursting can also be elicited by blocking the SK channel (Shepard and Bunney, 1988; Ji and Shepard, 2006), and stimulation of either nicotinic or muscarinic cholinergic receptors on DA neurons leads to depolarization and an increase in firing rate as well as to an increase in burst firing (Calabresi et al., 1989; Sorenson et al., 1998; Kitai et al., 1999). It is not inconceivable that there are multiple mechanisms interacting and/or acting independently to produce burst firing in nigral DA neurons in vivo (e.g., Canavier and Landry, 2006; Canavier et al., 2007).

Nevertheless, it is often assumed that the trigger for burst firing in vivo in SNc neurons is an excitatory glutamatergic input as it often is in other brain regions. Stimulation of nigral glutamate receptors in vivo, particularly NMDA receptors, induces burst firing whereas blocking NMDA receptors leads to a suppression of burst firing and a regularization of firing pattern (Grace and Bunney, 1984b, Charlety et al., 1991, Overton and Clark, 1992, 1997; Chergui et al., 1993, 1994). The principal glutamatergic afferents to SN come from the STN (Hammond et al., 1978), frontal cortex (Sesack and Carr, 2002) and pedunculopontine nucleus (that also provides cholinergic input to substantia nigra, Mena-Segovia et al., 2008) and electrical or chemical stimulation of these areas can increase burst firing in nigral DA neurons (but see above).

There is no little doubt that NMDA receptor stimulation, increases or causes burst firing in DA neurons in vivo although the mechanism remains controversial. An early hypothesis for this action identified a sodium-based mechanism dependent on an electrogenic sodium pump (Johnson et al., 1992) that was subsequently simulated in a compartmental model (Li et al., 1996; Canavier, 1999). However, the experimental data and the simulations replicate sustained plateau depolarizations far better than they do the burst itself, and both the experimental data and the model result in prolonged bouts of high frequency firing displaying reverse spike frequency adaptation riding on large plateau potentials that do not resemble in vivo bursts. Further the hypothesis cannot explain why NMDA receptor stimulation specifically promotes burst firing while depolarization of the membrane by non-NMDA glutamatergic agonists or current injection do not.

A more recent hypothesis, based on whole cell recording and calcium imaging studies of nigrostriatal neurons in vitro and supported by neuronal simulations suggests that DA neurons can be thought of as a series of coupled  $Ca^{2+}/$ Ca<sup>2+</sup>-activated K<sup>+</sup> channel oscillators with the soma and proximal dendrites oscillating at a lower frequency than the thinner, more distal dendrites due to the difference in calcium clearance time as a function of surface area to volume ratio (Wilson and Callaway, 2000; Kusnetsov et al., 2006). Because the amplitude of the subthreshold dendritic oscillation is small compared to that of the soma and the compartments are tightly coupled, the neuron normally oscillates near the low frequency of the soma. NMDA receptor activation, because of its voltage dependence, leads to an increase in amplitude of the fast dendritic oscillation allowing it to become larger than the somatic oscillation and compete with or completely suppress the slower somatic oscillation, setting up the subthreshold conditions for a high frequency burst. It is the hyperpolarizing phase of the amplified subthreshold dendritic oscillation that allows the repolarization necessary for high frequency

spiking, something that does not occur with depolarizing current injection or AMPA receptor stimulation (Kusnetsov et al., 2006). Among the strengths of the model are that it incorporates the obviously important crucial role of  $Ca^{2+}$  in burst firing and explains why NMDA receptor stimulation, but not depolarization by injected current or AMPA receptor stimulation is capable of including a burst. Finally, simulations with the model generate bursts that more closely resemble natural bursts in vivo in terms of the maximum firing frequency and the appearance of spike frequency adaptation during the burst (Kusnetsov et al., 2006) than other current models (Komendatov et al., 2004; Canavier and Landry, 2006; Canavier et al., 2007).

But a model that successfully explains the mechanism by which NMDA receptor activation leads to burst firing in nigrostriatal neurons need not necessarily imply that phasic glutamatergic afferent activity input is the only, or even the principal trigger for burst firing in vivo. As mentioned above, the overwhelming majority of afferents that contact neurons in the substantia nigra, perhaps up to 90%, make symmetric inhibitory GABA synapses (Rinvik and Grofova, 1970, Gulley and Smithberg, 1971) [note that this applies specifically to substantia nigra; the situation appears to be different in the VTA where the most common type of synaptic input is glutamatergic and excitatory (Smith et al., 1996)]. As reviewed above, blockade of GABA<sub>A</sub> receptors on nigrostriatal neurons or interruption of GABAergic input from the SNr causes almost all nigrostriatal neurons to fire bursts. Conversely, exogenously applied GABAergic agonists or increases in firing rate of SNr projection neurons can completely suppress burst firing occurring spontaneously in vivo or induced by NMDA in vitro. Given the overwhelming predominance of GABA synapses on nigral DA neurons, the rapid kinetics of most GABA<sub>A</sub> receptors, and the remarkable effectiveness of stimulation or blockade of GABAA receptors on nigrostriatal neurons in modulating firing pattern, it seems not only possible but rather likely that one important trigger for eliciting a burst in a nigrostriatal neuron is a transient interruption in SNr firing.

As a tentative hypothesis, assume that the glutamatergic afferents to nigrostriatal neurons, arising principally from the pedunculopontine nucleus and also the subthalamic nucleus are tonically active and provide a more or less constant input to DA neurons, keeping them "primed" and burst-capable. They do not burst fire all the time because of substantial GABA<sub>A</sub> tone provided by the GABA afferents, the most crucial of which is the SNr projection neuron.

The GABA tone suppresses the bursting, perhaps by altering the coupling between compartments as a result of increased membrane conductance. The SNr neuronal activity is tightly controlled by input from the GP. A transient increase in the activity of pallidonigral afferents (due to reduced striatal inhibition and/or increased STN input?) will lead to a similarly timed transient decrease in SNr GABAergic output, thereby disinhibiting the nigrostriatal neuron and allowing a burst. In contrast, brief decreases in output of pallidonigral neurons will disinhibit the SNr neurons, thereby increasing their activity and their GABAergic output to re-establish burst suppression in nigrostriatal neurons. In this model the glutamatergic/NMDA inputs are absolutely essential for burst firing, but do not trigger it.

### VI. CONCLUDING REMARKS

The normal functioning of nigrostriatal DA neurons is crucial to a large array of behaviors ranging from voluntary motor function to higher cognitive processes. This remarkable variety of functions is even more impressive when one considers how few SN DA neurons there are ( $\sim 25,000$ ; Nair-Roberts et al., 2008) relative to, say, the striatum (2.9 Million; Oorschot, 1996). Nigrostriatal neurons in vivo fire in three distinct patterns, but unlike many other CNS neurons that exhibit multiple firing patterns in vitro, nigrostriatal neurons in vitro exhibit only a very regular, pacemaker-like pattern. This suggests that while the cellular mechanisms capable of generating the different firing patterns seen in vivo are intrinsic, afferent input is required to manifest the different firing patterns. The sustained firing rate of nigrostriatal neurons is low, and constrained to a limited range, below 10 spikes/sec, and it seems likely that much or most of the important functional variability in these neurons is carried by firing pattern rather than firing rate. The most numerous afferents to nigral DA neurons are GABAergic, and manipulation of GABA receptors on nigrostriatal neurons, principally GABA<sub>A</sub> receptors, produces dramatic effects on firing pattern, and more modest effects on firing rate, in vivo. The GABA afferent that seems to be most efficacious at modulating the firing pattern of nigrostriatal neurons comes from the axon collaterals of the SNr projection neuron. Many or most of the afferents to SN contact both the DA and the GABA neurons, providing the basis for a complex series of mono- and polysynaptic responses to both excitatory and inhibitory afferents.

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### REFERENCES

- Bean AJ, Roth RH (1991) Extracellular dopamine and neurotensin in rat prefrontal cortex in vivo: effects of median forebrain bundle stimulation frequency, stimulation pattern, and dopamine autoreceptors. J Neurosci 11:2694–2702.
- Bevan MD, Booth PA, Eaton SA, Bolam JP (1998) Selective innervation of neostriatal interneurons by a subclass of neuron in the globus pallidus of the rat. J Neurosci 18:9438–9452.
- Björklund A, Lindvall O (1975) Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. Brain Res 83:531–537.
- Blythe SN, Atherton JF, Bevan MD (2007) Synaptic activation of dendritic AMPA and NMDA receptors generates transient high-frequency firing in substantia nigra dopamine neurons in vitro. J Neurophysiol 97:2837–2850.
- Bolam JP, Smith Y (1990) The GABA and substance P input to dopaminergic neurones in the substantia nigra of the rat. Brain Res 529:57–78.
- Bontempi B, Sharp FR (1997) Systemic morphine-induced Fos protein in the rat striatum and nucleus accumbens is regulated by  $\mu$ opioid receptors in the substantia nigra and ventral tegmental area. J Neurosci 17:8596–8612.
- Boyes J, Bolam JP (2003) The subcellular localization of  $GABA_B$  receptor subunits in the rat substantia nigra. Eur J Neurosci 18:3279–3293.
- Boyes J, Bolam JP (2007) Localization of GABA receptors in the basal ganglia. In: GABA in the Basal Ganglia: From Molecules to Systems (Tepper JM, Abercrombie ED, Bolam JP, eds) Prog Brain Res 160:229–243.
- Brazhnik E, Shah F, Tepper JM (2008) GABAergic afferents activate both GABA<sub>A</sub> and GABA<sub>B</sub> receptors in mouse substantia nigra dopaminergic neurons in vivo. J Neurosci 28:10386–10398.
- Bunney BS, Chiodo LA, Grace AA (1991) Midbrain dopamine system electrophysiological functioning: a review and new hypothesis. Synapse 9:79–94.
- Bunney BS, Aghajanian GK, Roth RH (1973a) Comparison of effects of I-dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurones. Nat New Biol 245:123–125.
- Bunney BS, Walters JR, Roth RH, Aghajanian GK (1973b) Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J Pharmacol Exp Ther 185:560–571.
- Caille I, Dumartin B, Bloch B (1996) Ultrastructural localization of D<sub>1</sub> dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. Brain Res 730:17–31.
- Calabresi P, Lacey MG, North RA (1989) Nicotinic excitation of rat ventral tegmental neurones in vitro studied by intracellular recording. Br J Pharmacol 98:135–140.

- Cameron DL, Williams JT (1993) Dopamine D<sub>1</sub> receptors facilitate transmitter release. Nature 366:344–347.
- Canavier CC (1999) Sodium dynamics underlying burst firing and putative mechanisms for the regulation of the firing pattern in midbrain dopamine neurons: a computational approach. J Comput Neurosci 6:49–69.
- Canavier CC, Landry RS (2006) An increase in AMPA and a decrease in SK conductance increase burst firing by different mechanisms in a model of a dopamine neuron in vivo. J Neurophysiol 96:2549–2563.
- Canavier CC, Oprisan SA, Callaway JC, Ji H, Shepard PD (2007) Computational model predicts a role for ERG current in repolarizing plateau potentials in dopamine neurons: implications for modulation of neuronal activity. J Neurophysiol 98:3006–3022.
- Celada P, Paladini CA, Tepper JM (1999) GABAergic control of rat substantia nigra dopaminergic neurons: role of globus pallidus and substantia nigra pars reticulata. Neuroscience 89:813–825.
- Charara A, Heilman TC, Levey AI, Smith Y (2000) Pre- and postsynaptic localization of GABA<sub>B</sub> receptors in the basal ganglia in monkeys. Neuroscience 95:127–140.
- Charlety PJ, Grenhoff J, Chergui K, De la Chapelle B, Buda M, Svensson TH, Chouvet G (1991) Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurenate. Acta Physiol Scand 142:105–112.
- Cheramy A, Leviel V, Glowinski J (1981) Dendritic release of dopamine in the substantia nigra. Nature 289:537–542.
- Chergui K, Akaoka H, Charlety PJ, Saunier CF, Buda M, Chouvet G (1994) Subthalamic nucleus modulates burst firing of nigral dopamine neurones via NMDA receptors. Neuroreport 5:1185–1188.
- Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH, Chouvet G (1993) Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. Eur J Neurosci 5:137–144.
- Chergui K, Suaud-Chagny MF, Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. Neuroscience 62:641–645.
- Chiodo LA (1988) Dopamine-containing neurons in the mammalian central nervous system: electrophysiology and pharmacology. Neurosci Biobehav Rev 12:49–91.
- Cobb WS, Abercrombie ED (2003) Differential regulation of somatodendritic and nerve terminal dopamine release by serotonergic innervation of substantia nigra. J Neurochem 84:576–584.
- Collingridge GL, Davies J (1981) The influence of striatal stimulation and putative neurotransmitters on identified neurones in the rat substantia nigra. Brain Res 212:345–359.
- Cragg SJ, Baufreton J, Xue Y, Bolam JP, Bevan MD (2004) Synaptic release of dopamine in the subthalamic nucleus. Eur J Neurosci 20:1788–1802.
- Dahlstrom A, Fuxe K (1964) Localization of monoamines in the lower brain stem. Experientia 20:398–399.
- Dai M, Tepper JM (1998) Do silent dopaminergic neurons exist in rat substantia nigra in vivo? Neuroscience 85:1089–1099.
- Deniau JM, Hammond C, Riszk A, Feger J (1978) Electrophysiological properties of identified output neurons of the rat substantia nigra (pars compacta and pars reticulata): evidences for the existence of branched neurons. Exp Brain Res 32:409–422.
- Deniau JM, Kitai ST, Donoghue JP, Grofova I (1982) Neuronal interactions in the substantia nigra pars reticulata through axon collaterals of the projection neurons. An electrophysiological and morphological study. Exp Brain Res 47:105–113.

- Deniau JM, Mailly P, Maurice N, Charpier S (2007) The pars reticulata of the substantia nigra: a window to basal ganglia output. Prog Brain Res 160:151–172.
- Deutch AY, Goldstein M, Roth RH (1986) The ascending projections of the dopaminergic neurons of the substantia nigra, zona reticulata: a combined retrograde tracer-immunohistochemical study. Neurosci Lett 71:257–263.
- Descarries L, Watkins KC, Garcia S, Bosler O, Doucet G (1996) Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: A quantitative autoradiographic and immunocytochemical analysis. J Comp Neurol 375:167–186.
- Diana M, Garcia-Munoz M, Richards J, Freed CR (1989) Electrophysiological analysis of dopamine cells from the substantia nigra pars compacta of circling rats. Exp Brain Res 74:625–630.
- Diana M, Tepper JM (2002). Electrophysiological pharmacology of mesencephalic dopaminergic neurons. In Dopamine in the CNS II, Handbook of Experimental Pharmacology (Di Chiara G ed) Springer-Verlag. 154/II: pp. 1–61.
- Engberg G, Kling-Petersen T, Nissbrandt H (1993) GABA<sub>B</sub>-receptor activation alters the firing pattern of dopamine neurons in the rat substantia nigra. Synapse 15:229–238.
- Erhardt S, Andersson B, Nissbrandt H, Engberg G (1998) Inhibition of firing rate and changes in the firing pattern of nigral dopamine neurons by gamma-hydroxybutyric acid (GHBA) are specifically induced by activation of GABA<sub>B</sub> receptors. Naunyn Schmiedebergs Arch Pharmacol 357:611–619.
- Erhardt S, Mathe JM, Chergui K, Engberg G, Svensson TH (2002) GABA<sub>B</sub> receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. Naunyn Schmiedebergs Arch Pharmacol 365:173–180.
- Erhardt S, Nissbrandt H, Engberg G (1999) Activation of nigral dopamine neurons by the selective GABA<sub>B</sub>-receptor antagonist SCH 50911. J Neural Transm 106:383–394.
- Fallon JH (1981) Collateralization of monoamine neurons: mesotelencephalic dopamine projections to caudate, septum, and frontal cortex. J Neurosci 1:1361–1368.
- Fallon JH, Moore RY (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545–580.
- Fallon JH, Riley N, Moore RY (1978) Substantia nigra dopamine neurons: Separate populations project to neostriatum and allocortex. Neurosci Lett 7:157–162.
- Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA<sub>A</sub> receptor signalling. Prog Brain Res 160:59–87.
- Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968–973.
- Francois C, Percheron G, Yelnik J, Heyner S (1979) Demonstration of the existence of small local circuit neurons in the Golgi-stained primate substantia nigra. Brain Res 172:160–164.
- Freeman AS, Meltzer LT, Bunney BS (1985) Firing properties of substantia nigra dopaminergic neurons in freely moving rats. Life Sci 36:1983–1994.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylaseimmunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 13:1189–1215.
- Fujiyama F, Fritschy JM, Stephenson FA, Bolam JP (2000) Synaptic localization of GABA<sub>A</sub> receptor subunits in the striatum of the rat. J Comp Neurol 416:158–172.

- Galarraga E, Bargas J (1995) Firing patterns in substantia nigra compacta identified neurons in vitro. Arch Med Res 26:191–199.
- Geffen LB, Jessell TM, Cuello AC, Iversen LL (1976) Release of dopamine from dendrites in rat substantia nigra. Nature 260:258–260.
- Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311:461–464.
- Gerfen CR, Baimbridge KG, Thibault J (1987a) The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7:3935–3944.
- Gerfen CR, Herkenham M, Thibault J (1987b) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and nondopaminergic systems. J Neurosci 7:3915–3934.
- Gerfen CR, Young WS 3rd (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161–167.
- Giralt MT, Bonanno G, Raiteri M (1990) GABA terminal autoreceptors in the pars compacta and in the pars reticulata of the rat substantia nigra are GABA<sub>B</sub>. Eur J Pharmacol 175:137–144.
- Goetz T, Arslan A, Wisden W, Wulff P (2007) GABA<sub>A</sub> receptors: structure and function in the basal ganglia. In: GABA in the Basal Ganglia: From Molecules to Systems (Tepper JM, Abercrombie ED, Bolam JP, eds). Prog Brain Res 160:21–41.
- Gonon FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 24:19–28.
- Gonon F (1997) Prolonged and extrasynaptic excitatory action of dopamine mediated by D<sub>1</sub> receptors in the rat striatum in vivo. J Neurosci 17:5972–5978.
- Gonon FG, Buda MJ (1985) Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in the rat striatum. Neuroscience 14:765–774.
- Grace AA (1990) Evidence for the functional compartmentalization of spike generating regions of rat midbrain dopamine neurons recorded in vitro. Brain Res 524:31–41.
- Grace AA (1991) Regulation of spontaneous activity and oscillatory spike firing in rat midbrain dopamine neurons recorded in vitro. Synapse 7:221–234.
- Grace AA, Bunney BS (1979) Paradoxical GABA excitation of nigral dopaminergic cells: Indirect mediation through reticulata inhibitory neurons. Eur J Pharmacol 59:211–218.
- Grace AA, Bunney BS (1983a) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 1. Identification and characterization. Neuroscience 10:301–315.
- Grace AA, Bunney BS (1983b) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 2. Action potential generating mechanisms and morphological correlates. Neuroscience 10:317–331.
- Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4:2866–2876.
- Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877–2890.
- Grace AA, Bunney BS (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333:271–284.
- Grace AA, Onn SP (1989) Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro. J Neurosci 9:3463–3481.
- Grofova I (1975) The identification of striatal and pallidal neurons projecting to substantia nigra. An experimental study by means of

retrograde axonal transport of horseradish peroxidase. Brain Res 91:286–291.

- Grofova I, Deniau JM, Kitai ST (1982) Morphology of the substantia nigra pars reticulata projection neurons intracellularly labeled with HRP. J Comp Neurol 208:352–368.
- Grofova I, Rinvik E (1970) An experimental electron microscopic study on the striatonigral projection in the cat. Exp Brain Res 11:249–262.
- Groves PM, Linder JC, Young SJ (1994) 5-Hydroxydopamine-labeled dopaminergic axons: Three-dimensional reconstructions of axons, synapses, and postsynaptic targets in rat neostriatum. Neuroscience 58:593–604.
- Groves PM, Wilson CJ, Young SJ, Rebec GV (1975) Self-inhibition by dopaminergic neurons. Science 190:522–528.
- Gulacsi A, Lee CR, Sik A, Viitanen T, Kaila K, Tepper JM, Freund TF (2003). Cell type-specific differences in chloride-regulatory mechanisms and GABA<sub>A</sub> receptor-mediated inhibition in rat substantia nigra. J Neurosci 23:8237–8246.
- Gulley RL, Smithberg M (1971) Synapses in the rat substantia nigra. Tissue Cell 3:691–700.
- Gulley RL, Wood RL (1971) The fine structure of the neurons in the rat substantia nigra. Tissue Cell 3:675–690.
- Guyenet PG, Aghajanian GK (1978) Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra. Brain Res 150:69–84.
- Hajós M, Greenfield SA (1993) Topographic heterogeneity of substantia nigra neurons: diversity in intrinsic membrane properties and synaptic inputs. Neuroscience 55:919–934.
- Hajós M, Greenfield SA (1994) Synaptic connections between pars compacta and pars reticulata neurones: electrophysiological evidence for functional modules within the substantia nigra. Brain Res 660:216–224.
- Hammond C, Deniau JM, Rizk A, Feger J (1978) Electrophysiological demonstration of an excitatory subthalamonigral pathway in the rat. Brain Res 151:235–244.
- Hattori T, Fibiger HC, McGeer PL (1975) Demonstration of a pallidonigral projection innervating dopaminergic neurons. J Comp Neurol 162:487–504.
- Hausser M, Stuart G, Racca C, Sakmann B (1995) Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. Neuron 15:637–647.
- Hausser MA, Yung WH (1994) Inhibitory synaptic potentials in guinea-pig substantia nigra dopamine neurones in vitro. J Physiol 479:401–422.
- Hebb MO, Robertson HA (2000) Identification of a subpopulation of substantia nigra pars compacta gamma-aminobutyric acid neurons that is regulated by basal ganglia activity. J Comp Neurol 416:30–44.
- Hikosaka O, Sesack SR, Lecourtier L, Shepard PD (2008) Habenula: crossroad between the basal ganglia and the limbic system. J Neurosci 28:11825–11829.
- Hyland BI, Reynolds JN, Hay J, Perk CG, Miller R (2002) Firing modes of midbrain dopamine cells in the freely moving rat. Neuroscience 114:475–492.
- Iribe Y, Moore K, Pang KC, Tepper JM (1999) Subthalamic stimulationinduced synaptic responses in substantia nigra pars compacta dopaminergic neurons in vitro. J Neurophysiol 82:925–933.
- Ji H, Shepard PD (2006) SK Ca<sup>2+</sup>-activated K<sup>+</sup> channel ligands alter the firing pattern of dopamine-containing neurons in vivo. Neuroscience 140:623–633.
- Ji H, Shepard PD (2007) Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA<sub>A</sub> receptor-mediated mechanism. J Neurosci 27:6923–6930.

- Johnson SW, North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol 450:455–468.
- Johnson SW, Seutin V (1997) Bicuculline methiodide potentiates NMDAdependent burst firing in rat dopamine neurons by blocking apaminsensitive Ca<sup>2+</sup>-activated K<sup>+</sup> currents. Neurosci Lett 231:13–16.
- Johnson SW, Seutin V, North RA (1992) Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. Science 258:665–667.
- Juraska JM, Wilson CJ, Groves PM (1977) The substantia nigra of the rat: A Golgi study. J Comp Neurol 172:585–599.
- Kang Y, Kitai ST (1993a) A whole cell patch-clamp study on the pacemaker potential in dopaminergic neurons of rat substantia nigra compacta. Neurosci Res 18:209–221.
- Kang Y, Kitai ST (1993b) Calcium spike underlying rhythmic firing in dopaminergic neurons of the rat substantia nigra. Neurosci Res 18:195–207.
- Kirmse K, Dvorzhak A, Kirischuk S, Grantyn R (2008) GABA transporter 1 tunes GABAergic synaptic transmission at output neurons of the mouse neostriatum. J Physiol 586:5665–5678.
- Kita H (2007) Globus pallidus external segment. In: GABA in the Basal Ganglia: From Molecules to Systems (Tepper JM, Abercrombie ED, Bolam JP, eds). Prog Brain Res160:111–133.
- Kita T, Kita H, Kitai ST (1986) Electrical membrane properties of rat substantia nigra compacta neurons in an in vitro slice preparation. Brain Res 372:21–30.
- Kitai ST, Shepard PD, Callaway JC, Scroggs R (1999) Afferent modulation of dopamine neuron firing patterns. Curr Opin Neurobiol 9:690–697.
- Kiyatkin EA, Rebec GV (1998) Heterogeneity of ventral tegmental area neurons: single-unit recording and iontophoresis in awake, unrestrained rats. Neuroscience 85:1285–1309.
- Komendantov AO, Komendantova OG, Johnson SW, Canavier CC (2004) A modeling study suggests complementary roles for GABA<sub>A</sub> and NMDA receptors and the SK channel in regulating the firing pattern in midbrain dopamine neurons. J Neurophysiol 91:346–357.
- Kuznetsov AS, Kopell NJ, Wilson CJ (2006) Transient high-frequency firing in a coupled-oscillator model of the mesencephalic dopaminergic neuron. J Neurophysiol 95:932–947.
- Lacey MG, Mercuri NB, North RA (1987) Dopamine acts on D<sub>2</sub> receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J Physiol 392:397–416.
- Lacey MG, Mercuri NB, North RA (1988) On the potassium conductance increase activated by  $GABA_B$  and dopamine  $D_2$  receptors in rat substantia nigra neurones. J Physiol 401:437–453.
- Lacey MG, Mercuri NB, North RA (1989) Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. J Neurosci 9:1233–1241.
- Lee CR, Abercrombie ED, Tepper JM (2004) Pallidal control of substantia nigra dopaminergic neuron firing pattern and its relation to extracellular neostriatal dopamine levels. Neuroscience 129:481–489.
- Lee CR, Tepper JM (2007) Morphological and physiological properties of parvalbumin- and calretinin-containing gamma-aminobutyric acidergic neurons in the substantia nigra. J Comp Neurol 500:958–972.
- Lee CR, Tepper JM (2009) Basal ganglia control of substantia nigra dopaminergic neurons. In: Birth, Life and Death of Dopaminergic Neurons in the Substantia Nigra (Di Giovanni G, et al. eds) J Neural Trans Suppl. 73 Springer pp. 71–90.
- Li YX, Bertram R, Rinzel J (1996) Modeling N-methyl-D-aspartateinduced bursting in dopamine neurons. Neuroscience 71:397–410.

- Lindvall O, Bjorkland A (1979) Dopaminergic innervation of the globus pallidus by collaterals from the nigrostriatal pathway. Brain Res 172:169–173.
- Lokwan SJ, Overton PG, Berry MS, Clark D (1999) Stimulation of the pedunculopontine tegmental nucleus in the rat produces burst firing in A9 dopaminergic neurons. Neuroscience 92:245–254.
- Lu XY, Ghasemzadeh MB, Kalivas PW (1999) Regional distribution and cellular localization of gamma-aminobutyric acid subtype 1 receptor mRNA in the rat brain. J Comp Neurol 407:166–182.
- MacNeil D, Gower M, Szymanska I (1978) Response of dopamine neurons in substantia nigra to muscimol. Brain Res 154:401–403.
- Manley LD, Kuczenski R, Segal DS, Young SJ, Groves PM (1992) Effects of frequency and pattern of medial forebrain bundle stimulation on caudate dialysate dopamine and serotonin. J Neurochem 58:1491–1498.
- Martin GE, Haubrich DR (1978) Striatal dopamine release and contraversive rotation elicited by intranigrally applied muscimol. Nature 275:230–231.
- Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, Kaneko T (2009) Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. J Neurosci 29:444–453.
- Matsumoto M, Hikosaka O (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447:1111–1115.
- Mailly P, Charpier S, Menetrey A, Deniau JM (2003) Three-dimensional organization of the recurrent axon collateral network of the substantia nigra pars reticulata neurons in the rat. J Neurosci 23:5247–5257.
- Mena-Segovia J, Winn P, Bolam JP (2008) Cholinergic modulation of midbrain dopaminergic systems. Brain Res Rev 58:265–271.
- Mereu G, Gessa GL (1985) Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect? Brain Res 360:325–330.
- Miller DW, Abercrombie ED (1999) Role of high-affinity dopamine uptake and impulse activity in the appearance of extracellular dopamine in striatum after administration of exogenous L-DOPA: studies in intact and 6-hydroxydopamine-treated rats. J Neurochem 72:1516–1522.
- Misgeld U, Drew G, Yanovsky Y (2007) Presynaptic modulation of GABA release in the basal ganglia. Prog Brain Res 160:245–259.
- Morikawa H, Khodakhah K, Williams JT (2003) Two intracellular pathways mediate metabotropic glutamate receptor-induced Ca<sup>2+</sup> mobilization in dopamine neurons. J Neurosci 23:149–157.
- Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA (2008) Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience 152:1024–1031.
- Nedergaard S, Flatman JA, Engberg I (1993) Nifedipine- and omegaconotoxin-sensitive Ca<sup>2+</sup> conductances in guinea-pig substantia nigra pars compacta neurones. J Physiol 466:727–747.
- Neuhoff H, Neu A, Liss B, Roeper J (2002)  $I_h$  channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. J Neurosci 22:1290–1302.
- Nitsch C, Riesenberg R (1988) Immunocytochemical demonstration of GABAergic synaptic connections in rat substantia nigra after different lesions of the striatonigral projection. Brain Res 461: 127–142.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a

stereological study using the cavalieri and optical dissector methods. J Comp Neurol 366:580–599.

- Overton P, Clark D (1992) Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse 10:131–140.
- Overton PG, Clark D (1997) Burst firing in midbrain dopaminergic neurons. Brain Res Brain Res Rev 25:312–334.
- Paden C, Wilson CJ, Groves PM (1976) Amphetamine-induced release of dopamine from the substantia nigra in vitro. Life Sci 19:1499–1506.
- Paladini CA, Celada P, Tepper JM (1999a) Striatal, pallidal, and pars reticulate evoked inhibition of nigrostriatal dopaminergic neurons is mediated by GABA<sub>A</sub> receptors in vivo. Neuroscience 89:799–812.
- Paladini CA, Iribe Y, Tepper JM (1999b) GABA<sub>A</sub> receptor stimulation blocks NMDA-induced bursting of dopaminergic neurons in vitro by decreasing input resistance. Brain Res 832:145–151.
- Paladini CA, Tepper JM (1999) GABA<sub>A</sub> and GABA<sub>B</sub> antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. Synapse 32:165–176.
- Pickel VM, Beckley SC, Joh TH, Reis DJ (1981) Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. Brain Res 225:373–385.
- Ribak CE, Vaughn JE, Saito K, Barber R, Roberts E (1976) Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra. Brain Res 116:287–298.
- Rice ME, Cragg SJ (2008) Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. Brain Res Rev 58:303–313.
- Rinvik E, Grofova I (1970) Observations on the fine structure of the substantia nigra in the cat. Exp Brain Res 11:229–248.
- Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K<sup>+</sup>/Cl<sup>-</sup> co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–255.
- Robledo P, Feger J (1990) Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res 518:47–54.
- Robledo P, Vezole I, Feger J (1988) Excitatory effect of subthalamo-nigral and subthalamo-pallidal efferent pathways in the rat. CR Acad Sci III 307:133–138.
- Ryan LJ, Young SJ, Groves PM (1986) Substantia nigra stimulation evoked antidromic responses in rat neostriatum. Exp Brain Res 63:449–460.
- Saitoh K, Isa T, Takakusaki K (2004) Nigral GABAergic inhibition upon mesencephalic dopaminergic cell groups in rats. Eur J Neurosci 19:2399–2409.
- Santiago M, Westerink BHC (1992) The role of GABA receptors in the control of nigrostriatal dopaminergic neurons: dual-probe microdialysis study in awake rats. Eur J Pharmacol 219:175–181.
- Scanziani M (2000) GABA spillover activates postsynaptic  $GABA_B$ receptors to control rhythmic hippocampal activity. Neuron 25:673–681.
- Schousboe A, Waagepetersen HS (2007) GABA: homeostatic and pharmacological aspects. In: GABA in the Basal Ganglia: From Molecules to Systems (Tepper JM, Abercrombie ED, Bolam JP, eds) Prog Brain Res 160:9–19.
- Schultz W (1997) Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 7:191–197.
- Schultz W (2007) Behavioral dopamine signals. Trends Neurosci 30:203–210.

- Sesack SR, Carr DB (2002) Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. Physiol Behav 77:513–517.
- Shepard PD, Bunney BS (1988) Effects of apamin on the discharge properties of putative dopamine-containing neurons in vitro. Brain Res 463:380–384.
- Shepard PD, Bunney BS (1991) Repetitive firing properties of putative dopamine-containing neurons in vitro: regulation by an apaminsensitive Ca<sup>2+</sup>-activated K<sup>+</sup> conductance. Exp Brain Res 86:141–150.
- Schwyn RC, Fox CA (1974) The primate substantia nigra: A Golgi and electron microscopic study. J Hirnforsch 15:95–126.
- Smith ID, Grace AA (1992) Role of the subthalamic nucleus in the regulation of nigral dopamine neuron activity. Synapse 12:287–303.
- Smith Y, Bolam JP (1989) Neurons of the substantia nigra reticulata receive a dense GABA-containing input from the globus pallidus in the rat. Brain Res 493:160–167.
- Smith Y, Bolam JP (1990) The output neurones and the dopaminergic neurones of the substantia nigra receive a GABA-containing input from the globus pallidus in the rat. J Comp Neurol 296:47–64.
- Smith Y, Charara A, Parent A (1996) Synaptic innervation of midbrain dopaminergic neurons by glutamate-enriched terminals in the squirrel monkey. J Comp Neurol 364:231–253.
- Somogyi P, Bolam JP, Totterdell S, Smith AD (1981) Monosynaptic input from the nucleus accumbens – ventral striatum region to retrogradely labelled nigrostriatal neurones. Brain Res 217:245–263.
- Sorenson EM, Shiroyama T, Kitai ST (1998) Postsynaptic nicotinic receptors on dopaminergic neurons in the substantia nigra pars compacta of the rat. Neuroscience 87:659–673.
- Suaud-Chagny MF, Chergui K, Chouvet G, Gonon F (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. Neuroscience 49:63–72.
- Takada M, Hattori T (1986) Collateral projections from the substantia nigra to the cingulate cortex and striatum in the rat. Brain Res 380:331–335.
- Tepper JM, Bolam JP (2004) Functional diversity and specificity of neostriatal interneurons. Curr Opin Neurobiol 14:685–692.
- Tepper JM, Celada P, Iribe Y, Paladini CA (2003) Afferent control of nigral dopaminergic neurons: The role of GABAergic afferents. In: Basal Ganglia VI (Graybiel AM, Delong MR, Kitai ST, eds) Adv Behav Biol 54:641–651.
- Tepper JM, Damlama M, Trent F (1994) Postnatal changes in the distribution and morphology of rat substantia nigra dopaminergic neurons. Neuroscience 60:469–477.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tepper JM, Lee CR (2007) GABAergic control of substantia nigra dopaminergic neurons. In: GABA in the Basal Ganglia: From Molecules to Systems (Tepper JM, Abercrombie ED, Bolam JP, eds) Prog Brain Res 160:189–208.
- Tepper JM, Martin LP, Anderson DR (1995) GABA<sub>A</sub> receptor-mediated inhibition of rat substantia nigra dopaminergic neurons by pars reticulata projection neurons. J Neurosci 15:3092–3103.
- Tepper JM, Nakamura S, Spanis CW, Squire LR, Young SJ, Groves PM (1982) Subsensitivity of catecholaminergic neurons to direct acting agonists after single or repeated electroconvulsive shock. Biol Psychiatry 17:1059–1070.
- Tepper JM, Nakamura S, Young SJ, Groves PM (1984a) Autoreceptormediated changes in dopaminergic terminal excitability: effects of striatal drug infusions. Brain Res 309:317–333.

- Tepper JM, Sawyer SF, Young SJ, Groves PM (1986) Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of potassium channel blockers. Brain Res 367:230–237.
- Tepper JM, Sawyer SF, Groves PM (1987) Electrophysiologically identified nigral dopaminergic neurons intracellularly labeled with HRP: Light-microscopic analysis. J Neurosci 7:2794–2806.
- Tepper JM, Sharpe NA, Koos TZ, Trent F (1998) Postnatal development of the rat neostriatum: electrophysiological, light- and electronmicroscopic studies. Dev Neurosci 20:125–145.
- Tepper JM, Sun B-C, Martin LP, Creese I (1997) Functional roles of dopamine  $D_2$  and  $D_3$  autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo. J Neurosci 17:2519–2530.
- Tepper JM, Trent F (1993) In vivo studies of the postnatal development of rat neostriatal neurons. In: Chemical Signalling in the Basal Ganglia (Arbuthnott GW, Emson PC, eds) Prog Brain Res 99:35–50.
- Tepper JM, Trent F, Nakamura S (1990) Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons. Develop Brain Res 54:21–33.
- Tepper JM, Trent F, Nakamura S (1991) In vivo development of the spontaneous activity of rat nigrostriatal dopaminergic neurons. In: Basal Ganglia III (Bernardi G, Carpenter MB, DiChiara G, Morelli M, Stanzione P, eds) Adv Behav Biol 39:259–268.
- Tepper JM, Wilson CJ, Koos T (2008) Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons. Brain Res Rev 58:272–281.
- Tepper JM, Young SJ, Groves PM (1984b) Autoreceptor-mediated changes in dopaminergic terminal excitability: effects of increases in impulse flow. Brain Res 309:309–316.
- Tong ZY, Overton PG, Clark D (1996) Antagonism of NMDA receptors but not AMPA/kainate receptors blocks bursting in dopaminergic neurons induced by electrical stimulation of the prefrontal cortex. J Neural Transm 103:889–904.
- Totterdell S, Bolam JP, Smith AD (1984) Characterization of pallidonigral neurons in the rat by a combination of Golgi impregnation and retrograde transport of horseradish peroxidase: their monosynaptic input from the neostriatum. J Neurocytol 13:593–616.
- Tower DB (1954) Structural and functional organization of mammalian cerebral cortex; the correlation of neurone density with brain size; cortical neurone density in the fin whale (*Balaenoptera physalus* L.) with a note on the cortical neurone density in the Indian elephant. J Comp Neurol 101:19–51.
- Trent F, Tepper JM (1991) Dorsal raphé stimulation modifies striatalevoked antidromic invasion of nigral dopaminergic neurons in vivo. Exp Brain Res 84:620–630.
- Walters JR, Lakoski JM (1978) Effect of muscimol on single unit activity of substantia nigra dopamine neurons. Eur J Pharmacol 47:469–471.
- Waszczak BL, Eng N, Walters JR (1980) Effects of muscimol and picrotoxin on single unit activity of substantia nigra neurons. Brain Res 188:185–197.
- Waszczak BL, Hume C, Walters JR (1981) Supersensitivity of substantia nigra pars reticulata neurons to GABAergic drugs after striatal lesions. Life Sci 28:2411–2420.
- Wilson CJ (1993) The Generation of Natural Firing Patterns in Neostriatal Neurons. In: Chemical Signalling in the Basal Ganglia (Arbuthnott GW, Emson PC, eds) Prog Brain Res 99:277–297.
- Wilson CJ, Callaway JC (2000) Coupled oscillator model of the dopaminergic neuron of the substantia nigra. J Neurophysiol 83:3084–3100.
- Wilson CJ, Groves PM, Fifkova E (1977a) Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. Exp Brain Res 30:161–174.

- Wilson CJ, Young SJ, Groves PM (1977b) Statistical properties of neuronal spike trains in the substantia nigra: cell types and their interactions. Brain Res 136:243–260.
- Yoshida M, Precht W (1971) Monosynaptic inhibition of neurons of the substantia nigra by caudato-nigral fibers. Brain Res 32: 225–228.
- Yung WH, Hausser MA (1993) Evoked and spontaneous inhibitory postsynaptic potentials in guinea-pig substantia nigra dopamine neurones in vitro. J Physiol 459:431P.
- Yung WH, Hausser MA, Jack JJ (1991) Electrophysiology of dopaminergic and non-dopaminergic neurones of the guinea-pig substantia nigra pars compacta in vitro. J Physiol 436:643–667.
- Zhang J, Chiodo LA, Freeman AS (1992) Electrophysiological effects of MK-801 on rat nigrostriatal and mesoaccumbal dopaminergic neurons. Brain Res 590:153–163.
- Zhang J, Chiodo LA, Freeman AS (1993) Effects of phencyclidine, MK-801 and 1,3-di(2-tolyl)guanidine on non-dopaminergic midbrain neurons. Eur J Pharmacol 230:371–374.

# Regulation of Extracellular Dopamine: Release and Reuptake

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## I. INTRODUCTION

This review deals with one central question: How is the discharge activity of dopamine neurons translated into a chemical signal, i.e., into dynamic changes in the extracellular dopamine level? The extracellular dopamine level results from an equilibrium between dopamine release and dopamine clearance. In the first section, we discuss how dopamine release is achieved and regulated. The second section focuses on dopamine reuptake, which is the main mechanism of dopamine clearance. Finally, both release and reuptake are integrated to describe the relationship between the firing of dopamine neurons and the extracellular dopamine level. We do not discuss here how the discharge activity of dopamine neurons is generated and regulated (recently reviewed in Grace et al., 2007; see Chapter 16).

## **II. REGULATION OF DOPAMINE RELEASE**

Dopamine neurotransmission is generally initiated by synaptic vesicle fusion, which can be modulated at different levels including dopamine synthesis, uptake and vesicular transport as well as  $Ca^{2+}$  homeostasis and exocytotic proteins. In addition, dopamine autoreceptors expressed on dopamine neurons and presynaptic axon terminals provide feedback and regulate dopamine release.

## A. Exocytotic Processes

#### 1. Quantal Dopamine Release

In 1950, Bernard Katz and Paul Fatt at University College London published recordings of random electrical "noise" consisting of spontaneous small, rapid "action potentials" at the frog neuromuscular junction they compared to "fluctuations in the number of light quanta which strike the [photo]receptor cells" (Fatt and Katz, 1950). These "miniature end plate potentials" required extracellular calcium and were exacerbated in high osmolarity solution (Fatt and Katz, 1952). The events fitted well with a Poisson distribution, which simulates the probability of random occurrences of a multiple basic event (Del Castillo and Katz, 1954), indicating that neurotransmission occurs in multiples of a basic "quantal" unit. From these data and others, Fatt conjectured that "the apparatus for the release of acetylcholine at a junction is subdivided into a large number of units (at least 100), each of which is able to operate independently of the rest" (Fatt, 1954).

While adrenal cell extracts provided the subject of the original studies of secretory transmission (Oliver and Schafer, 1895), catecholamines activate G protein-coupled receptors that do not produce rapid potential changes, and four decades transpired until the measure of the quantal catecholamine release event. In 1990, Mark Wightman and colleagues (Leszczyszyn et al., 1990) used carbon fiber electrodes, originally designed by Gonon et al. (1978), to provide amperometric detection of quantal catecholamine release from adrenal cells. In contrast to postsynaptic recording, amperometric recordings directly measure the number of molecules released and the duration of the quantal release event, which in adrenal chromaffin cells were found to release about  $\sim 10^6$  molecules over the course of  $\sim 10^{-1}$  sec.

Amperometric recordings were adapted to record from the axonal terminals of cultured midbrain dopamine neurons, which produced quantal events that were about three orders of magnitude smaller and of shorter duration than that from adrenal cells (Pothos et al., 1998a; Staal et al., 2004). The material measured by the amperometric recordings was identified as dopamine based on (1) reserpine blockade; (2) colocalization with tyrosine hydroxylase immunolabel; (3) dependence on sufficient oxidation potential; (4) absence of events recorded in neurons that lack dopamine; (5) elevation of quantal size following L-DOPA or increased vesicular catecholamine transporter (VMAT2) expression. The shape of the majority of quantal dopamine events in neurons closely fit a simulation of transmitter diffusion through a pore (Sulzer and Pothos, 2000), but there are events that deviate from such simple shapes (see below).

In cultured dopamine neurons, quantal events have been observed to date in axons and not from cell bodies, but similar events have been recorded from acutely dissociated substantia nigra neuronal cell bodies (Kim et al., 2008), which may represent either normal dendritic quantal release events or synaptic vesicles that would have otherwise been trafficked to axons. Similar events have been found in acute midbrain slices, although it is difficult to exclude release from nearby dopamine or serotonergic terminals (Jaffe et al., 1998). In any case, while dopamine is well established to be released from dendrites, normal dendrites have few obvious synaptic vesicles in electron microscopy, and VMAT2 is mostly found in tubular structures (Nirenberg et al., 1996b). Quantal dopamine release from synaptic vesicles has also been recorded by amperometry from retinal bipolar cells (Puopolo et al., 2001) and invertebrate neuronal cell bodies (Chen et al., 1995; Sulzer et al., 1995).

#### 2. The Synaptic Vesicle Cycle

The fundamental difference between the quantal release of catecholamines from adrenal and other secretory glands and central dopamine neurons is due to differences in the storage vesicle. In adrenal medullary cells, the large (150–300 nm diameter) "chromaffin granules" (Cramer, 1918) that accumulate catecholamines (Blaschko and Welch, 1953; Hillarp et al., 1953) fuse with the plasma membrane to exocytose transmitter, but do not recycle locally to produce new storage vesicles.

In contrast, the relatively complex "cycle" of synaptic vesicles leads to a range of means to regulate synaptic transmission. Soon after early electron microscope images of synapses demonstrated the presence of small ( $\sim 40 \text{ nm}$ diameter) "synaptic vesicles" in axonal terminals (Palade, 1954; De Robertis and Bennett, 1955), Sanford Palay made a link between Fatt's conjecture from quantal recording, writing, "The heretofore unrecognized structure demanded by these physiological data may be the small vesicles which crowd the axon terminals, cluster at the junctional surface, and open onto the intrasynaptic space" (Palay, 1956). Indeed, catecholamine synaptic vesicles played an important role in confirming the hypothesis that synaptic vesicles store and release neurotransmitter, as they accumulate osmophilic catecholamine reaction products (Wood, 1966) and the osmophilic false transmitter 5-hydroxydopamine (Tranzer and Thoenen, 1967).

Eric Holtzman (Holtzman et al., 1971) and subsequently Bruno Ceccarelli (Ceccarelli et al., 1972) showed that fluid phase endocytotic tracers such as horseradish peroxidase are accumulated by synaptic vesicles during stimulation, and that after the tracer is removed, further stimulation eliminates the label. This demonstrated that small synaptic vesicle membrane was endocytosed from the plasma membrane following full fusion and that synaptic vesicles were reformed (i.e., recycled) and then underwent further bouts of fusion to release the tracer. Early studies indicated that some vesicles may fuse transiently via a fusion pore without full fusion (Ceccarelli et al., 1973; Heuser and Reese, 1973; Valtorta et al., 2001), a process typically known as 'kiss-and-run" fusion, and although clearly demonstrated for large dense core vesicles (Williams and Webb, 2000), this continues to be controversial for small synaptic vesicles (Klyachko and Jackson, 2002; Gandhi and Stevens, 2003; Mitchell and Ryan, 2004; Staal et al., 2004; Zhang et al., 2009a), although amperometric recordings of quantal dopamine release are consistent with "flickering" reversible fusion pore formation (see following section).

The synaptic vesicle cycle introduced by Holtzman and Ceccarelli can be modeled as a series of kinetic steps, including the uptake of neurotransmitter by specific transporters that utilize an energy gradient formed by an ATP driven proton pump, trafficking of vesicles to a presynaptic release site, a "docking" step which tethers the vesicle to its eventual site of fusion with the plasma membrane, a "priming" step during which the docked vesicle is placed in a fusion-ready state, a fusion step which may proceed via full fusion with the membrane or transient fusion, and a series of recycling steps leading to vesicle reformation (Edwards, 2007).

## 3. Dopamine Vesicle Fusion Events and Quantal Size

Amperometric recordings from chromaffin and mast cells suggest that the fusion pore during large dense cored vesicle fusion can exist in at least two states, a "foot" that represents a reversible fusion pore, and a full event that often indicates full fusion (Alvarez de Toledo and Fernandez, 1990; Chow et al., 1992; Albillos et al., 1997; Xu and Tse, 1999). Such findings suggest that fusion pore modulation is capable of affecting the amount and kinetics of transmitter release.

Amperometric recordings at high time resolution (~50µsec) demonstrate that dopamine small synaptic vesicle fusion pores flicker either once (*simple* events) or multiple times in rapid succession (*complex* events), with each flicker releasing on average ~25–30% of total vesicular dopamine. The type of event is apparently regulated by PKC activity (Staal et al., 2004), as drugs that enhance PKC activity increase the number of events per stimulus but decrease the fraction of complex events, whereas staurosporine, a broad spectrum kinase inhibitor, decreases the number of events.

Complex events may provide a means by which neurons can rapidly reuse vesicles without undergoing the comparatively slow process of recycling. As complex events release a higher quantal size, complex events could regulate the spillover of neurotransmitter (see below). Transient flickering of the fusion pore also appears to occur in adrenal chromaffin and other large dense cored vesicles (Alvarez de Toledo and Fernandez, 1990; Zhou et al., 1996) but the duration of dopamine synaptic vesicles subunits is considerably shorter (100–150 $\mu$ s vs. 10,000–500,000 $\mu$ s respectively), occurs at a much higher frequency than in LDCVs (4000 Hz vs. 170 Hz) (Zhou et al., 1996), and releases a far greater fraction of the vesicle's neurotransmitter (25–30% vs. <1%) (Zhou et al., 1996).

## B. Regulation of Quantal Size

In addition to the mode of fusion, a variety of means to regulate steps in synaptic vesicle cycling that modify the quantal size of dopamine neurotransmission have been identified: detail is provided in extensive reviews (Sulzer and Pothos, 2000; Edwards, 2007). Pertinently, there has been a long-standing parallel effort on the part of William Van der Kloot to detail presynaptic mechanisms that lead to altered quantal size in the neuromuscular junction, and his reviews, which precede the advent of amperometric quantal detection in the CNS, are highly recommended (Van der Kloot, 1991; Van der Kloot and Molgo, 1994).

## 1. Altered Free Energy for Vesicular Dopamine Sequestration

In the 1980s, work by several groups characterized how chromaffin granules maintain high levels of monoamines against a large concentration gradient (Njus et al., 1986; Johnson, 1988). In isolated chromaffin granules, monoamines (A) distribute according to the electrochemical gradient composed of the voltage gradient DY and pH gradient as:

$$\log([A]_{in}/[A]_{out}) = \Delta \Psi F/RT + 2\Delta pH$$

In chromaffin granules, granule pH is often estimated to be ~5.6, the cytosolic pH ~7.2, and DY ~ + 80 mV. Assuming RT/F = 59 mV, this indicates an equilibrium transvesicular catecholamine gradient of ~36,000:1.

This relationship hints at multiple interventions that might alter quantal size. First, we will discuss effects on the *right* hand side of the equation the pH and electrical gradients. Then, we will discuss effects of the *left* side of the equation, the dopamine concentration gradient across the membrane.

### 2. pH, Electrical Gradients, and Amphetamines

The pH gradient is provided by the vacuolar H<sup>+</sup>-ATPase, which consists of V0 and V1 subunits (Drory and Nelson, 2006; Nakanishi-Matsui and Futai, 2006). [Some data implicate the V0 domain in the process of vesicle fusion as well (Peters et al., 2001; Hiesinger et al., 2005).]

Mani and Ryan have used "synaptopHlorin", a fluorescent pH sensitive mutation of the synaptic vesicle protein synaptobrevin, to determine that the internal pH of dopamine synaptic vesicles in situ is about 5.6 (Mani and Ryan, 2009): while the pKa of the protein is neutral, they took advantage of the dependence of total fluorescence on the contribution of plasma membrane synaptopHlorin to solve a simultaneous equation that would describe changes due to weak base induced synaptic vesicle pH collapse and quenching of the external signal using acidic buffer.

Exposure of isolated catecholamine vesicles to protonophores collapses the pH gradient and rapidly redistributes transmitter from inside to outside the vesicle (Johnson, 1988; Sulzer and Rayport, 1990), while the proton pump inhibitor bafilomycin reduces quantal size in chromaffin cells (Pothos et al., 2002). Lipophilic weak bases such as chloroquine are distributed across membranes according to the pH gradient (Maron et al., 1983). As their concentration becomes sufficiently high, they exceed the buffering capacity of the vesicle interior and collapse the pH gradient. Thus, lipophilic weak bases collapse the pH gradient, leading to decreases in quantal size (Sulzer et al., 1995). The amphetamines are weak base compounds that are the only widely used class of drugs that elicit transmitter release by a non-exocytic mechanism (Sulzer et al., 2005). It seems likely that as both VMAT and DAT substrates (Amara and Kuhar, 1993; Pifl et al., 1995), amphetamines are effectively sequestered in vesicles. Amphetamine provided a first instance of pharmacological manipulation of dopamine quantal size, as seen in an adrenal derived cell line (PC12 cells) (Sulzer et al., 1995). Cyclic voltammetry (CV) recordings in the acute striatal slice strongly suggest that similar actions occur in intact tissue (Jones et al., 1998a; Schmitz et al., 2001). Interestingly, two classes of dopamine vesicles are detected in the giant dopamine neuron of Planorbis corneus, and they are differentially depleted by amphetamine (Anderson et al., 1998).

An unexpected effect of prolonged amphetamine or weak base exposure, at least in adrenal vesicles, is a

delayed rebound hyperacidification that eventually leads to an enhanced quantal size (Markov et al., 2008), although this has not been explored for small synaptic vesicles. Likewise, extensive depolarization also acidifies chromaffin vesicles (Pothos et al., 2002) and increases quantal size (Finnegan and et al., 1996; Pothos et al., 2002), in tandem with a greater proportion of larger "active" vesicles that contain a halo around the dense core (Colliver et al., 2000; Pothos et al., 2002), [see also (Han et al., 1999; Elhamdani et al., 2001; Camacho et al., 2006; Camacho et al., 2008)]. The means by which prolonged weak base exposure or stimulation regulate enhanced acidification are unknown, although PKA and PKC effects on quantal size and exocytosis (Machado et al., 2001; Staal et al., 2008) may be involved, as these kinases can be regulated by activity, via by calcium-dependent mechanisms. Calcium gradients may further regulate vesicle trafficking (Camacho et al., 2008).

It appears that additional regulation of ionic conductances, particularly via chloride channels (Jentsch et al., 2005), trp channels (Krapivinsky et al., 2006) and glutamate accumulated by a vesicular vGluT transporter (R.H. Edwards et al., under submission) across the synaptic vesicle also control the net accumulation of dopamine, by regulating the electrical gradient, although this has to date been explored mostly in adrenal chromaffin and other large dense cored vesicles (Barasch et al., 1988; Tamir et al., 1996; Pothos et al., 2002).

#### 3. Transmitter Concentration Gradients

If the electrochemical gradient is unchanged, a 2-fold increase in cytosolic levels of transmitter should produce a 2-fold increase in quantal size. Typically, the synthesis of L-DOPA from tyrosine via tyrosine hydroxylase provides the rate-limiting step in catecholamine synthesis, and so the dopamine precursor L-DOPA is the most widely used clinical intervention for Parkinson's disease. Amperometric recordings demonstrate that L-DOPA rapidly elevates quantal size in midbrain neurons (Pothos et al., 1998a; Puopolo et al., 2001; Kim et al., 2008) and secretory cells (Pothos et al., 1996) (Pothos et al., 2002). The effects on quantal size appear consistent with in vivo cyclic voltammetry results, where L-DOPA rapidly increased evoked dopamine release (Garris et al., 1994). Tyrosine hydroxylase activity appears to underlie changes in quantal size mediated by D2 autoreceptors in PC12 cells (Pothos et al., 1998b) in addition to autoreceptor-mediated effects on the number of events released (see below).

### 4. VMAT2 Activity

The level of VMAT expression further regulates dopamine accumulation. As originally reported by Carlsson and Kirshner in chromaffin vesicles, inhibition of VMAT1 with reserpine decreases catecholamine content (Carlsson et al., 1962; Kirshner, 1962), and consistently, amperometry showed that it decreased quantal size (Kozminski et al., 1998). More surprisingly, reserpine decreased the volume of large dense cored vesicles, while L-DOPA exposure increased the vesicle volume, with the resulting catecholamine concentration apparently remaining constant (Colliver et al., 2000; Pothos et al., 2002; Gong et al., 2003). The means by which large dense cored vesicle volume changes occur remain obscure.

Expression of the CNS transporter, VMAT2, can convert even hippocampal neurons to secrete dopamine in the presence of L-DOPA (Li et al., 2005). In cultured dopamine neurons, overexpression of VMAT2 markedly increases quantal size, and also increases the number of events per stimulus, likely by revealing events that were otherwise buried in the noise (Pothos et al., 2000). While quantal recordings have not been conducted in neurons underexpressing VMAT2, mutants with low VMAT2 activity release less dopamine (Patel et al., 2003; Croft et al., 2005), whereas VMAT2 overexpressing mice release more (H. Zhang, R. Edwards, et al., unpublished results), suggesting that corresponding changes in quantal size occur. Interestingly, VMAT2 knockout mice appear to recycle synaptic vesicles in dopamine terminals normally, indicating that recycling steps may be independent of the rate of transmitter accumulation (Croft et al., 2005).

#### 5. Growth Factors

Exposure to glial-derived neurotrophic factor (GDNF) increases the quantal size of dopamine release neurons (Pothos et al., 1998a), although whether this occurs by altering energy or substrate concentration gradients, transporter activity, vesicle fusion, or vesicle volume remains unknown.

## 6. Regulation of the Number of Quanta Released

As apparent from the steps involved in the synaptic vesicle cycle, there are likely to be multiple presynaptic means to regulate the number of dopamine quantal events evoked per stimulus. As demonstrated by Fatt and Katz (1952), extracellular calcium regulates quantal release; the role of presynaptic calcium channels in dopamine release has been characterized in axon terminals (Phillips and Stamford, 2000), which contain N and P/Q type calcium channels, as well as dendrites (Chen et al., 2006b; Beckstead et al., 2007), where CaV1.3 channels in the substantial nigra (Nedergaard et al., 1993; Chan et al., 2007) regulate pacemaking. A variety of heteroreceptors and the D2 autoreceptor appear to regulate these calcium currents (Cardozo and Bean, 1995) (see below).

While there has been little analysis of the regulation of dopamine synaptic vesicle recycling and reacidification, there is evidence suggesting that this occurs at somewhat higher rates than for hippocampal terminals under high stimulus (Mani and Ryan, 2009). The fusion of chromaffin large dense cored vesicles is regulated by the adaptor protein AP-3 (Grabner et al., 2005; Grabner et al., 2006) and over-expression of the neuronal isoform reduces quantal size while the loss of AP-3 increases quantal size with consistent changes in vesicle volume. Although AP-2 and AP-3 are involved in synaptic vesicle recycling (VogImaier et al., 2006) their effects on dopamine quantal size are unreported.

A relatively surprising means to regulate quantal dopamine release is via alpha-synuclein, which inhibits dopamine release. The inhibition of dopamine release by alpha-synuclein is attenuated with high activity and high calcium (Abeliovich et al., 2000; Yavich et al., 2004; Yavich et al., 2005), possibly as high calcium redistributes alpha-synuclein away from synaptic vesicles (Fortin et al., 2004). In chromaffin cells, this inhibitory effect appears to take place at a late pre-fusion "priming" step (Larsen et al., 2006).

Relatively little research has examined quantal dopamine release for mutations of proteins directly involved in exocytosis, such as the SNARE proteins or synaptotagmin, as most efforts have examined such roles in adrenal cells or the adrenal-derived PC12 cell line (Wang et al., 2003). Examination of synapsin I/II/III triple knock-out mice, however, revealed that these proteins regulate dopamine synaptic vesicle "reserve pools" (Venton et al., 2006).

## C. Regulation of Release by Autoreceptors

Dopamine autoreceptors expressed on dopamine neurons and presynaptic axon terminals provide feedback and regulate dopamine release. While activation of dopamine autoreceptors decrease release by inhibiting dopamine synthesis and enhancing dopamine reuptake by the dopamine transporter as well as regulating VMAT expression (Schmitz et al., 2003), here we specifically review data on effects on the probability of release events.

Dopamine autoreceptors belong to the D2-family (D2, D3, D4) of dopamine receptors that are coupled to inhibitory G proteins and modulate ion channel activity and/or inhibit adenylyl cyclase. D2 receptors are expressed along the somatodendritic extent of midbrain dopamine neurons, as well as at their axon terminals in the striatum and nucleus accumbens (Sesack et al., 1994). Roles for D2 autoreceptors in presynaptic regulation are clearly established, but a role for D3 autoreceptors has been controversial. D3 immunoreactivity was found in almost all midbrain dopamine neurons, but is undetectable in the terminal regions (Diaz et al., 2000). While it has been suggested that both D2 and D3 receptors function as autoreceptors (Sokoloff et al., 1990; Gainetdinov et al., 1994; Tepper et al., 1997; Zapata et al., 2001), no deficits in autoreceptor functions were apparent in D3 receptor knockout mice, although extracellular dopamine was elevated in the ventral striatum (Koeltzow et al., 1998). D2 receptor knockout mice exhibited no detectable autoreceptor response to D2-class receptor agonists in firing rate, dopamine release or dopamine synthesis. Thus, results implicated the D2 receptor as the only functional autoreceptor in the D2-family (Mercuri et al., 1997; L'Hirondel et al., 1998; Benoit-Marand et al., 2001; Schmitz et al., 2002). Nevertheless, one study of D3 KO mice using CV in striatal slices has demonstrated a small D3 role for regulation of secretion, but not synthesis, in the striatum (Joseph et al., 2002).

Studies on transfected cells demonstrated that D3 receptors can modulate the same ion channels G protein-activated inwardly rectifying potassium channels (GIRKs) as D2 receptors (Kuzhikandathil et al., 1998; Kuzhikandathil and Oxford, 1999, 2000), and have the potential to affect dopamine release (Tang et al., 1994). However, a study on acutely dissociated midbrain neurons from D2 receptor knockout mice did not find evidence for D3 receptor-activation of GIRK currents (Davila et al., 2003).

There is little evidence supporting a role of D4 receptors as autoreceptors beyond an immunohistochemistry study that demonstrated presynaptic D4 receptor localization in a subset of mesoaccumbal terminals in the nucleus accumbens shell (Svingos et al., 2000).

In summary, it appears that dopamine autoreceptor function is predominantly carried out by D2 receptors. In recent studies, it has been suggested that of two isoforms of the D2 receptor generated by alternative splicing, D2S and D2L, D2S serves presynaptic autoreceptor functions regulating dopamine release while D2L acts mainly at postsynaptic sites (Usiello et al., 2000; Wang et al., 2000; Centonze et al., 2002; Rouge-Pont et al., 2002).

### 1. In Vitro Studies

The regulation of dopamine release by dopamine autoreceptors was initially studied *in vitro* with neurochemical approaches (Starke et al., 1989). D2 autoreceptor activation inhibits axon terminal (Starke K et al., 1978; Cubeddu and Hoffmann, 1982; Dwoskin and Zahniser, 1986; Mayer et al., 1988; Palij et al., 1990; Kennedy et al., 1992; Cragg and Greenfield, 1997) and somatodendritic dopamine release (Cragg and Greenfield, 1997).

The molecular mechanism underlying the inhibition of dopamine release through terminal D2 autoreceptor is unknown. One possibility is that D2 autoreceptors inhibit the voltage-gated Ca<sup>2+</sup> channels in axon terminals, thus directly inhibiting Ca<sup>2+</sup> -dependent release of dopamine. Patch clamp studies on dopamine midbrain neurons in vitro have revealed a D2 receptor regulation of voltage-gated calcium currents (Cardozo and Bean, 1995) and axon terminal dopamine release is dependent on N and P/Q type calcium channels (Phillips and Stamford, 2000; Chen et al., 2006a). This has not been proven directly, however, and a study on autapses (i.e., synapses that a neuron makes on itself) of midbrain neurons in culture found no evidence for a D2 autoreceptor regulation of calcium influx (Congar et al., 2002) but that 4-aminopyridine-sensitive  $K^+$  channels acting downstream from calcium influx are involved. Autapses of cultured dopamine neurons are glutamatergic (Sulzer et al., 1998), however, and it is not known whether this finding can be extrapolated to dopamine release. Nevertheless, the broad-spectrum K<sup>+</sup> channel blockers 4-aminopyridine and tetraethylammonium limit quinpirole ability to inhibit evoked dopamine release in slices (Cass and Zahniser, 1991), supporting a role for presynaptic  $K^+$ channels.

#### 2. In Vivo Studies

The regulation of dopamine autoreceptors was subsequently shown in vivo with microdialysis. Indeed, systemic administration of D2 antagonists enhances the extracellular dopamine level (Imperato and Di Chiara, 1985). Moreover, intrastriatal infusion of D2 agonists or antagonists decreases or enhances extracellular dopamine, respectively (Imperato and Di Chiara, 1988). This autoregulation acts on the impulse flow-dependent dopamine release (Imperato and Di Chiara, 1985) and is not mediated by an indirect action on striatal neurons (Westerink and De Vries, 1989). However, the extracellular dopamine level results from a dynamic equilibrium between dopamine release and dopamine reuptake, and microdialysis is not suitable for distinguishing between changes in release and reuptake.

In vivo electrochemical studies have shed light on the D2 autoregulation of dopamine release and of dopamine reuptake. These studies confirmed that the tonic level of extracellular dopamine concentration is high enough to exert a tonic stimulation of D2 autoreceptors, which inhibits the impulse flow-dependent dopamine release (May and Wightman, 1989; Suaud-Chagny et al., 1991). However, the electrochemical techniques used in these initial studies were too slow to describe the kinetics of the D2 autoregulation. The use of a faster technique and of control experiments with mice lacking D2 receptors made it possible to describe the dynamic characteristics of D2 autoregulation (Benoit-Marand et al., 2001). The amplitude of the dopamine release per pulse was tested with brief electrical stimulations (three pulses at 100 Hz) and the inhibitory effect of conditioning stimulation on the response to the test stimulation was investigated. The onset of the D2 effect is between 50 ms and 100 ms. The D2 inhibition reaches a maximum between 150 and 300 ms after the end of the conditioning stimulation and disappears within 800ms (Benoit-Marand et al., 2001). Similar kinetics have been described with in vitro experiments (Phillips et al., 2002; Schmitz et al., 2002) with the maximum effect around 500ms and a duration of less than 5 sec. The slightly longer time course determined in the in vitro studies is likely due to the larger amount of dopamine released per stimulation in slice preparations.

#### D. Regulation of Release by Heteroreceptors

In addition to D2 autoreceptors, there are additional neurotransmitter receptors on dopamine cell bodies and terminals, but their modulatory roles in dopamine release are less well characterized. Although modulation of dopamine transmission can occur at both the midbrain dopamine cell bodies and at the presynaptic terminals, we focus this discussion on the terminal level. For effects on cell bodies, we refer to other studies (e.g., Bonci et al., 2003; Mansvelder et al., 2003; Margolis et al., 2003; Cruz et al., 2004) (see also Chapter 16).

Most drugs including nicotine, ethanol, opioids, morphine, and cannabinoids enhance dopamine neuronal activity by acting directly on dopamine neurons or indirectly on GABA interneurons through the mechanism of disinhibition (Cheer et al., 2004). Electron microscopic demonstration of heteroreceptor immunolabel in dopamine terminals is invaluable for establishing their presence, but there are relatively few studies in this area due to technical difficulties. To date, only the GDNF receptor (Araujo et al., 1997), nicotinic receptors (nAChRs) (Wonnacott et al., 2000), delta and kappa opioid receptors (Svingos et al., 1999; Svingos et al., 2001), the metabotropic receptor mGluR1 (Paquet et al., 1997), and possibly the GABA(B) receptor (Charara et al., 2000) have been observed by ultrastructural immunolabel in dopamine terminals. Although the presence of other presynaptic receptors in dopamine terminals remains to be fully elucidated, studies using synaptosome preparations, microdialysis, and CV recordings in vivo and in vitro suggest important roles for heteroreceptor modulation on dopamine release, albeit with some conflicting results.

#### 1. Glutamate Receptors

While microdialysis studies indicated a stimulatory effect for ionotropic glutamate receptors on dopamine release, CV recordings demonstrated an inhibitory role on evoked dopamine release in the terminal region (Wu et al., 2000; Kulagina et al., 2001; Avshalumov et al., 2003). The effects of ionotropic glutamate-receptor activation on dopamine release are most likely indirect given that dopamine terminals lack these receptors (Bernard and Bolam, 1998; Chen et al., 1998). Recent studies by Margaret Rice's group suggest that glutamatergic regulation of dopamine release are indeed indirect and mediated by AMPA receptors on striatal cells, which is in turn mediated through retrograde signaling by diffusible H<sub>2</sub>O<sub>2</sub> generated in striatal cells, rather than in dopamine axons (Avshalumov et al., 2008). In contrast to ionotropic glutamate receptors, the metabotropic receptor mGluR1 has been detected by ultrastructural immunolabel on striatal dopamine terminals (Paquet and Smith, 2003), and evoked dopamine release can be inhibited via metabotropic glutamate receptors on dopamine terminals (Zhang and Sulzer, 2003).

#### 2. GABA Receptors

By local infusion of GABA(A) and GABA(B) receptor agonists and antagonists in the striatum of intact and kainic acid lesioned rats, microdialysis data support a direct influence of GABA on the dopamine terminals via presynaptic GABA(B) receptors, while the effects via the GABA(A) receptor seem to be postsynaptic and mediated by striatal interneurons (Smolders et al., 1995). The direct effect of GABA(B) receptors on dopamine release is further supported by a study using CV recordings, which shows that GABA(B) receptor agonists inhibit single pulse evoked dopamine release in the striatal slice with kinetic parameters similar to those of the D2 autoreceptor (Schmitz et al., 2002). There is ultrastructural evidence consistent with expression of GABA(B) receptors on dopamine terminals (Charara et al., 2000).

Functional studies suggest that GABA(A) receptors might be colocalized on the dopamine terminals. Muscimol, a GABA(A) receptors agonist, inhibits the evoked dopamine release in striatal synaptosomes (Ronken et al., 1993) and muscimol also inhibits evoked dopamine release by single pulse stimulation measured by CV in striatal slices (Zhang and Sulzer, unpublished observations).

### 3. Acetylcholine Receptors

Classical pharmacological studies of the effects of muscarinic acetylcholine receptors (mAChRs) on dopamine release in the striatum have led to contradictory results (Lehmann and Langer, 1982; Raiteri et al., 1984; Schoffelmeer et al., 1986; Kemel et al., 1989; Xu et al., 1989; De Klippel et al., 1993; Smolders et al., 1997), likely due in part to the diversity of the mAChR subtypes involved in this activity and the limited receptor subtype selectivity of the muscarinic agonists and antagonists used in these studies (Wess, 1996). Activation of mAChRs was shown to inhibit dopamine release in slices examined by CV (Kudernatsch and Sutor, 1994). A humanbrain imaging study indicated a tonic muscarinic inhibition of dopamine release (Dewey et al., 1993). A study using genetically altered mice that lacked functional M1-M5 mAChRs provides evidence of the different physiological roles of individual AChRs in a direct manner (Zhang et al., 2002). The results show that M3 receptors inhibit release, whereas M4 and M5 receptors facilitate release, and M1 and M2 receptors had no effect on dopamine release. It seems that the modulating effects of M3 and M4 receptors are mediated via striatal GABA release. M5 receptor mRNA is the only mAChR subtype mRNA detectable in the dopamine-containing cells of the substantia nigra pars compacta (Vilaro et al., 1990; Weiner et al., 1990), strongly suggesting that the dopamine release-facilitating M5 receptors are located on dopamine nerve terminals.

Nicotinic acetylcholine receptors (nAChRs) are expressed on dopamine terminals in the striatum (Wonnacott et al., 2000; Zoli et al., 2002). Nicotine enhanced the extracellular dopamine level by microdialysis (Pontieri et al., 1996) and results in vivo indicate that nicotine, like cocaine and alcohol, increase the frequency of non-evoked dopamine transients in the nucleus accumbens (Cheer et al., 2007). CV studies demonstrated an inhibition of evoked dopamine release in slices by nicotinic agonists (Kudernatsch and Sutor, 1994; Zhou et al., 2001). Because nAChRs antagonists also inhibit dopamine release, it appears that nicotine is excitatory for dopamine release, but the receptor is rapidly desensitized by nicotine application (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004).

## 4. Opioid Receptors

Kappa opioid receptors are located on dopamine axon terminals (Svingos et al., 2001), while mu opioid receptors are not expressed on striatal dopamine axon terminals (Trovero et al., 1990). Using CV, Schlosser et al. (1995) demonstrated that mu, delta, and kappa opioid receptors exerted an inhibitory control on striatal dopamine release (see also Chapter 29). It seems that the effect of kappa opioid receptors on dopamine overflow is likely to be direct, while the influence of mu opioid receptors is indirect, mediated by an inhibition of cholinergic interneuron activity (Svingos et al., 2001; Britt and McGehee, 2008). Whether the effect of delta opioid receptors on dopamine release is direct remains unclear since ultrastructural studies show that delta opioid receptors are present on dopamine terminals (Svingos et al., 1999).

#### 5. Adenosine Receptors

It is unclear whether there is a direct heteroreceptor modulation by adenosine on dopamine release. Activation of A1 receptors inhibits dopamine release (Jin et al., 1993; Quarta et al., 2004) and activation of A2A receptors increases dopamine release in the striatum (Golembiowska and Zylewska, 1998). CV studies also demonstrate that A1 receptor agonists decrease single pulse evoked dopamine release in vitro, but the inhibition is dependent at least in part on the simultaneous activation of D1 dopamine receptors. While the mechanism underlying this interaction remains to be determined, it does not appear to involve an intramembrane interaction between A1 and D1 receptors (O'Neill et al., 2007). To date, the morphological evidence of presynaptic localization of A1 receptors on dopamine terminals is still indirect (Mahan et al., 1991; Rivkees et al., 1995; Glass et al., 1996). Furthermore, the lesion of glutamatergic, but not dopamine, striatal afferents significantly decreases striatal A1 receptor function and agonist binding (Alexander and Reddington, 1989). In addition, there are no A2A receptors on dopamine terminals (Hettinger et al., 2001; Rosin et al., 2003). Therefore, the main mechanism underlying adenosine-mediated modulation of striatal dopamine release may be indirect (see also Chapter 11).

### 6. Cannabinoid Receptors

CV studies have not identified a direct presynaptic modulation of dopamine release by type 1 cannabinoid (CB1) receptors (Szabo et al., 1999; Zhang and Sulzer, 2003; Sidlo et al., 2008), in agreement with the lack of anatomical evidence of CB1 receptor on dopamine terminals (Matsuda et al., 1993; Romero et al., 1997; Fusco et al., 2004). Nevertheless, there are indirect effects by cannabinoids on dopamine release. Ventral tegmental area dopamine neurons are thought to produce the cannabinoids (Riegel and Lupica, 2004; Lupica and Riegel, 2005; Matyas et al., 2008), which would be expected to activate local receptors. CB1 agonists decrease evoked dopamine release while increasing the frequency of non-evoked dopamine concentration transients in rat striatum, responses that may be related to effects on neuronal firing (Cheer et al., 2004). Additional effects can be seen in striatal slices, where a CB1 agonist decreased DA released over trains of stimuli, suggesting cannabinoids exert indirect changes via a local striatal circuit. Rice and colleagues have suggested this could occur via a nonsynaptic mechanism involving inhibiton of GABA release, generation of hydrogen peroxide, and activation of KATP channels to inhibit DA release (Sidlo et al., 2008).

In addition, there are additional ionotropic and G-protein-linked receptor candidates that may act as heteroreceptors on dopamine terminals, and elucidation of their effects is fundamental for understanding their roles in modulating dopamine transmission.

## E. Relationship Between Impulse Flow and Vesicular Release

## 1. Frequency Dependent Modulation of Dopamine Release

As detailed above, numerous studies provide evidence for heteroreceptor regulation of dopamine release, although some results are controversial due in large part to different preparations, stimulation and recording paradigms. In vivo and in vitro data in slices with local stimulation cannot exclude circuit effects. There are striking differences between such results in the slice preparation where there is significant paired pulse depression, and in vivo, where there is typically no detectable depression. While the basis for these differences remain unclear one factor is presumably due to the loss of most ongoing synaptic activity in the slice. Furthermore, most voltammetry studies in striatal slice only examine the effects evoked by single pulse stimuli mimicking the tonic firing mode, while recent studies have demonstrated the frequency dependent modulation of dopamine release by nAChRs (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009b).

Nicotine shifts VTA neurons from tonic to burst firing modes (Grenhoff et al., 1986; Mansvelder et al., 2003) and enhances extracellular dopamine as measured by microdialysis (Pontieri et al., 1996). On the other hand, nicotine at levels thought to be experienced by smokers (~250–300 nM) desensitizes nAChRs so rapidly that tonic ACh activation is blocked and evoked dopamine release is potently inhibited (Kudernatsch and Sutor, 1994; Zhou et al., 2001).

In order to resolve the question of how nicotine both elevates extracellular dopamine and depresses evoked dopamine release, the effect of nicotine on the modulation of evoked dopamine release were compared under different firing patterns and found to be dependent on the firing pattern of dopamine neurons. While desensitization of nAChRs indeed curbs dopamine released by stimuli emulating tonic firing, it allows a rapid rise in dopamine from stimuli emulating the phasic firing patterns associated with incentive/ salience paradigms. Nicotine may thus enhance the contrast of dopamine signals associated with behavioral cues (Rice and Cragg, 2004; Zhang and Sulzer, 2004) (Fig. 17.1). Interestingly, mu opioid agonists also inhibit dopamine overflow elicited with single-spike stimuli while leaving that produced by burst stimuli unaffected, but these differential effects are mediated by nAChRs and caused by inhibition of cholinergic interneurons (Britt and McGehee, 2008).

Remarkably, most heteroreceptors studied to date have been found to depress dopamine release. Therefore, in addition to D2 autoreceptors, most heteroreceptors in vivo may maintain the dopamine release probability at a low level, which may then provide a marked and rapid increase in dopamine concentration during phasic firing. A range of drugs that affect the dopamine system may exert their actions via altering the signal/noise ratio of dopamine by affecting heteroreceptors on dopamine terminals as well as on cell bodies (Zhang and Sulzer, unpublished observations).

## 2. Failure of Vesicular Release at Individual Dopamine Terminals

In the sympathetic nervous system the release of noradrenaline exhibits a very low probability per release site (about 1%) (Msghina et al., 1993). In other words, although electrically evoked action potentials always reach sympathetic terminals, very often they do not trigger noradrenaline release. Despite this very high rate of failure (99%), the contraction of certain smooth muscles (e.g. the rat tail artery) is entirely due to the stimulation of extracellular noradrenergic receptors by the impulse flow-dependent noradrenaline release. Indeed, robust contraction is achieved by a burst of action potentials, which triggers an extracellular accumulation of released noradrenaline because noradrenaline reuptake is too slow to clear noradrenaline during this burst (Gonon et al., 1993).

The high rate of failure at single sympathetic terminals has been precisely estimated with electrophysiological techniques but similar data are not available regarding the dopamine transmission. The use of a fluorescent dopamine analog as a fluorescent false neurotransmitter shows that the fraction of total presynaptic dopamine released per action potential is far less than 1% and is regulated by the frequency of activity (Gubernator et al., 2009). This, however, does not directly indicate the failure rate of vesicular release at individual terminals because each terminal contains hundreds of vesicles (Hersch et al., 1995).

However, it is likely that the rate of failure of dopamine terminals is much lower than regarding the sympathetic terminals. First, failure of noradrenaline release has been also observed with carbon fiber electrodes coupled with electrochemical techniques, which monitored the noradrenaline released from some tens of terminals (Msghina et al., 1993). In contrast, with a similar approach no failure of dopamine release was observed in the rat nucleus accumbens (Garris et al., 1994). Second, the averaged amplitude of the noradrenaline overflow evoked in a sympathetic preparation by a single pulse stimulation is about 50 times smaller than that of the dopamine overflow evoked in vivo in the striatum by a single pulse stimulation (compare data from (Gonon et al., 1993) with those from (Benoit-Marand et al., 2000)).



**FIGURE 17.1** Modulation of evoked dopamine (DA) release by nAChRs depends on firing pattern. (A, B) Voltammetric responses before and after 10-min bath application of nicotine (Nic, 300 nM) or mecamylamine (Meca, 2 $\mu$ M) at different stimuli. The inhibition of evoked release was not blocked by D2 dopamine, GABA(A), GABA(B) or ionotropic glutamate receptor antagonists. (C, D) Frequency modulation of nicotine and mecamylamine effects on dopamine release (mean ± s.e.m, *n* = 8 for control; *n* = 5 for nicotine, *n* = 4 for mecamylamine; \**P* < 0.05 compared with respective control values by Student's *t*-test). Top panels: evoked dopamine release normalized to that elicited by 1p stimulation under control condition. Bottom panels: relative evoked dopamine release after nicotine and mecamylamine at different stimulation frequencies. (E–H) Effects of number of pulses, nicotine and mecamylamine on dopamine release at 20 Hz (E, F) and 100 Hz (G,H) (mean ± s.e.m., *n* = 4–8, \**P* < 0.05 compared with respective control values by Student's *t*-test). Data from Zhang and Sulzer (2004), copyright by Nature Publishing Group.

## **III. DOPAMINE REUPTAKE**

During the 1960s it was shown that dopamine neurons are equipped with a dopamine transporter (DAT) and it was hypothesized that DAT might control the intensity and duration of the dopamine transmission. Here, we focus on this functional role of dopamine reuptake, while other aspects of DAT are reviewed elsewhere (Uhl, 2003).

Microdialysis and voltammetric techniques coupled with carbon fiber electrodes were developed during the 1980s and enabled monitoring the extracellular level of dopamine in vivo. Both technical approaches showed that in resting conditions, this level was low (10-20 nM) and that dopamine reuptake strongly contributed to clearing dopamine from the extracellular space. Indeed, pharmacological inhibition of dopamine reuptake potently enhanced the extracellular dopamine level (Gonon and Buda, 1985; Church et al., 1987) and this enhancement was no longer observed after blocking the impulse flow-dependent dopamine release (Carboni et al., 1989). These approaches, however, were too slow to accurately describe the kinetics of dopamine clearance and could not distinguish between an increase in dopamine release and a decrease in dopamine clearance. Both issues were resolved with improvements in voltammetric techniques (Wightman and Zimmerman, 1990; Dugast et al., 1994). Moreover, the design of mice lacking DAT (Giros et al., 1996) made it possible to investigate the relative contribution of dopamine reuptake to dopamine clearance versus extracellular degradation, non-neuronal uptake and diffusion.

## A. Reuptake Replenishes the Releasable Pool

In dopamine terminals, dopamine is synthesized from L-DOPA by a decarboxylase and L-DOPA is synthesized from tyrosine by tyrosine hydroxylase (TH), an enzyme that is specifically expressed by catecholaminergic neurons. However, pharmacological inhibition of TH activity by alpha-methyl-para-tyrosine (AMPT) only partly and very slowly depresses the dopamine tissue content (Jones et al., 1998b) and in vivo dopamine release (Benoit-Marand et al., 2000) in wild-type (WT) mice. In DAT -/- mice, although TH activity is doubled, AMPT induces a profound and rapid decrease of the dopamine tissue content (Jones et al., 1998b) and of in vivo dopamine release (Benoit-Marand et al., 2000). These observations demonstrated that in brain structures densely innervated by dopamine terminals, recycling of released dopamine by reuptake plays a major role in replenishing the releasable pool of dopamine.

## **B.** Extracellular Elimination of the Released Dopamine is Achieved by Reuptake

Electrical stimulation of the dopamine axons either with a single pulse or with a brief train (e.g. four pulses at 100Hz) induces a brief dopamine overflow that can be detected with rapid electrochemical techniques either in vitro (Schmitz et al., 2001) or in vivo (Dugast et al., 1994; Garris et al., 1994). The decay phase of this evoked dopamine overflow reflects the clearance of released dopamine. Dopamine reuptake inhibitors slow down the clearance kinetics by one order of magnitude (Garris et al., 1994; Suaud-Chagny et al., 1995; Schmitz et al., 2001). However, in the striatum of DAT -/- mice the decay phase is slowed down by two orders of magnitude both in vitro (Giros et al., 1996; Jones et al., 1998b) and in vivo (Benoit-Marand et al., 2000). Pharmacological inhibition of dopamine degradation does not further slow the decay phase in DAT -/mice in vitro. However, in vivo, inhibition of dopamine degradation by monoamine oxidase slightly slows the decay phase in the striatum of DAT -/- mice but not in WT mice. Therefore, in brain structures densely innervated by dopamine terminals, dopamine reuptake represents the only mechanism responsible for the clearance of released dopamine (Jones et al., 1998b; Benoit-Marand et al., 2000). The roles of extracellular dopamine degradation and of non-neuronal uptake are negligible compared to dopamine reuptake. In DAT -/mice, dopamine clearance is mainly due to dopamine diffusion (Jones et al., 1998b; Benoit-Marand et al., 2000).

In brain structures weakly innervated by dopamine terminals, such as the amygdala, the prefrontal cortex (PfC) and the cingulated cortex (CgC), the clearance of released dopamine is much slower than in the striatum (Garris and Wightman, 2004). Indeed, the half-life for dopamine uptake is about 2 sec in these structures whereas it was estimated to be 60 ms in the striatum (Garris and Wightman, 1994). Moreover, inhibitors of dopamine reuptake are less efficient at slowing dopamine clearance in these brain structures than in the striatum (Garris and Wightman, 1995; Mundorf et al., 2001). At least in PfC and CgC, the released dopamine is partly cleared by the noradrenergic transporter (Mundorf et al., 2001).

## C. Reuptake Limits Dopamine Diffusion in the Extracellular Fluid

## 1. Diffusion and Reuptake of Dopamine Quanta in the Extracellular Fluid

In the striatum, most dopamine terminals form small symmetrical synaptic contacts on the neck of dendritic spines of medium sized spiny neurons (Freund et al., 1984) (Fig. 17.2A). Striatal postsynaptic dopamine receptors are either of the D1 or of the D2 type and are not located in front of the dopaminergic contacts (Hersch et al., 1995; Caille et al., 1996) but are distributed along the dendritic membrane with a higher density in the perisynaptic zone of the asymmetric synapses formed by corticostriatal glutamatergic terminals on the head of the dendritic spine (Fig. 17.2A). Moreover, reuptake sites are evenly distributed on the membrane surface of dopamine fibers and are rarely observed on the dopaminergic synaptic membrane (Nirenberg et al., 1996a; Pickel et al., 1996; Hersch et al., 1997). These subcellular observations strongly suggest that the dopamine transmission is mainly extrasynaptic (Pickel et al., 1996) and that dopamine diffuses from release sites within the synaptic cleft to extrasynaptic dopamine receptors. Moreover, all

dopamine receptors belong to the family of G-protein coupled receptors. Stimulation of G-protein receptors requires a minimal level of neurotransmitter, which depends on the receptor affinity, present for several tens of milliseconds (Hille, 1992). In the striatum D1 receptors appear to be in a low affinity state (1 $\mu$ M) whereas D2 receptors are in the high affinity state (10nM) (Richfield et al., 1989). Indeed, the basal extracellular dopamine level due to the tonic discharge activity of dopamine neurons is in the range of 10–20nM and is high enough to exert a tonic stimulation of presynaptic (Gonon and Buda, 1985) and postsynaptic (Svenningsson et al., 1999) D2 receptors.

Cragg and Rice (2004) have thoroughly investigated the diffusion of dopamine in the extracellular space. Assuming that a quantal release event of dopamine occurs at time t = 0 they calculated the change in extracellular dopamine



**FIGURE 17.2** Kinetics of dopamine diffusion and reuptake in the extracellular space. (A) This drawing summarizes morphological data concerning the dopamine transmission on striatal medium sized spiny neurons. The extracellular volume represents about 15-20% of the whole tissue volume. Dopamine terminals form symmetric contact with the neck of dendritic spines, whereas glutamatergic excitatory synapses are characterized by asymmetric contacts on the head of the spines. Postsynaptic dopamine receptors are rarely observed inside the dopaminergic synaptic cleft and are denser in the perisynaptic zone of the glutamatergic synapses. (B) When a quantum of dopamine (Q = 9800 dopamine molecules) is released in the synaptic cleft at time t = 0 (arrows), dopamine diffuses outside the synaptic cleft. At increasing distances from the release site the simulated transient changes in dopamine concentration are increasingly affected by dopamine reuptake. Note progressive changes in concentration and time scales with increasing distances. (C) Effect of reuptake inhibition by nomifensine (20 mg/kg, s.c.) on the dopamine overflow evoked by single pulse stimulation. Single pulse stimulations of the medial forebrain bundle were applied every 15 s. The resulting dopamine overflow was monitored in vivo in the striatum with a carbon fiber electrode coupled with continuous amperometry. The traces show the averaging of 20 recordings before (control) and 20 min after nomifensine injection. (A and C) data from Gonon (1997), copyright by Society for Neuroscience. (B) data from Cragg and Rice (2004), copyright by Elsevier.

concentration at variable distances from the release site (Fig. 17.2B). They showed that at short distance (1 and 2µm) diffusion entirely governs the dopamine overflow, whereas at increasing distance from release site (5 to  $20 \mu m$ ) dopamine reuptake increasingly limits the dopamine overflow both in term of maximal amplitude and duration. However, in the latter distance range, the maximal amplitude of the dopamine overflow is below 100 nM. Therefore, Cragg and Rice suggested that dopamine transmission mediated by D1 receptors occurs at a maximal distance of 2µm from the release site and is not affected by dopamine reuptake. In contrast, the dopamine transmission mediated by D2 receptors might be effective at a distance of 7µm from release sites. This distance as well as the duration of effective D2 stimulation (i.e. time during which the extracellular dopamine concentration exceeds 10nM) is limited by dopamine reuptake (Cragg and Rice, 2004).

This view that released dopamine escapes the extrasynaptic cleft and that its diffusion is not strongly affected by dopamine reuptake in the vicinity of release sites (i.e. at a distance  $<5\mu$ m) has been experimentally supported by electrochemical measurements of the electrically evoked dopamine overflow. Indeed, inhibition of dopamine reuptake strongly slows dopamine clearance but only moderately enhances the maximal amplitude of the dopamine overflow evoked by a single pulse (Fig. 17.2C) (Garris et al., 1994; Gonon, 1997; Schmitz et al., 2001). This is due to the fact that diffusion at short distance ( $<5\mu$ m) is faster than dopamine reuptake. Indeed, the half-life for dopamine clearance, which has been calculated from in vivo recordings, is about 30 ms (Garris et al., 1994; Gonon et al., 2000).

## 2. Extracellular Summation of Multiple Quanta: Role of Reuptake

In the striatum, the density of dopamine terminals is very high and the average distance between two terminals is about  $4\mu$ m (Doucet et al., 1986). If single pulse stimulation triggers dopamine release in a small fraction of dopamine terminals, every active single release site and its sphere of influence must be considered independently as discussed by Gragg and Rice (2004). Alternatively, if most terminals synchronously release single dopamine quanta in response to single pulse stimulation, the extracellular dopamine level largely results from a spatial summation of released quanta. As discussed above under the section on *Failure of vesicular release* (p. 305), it is likely that the failure rate of vesicular release is low regarding dopamine terminals in vivo.

This view that the extracellular dopamine level largely results from a spatial summation of quanta from neighboring terminals is further supported by in vivo estimates. Indeed, the maximal amplitude of the dopamine overflow evoked by a single pulse in the rodent striatum and measured in vivo by a carbon fiber electrode, is in the range of 100-400 nM (Fig. 17.2C) (Dugast et al., 1994; Garris et al., 1994; Benoit-Marand et al., 2000; Venton et al., 2003). However, this observed value represents an underestimate of the genuine change in the intact tissue. Models of electrochemical monitoring assume that between the intact tissue and the electrode surface, there is a dead zone with a thickness of  $6-8\mu m$ (Gonon et al., 2000; Schmitz et al., 2001; Venton et al., 2003). This estimate is consistent with an electron microscopy study showing the extent of the tissue damage generated by the implantation of a carbon fiber electrode for 4h in the striatum of anesthetized rats (Peters et al., 2004). This dead zone slows the kinetics of the evoked dopamine overflow and diminishes their maximal amplitude. Therefore, the dopamine overflow recorded by a carbon fiber electrode is much larger than expected by Cragg and Rice's calculation unless dopamine overflow summation from several dopamine terminals is taken into account (compare Figs 17.2B and C). Nevertheless, this apparent spatial summation from multiple release sites is limited by dopamine reuptake (Gonon, 1997).

In summary, dopamine overflow evoked in the striatum by single pulse stimulation exhibits two phases. The rising phase is rapid and mainly reflects dopamine release. The decay phase is slower and reflects dopamine reuptake. The kinetics of release and reuptake can be obtained by simulation taking into account the diffusion of dopamine from the intact tissue to the electrode surface through the dead zone. These simulations show that the half-life of released dopamine in the striatum in vivo is about 30 ms (Garris et al., 1994; Gonon et al., 2000; Schmitz et al., 2001; Venton et al., 2003). In this period of time the diffusion distance for dopamine is about 7 µm. Therefore, in a given point of the extracellular space, release sites at a distance  $>7 \,\mu m$  do not significantly contribute to the dopamine overflow. Thus, in the striatum and other brain structure densely innervated by dopamine terminals the dopamine transmission is extrasynaptic but, nevertheless, spatially constricted by dopamine reuptake.

In brain structures with a much lower DAT density, such as the PfC and CgC, diffusion plays a larger role in the clearance of released dopamine. However, even in these regions, pharmacological inhibition of DAT strongly slows the clearance of released dopamine (Mundorf et al., 2001).

# **D.** Regulation of Dopamine Reuptake by D2 Autoreceptors

In vivo studies (Cass and Gerhardt, 1994; Rothblat and Schneider, 1997) show that D2 antagonists slow the clearance of exogenously applied dopamine. Moreover, D2 antagonists decrease the rate of elimination of endogenous dopamine released in vivo by electrical stimulation (Benoit-Marand et al., 2001; Wu et al., 2002). Kinetics analysis of reuptake parameters shows that haloperidol reduces the Vm but does not affect Km (Wu et al., 2002). This inhibition of dopamine reuptake is not due to the direct effect of D2 antagonists on DAT activity because it is not observed in mice lacking D2 receptors (Dickinson et al., 1999).

The stimulation of D2 autoreceptors by the basal extracellular dopamine level exerts a tonic inhibition of the impulse flow-dependent dopamine release and, therefore, D2 antagonists facilitate dopamine release by blocking this D2 inhibition (see Section IIC). In contrast, the inhibitory effect of D2 antagonists on DAT activity does not seem to be due to the blockade of a tonic stimulation of D2 receptors. Indeed, the rate of elimination of electrically evoked dopamine release is not altered in D2 -/- mice compared to WT mice (Rouge-Pont et al., 2002). Whatever the mechanism, the inhibition of DAT activity acts in synergy with the facilitation of dopamine release by D2 antagonists to enhance impulse-flow dependent dopamine overflow. Both mechanisms should be taken into account when considering the therapeutic effects of D2 antagonists as well as their side effects.

## IV. RELATIONSHIP BETWEEN THE FIRING OF DOPAMINE NEURONS AND EXTRACELLULAR DOPAMINE

Dopamine neurons exhibit two patterns of discharge activity: a continuous mode with regularly spaced spikes at a frequency between 2 and 5 Hz, and a bursting activity characterized by brief bursts of 2 to 6 action potentials (Grace and Bunney, 1984a,b) (see also Chapter 16). Single dopamine neurons switch between the patterns. In resting condition and during sleep most dopamine neurons discharge with the tonic mode, but rewarding or sensorial stimuli predicting a reward trigger in most dopamine neurons a single burst both in rats (Hyland et al., 2002) and monkeys (Schultz et al., 1993; Mirenowicz and Schultz, 1996). In rats the intra-burst frequency is 15–30 Hz (Grace and Bunney, 1984b; Hyland et al., 2002). Grace and Bunney hypothesized that the bursting mode would be more potent than the tonic pattern to induce dopamine release (Grace and Bunney, 1984b). Accordingly, electrical stimulations mimicking the bursting mode were twice as potent as regularly spaced stimulation having the same whole duration and number of pulse to enhance the extracellular dopamine level (Gonon, 1988). However, these data were erroneously interpreted as a non-linear relationship between dopamine release and the impulse flow frequency.

## A. The Tonic Extracellular Dopamine Level

When most dopamine neurons discharge in the tonic mode, the pause between two successive action potentials reaching the same release site exceeds 200ms. Therefore, the dopamine released by one action potential is completely cleared by reuptake before the next action potential. This view has been experimentally demonstrated in vivo with electrical stimulation of the dopamine fiber at 4Hz (Chergui et al., 1994). Although this point has not been extensively studied to our knowledge, the degree of synchronous activity of dopamine neurons is probably low during tonic activity. Indeed, synchronous activity has been observed between pairs of adjacent neurons recorded with the same electrode (Wilson et al., 1977; Grace and Bunney, 1983) but not between pairs of distant neurons recorded with two electrodes (Wilson et al., 1977) and this synchrony has been reported to be more prominent during bursting activity (Grace and Bunney, 1983). Thus, taking into account the very high density of dopamine terminals in the striatum, the tonic activity induces a steady-state extracellular dopamine level, in the range of 10-30nM, which is stable with time and spatially homogenous (Venton et al., 2003). However, this steady-state is almost entirely firing dependent (Gonon and Buda, 1985; Svenningsson et al., 1999). A pause in the tonic discharge activity of dopamine neurons induces a rapid and profound (-80 %) decrease in the extracellular dopamine level (Gonon, 1988; Suaud-Chagny et al., 1992). Dopamine reuptake limits this tonic extracellular level. Indeed, pharmacological inhibition of dopamine reuptake increases this level by +300% (Gonon and Buda, 1985; Carboni et al., 1989; Venton et al., 2003) while in DAT -/mice, the basal extracellular dopamine level is five times as large as in WT mice (Jones et al., 1998b).

## **B.** Phasic Changes in Extracellular Dopamine

### 1. Electrically Evoked Dopamine Overflow

Electrical stimuli mimicking the bursting mode are more potent than regularly spaced stimuli in evoking dopamine

overflow, but this is not due to a facilitation of the release per se. Indeed, in the frequency range of 15-100 Hz brief train pulse stimuli (2 to 6 pulses) induce in vivo a larger dopamine overflow than that evoked by a single pulse stimulation, mostly due to extracellular accumulation of the released dopamine with successive pulses (Chergui et al., 1994; Garris et al., 1994; Venton et al., 2003). Indeed, in DAT -/- mice the maximal amplitude of the dopamine overflow evoked by a four-pulse stimulation is independent of frequency (15 or 4Hz), whereas in WT mice the ratio S(15Hz)/S(4Hz) is 2.4 in the dorsal striatum and 3.0 in the nucleus accumbens (Fig. 17.3) (Benoit-Marand et al., 2000). Burst-induced extracellular accumulation is higher in the nucleus accumbens than in the dorsal striatum because dopamine reuptake is about twice as slow in the former than in the latter region. Therefore, the specificity of the bursting mode over the tonic pattern results from the kinetics of dopamine reuptake rather than from changes in the quantal release per pulse. As expected, pharmacological inhibition of DAT activity increases the amplitude and duration of the phasic dopamine overflow evoked by a train pulse stimulation (Garris et al., 1994; Suaud-Chagny et al., 1995; Venton et al., 2003). Indeed, DAT inhibition facilitates the extracellular accumulation of dopamine with successive pulses.

#### 2. Dopamine Transients in Behaving Animals

Rewards or stimuli predicting a reward evoke a burst of action potential in most dopamine neurons with a similar delay and duration both in monkeys (Mirenowicz and Schultz, 1996) and rats (Pan et al., 2005) (see also Chapter 31). Therefore, in behaving rats, brief changes in extracellular dopamine, similar in kinetic and amplitude to dopamine overflow evoked by brief electrical stimulation, can be observed (Robinson et al., 2001; Robinson et al., 2002). As expected, DAT inhibition increases the maximal amplitude and duration of these dopamine transients to the same extent as dopamine overflow evoked by brief electrical stimulations (Robinson and Wightman, 2004). Moreover, in the nucleus accumbens dopamine transients clearly result from the bursting discharge activity of VTA dopamine neurons. Indeed, NMDA-mediated excitatory inputs to VTA dopamine neurons generate their bursting activity (Suaud-Chagny et al., 1992; Chergui et al., 1993; Overton and Clark, 1997). Specific manipulations of these inputs by local VTA injections of NMDA and of NMDA antagonists in freely moving rats alter the frequency of dopamine transients in the expected way (Sombers et al., 2009). Therefore, these dopamine transients represent dopamine overflow evoked by naturally occurring bursts of action potentials.

While dopamine neurons initially respond with a burst to unpredicted reward, after intense training they respond to a cue predicting a reward but no longer to the expected reward, both in monkeys (Schultz et al., 1993; Mirenowicz and Schultz, 1996) and rats (Pan et al., 2005). Electrochemical studies show the same relationship between dopamine transients and reward or cue predicting a reward (Day et al., 2007) (Fig. 17.4). Dopamine neurons



**FIGURE 17.3** Dopamine overflows evoked in nucleus accumbens (NAc) by stimulations mimicking the spontaneous activity of dopamine neurons. Medial forebrain bundle electrical stimulations consisted of four pulses either at 4 or at 15 Hz. The dopamine overflow was recorded with a carbon fiber electrode coupled with continuous amperometry. Electrodes were calibrated for dopamine concentration after in vivo recordings. The figure shows typical recordings obtained from one DAT +/+ and one DAT -/- mouse. Notice that the time scale is not the same with DAT +/+ and DAT -/- because dopamine clearance is much slower in DAT -/- mice. Stimulations mimicking a spontaneous burst (four pulses at 15 Hz) were more efficient than stimulations mimicking the single spike discharge mode (four pulses at 4 Hz) to evoke dopamine overflow (Benoit-Marand et al., 2000), copyright by Elsevier.



**FIGURE 17.4** Dopamine transients in the nucleus accumbens in response to reward and to its prediction. Dopamine transients were recorded with a carbon fiber electrode coupled with fast scan cyclic voltammetry. Before training dopamine transients are observed just after reward retrieval, whereas after training they appear just after the cue onset but disappear after reward. Figure prepared by R. Carelli from Day et al. (2007), copyright by Nature Publishing Group.

are preferentially activated with a burst by appetitive stimuli and are inhibited by aversive stimuli both in monkeys (Mirenowicz and Schultz, 1996) and rats (Ungless et al., 2004; Pan et al., 2005). Likewise, appetitive stimuli trigger dopamine transients whereas aversive stimuli briefly decrease the extracellular dopamine level (Roitman et al., 2008). Finally, a subpopulation of dopamine neurons in the ventral VTA respond with bursts to noxious stimuli in anesthetized rats (Brischoux et al., 2009). In line with these observations, intense stress induced in freely moving rats by social defeat enhances burst firing in a subpopulation of VTA dopamine neurons and is associated with an increase in the frequency of dopamine transients in the nucleus accumbens (Anstrom et al., 2009). Therefore, data obtained with electrochemical recording of dopamine transients are highly consistent with electrophysiological data obtained from dopamine neurons.

## **V. CONCLUSIONS**

The extracellular dopamine level results from a dynamic equilibrium between two processes: release and reuptake by dopamine terminals. Both processes are highly regulated by several mechanisms. Apart from these sophisticated means of regulation, the relationship between the discharge activity of dopamine neurons and the extracellular dopamine level is, in first approximation, quite simple. The tonic activity of dopamine neurons is translated into a tonic level of extracellular dopamine and a pause is translated into a profound depression of this tonic level. The bursting activity is translated into transient dopamine overflow, which largely exceeds the tonic level, due to accumulation of released dopamine into the extracellular space. This relationship was first described using electrical stimulation and lesion to manipulate the impulse flow. Recent data obtained in behaving rata fully confirm this relationship.

## REFERENCES

- Abeliovich A, Schmitz Y, Farinas I, et al. (2000) Mice lacking alphasynuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25:239–252.
- Albillos A, Dernick G, Horstmann H, Almers W, Alvarez de Toledo G, Lindau M (1997) The exocytotic event in chromaffin cells revealed by patch amperometry. Nature 389:509–512.
- Alexander SP, Reddington M (1989) The cellular localization of adenosine receptors in rat neostriatum. Neuroscience 28:645–651.
- Alvarez de Toledo G, Fernandez JM (1990) Compound versus multigranular exocytosis in peritoneal mast cells. J Gen Physiol 95:397–409.
- Amara SG, Kuhar MJ (1993) Neurotransmitter transporters: Recent progress. Ann Rev Neurosci 16:73–93.
- Anderson BB, Chen G, Gutman DA, Ewing AG (1998) Dopamine levels of two classes of vesicles are differentially depleted by amphetamine. Brain Res 788:294–301.
- Anstrom KK, Miczek KA, Budygin EA (2009) Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. Neuroscience 161:3–12.
- Araujo DM, Hilt DC, Miller PJ, Wen D, Jiao S, Lapchak PA (1997) ret receptor tyrosine kinase immunoreactivity is altered in glial cell linederived neurotrophic factor-responsive neurons following lesions of the nigrostriatal and septohippocampal pathways. Neuroscience 80:9–16.
- Avshalumov MV, Patel JC, Rice ME (2008) AMPA receptor-dependent H<sub>2</sub>O<sub>2</sub> generation in striatal medium spiny neurons but not dopamine axons: one source of a retrograde signal that can inhibit dopamine release. J Neurophysiol 100:1590–1601.
- Avshalumov MV, Chen BT, Marshall SP, Pena DM, Rice ME (2003) Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H<sub>2</sub>O<sub>2</sub>. J Neurosci 23:2744–2750.
- Barasch J, Gershon MD, Nunez EA, Tamir H, Al-Awqati Q (1988) Thyrotropin induced the acidification of secretory granules of parafollicular cells by increasing the chloride conductance of the granular membrane. J Cell Biol 107:2137–2147.
- Beckstead MJ, Ford CP, Phillips PE, Williams JT (2007) Presynaptic regulation of dendrodendritic dopamine transmission. Eur J Neurosci 26:1479–1488.
- Benoit-Marand M, Jaber M, Gonon F (2000) Release and elimination of dopamine in vivo in mice lacking the dopamine transporter: functional consequences. Eur J Neurosci 12:2985–2992.
- Benoit-Marand M, Borrelli E, Gonon F (2001) Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics in vivo. J Neurosci 21:9134–9141.
- Bernard V, Bolam JP (1998) Subcellular and subsynaptic distribution of the NR1 subunit of the NMDA receptor in the neostriatum and globus

pallidus of the rat: co-localization at synapses with the GluR2/3 subunit of the AMPA receptor. Eur J Neurosci 10:3721–3736.

- Blaschko H, Welch AD (1953) Localization of adrenaline in cytoplasmic particles of the bovine adrenal medulla. Naunyn-Schmiedebergs Arch Exp Pathol Pharmacol 219:17–22.
- Bonci A, Bernardi G, Grillner P, Mercuri NB (2003) The dopaminecontaining neuron: maestro or simple musician in the orchestra of addiction? Trends Pharmacol Sci 24:172–177.
- Brischoux F, Chakraborty S, Brierley DI, Ungless MA (2009) Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci USA 106:4894–4899.
- Britt JP, McGehee DS (2008) Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. J Neurosci 28:1672–1681.
- Caille I, Dumartin B, Bloch B (1996) Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. Brain Res 730:17–31.
- Camacho M, Machado JD, Alvarez J, Borges R (2008) Intravesicular calcium release mediates the motion and exocytosis of secretory organelles: a study with adrenal chromaffin cells. J Biol Chem 283:22383–22389.
- Camacho M, Machado JD, Montesinos MS, Criado M, Borges R (2006) Intragranular pH rapidly modulates exocytosis in adrenal chromaffin cells. J Neurochem 96:324–334.
- Carboni E, Imperato A, Perezzani L, Di-Chiara G (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661.
- Cardozo DL, Bean BP (1995) Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and GABAB receptors. J Neurophysiol 74:1137–1148.
- Carlsson A, Hillarp N-A, Waldeck A (1962) A Mg<sup>++</sup>-ATP dependent storage mechanism in the amine granules of the adrenal medulla. Med Exp 6:47–53.
- Cass WA, Zahniser NR (1991) Potassium channel blockers inhibit D2 dopamine, but not A1 adenosine, receptor-mediated inhibition of striatal dopamine release. J Neurochem 57:147–152.
- Cass WA, Gerhardt GA (1994) Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. Neurosci Lett 176:259–263.
- Ceccarelli B, Hurlbut WP, Mauro A (1972) Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation. J Cell Biol 54:30–38.
- Ceccarelli B, Hurlbut WP, Mauro A (1973) Turnover of transmitter and synaptic vesicles at the frog neuromuscular junction. J Cell Biol 57:499–524.
- Centonze D, Usiello A, Gubellini P, Pisani A, Borrelli E, Bernardi G, Calabresi P (2002) Dopamine D2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D2L receptors. Neuropsychopharmacology 27:723–726.
- Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ (2007) "Rejuvenation" protects neurons in mouse models of Parkinson's disease. Nature 447:1081–1086.
- Charara A, Heilman C, Levey AI, Smith Y (2000) Pre- and postsynaptic localization of GABAB receptors in the basal ganglia in monkeys. Neuroscience 95:127–140.
- Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. J Neurosci 24:4393–4400.
- Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release

evoked by abused substances requires cannabinoid receptor activation. J Neurosci 27:791–795.

- Chen BT, Moran KA, Avshalumov MV, Rice ME (2006a) Limited regulation of somatodendritic dopamine release by voltage-sensitive Ca channels contrasted with strong regulation of axonal dopamine release. J Neurochem 96:645–655.
- Chen G, Gavin PF, Luo G, Ewing AG (1995) Observation and quantitation of exocytosis from the cell body of a fully developed neuron in Planorbis corneus. J Neurosci 15:7747–7755.
- Chen Q, Veenman L, Knopp K, Yan Z, Medina L, Song WJ, Surmeier DJ, Reiner A (1998) Evidence for the preferential localization of glutamate receptor-1 subunits of AMPA receptors to the dendritic spines of medium spiny neurons in rat striatum. Neuroscience 83:749–761.
- Chen Y, Yu FH, Surmeier DJ, Scheuer T, Catterall WA (2006b) Neuromodulation of Na<sup>+</sup> channel slow inactivation via cAMP-dependent protein kinase and protein kinase C. Neuron 49:409–420.
- Chergui K, Suaud-Chagny MF, Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. Neuroscience 62:641–645.
- Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH, Chouvet G (1993) Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. Eur J Neurosci 5:137–144.
- Chow RH, Von Rueden L, Neher E (1992) Delay in vesicle fusion revealed by electrochemical monitoring of single secretory events in adrenal chromaffin cells. Nature 356:60–63.
- Church WH, Justice JB Jr., Neill DB (1987) Detecting behaviorally relevant changes in extracellular dopamine with microdialysis. Brain Res 412:397–399.
- Colliver TL, Pyott SJ, Achalabun M, Ewing AG (2000) VMAT-mediated changes in quantal size and vesicular volume. J Neurosci 20:5276–5282.
- Congar P, Bergevin A, Trudeau LE (2002) D2 receptors inhibit the secretory process downstream from calcium influx in dopaminergic neurons: implication of K(+) channels. J Neurophysiol 87:1046–1056.
- Cragg SJ, Greenfield SA (1997) Differential autoreceptor control of somatodendritic and axon terminal dopamine release in substantia nigra, ventral temental area, and striatum. J Neurosci 17:1746–5738.
- Cragg SJ, Rice ME (2004) DAncing past the DAT at a DA synapse. Trends Neurosci 27:270–277.
- Cramer W (1918) Further observations on the thyroid-adrenal apparatus. A histochemical method for the demonstration of adrenalin granules in the suprarenal gland. J Physiol 52:7–10.
- Croft BG, Fortin GD, Corera AT, Edwards RH, Beaudet A, Trudeau LE, Fon EA (2005) Normal biogenesis and cycling of empty synaptic vesicles in dopamine neurons of vesicular monoamine transporter 2 knockout mice. Mol Biol Cell 16:306–315.
- Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C (2004) Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7:153–159.
- Cubeddu LX, Hoffmann IS (1982) Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors. J Pharmacol Exp Ther 223:497–501.
- Davila V, Yan Z, Craciun LC, Logothetis D, Sulzer D (2003) D3 dopamine autoreceptors do not activate G-protein-gated inwardly rectifying potassium channel currents in substantia nigra dopamine neurons. J Neurosci 23:5693–5697.
- Day JJ, Roitman MF, Wightman RM, Carelli RM (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10:1020–1028.

- De Klippel N, Sarre S, Ebinger G, Michotte Y (1993) Effect of M1- and M2-muscarinic drugs on striatal dopamine release and metabolism: an in vivo microdialysis study comparing normal and 6-hydroxydopamine-lesioned rats. Brain Res 630:57–64.
- De Robertis ED, Bennett HS (1955) Some features of the submicroscopic morphology of synapses in frog and earthworm. J Biophys Biochem Cytol 1:47–58.
- Del Castillo J, Katz B (1954) Quantal components of the end-plate potential. J Physiol 124:560–573.
- Dewey SL, Smith GS, Logan J, et al. (1993) Effects of central cholinergic blockade on striatal dopamine release measured with positron emission tomography in normal human subjects. Proc Natl Acad Sci USA 90:11816–11820.
- Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz JC, Sokoloff P (2000) Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. J Neurosci 20:8677–8684.
- Dickinson SD, Sabeti J, Larson GA, et al. (1999) Dopamine D2 receptordeficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J Neurochem 72:148–156.
- Doucet G, Descarries L, Garcia S (1986) Quantification of the dopamine innervation in adult rat neostriatum. Neuroscience 19:427–445.
- Drory O, Nelson N (2006) The emerging structure of vacuolar ATPases. Physiology (Bethesda) 21:317–325.
- Dugast C, Suaud-Chagny MF, Gonon F (1994) Continuous in vivo monitoring of evoked dopamine release in the rat nucleus accumbens by amperometry. Neuroscience 62:647–654.
- Dwoskin LP, Zahniser NR (1986) Robust modulation of [3H]dopamine release from rat striatal slices by D-2 dopamine receptors. J Pharmacol Exp Ther 239:442–453.
- Edwards RH (2007) The neurotransmitter cycle and quantal size. Neuron 55:835–858.
- Elhamdani A, Palfrey HC, Artalejo CR (2001) Quantal size is dependent on stimulation frequency and calcium entry in calf chromaffin cells. Neuron 31:819–830.
- Fatt P (1954) Biophysics of junctional transmission. Physiol Rev 34:674–710.
- Fatt P, Katz B (1950) Some observations on biological noise. Nature 166:597–598.
- Fatt P, Katz B (1952) Spontaneous subthreshold activity at motor nerve endings. J Physiol 117:109–128.
- Finnegan JM, et al. (1996) Vesicular quantal size measured by amperometry at chromaffin, mast, pheochromocytoma, and pancreatic beta-cells. J Neurochem 66:1914–1923.
- Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH (2004) Lipid rafts mediate the synaptic localization of alphasynuclein. J Neurosci 24:6715–6723.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylaseimmunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 13:1189–1215.
- Fusco FR, Martorana A, Giampa C, De March Z, Farini D, D'Angelo V, Sancesario G, Bernardi G (2004) Immunolocalization of CB1 receptor in rat striatal neurons: a confocal microscopy study. Synapse 53:159–167.
- Gainetdinov RR, Grekhova TV, Sotnikova TD, Rayevsky KS (1994) Dopamine D2 and D3 receptor preferring antagonists differentially affect striatal dopamine release and metabolism in conscious rats. Eur J Pharmacol 261:327–331.

- Gandhi SP, Stevens CF (2003) Three modes of synaptic vesicular recycling revealed by single-vesicle imaging. Nature 423:607–613.
- Garris PA, Wightman RM (1994) Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study. J Neurosci 14:442–450.
- Garris PA, Wightman RM (1995) Distinct pharmacological regulation of evoked dopamine efflux in the amygdala and striatum of the rat in vivo. Synapse 20:269–279.
- Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. J Neurosci 14:6084–6093.
- Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612.
- Glass M, Faull RL, Dragunow M (1996) Localisation of the adenosine uptake site in the human brain: a comparison with the distribution of adenosine A1 receptors. Brain Res 710:79–91.
- Golembiowska K, Zylewska A (1998) Agonists of A1 and A2A adenosine receptors attenuate methamphetamine-induced overflow of dopamine in rat striatum. Brain Res 806:202–209.
- Gong LW, Hafez I, Alvarez de Toledo G, Lindau M (2003) Secretory vesicles membrane area is regulated in tandem with quantal size in chromaffin cells. J Neurosci 23:7917–7921.
- Gonon F (1988) Nonlinear relationship between impulse flow and dopamine release by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 24:19–28.
- Gonon F (1997) Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. J Neurosci 17:5972–5978.
- Gonon F, Buda M (1985) Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in the rat striatum. Neuroscience 14:765–774.
- Gonon F, Msghina M, Stjarne L (1993) Kinetics of noradrenaline released by sympathetic nerves. Neuroscience 56:535–538.
- Gonon F, Burie JB, Jaber M, Benoit-Marand M, Dumartin B, Bloch B (2000) Geometry and kinetics of dopaminergic transmission in the rat striatum and in mice lacking the dopamine transporter. Prog Brain Res 125:291–302.
- Gonon F, Cespuglio R, Ponchon JL, Buda M, Jouvet M, Adams RN, Pujol JF (1978) [In vivo continuous electrochemical determination of dopamine release in rat neostriatum]. C R Acad Sci Hebd Seances Acad Sci D 286:1203–1206.
- Grabner CP, Price SD, Lysakowski A, Fox AP (2005) Mouse chromaffin cells have two populations of dense core vesicles. J Neurophysiol 94:2093–2104.
- Grabner CP, Price SD, Lysakowski A, Cahill AL, Fox AP (2006) Regulation of large dense-core vesicle volume and neurotransmitter content mediated by adaptor protein 3. Proc Natl Acad Sci USA 103:10035–10040.
- Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons. 1. Identification and characterization. Neuroscience 10:301–315.
- Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4:2866–2876.
- Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877–2890.
- Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220–227.

- Grenhoff J, Aston-Jones G, Svensson TH (1986) Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol Scand 128:351–358.
- Gubernator NG, Zhang H, Staal RG, Mosharov EV, Pereira DB, Yue M, Balsanek V, Vadola PA, Mukherjee B, Edwards RH, Sulzer D, Sames D (2009) Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. Science 324:1441–1444.
- Han W, Li D, Stout AK, Takimoto K, Levitan ES (1999) Ca<sup>2+</sup>-induced deprotonation of peptide hormones inside secretory vesicles in preparation for release. J Neurosci 19:900–905.
- Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI (1997) Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. J Comp Neurol 388:211–227.
- Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Hettinger BD, Lee A, Linden J, Rosin DL (2001) Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. J Comp Neurol 431:331–346.
- Heuser JE, Reese TS (1973) Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J Cell Biol 57:315–344.
- Hiesinger PR, Fayyazuddin A, Mehta SQ, et al. (2005) The v-ATPase V0 subunit a1 is required for a late step in synaptic vesicle exocytosis in Drosophila. Cell 121:607–620.
- Hillarp N-A, Lagerstedt S, Nilson B (1953) The isolation of a granular fraction from the rurarenal medulla, containing the sympathomimetic catecholamines. Acta Physiol Scand 29:251–263.
- Hille B (1992) G-Protein-coupled mechanisms and nervous signaling. Neuron 9:187–195.
- Holtzman E, Freeman AR, Kashner LA (1971) Stimulation-dependent alterations in peroxidase uptake at lobster neuromuscular junctions. Science 173:733–736.
- Hyland BI, Reynolds JN, Hay J, Perk CG, Miller R (2002) Firing modes of midbrain dopamine cells in the freely moving rat. Neuroscience 114:475–492.
- Imperato A, Di Chiara G (1985) Dopamine release and metabolism in awake rats after systemic neuroleptics as studied by trans-striatal dialysis. J Neurosci 5:297–306.
- Imperato A, Di Chiara G (1988) Effects of locally applied D-1 and D-2 receptor agonists and antagonists studied with brain dialysis. Eur J Pharmacol 156:385–393.
- Jaffe EH, Marty A, Schulte A, Chow RH (1998) Extrasynaptic vesicular transmitter release from the somata of substantia nigra neurons in rat midbrain slices. J Neurosci 18:3548–3553.
- Jentsch TJ, Poet M, Fuhrmann JC, Zdebik AA (2005) Physiological functions of CLC Cl<sup>-</sup> channels gleaned from human genetic disease and mouse models. Annu Rev Physiol 67:779–807.
- Jin S, Johansson B, Fredholm BB (1993) Effects of adenosine A1 and A2 receptor activation on electrically evoked dopamine and acetylcholine release from rat striatal slices. J Pharmacol Exp Ther 267:801–808.
- Johnson RG (1988) Accumulation of biological amines into chromaffin granules: a model for hormone and neurotransmitter transport. Physiol Rev 68:232–307.
- Jones SR, Gaindetdinov RR, Wightman RM, Caron MG (1998a) Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci 18:1979–1986.

- Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG (1998b) Profound neuronal plasticity in response to inactivation of the dopamine transporter. Proc Natl Acad Sci USA 95:4029–4034.
- Joseph JD, Wang YM, Miles PR, Budygin EA, Picetti R, Gainetdinov RR, Caron MG, Wightman RM (2002) Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D(3) receptors. Neuroscience 112:39–49.
- Kemel ML, Desban M, Glowinski J, Gauchy C (1989) Distinct presynaptic control of dopamine release in striosomal and matrix areas of the cat caudate nucleus. Proc Natl Acad Sci USA 86:9006–9010.
- Kennedy RT, Jones SR, Wightman RM (1992) Dynamic observation of dopamine autoreceptor effects in rat striatal slices. J Neurochem 59:449–455.
- Kim Y, Park MK, Chung S (2008) Voltage-operated Ca<sup>2+</sup> channels regulate dopamine release from somata of dopamine neurons in the substantia nigra pars compacta. Biochem Biophys Res Commun 373:665–669.
- Kirshner N (1962) Uptake of catecholamines by a particulate fraction of the adrenal medulla. J Biol Chem 237:2311–2317.
- Klyachko VA, Jackson MB (2002) Capacitance steps and fusion pores of small and large-dense-core vesicles in nerve terminals. Nature 418:89–92.
- Koeltzow TE, Xu M, Cooper DC, Hu XT, Tonegawa S, Wolf ME, White FJ (1998) Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. J Neurosci 18:2231–2238.
- Kozminski KD, Gutman DA, Davila V, Sulzer D, Ewing AG (1998) Voltammetric and pharmacological characterization of dopamine released from single quantal events in PC12 cells. Analyt Chem 70:3123–3130.
- Krapivinsky G, Mochida S, Krapivinsky L, Cibulsky SM, Clapham DE (2006) The TRPM7 ion channel functions in cholinergic synaptic vesicles and affects transmitter release. Neuron 52:485–496.
- Kudernatsch M, Sutor B (1994) Cholinergic modulation of dopamine overflow in the rat neostriatum: a fast cyclic voltammetric study in vitro. Neurosci Lett 181:107–112.
- Kulagina NV, Zigmond MJ, Michael AC (2001) Glutamate regulates the spontaneous and evoked release of dopamine in the rat striatum. Neuroscience 102:121–128.
- Kuzhikandathil EV, Oxford GS (1999) Activation of human D3 dopamine receptor inhibits P/Q-type calcium channels and secretory activity in AtT-20 cells. J Neurosci 19:1698–1707.
- Kuzhikandathil EV, Oxford GS (2000) Dominant-negative mutants identify a role for GIRK channels in D3 dopamine receptormediated regulation of spontaneous secretory activity. J Gen Physiol 115:697–706.
- Kuzhikandathil EV, Yu W, Oxford GS (1998) Human dopamine D3 and D2L receptors couple to inward rectifier potassium channels in mammalian cell lines. Mol Cell Neurosci 12:390–402.
- L'Hirondel M, Cheramy A, Godeheu G, Artaud F, Saiardi A, Borrelli E, Glowinski J (1998) Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice. Brain Res 792:253–262.
- Larsen KE, Schmitz Y, Troyer M, Mosharov E, Dietrich P, Savalle M, Edwards RH, Stefanis L, Sulzer D (2006) Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. J Neurosci 26:11915–11922.
- Lehmann J, Langer SZ (1982) Muscarinic receptors on dopamine terminals in the cat caudate nucleus: neuromodulation of [3H]dopamine release in vitro by endogenous acetylcholine. Brain Res 248:61–69.

- Leszczyszyn DJ, Jankowski JA, Viveros OH, Diliberto EJ Jr., Near JA, Wightman RM (1990) Nicotinic receptor-mediated catecholamine secretion from individual chromaffin cells. Chemical evidence for exocytosis. J Biol Chem 265:14736–14737.
- Li H, Waites CL, Staal RG, Dobryy Y, Park J, Sulzer DL, Edwards RH (2005) Sorting of vesicular monoamine transporter 2 to the regulated secretory pathway confers the somatodendritic exocytosis of monoamines. Neuron 48:619–633.
- Lupica CR, Riegel AC (2005) Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. Neuropharmacology 48:1105–1116.
- Machado JD, Morales A, Gomez JF, Borges R (2001) cAmp modulates exocytotic kinetics and increases quantal size in chromaffin cells. Mol Pharmacol 60:514–520.
- Mahan LC, McVittie LD, Smyk-Randall EM, Nakata H, Monsma FJ Jr, Gerfen CR, Sibley DR (1991) Cloning and expression of an A1 adenosine receptor from rat brain. Mol Pharmacol 40:1–7.
- Mani M, Ryan TA (2009) Live imaging of synaptic vesicle release and retrieval in dopaminergic neurons. Front Neural Circuits 3:3.
- Mansvelder HD, De Rover M, McGehee DS, Brussaard AB (2003) Cholinergic modulation of dopaminergic reward areas: upstream and downstream targets of nicotine addiction. Eur J Pharmacol 480:117–123.
- Margolis EB, Hjelmstad GO, Bonci A, Fields HL (2003) Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 23:9981–9986.
- Markov D, Mosharov EV, Setlik W, Gershon MD, Sulzer D (2008) Secretory vesicle rebound hyperacidification and increased quantal size resulting from prolonged methamphetamine exposure. J Neurochem 107:1709–1721.
- Maron R, Stern Y, Kanner BI, Schuldiner S (1983) Functional asymmetry of the amine transporter from chromaffin granules. J Biol Chem 258:11476–11481.
- Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327:535–550.
- Matyas F, Urban GM, Watanabe M, Mackie K, Zimmer A, Freund TF, Katona I (2008) Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. Neuropharmacology 54:95–107.
- May LJ, Wightman RM (1989) Effects of D-2 antagonists on frequencedependent stimulated dopamine overflow in nucleus accumbens and caudate-putamen. J Neurochem 53:898–906.
- Mayer A, Limberger N, Starke K (1988) Transmitter release patterns of noradrenergic, dopaminergic, and cholinergic axons in rabbit brain slices during short pulse trains, and the operation of presynaptic autoreceptors. Naunyn Schmiedegergs Arch Pharmacol 338:632–643.
- Mercuri NB, Saiardi A, Bonci A, Picetti R, Calabresi P, Bernardi G, Borrelli E (1997). Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 79:323–327.
- Mirenowicz J, Schultz W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379:449–451.
- Mitchell SJ, Ryan TA (2004) Syntaxin-1A is excluded from recycling synaptic vesicles at nerve terminals. J Neurosci 24:4884–4888.
- Msghina M, Gonon F, Stjarne L (1993) Intermittent release of noradrenaline by single pulses and release during short trains at high frequencies from sympathetic nerves in rat tail artery. Neuroscience 57:887–890.

- Mundorf ML, Joseph JD, Austin CM, Caron MG, Wightman RM (2001) Catecholamine release and uptake in the mouse prefrontal cortex. J Neurochem 79:130–142.
- Nakanishi-Matsui M, Futai M (2006) Stochastic proton pumping ATPases: from single molecules to diverse physiological roles. IUBMB Life 58:318–322.
- Nedergaard S, Flatman JA, Engberg I (1993) Nifedipine- and omegaconotoxin-sensitive Ca<sup>2+</sup> conductances in guinea-pig substantia nigra pars compacta neurones. J Physiol 466:727–747.
- Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1996a) The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. J Neurosci 15:436–447.
- Nirenberg MJ, Chan J, Liu Y, Edwards RH, Pickel VM (1996b) Ultrastructural localization of the vesicular monoamine transporter-2 in midbrain dopaminergic neurons: potential sites for somatodendritic storage and release of dopamine. J Neurosci 16:4135–4145.
- Njus D, Kelley PM, Harnadek GJ (1986) Bioenergetics of secretory vesicles. Biochimica et Biophysica Acta 853:237–265.
- O'Neill C, Nolan BJ, Macari A, O'Boyle KM, O'Connor JJ (2007) Adenosine A1 receptor-mediated inhibition of dopamine release from rat striatal slices is modulated by D1 dopamine receptors. Eur J Neurosci 26:3421–3428.
- Oliver G, Schafer EA (1895) The physiological effects of extracts of the suprarenal capsules. J Physiol 18:230–276.
- Overton PG, Clark D (1997) Burst firing in midbrain dopaminergic neurons. Brain Res Brain Res Rev 25:312–334.
- Palade GE (1954) Electron microscope observations of intraneuronal and neuromuscular synapses. Anat Rec 118:335–336.
- Palay SL (1956) Synapses in the central nervous system. J Biophys Biochem Cytol 2:193–202.
- Palij P, Bull DR, Sheehan MJ, Millar J, Stamford J, Kruk ZL, Humphrey PP (1990) Presynaptic regulation of dopamine release in corpus striatum monitored in vitro in real time by fast cyclic voltammetry. Brain Res 509:172–174.
- Pan WX, Schmidt R, Wickens JR, Hyland BI (2005) Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. J Neurosci 25:6235–6242.
- Paquet M, Smith Y (2003) Group I metabotropic glutamate receptors in the monkey striatum: subsynaptic association with glutamatergic and dopaminergic afferents. J Neurosci 23:7659–7669.
- Paquet M, Tremblay M, Soghomonian JJ, Smith Y (1997) AMPA and NMDA glutamate receptor subunits in midbrain dopaminergic neurons in the squirrel monkey: an immunohistochemical and in situ hybridization study. J Neurosci 17:1377–1396.
- Patel J, Mooslehner KA, Chan PM, Emson PC, Stamford JA (2003) Presynaptic control of striatal dopamine neurotransmission in adult vesicular monoamine transporter 2 (VMAT2) mutant mice. J Neurochem 85:898–910.
- Peters C, Bayer MJ, Buhler S, Andersen JS, Mann M, Mayer A (2001) Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. Nature 409:581–588.
- Peters JL, Miner LH, Michael AC, Sesack SR (2004) Ultrastructure at carbon fiber microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats. J Neurosci Methods 137:9–23.
- Phillips PE, Stamford JA (2000) Differential recruitment of N-, P- and Qtype voltage-operated calcium channels in striatal dopamine release evoked by 'regular' and 'burst' firing. Brain Res 884:139–146.

- Phillips PE, Hancock PJ, Stamford JA (2002) Time window of autoreceptor-mediated inhibition of limbic and striatal dopamine release. Synapse 44:15–22.
- Pickel VM, Nirenberg MJ, Milner TA (1996) Ultrastructural view of central catecholaminergic transmission: immunocytochemical localization of synthesizing enzymes, transporters and receptors. J Neurocytol 25:843–856.
- Pifl C, Drobny H, Reither H, et al. (1995) Mechanism of the dopaminereleasing actions of amphetamine and cocaine: plasmalemmal dopamine transporter versus vesicular monoamine transporter. Mol Pharmacol 47:368–373.
- Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255–257.
- Pothos E, Desmond M, Sulzer D (1996) L-3,4-Dihydroxyphenylalanine increases the quantal size of exocytic dopamine release in vitro. J Neurochem 66:629–636.
- Pothos EN, Davila V, Sulzer D (1998a) Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size. J Neurosci 18:4106–4118.
- Pothos EN, Przedborski S, Davila V, Schmitz Y, Sulzer D (1998b) D2-Like dopamine autoreceptor activation reduces quantal size in PC12 cells. J Neurosci 18:5575–5585.
- Pothos EN, Larsen KE, Krantz DE, Liu Y, Haycock JW, Setlik W, Gershon MD, Edwards RH, Sulzer D (2000) Synaptic vesicle transporter expression regulates vesicle phenotype and quantal size. J Neurosci 20:7297–7306.
- Pothos EN, Mosharov E, Liu KP, Setlik W, Haburcak M, Baldini G, Gershon MD, Tamir H, Sulzer D (2002) Stimulation-dependent regulation of the pH, volume and quantal size of bovine and rodent secretory vesicles. J Physiol 542:453–476.
- Puopolo M, Hochstetler SE, Gustincich S, Wightman RM, Raviola E (2001) Extrasynaptic release of dopamine in a retinal neuron: activity dependence and transmitter modulation. Neuron 30:211–225.
- Quarta D, Borycz J, Solinas M, et al. (2004) Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation. J Neurochem 91:873–880.
- Raiteri M, Leardi R, Marchi M (1984) Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. J Pharmacol Exp Ther 228:209–214.
- Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7:583–584.
- Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 30:767–777.
- Riegel AC, Lupica CR (2004) Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. J Neurosci 24:11070–11078.
- Rivkees SA, Price SL, Zhou FC (1995) Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. Brain Res 677:193–203.
- Robinson DL, Wightman RM (2004) Nomifensine amplifies subsecond dopamine signals in the ventral striatum of freely-moving rats. J Neurochem 90:894–903.
- Robinson DL, Heien ML, Wightman RM (2002) Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. J Neurosci 22:10477–10486.

- Robinson DL, Phillips PE, Budygin EA, Trafton BJ, Garris PA, Wightman RM (2001) Sub-second changes in accumbal dopamine during sexual behavior in male rats. Neuroreport 12:2549–2552.
- Roitman MF, Wheeler RA, Wightman RM, Carelli RM (2008) Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci 11:1376–1377.
- Romero J, Garcia-Palomero E, Castro JG, Garcia-Gil L, Ramos JA, Fernandez-Ruiz JJ (1997) Effects of chronic exposure to delta9tetrahydrocannabinol on cannabinoid receptor binding and mRNA levels in several rat brain regions. Mol Brain Res 46:100–108.
- Ronken E, Mulder AH, Schoffelmeer AN (1993) Interacting presynaptic kappa-opioid and GABAA receptors modulate dopamine release from rat striatal synaptosomes. J Neurochem 61:1634–1639.
- Rosin DL, Hettinger BD, Lee A, Linden J (2003) Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. Neurology 61:S12–S18.
- Rothblat DS, Schneider JS (1997) Regionally specific effects of haloperidol and clozapine on dopamine reuptake in the striatum. Neurosci Lett 228:119–122.
- Rouge-Pont F, Usiello A, Benoit-Marand M, Gonon F, Piazza PV, Borrelli E (2002) Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J Neurosci 22:3293–3301.
- Schlosser B, Kudernatsch MB, Sutor B, ten Bruggencate G (1995) Delta, mu and kappa opioid receptor agonists inhibit dopamine overflow in rat neostriatal slices. Neurosci Lett 191:126–130.
- Schmitz Y, Schmauss C, Sulzer D (2002) Altered dopamine release and uptake kinetics in mice lacking D2 receptors. J Neurosci 15:8002–8009.
- Schmitz Y, Benoit-Marand M, Gonon F, Sulzer D (2003) Presynaptic regulation of dopaminergic neurotransmission. J Neurochem 87:273–289.
- Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D (2001) Amphetamine distorts synaptic dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. J Neurosci 21:5916–5924.
- Schoffelmeer AN, Van Vliet BJ, Wardeh G, Mulder AH (1986) Muscarine receptor-mediated modulation of [3H]dopamine and [14C]acetylcholine release from rat neostriatal slices: selective antagonism by gallamine but not pirenzepine. Eur J Pharmacol 128:291–294.
- Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci 13:900–913.
- Sesack SR, Aoki C, Pickel VM (1994) Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. J Neurosci 14:88–106.
- Sidlo Z, Reggio PH, Rice ME (2008) Inhibition of striatal dopamine release by CB1 receptor activation requires nonsynaptic communication involving GABA, H<sub>2</sub>O<sub>2</sub>, and KATP channels. Neurochem Int 52:80–88.
- Smolders I, Bogaert L, Ebinger G, Michotte Y (1997) Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with in vivo microdialysis. J Neurochem 68:1942–1948.
- Smolders I, De Klippel N, Sarre S, Ebinger G, Michotte Y (1995) Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat. Eur J Pharmacol 284:83–91.
- Sokoloff P, Giros B, Martres M, Bouthene M, Schwartz J (1990) Molecular cloning and characterization of a novel dopamine receptor  $(D_3)$  as a target for neuroleptics. Nature 347:146–151.
- Sombers LA, Beyene M, Carelli RM, Wightman RM (2009) Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. J Neurosci 29:1735–1742.

- Staal RG, Mosharov EV, Sulzer D (2004) Dopamine neurons release transmitter via a flickering fusion pore. Nat Neurosci 7:341–346.
- Staal RG, Hananiya A, Sulzer D (2008) PKC theta activity maintains normal quantal size in chromaffin cells. J Neurochem 105:1635–1641.
- Starke K, Gother M, Kilbinger H (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol Rev 69:864–989.
- Starke K, Reimann W, Zumstein A, Hertting G (1978) Effect of dopamine receptor agonists and antagonists on release of. Naunyn Schmiedebergs Arch Pharmacol 305:27–36.
- Suaud-Chagny MF, Ponec J, Gonon F (1991) Presynaptic autoinhibition of the electrically evoked dopamine release studied in the rat olfactory tubercle by in vivo electrochemistry. Neuroscience 45:641–652.
- Suaud-Chagny MF, Chergui K, Chouvet G, Gonon F (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. Neuroscience 49:63–72.
- Suaud-Chagny MF, Dugast C, Chergui K, Msghina M, Gonon F (1995) Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems in vivo. J Neurochem 65:2603–2611.
- Sulzer D, Rayport S (1990) Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. Neuron 5:797–808.
- Sulzer D, Pothos EN (2000) Regulation of quantal size by presynaptic mechanisms. Rev Neurosci 11:159–212.
- Sulzer D, Sonders MS, Poulsen NW, Galli A (2005) Mechanisms of neurotransmitter release by amphetamines: a review. Prog Neurobiol 75:406–433.
- Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci 15:4102–4108.
- Sulzer D, Joyce MP, Lin L, Geldwert D, Haber SN, Hattori T, Rayport S (1998) Dopamine neurons make glutamatergic synapses in vitro. J Neurosci 18:4588–4602.
- Svenningsson P, Fourreau L, Bloch B, Fredholm BB, Gonon F, Le Moine C (1999) Opposite tonic modulation of dopamine and adenosine on c-fos gene expression in striatopallidal neurons. Neuroscience 89:827–837.
- Svingos AL, Clarke CL, Pickel VM (1999) Localization of the deltaopioid receptor and dopamine transporter in the nucleus accumbens shell: implications for opiate and psychostimulant cross-sensitization. Synapse 34:1–10.
- Svingos AL, Periasamy S, Pickel VM (2000) Presynaptic dopamine D(4) receptor localization in the rat nucleus accumbens shell. Synapse 36:222–232.
- Svingos AL, Chavkin C, Colago EE, Pickel VM (2001) Major coexpression of kappa-opioid receptors and the dopamine transporter in nucleus accumbens axonal profiles. Synapse 42:185–192.
- Szabo B, Muller T, Koch H (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens in vitro. J Neurochem 73:1084–1089.
- Tamir H, Liu KP, Adlersberg M, Hsuing S, Gershon MD (1996) Acidification of serotonin-containing secretory vesicles induced by a plasma membrane calcium receptor. J Biol Chem 271:6441–6450.
- Tang L, Todd RD, O'Malley KL (1994) Dopamine D2 and D3 receptors inhibit dopamine release. J Pharmacol Exp Ther 270:475–479.
- Tepper JM, Sun BC, Martin LP, Creese I (1997) Functional roles of dopamine D2 and D3 autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo. J Neurosci 17:2519–2530.

- Tranzer JP, Thoenen H (1967) Electron microscopic localization of 5-hydroxy-dopamine (3,4,5-trihydroxy-phenyl-ethylamine) a new 'false' sympathetic transmitter. Experimentia 23:743–745.
- Trovero F, Herve D, Desban M, Glowinski J, Tassin JP (1990) Striatal opiate mu-receptors are not located on dopamine nerve endings in the rat. Neuroscience 39:313–321.
- Uhl GR (2003) Dopamine transporter: basic science and human variation of a key molecule for dopaminergic function, locomotion, and parkinsonism. Mov Disord 18(Suppl 7):S71–S80.
- Ungless MA, Magill PJ, Bolam JP (2004) Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. Science 303:2040–2042.
- Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, Piazza PV, Borrelli E (2000) Distinct functions of the two isoforms of dopamine D2 receptors. Nature 408:199–203.
- Valtorta F, Meldolesi J, Fesce R (2001) Synaptic vesicles: is kissing a matter of competence? Trends Cell Biol 11:324–328.
- Van der Kloot W (1991) The regulation of quantal size. Progr Neurobiol 36:93–130.
- Van der Kloot W, Molgo J (1994) Quantal acetylcholine release at the vertebrate neuromuscular junction. Physiol Rev 74:899–991.
- Venton BJ, Zhang H, Garris PA, Phillips PE, Sulzer D, Wightman RM (2003) Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. J Neurochem 87:1284–1295.
- Venton BJ, Seipel AT, Phillips PE, Wetsel WC, Gitler D, Greengard P, Augustine GJ, Wightman RM (2006) Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. J Neurosci 26:3206–3209.
- Vilaro MT, Palacios JM, Mengod G (1990) Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci Lett 114:154–159.
- Voglmaier SM, Kam K, Yang H, Fortin DL, Hua Z, Nicoll RA, Edwards RH (2006) Distinct endocytic pathways control the rate and extent of synaptic vesicle protein recycling. Neuron 51:71–84.
- Wang CT, Lu JC, Bai J, Chang PY, Martin TF, Chapman ER, Jackson MB (2003) Different domains of synaptotagmin control the choice between kiss-and-run and full fusion. Nature 424:943–947.
- Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, Sankoorikal EB (2000) Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. J Neurosci 20:8305–8314.
- Weiner DM, Levey AI, Brann MR (1990) Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc Natl Acad Sci USA 87:7050–7054.
- Wess J (1996) Molecular biology of muscarinic acetylcholine receptors. Crit Rev Neurobiol 10:69–99.
- Westerink BHC, De Vries JB (1989) On the mechanism of neuroleptic induced increase in striatal dopamine release: brain dialysis provides direct evidence for mediation by autoreceptors localized on nerve terminals. Neurosci Lett 99:197–202.
- Wightman RM, Zimmerman JB (1990) Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. Brain Res Rev 15:135–144.
- Williams RM, Webb WW (2000) Single granule pH cycling in antigen induced mast cell secretion. J Cell Sci 21:3839–3850.
- Wilson CJ, Young SJ, Groves PM (1977) Statistical properties of neuronal spike trains in the substantia nigra: cell types and their interactions. Brain Res 136:243–260.

- Wonnacott S, Kaiser S, Mogg A, Soliakov L, Jones IW (2000) Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur J Pharmacol 393:51–58.
- Wood JG (1966) Electron localization of amines in central nervous tissue. Nature 209:1131–1133.
- Wu Q, Reith ME, Walker QD, Kuhn CM, Carroll FI, Garris PA (2002) Concurrent autoreceptor-mediated control of dopamine release and uptake during neurotransmission: an in vivo voltammetric study. J Neurosci 22:6272–6281.
- Wu Y, Pearl SM, Zigmond MJ, Michael AC (2000) Inhibitory glutamatergic regulation of evoked dopamine release in striatum. Neuroscience 96:65–72.
- Xu J, Tse FW (1999) Brefeldin A increases the quantal size and alters the kinetics of catecholamine release from rat adrenal chromaffin cells. J Biol Chem 274:19095–19102.
- Xu M, Mizobe F, Yamamoto T, Kato T (1989) Differential effects of M1- and M2-muscarinic drugs on striatal dopamine release and metabolism in freely moving rats. Brain Res 495:232–242.
- Yavich L, Tanila H, Vepsalainen S, Jakala P (2004) Role of alphasynuclein in presynaptic dopamine recruitment. J Neurosci 24:11165–11170.
- Yavich L, Oksman M, Tanila H, et al. (2005) Locomotor activity and evoked dopamine release are reduced in mice overexpressing A30Pmutated human alpha-synuclein. Neurobiol Dis 20:303–313.
- Zapata A, Witkin JM, Shippenberg TS (2001) Selective D3 receptor agonist effects of (+)-PD 128907 on dialysate dopamine at low doses. Neuropharmacology 41:351–359.

- Zhang H, Sulzer D (2003) Glutamate spillover in the striatum depresses dopaminergic transmission by activating group I metabotropic glutamate receptors. J Neurosci 23:10585–10592.
- Zhang H, Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7:581–582.
- Zhang Q, Li Y, Tsien RW (2009a) The dynamic control of kiss-andrun and vesicular reuse probed with single nanoparticles. Science 323:1448–1453.
- Zhang T, Zhang L, Liang Y, Siapas AG, Zhou FM, Dani JA (2009b) Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. J Neurosci 29:4035–4043.
- Zhang W, Yamada M, Gomeza J, Basile AS, Wess J (2002) Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. J Neurosci 22:6347–6352.
- Zhou FM, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4:1224–1229.
- Zhou Z, Misler S, Chow RH (1996) Rapid fluctuations in transmitter release from single vesicles in bovine adrenal chromaffin cells. Biophysical Journal 70:1543–1552.
- Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C (2002) Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. J Neurosci 22:8785–8789.

# Organization of Corticostriatal Projection Neuron Types

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## I. INTRODUCTION

Models of neuronal circuitry have been developed to account for the role of the basal ganglia in motor control the so-called direct and indirect pathway model of basal ganglia function (Albin et al., 1989; DeLong, 1990) (see Chapter 1). This was a breakthrough in the understanding of basal ganglia function, and was effective at globally explaining the motor defects in ballismus, Parkinson's disease (PD) and Huntington's disease (HD), and predicting the palliative effects of internal pallidal segment lesions in PD. This model, however, did not consider how the direct and indirect pathway striatal neurons might differ in their cortical input, a key issue in explaining their differing roles in motor control. Additionally, it seems clear that the basal ganglia participates in the learning of reward-motivated behavioral routines, and that corticostriatal plasticity in the form of potentiation of some synaptic inputs and inhibition of others underlies this process. Here too understanding differences between direct and indirect pathway striatal neurons in the types of cortical input they receive is important for understanding how corticostriatal plasticity shapes motor routines. In our studies, we have found that direct and indirect pathway striatal neurons do, in fact, appear

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. to differ in the types of cortical input they receive. In this paper, we review our findings on corticostriatal organization, and discuss how they contribute to understanding the role of the basal ganglia in motor learning and movement selection.

## II. CORTICAL PROJECTIONS TO BASAL GANGLIA – HISTORICAL OVERVIEW

Widespread and functionally diverse areas of the cerebral cortex, including sensory, motor, and association regions, project to the striatum in all mammals studied, which include rodents, carnivores and primates (Kemp and Powell, 1970; Jones et al., 1977; Oka, 1980; Veening et al., 1980; Royce, 1982; Goldman-Rakic and Selemon, 1986; Tanaka, 1987; McGeorge and Faull, 1989). This input is bilateral, with an ipsilateral predominance, and it is thought to provide the striatum with the sensory and motor planning information it needs to execute its role in motor control (see also Chapters 19 and 20). The cortical projection is known to be glutamatergic and to end as terminals that make asymmetric synaptic contacts, primarily with the spines of striatal projection neurons, which are by far the most abundant type of striatal neuron (Albin et al., 1989; Gerfen, 1992). The projection targets both the matrix and striosomal compartments of the striatum and tends to be topographically ordered in the mammals studied (Kemp and Powell, 1970; Selemon and Goldman-Rakic, 1985; McGeorge and Faull, 1989). Of note with respect to the role of the basal ganglia in motor control, the dorsolateral striatum (part of the putamen) receives the striatal input from the somatosensory - somatomotor cortices, and somatotopy is preserved in this input (Jones et al., 1977; Goldman-Rakic and Selemon, 1986). Moreover, while the cortical input to striatum is broadly topographically organized, any given region of striatum receives overlapping, convergent input from multiple, often related, cortical areas (Goldman-Rakic and Selemon, 1986; Brown et al., 1998; Hoffer and Alloway, 2001). Additionally, the input to striatum from any given region of cortex is not uniform, and instead exhibits discontinuities (Tanaka et al., 1981; Goldman-Rakic and Selemon, 1986; Flaherty and Graybiel, 1993; Alloway et al., 1998). The discontinuity in some cases represents cortical input to striosomal patches, since cortical areas receiving prominent hippocampal and amygdaloid input (e.g., prelimbic frontal cortex) have been reported to preferentially project to the striosomal compartment (Gerfen, 1984; Donoghue and Herkenham, 1986). Nonetheless, in many cases the discontinuities represent separate fields of terminals within the matrix compartment of striatum – referred to as matrisomes (Flaherty and Graybiel, 1993). The basis of such inhomogeneities was initially uncertain, but it seemed likely that it reflects the separate terminal fields of different cortical layers, or different cortical neuron types irrespective of their laminar location.

The laminar source(s) and the neuron type-specific source(s) of the corticostriatal projection have been the subject of interest for many years. In an early description of the corticostriatal projection based on Golgi material, Ramon y Cajal (1911) suggested that it arose as a collateral projection of the corticofugal fibers arising from pyramidal neurons of deep layer 5, as their axons descended through the striatum via the internal capsule. Later studies in rats using retrograde labeling, by contrast, reported a large and widespread population of neurons in ipsilateral cortical layer 3 and in ipsilateral upper layer 5 following tracer injection into the striatum, with few of the large deep layer 5 pyramidal neurons labeled (Kitai et al., 1976; Hedreen, 1977; Wise and Jones, 1977; Veening et al., 1980; Arikuni and

Kubota, 1986). This initially led to the view that corticostriatal neurons were largely limited to layer 3 and upper layer 5, and did not notably include the pyramidal tract neurons projecting out of the telencephalon. The input from layer 3 and upper 5 was mainly thought to end in the matrix compartment. Input to striosomes from the prelimbic cortices was thought to be an exception to the predominance of the layer 3 and upper layer 5 input to striatum, and arise from deep layer 5 neurons (Gerfen, 1989; Kincaid and Wilson, 1996). Some studies, however, challenged the view that layer 3 and upper 5 were the main source of cortical input to striatum, and reported that cortical neurons projecting to matrix compartment include two types by electrophysiological criteria. For example, Jinnai and Matsuda (1979) reported that two types of cortical neurons could be activated antidromically from striatum in cats, one type that only responded to caudate activation and one type that responded to both caudate and pyramidal tract (PT) activation. Control experiments precluded the possibility for the latter cortical neuron type that fibers of passage through the striatum had been activated during caudate stimulation. While the conduction velocity of the PT-type and non-PT type was overlapping, the mean latency of antidromic activation from caudate was less for the PTtype, again supporting the view of these as two separate corticostriatal neuron types. About 40% of the corticostriatal neurons identified in this study were of the PT-type. Similarly, Wilson (1986) recorded intracellularly from striatal neurons in rats, and showed that the EPSP response latencies to ipsilateral motor cortex stimulation overlapped those to contralateral motor cortex stimulation. Those in response to ipsilateral stimulation, however, included a short latency component that was absent in response to contralateral stimulation. Given the more rapid conduction velocity of PT-type neurons, Wilson interpreted this as evidence that striatum receives input from both PT-type cortical neurons as well as from non-PT type cortical neurons. This was consistent with his prior evidence that stimulation of the pyramidal tract at midbrain levels evoked monosynaptic EPSPs in many striatal neurons (Wilson et al., 1982). Both Jinnai and Matsuda (1979) and Wilson (1986) noted that the PT collateral in striatum is thin and conducts much more slowly than does the main PT axon descending to the brainstem. The projection of PT-type neurons of motor and somatosensory cortex in rats to striatum via collaterals of the main descending extratelecephalic axon was confirmed by intracellular filling of PT-type neurons (Donoghue and Kitai, 1981; Landry et al., 1984).
### **III. CORTICOSTRIATAL NEURON TYPES**

A series of more recent studies in rats and monkeys employing neuron-type specific pathway tracing, or intracellular recording and neuron filling have further clarified the morphology and laminar location of the cortical neurons projecting to striatum, and made it clear that in each cortical region projecting to striatum at least two main types of corticostriatal projection neuron can be distinguished by their connections within the cortex and their projections to other subcortical areas. One is, in fact, the type whose main axon projects extratelencephalically (brainstem-projecting neurons) identified by Ramon y Cajal, whereas the second is a type that projects to the basal ganglia and cortex but not outside the telencephalon, namely the intratelencephalically projecting neurons, or IT-type (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998; Reiner et al., 2003; Parent and Parent, 2006). Many but not all IT-type neurons project to contralateral cortex and striatum, and this appears to be region-specific, since motor cortex but not somatosensory cortex projects heavily contralaterally. By contrast, PT-type neurons project only ipsilaterally to the striatum. Brainstem-projecting corticostriatal neurons (i.e., PT-type) are typically larger than IT-type corticostriatal neurons and mainly found in lower cortical layer 5, whereas intratelencephalically projecting corticostriatal neurons are mainly found in layer 3 and upper layer 5 (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998; Reiner et al., 2003; Parent and Parent, 2006). For rats, IT-type neurons have a mean diameter of  $12-13\,\mu\text{m}$ , while PT-type have a mean diameter of 18-19µm (Reiner et al., 2003). These neurons differ too in their dendritic arborization patterns. PT-type neurons have a prominent apical dendrite that ascends and branches profusely in layer 1 of cortex, while the dendrites of IT-type neurons are more slender and the arborization in layer 1 is sparser. These various anatomical features of IT-type and PT-type neurons were initially demonstrated in rats, and more recently for monkeys as well (Parent and Parent, 2006). It should be noted that layer 5 of cerebral cortex is recognized to broadly consist of two types of neurochemically, morphologically, and physiologically distinct pyramidal neuron types matching the description of IT-type and PT-type neurons (Molnar and Cheung, 2006). Thus, IT-type and PT-type corticostriatal neurons do not merely represent a subset of layer 5 pyramidal neurons. Rather, layer 5 pyramidal neurons fall into two types – an intratelencephalically and an extratelencephalically projecting type, with each possessing a projection to striatum. It may be, but it is not definitively established, that all layer 5 pyramidal neurons of each type have input to striatum.

The laminar distribution of IT-type and PT-type perikarya differs slightly between the motor and somatosensory cortices in rats (Wilson, 1987; Cowan and Wilson, 1994; Reiner et al., 2003). In the somatosensory cortex, the vast majority of IT-type perikarya are in layer 3 and upper layer 5, with the neurons being nearly equally abundant in the two. By contrast, in motor cortex, the predominant location of IT-type perikarya is in upper layer 5, with additional IT-type perikarya being more abundant in lower layer 5 than in layer 3. The laminar distribution of IT-type neurons may differ in different mammalian species. For example, in cats, layer 3 seems to be the more prevalent location of retrogradely labeled IT-type neurons in the ipsilateral cortex after intrastriatal injection of HRP (Oka, 1980; Royce, 1982). Monkeys, however, appear to be more similar to rats, because upper layer 5 has been reported to be the predominant location of retrogradely labeled IT-type neurons in the ipsilateral monkey cortex after intrastriatal HRP injection (Jones and Wise, 1977; Jones et al., 1977; Goldman-Rakic and Selemon, 1986), and in single neuron tracing studies (Parent and Parent, 2006). Using retrograde labeling from the pontine pyramidal tract to identify PT-type neurons in rats (Fig. 18.1), we also observed a slight difference between the motor cortex and somatosensory cortex in the location of PT-type neuronal perikarya, with about 90% of the PT-type neurons of somatosensory cortex in deep layer 5, but only 65% of the PT-type neurons of motor cortex situated in deep layer 5 (Fig. 18.2) (Reiner et al., 2003). Most of the PT-type neurons not in deep layer 5 were located in upper layer 5. Several studies have shown by morphological and/or electrophysiological means that all or nearly all cortical pyramidal neurons projecting to the brainstem and/or spinal cord send a collateral into the striatum (Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998). On this basis, then, it would seem likely that the distribution of retrogradely labeled neurons observed by us in the ipsilateral cortex after pyramidal tract injection of BDA3k represents the distribution of PT-type corticostriatal neurons, with minimal labeling of pyramidal tract neurons not having a striatal collateral (Reiner et al., 2003).

The input of PT-type neurons of motor cortices to the striatum has attracted interest because of its potential for providing the striatum with a copy of the cortical motor



**FIGURE 18.1** Schematic depiction of the strategies employed for selective retrograde labeling of intratelencephalically projecting (IT)-type (A) and pyramidal tract (PT)-type (B) corticostriatal neurons in Reiner et al. (2003). To directly compare IT- and PT-type perikarya in the cortex of the right cerebral hemisphere, we retrogradely labeled them in the same hemisphere by injecting tetramethylrhodamine-dextran amine (RDA)3k into the left striatum (A) and biotinylated dextran amine (BDA)3k into the right pyramidal tract at caudal pontine levels (B) in some rats.

signal transmitted to the hindbrain and spinal cord. The intrastriatal axon of PT-type corticostriatal neurons arises as a collateral of the main descending axon as it traverses the striatum via the internal capsule (primates) or within the pencil bundles (rodents). To a variable extent, these neurons also have collaterals in globus pallidus, the sub-thalamic nucleus, midline or posterior thalamic nuclei (depending upon the cortical region of origin), and the substantia nigra (Canteras et al., 1990; Levesque et al., 1996a,b; Levesque and Parent, 1998). The intrastriatal branching pattern of the PT-type input is not entirely certain. Cowan and Wilson (1994) used intracellular filling of electrophysiologically identified PT-type neurons in rats



**FIGURE 18.2** Low-power images of the laminar distribution in primary somatosensory cortex of intratelencephalically projecting (IT)-type (A) and pyramidal tract (PT)-type (B) perikarya in the same rat. The IT-type perikarya were retrogradely labeled from the contralateral striatum with RDA3k. As is evident in A, they are 12–13  $\mu$ m in size, and largely localized to layer 3 and upper layer 5. By contrast, the PT-type perikarya retrogradely labeled by BDA3k injection into the ipsilateral pontine pyramidal tract are largely localized to deep layer 5 (B) and are larger (18–19  $\mu$ m) than the IT-type perikarya. Scale bar = 200  $\mu$ m in A (applies to A and B). Images C and D show high power views of images IT-type (C) and PT-type perikarya (D) in cortex. The PT-type perikarya are larger and possess a more prominent apical dendrite than the IT-type perikarya. Scale bars = 50  $\mu$ m in C and D.

(Fig. 18.3), and reported that individual neurons of this type give rise to an intrastriatal arborization that consists of scattered small, dense focal clusters of fine processes and terminals (about  $250 \,\mu\text{m}$  in diameter per focal cluster) scattered over a 1–2 mm region of the striatum (Cowan and Wilson, 1994). Using single axon tracing, a similar result was reported for the PT-type input in monkeys (Parent and Parent, 2006). More recently, however, Zheng and Wilson (2001) used juxtacellular labeling to study the intrastriatal arborization of PT-type neurons of motor and cingulate cortex in rats. They reported that the PT-type neurons identified in that study possessed a broader and more diffuse striatal arborization and termination pattern. It is uncertain whether PT-type neurons vary in their intrastriatal arborization, with perhaps different cortical areas differing in



**FIGURE 18.3** Intracortical dendritic (A) and axonal arborizations (B), overall axonal projections (C), and intrastriatal projections (D) for a PT-type neuron of rat motor cortex labeled by intracellular filling and identified by antidromic activation from the brainstem pyramidal tract. The intracortical axonal projections of the neuron are confined to the vicinity of the cell body, and the intrastriatal arborizations (marked as a and b) shows scattered pockets of dense terminal fields. This illustration is a modified version of Figure 3 in Cowan and Wilson (1994) and is reprinted with permission of the authors. The descending axon to the cerebral peduncle beyond the GPe (CP) is highlighted. GPe – globus pallidus externus.

the PT-type varieties they possess, or if the differences observed between the intracellular and juxtacellular labeling studies in rodents stems from methodological differences. In any event, PT-type neurons with a discontinuous arborization pattern would explain why individual regions of cerebral cortex have a discontinuous projection to striatum. Moreover, PT-type neurons of prelimbic cortex with scattered terminals are thought to account for the cortical input targeting striosomes (Kincaid and Wilson, 1996; Levesque and Parent, 1998). Part of the terminal field of each PT-type neuron of motor and somatosensory cortex has a discrete ending in dorsolateral striatum, which appears to account for the topographically and somatotopically ordered input of these cortical regions to dorsolateral motor striatum (Wright et al., 1999).

The other major type of corticostriatal projection neuron, the IT-type, projects to the contralateral (in many cases) as well as ipsilateral cortex and striatum, and neurons of the bilaterally projecting type are numerous in the motor cortex (Wilson, 1987; Cowan and Wilson, 1994; Gerfen and Wilson, 1996; Kincaid and Wilson, 1996; Wright et al., 2001; Parent and Parent, 2006). This neuron type does not project out of the telencephalon, but does have extensive intratelencephalic projections, which include cerebral cortex and striatum, in many cases on both sides of the brain. In contrast to the scattered focal arborization of the PT-type neuron, the intrastriatal axon of individual ITtype neurons has been reported to give rise to an extended and uniform arborization (Fig. 18.4) that has sparse en passant terminals over a wide (about 1.5 mm in diameter) striatal expanse (Cowan and Wilson, 1994; Kincaid and Wilson, 1996). While Cowan and Wilson (1994) had reported that PT-type neurons and IT-type neurons differed markedly in their intrastriatal arborization, with PT input being discontinuous and IT input being uniform, the later study of Zheng and Wilson (2001) indicated that at least for some cortical regions and some PT-type neurons both IT-type and PT-type possess a broad striatal arborization and sparse termination pattern. Regardless of this detail of arborization, IT-type and PT-type neurons are clearly dichotomous in terms of their size, shape, dendritic branching and typical laminar location in cerebral cortex (Fig. 18.2). Moreover, PT-type and IT-type neurons appear to convey different signals to striatum. For example, for motor cortex in primates, the PT-type neurons fire during movement and the IT-type neurons more typically fire in relation to movement planning prior to movement (Beloozerova et al., 2003; Turner and DeLong, 2000; Bauswein et al., 1989). In addition, the conduction velocities of the parent PT-type axons are about 3-4 times more rapid than those of the parent IT-type axons (Wilson, 1986, 1987; Bauswein et al., 1989; Wilson and Cowan, 1994; Turner and DeLong, 2000). Even with the conduction velocity slowing that occurs for the thin



**FIGURE 18.4** Dendritic and ipsilateral axonal fields of an IT-type neuron in rat motor cortex. Two axonal branches in the subcortical white matter (WM) marked by arrows were traced to the contralateral hemisphere. Other axonal branches arborized in the ipsilateral striatum, where they formed a broad arborization (about 1 mm diameter) with a sparse density of boutons. Intracortical axon collaterals ramified mainly in the layers containing corticostriatal neurons of the same type (layer 3 and upper half of layer 5), but some collaterals extended as far as layer I. Some collaterals projected across the boundary (arrow) between the medial agranular field (AGm) and the lateral agranular field (AGI), and formed a terminal arborization in AGI (primary motor cortex). This illustration appears as Figure 4 in Cowan and Wilson (1994) and is reprinted with permission of the authors. The illustration has been modified.

PT-type collateral in striatum, PT-type signals reach their striatal target neurons a few milliseconds before IT-type signals do upon PT-type and IT-type neuron co-activation in cortex.

Earlier studies in rats had reported retrograde labeling of a large and widespread population of neurons in upper layer 5 following HRP injection into the striatum (Hedreen, 1977; Hedreen and McGrath, 1977; Wise and Jones, 1977; Veening et al., 1980). It is now evident that these retrogradely labeled perikarya in layers 3 and 5 mainly represented IT-type corticostriatal projection neurons (Wilson, 1987; Cowan and Wilson, 1994). A relatively insensitive retrograde tracer such as HRP would be likely to label many neurons of this type preferentially in the ipsilateral striatum because individual neurons of the IT-type give rise to a more uniform and widespread intrastriatal arborization than individual PT-type neurons typically do (Wilson, 1987; Cowan and Wilson, 1994; Wright et al., 2001). By contrast, the scattered focal terminations of many PT-type neurons in striatum would result in fewer of them being labeled than IT-type neurons after any given intrastriatal retrograde tracer injection. Nonetheless, since morphological and electrophysiological data suggest that all or nearly all cortical pyramidal neurons projecting to the brainstem and/or spinal cord send a collateral into the striatum and since PT-type neurons are found in all cortical regions (Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998), the PT-type input to striatum appears to be substantial.

# IV. ULTRASTRUCTURE OF CORTICAL INPUT TO STRIATUM

Given their differing neuronal morphologies, laminar location and physiologies, the possible ultrastructural differences between IT-type and PT-type terminals in striatum is of interest. We examined this issue by using selective labeling of IT-type and PT-type terminals in rat striatum (Reiner et al., 2003). The IT-type intrastriatal terminals were labeled anterogradely by biotinylated dextran amine (BDA)10k injection into the contralateral motor or primary somatosensory cortex (Figs 18.5, 18.6). Because IT-type but not PT-type neurons have crossed projections to striatum, BDA10k-labeled terminals in striatum contralateral to cortical injection are all of the IT-type. We were able to selectively labeled PT-type terminals by using the sensitivity of BDA3k and its tendency to label axonal collaterals of retrogradely labeled neurons. In brief, BDA3k injections into pontine pyramidal tract yielded retrograde labeling of ipsilateral PT-type cortical perikarya, as well as the intrastriatal collaterals of these neurons (Figs. 18.5, 18.6). At the electron microscopic level, the intrastriatal terminals of both IT-type and PT-type corticostriatal neurons made asymmetric synaptic contact with spine heads and less frequently with dendrites (Fig. 18.7). We observed that IT-type terminals tend to be round and relatively small, and the postsynaptic density (PSD) at their axospinous contacts was rarely perforated (Reiner et al., 2003). By contrast, PT-type terminals tend to be more variable in shape and about twice as large as IT-type terminals, and the PSD at their axospinous contacts was commonly perforated. In this prior paper, we reported that contralateral IT-type axospinous terminals of dorsolateral striatum arising from somatosensory and motor cortices had a mean diameter of 0.41 µm, whereas PT-type terminals of dorsolateral striatum, presumably mainly arising from somatosensory and



**FIGURE 18.5** Schematic depiction of the strategies employed for selective anterograde labeling of the intrastriatal terminals of intratelencephalically projecting (IT)-type (A) and pyramidal tract (PT)-type (B) neurons. To label IT-type terminals in striatum selectively, we relied on the fact that this projection, but not the PT-type projection, is bilateral. Accordingly, BDA10k was injected into the right motor or sensory cortex, and anterogradely labeled IT-type terminals were examined in the contralateral (i.e., left) striatum (A). To label PT-type terminals in the striatum selectively, the intrastriatal collaterals of the PT-type neurons were retrogradely labeled by injection of BDA3k into the ipsilateral pyramidal tract at caudal pontine levels (B).

motor cortices, had a mean diameter of  $0.82 \,\mu\text{m}$ . More recently, we re-measured the diameters of 240 IT-type and 220 PT-type axospinous synaptic terminals from our prior study, consistently measuring the diameter of the terminal parallel to and  $0.1 \,\mu\text{m}$  behind the PSD. We found that the mean diameters for axospinous synaptic IT-type and PT-type terminals measured in this standardized way were  $0.52 \,\mu\text{m}$  and  $0.91 \,\mu\text{m}$ , respectively, with only 3.3% of IT-type terminals associated with a perforated postsynaptic density (PSD) and 40% of PT-type terminals associated with a perforated PSD.



**FIGURE 18.6** Images of IT-type (A) and PT-type (B) axons and varicosities in the striatum. IT-type terminals enter the striatum from the external capsule (A), whereas PT-type terminals arise as collaterals of corticofugal axons as they course through the striatum (B). Both give rise to varicosities within the striatal neuropil, with the IT-type appearing somewhat smaller. Scale bars =  $50 \,\mu$ m in A, B.



**FIGURE 18.7** A–D: Examples of the BDA-labeled intrastriatal terminals of IT-type corticostriatal neurons (A, B) and of PT-type corticostriatal neurons (C, D) at the electron microscopic level. The IT-type terminals and PT-type terminals shown make asymmetric synaptic contact with a spine (s), as revealed by their size and the presence of spine apparatus (asterisk), presumably that of a striatal projection neuron. Note that the IT-type terminals are round, largely regular in shape, and about 0.5  $\mu$ m in diameter, while the PT-type terminals shown are typically large, irregular in shape, and in some cases envelop their postsynaptic target structure. Scale bars = 0.5  $\mu$ m in A–D.

were made in random sections that did not necessarily pass through the widest point of each terminal, they underestimate the size of IT-type and PT-terminals. For the smaller IT-type terminals, this underestimate is likely to be small. To address the underestimate for PT-type terminals, we have analyzed several PT-type terminals in semi-serial sections, and found that their peak size was about  $1 \,\mu\text{m}$ . Thus, PT-type axospinous terminals are about twice the size of the IT-type axospinous terminals, with PT-type terminals at their widest being about  $1\,\mu$ m. Although we did not examine it systematically, PT-type axodendritic synaptic terminals also appeared larger than IT-type axodendritic synaptic terminals.

Concerns can be raised about our approach for selective IT-type and PT-type terminal labeling: (1) Our IT-type labeling was limited to the set of IT-type axons that projects contralaterally, which may not be representative of those that project ipsilaterally; and (2) The precise source of the PT-type terminals is uncertain, and the phenomenon of retrograde collateral labeling may be selective for axons (e.g., possibly larger ones) that are not representative of the PT-type population as a whole. The work of Wright et al. (1999, 2001) on the intrastriatal terminals of IT-type and PT-type corticostriatal neurons of the primary somatosensory cortex of rat addresses these concerns. In brief, their findings are consistent with those of Reiner et al. (2003) that intrastriatal PT-type terminals differ in several respects from IT-type terminals. Wright et al used two anterograde pathway tracers, PHA-L and BDA, and found that two distinct types of corticostriatal pathways arise from the barrel cortex. One system was reported to give rise to a nontopographic projection to the striatum with an intrastriatal arborization that was termed a "diffuse" system (Wright et al., 1999). This projection was found to arise largely from neurons located between barrel columns that also project contralaterally to the striatum (Wright et al., 2001). These neurons resemble IT-type neurons in their size, shape, and preferential localization in upper layer 5. The other corticostriatal projection arising from the barrel cortex was reported to give rise to a topographically ordered projection that was termed the "discrete pathway." This corticostriatal projection arose as collaterals of corticofugal axons descending through the striatum and only projected to the ipsilateral striatum. Moreover, the intrastriatal arborization of the discrete system gave rise to scattered patches of dense focal innervation. Because of these features, the authors concluded that the diffuse system arose from the IT-type corticostriatal projection neurons described by Wilson and coworkers (Wilson, 1987; Wilson and Cowan, 1994), whereas the discrete system arose from the PT-type corticostriatal projection neurons described by Wilson and coworkers (Wilson, 1987; Wilson and Cowan, 1994). Wright et al. (1999) examined the light microscopic (LM) and electron microscopic (EM) morphology of these two types of intrastriatal terminals. The fibers of the diffuse pathway were noted to be fine caliber, and at the EM level the terminals of this pathway were described as small, with a mean diameter of about 0.55 µm. These terminals were reported to make asymmetric synaptic contacts with the dendritic spines of striatal projection neurons, and the contacts possessed an unperforated PSD. By contrast, the discrete system was made of thicker axons that gave rise to large terminals, whose mean diameter was 0.89 µm. The terminals of the discrete system also made asymmetric synaptic contacts with the dendritic spines of striatal projection neurons, but these contacts were complex and the spines typically possessed a perforated PSD. The findings of this study are consistent with our own, and they obviate possible concerns that our techniques labeled atypical subsets of IT-type and PT-type terminals. Thus, our findings and those of Wright et al. (1999, 2001) indicate that corticostriatal neurons projecting into the pontine pyramidal tract give rise to intrastriatal terminals that are considerably larger and more complex than the corticostriatal neurons of the motor and sensory cortex that project only within the telencephalon. LM studies suggest the same is true in monkeys (Parent and Parent, 2006).

# V. DIFFERENTIAL INPUT OF CORTEX TO STRIATAL NEURONS

We next assessed whether the two types of corticostriatal neurons project differentially to the two types of striatal output neurons, namely, those that give rise to the indirect output pathway, projecting to the external segment of the globus pallidus (GPe) (i.e., striato-GPe neurons), and those that form the direct pathway by projecting to the internal segment of the globus pallidus (GPi) and/or the substantia nigra pars reticulata (SNr) (i.e., striato-GPi/SNr neurons) (see Chapter 1). We reasoned that the different roles of these output neurons in motor control might in part be enabled by differing cortical inputs. Consistent with this possibility, prior studies had shown that these two striatal neuron types differed in their gene expression responses to cortical stimulation or ablation (Uhl et al., 1988; Parthasarathy and Graybiel, 1997).

## A. Anatomical Evidence

In our first approach for assessing this, we examined whether axospinous terminals differed on these two striatal projection neuron types in size. We reasoned that since axospinous IT-type terminals differed from PT-type in size,



**FIGURE 18.8** Electron microscopic images of dendrite (+d) and spine (+s) labeling of striatonigral (A) and striato-GPe (B) neurons that had been retrogradely labeled with BDA3k from their target areas. Note that striatonigral (A) spines receive asymmetric synaptic contact from smaller unlabeled terminals (-t) than do striato-GPe neuron spines (B).

if either preferentially targeted a given projection neuron type, then there should be a difference in the mean size of axospinous terminals on them. For this approach, we identified direct pathway neurons either by retrogradely labeling them with BDA3k from the substantia nigra or by immunolabeling for the D1 dopamine receptor, and we identified indirect pathway neurons by retrogradely labeling them with BDA3k from the GPe or by immunolabeling for the D2 dopamine receptor (Lei et al., 2004). Since striato-GPi neurons in rats have a collateral in SNr, our BDA3k injection into substantia nigra yielded retrograde labeling of striato-SNr and striato-GPi neurons. We thus refer to the neurons BDA3k-labeled from substantia nigra as striato-GPi/SNr. Regarding D1 and D2 immunolabeling, numerous studies suggest that D1+ versus D2+ neurons in striatum largely represent striato-GPi/SNr versus striato-GPe projection neurons, respectively, since striato-GPi/SNr neurons are enriched in D1 receptors while striato-GPe neurons are enriched in D2 receptors (Deng et al., 2006) (see Chapter 1). We then measured the diameter of asymmetric axospinous synaptic terminals on either striatal projection neuron type (in random sections not necessarily measuring individual terminals at their widest point). We limited our attention to dorsolateral striatum. Electron microscopic viewing revealed that asymmetric synaptic terminals on BDA3k-labeled striato-GPi/SNr neuron spines were characteristically small (0.43 µm, based on 309 terminals) and rounded (Fig. 18.8). Similarly, we found that the mean size of terminals making asymmetric synaptic contact with D1+ spines was 0.45 µm, based on 867 terminals. Given that 20-30% of the axospinous input to striato-GPi/SNr neurons represents the small-sized terminals of the thalamostriatal input (Chung et al., 1977; Smith et al., 2004), these results are consistent with the interpretation that D1+ striato-GPi/SNr neurons preferentially receive axospinous IT-type input and some axospinous thalamic input. By contrast, asymmetric synaptic terminals on BDA3k-labeled striato-GPe neuron spines tended to be notably larger (0.69 µm, based on 342 terminals), irregular in shape, and in many cases associated with a perforated postsynaptic density. Given the sparseness of the intra-GPe collateral of striato-GPi/SNr neurons, which contain substance P (SP) (Kawaguchi et al., 1990; Wu et al., 2000), and given that we have previously found by single-cell RT-PCR that the vast majority of neurons retrogradely labeled from GPe contain enkephalin (ENK) but not SP (Wang et al., 2006), the vast majority of the striatal neurons labeled from GPe with BDA3k must have been ENK+ striato-GPe neurons. The mean size of terminals making asymmetric synaptic contact with D2+ spines was 0.61 µm, based on 519 terminals. Thus, the size of axospinous synaptic terminals on striatal neurons retrogradely labeled from GPe or by D2 immunolabeling is greater than that of asymmetric axospinous synaptic terminals on D1+ striato-GPi/SNr neurons, but not equal to the mean size of PT-type axospinous synaptic terminals, as reported in Reiner et al. (2003). This suggests that indirect pathway neurons receive a substantial input from PT-type terminals, as well as from possibly thalamic and IT-type terminals.

To directly assess the cortical input to the two striatal projection neuron types, we combined BDA-labeling of IT-type or PT-type terminals with D1 or D2 immunolabeling (Lei et al., 2004). We found that of all axospinous ITtype synaptic terminals labeled with BDA10k in tissue immunolabeled for D1, 50.9% made synaptic contact with D1+ spines (Fig. 18.9). By contrast, of all axospinous ITtype synaptic terminals labeled with BDA10k in tissue immunolabeled for D2, only 12.6% made synaptic contact with D2+ spines. Double-labeling for PT-type terminals and striato-GPi/SNr (D1+) or striato-GPe (D2+) spines showed a different trend-of all axospinous PT-type synaptic terminals labeled with BDA3k in tissue immunolabeled for D1, only 21.3% synaptically contacted D1+ spines, while of all axospinous PT-type synaptic terminals labeled with BDA3k in tissue immunolabeled for D2, 50.5% synaptically contacted D2+ spines (Fig. 18.9). Thus, IT-type terminals preferentially contact D1+ spines whereas PT-type terminals preferentially contact D2+ spines. The preference of IT-type terminals for D1+ spines and PT-type terminals for



FIGURE 18.9 Electron microscopic images showing double labeling for: (A) BDA10k-labeled IT-type corticostriatal terminals and D1-immunolabeled (+s) (striatonigral) spines in rat striatum; (B) for BDA10k-labeled IT-type corticostriatal terminals and D2-immunolabeled (striato-GPe) spines; (C) for BDA3k-labeled PT-type corticostriatal terminals and D1-immunolabeled (striatonigral) spines (+s) in rat striatum and for BDA3k-labeled PT-type corticostriatal terminals; and (D) for BDA3k-labeled PT-type corticostriatal terminals and D2-immunolabeled (striato-GPe) spines. These images show that IT-type terminals make asymmetric synaptic contact with D1-immunolabeled spines, as well as D2-immunolabeled spines, and that PT-type terminals also make asymmetric synaptic contact with both D1-immunolabeled spines, as well as D2-immunolabeled spines. The IT-type terminals, however, about three times as commonly contact D1+ than D2+ spines, and PT-type terminals about three times more commonly contact D2+ than D1+ spines. Abbreviations: +d = labeled dendrites; -t = unlabeled terminals; +t = labeled terminals.

D2+ spines was also evident in the percent of spines of a given type observed to receive BDA-labeled IT-type or PTtype synaptic input. For example, 23.7% of D1+ spines in the analyzed fields of view received BDA-labeled IT-type terminals, but only 10.2% of D1+ spines received BDAlabeled PT terminals. By contrast, 40.1% of D2+ spines received BDA-labeled PT-type input, while only 12.4% of the D2+ spines received BDA-labeled IT-type terminals. Note that fewer than 100% of either the D1+ or D2+spines received labeled terminals because not all axospinous terminals of a given type were labeled with BDA in the fields examined, and some spines receive thalamic input. Irrespective of how the results are considered, the findings show that IT-type terminals preferentially contact D1+ (striato-GPi/SNr) spines and PT-type terminals preferentially target D2+ (striato-GPe) spines (Fig. 18.10). Note that this preference does not appear to be absolute, since our results indicate that a minority of PT-type terminals contact



**FIGURE 18.10** Schematic illustration of the differential projections of IT-type and PT-type cortical neurons to the two main types of striatal projection neurons – the direct pathway type containing substance P (SP) and possessing D1-type receptors and the indirect pathway type containing enkephalin (ENK) and possessing D2-type dopamine receptors. The major functions and/or projection targets of each of the two main types of cortical pyramidal and striatal neurons is indicated.

D1+ (striato-GPi/SNr) spines and a minority of IT-type terminals target D2+ (striato-GPe) spines.

Our studies focused on dorsolateral striatum, a part of the striatal matrix. As noted earlier, the input to the striosomal compartment is thought to arise from PT-type neurons. One study has used BDA anterograde labeling to examine the input of motor and cingulate cortex to striosomes as identified by mu opiate receptor (MOR) immunolabeling in rat (Wang and Pickel, 1998). They found that the BDA-labeled corticostriatal terminals ending on MOR+ spines tended to be large and frequently exhibited a perforated postsynaptic density. Based on measurements of terminals shown in that paper 0.1 µm behind the PSD, the mean size of nine terminals making axospinous synaptic contact on

MOR+spines (thus establishing them as within striosomes) was  $0.88 \mu m$  and a third of these possessed perforated PSDs. These results are similar to those we have found for BDA-labeled PT-type terminals in the matrix compartment of dorsolateral striatum, and thus the results of Wang and Pickel (1998) are consistent with the view that striosomes are innervated by PT-type input ending as large axospinous terminals.

### **B.** Electrophysiological Evidence

Our findings that IT-type terminals preferentially target direct pathway neurons and PT-type terminals preferentially target indirect pathway neurons are consistent with the results of several electrophysiological studies. For example, Kreitzer and Malenka (2007) have shown that indirect pathway neurons have a lower paired-pulse ratio and a higher mEPSC frequency than do direct pathway neurons in mouse striatum. These results suggest that excitatory synapses on indirect pathway neurons have a higher probability of transmitter release than do those on direct pathway neurons. Since large terminals and perforated postsynaptic densities are characteristic of more efficacious synaptic contacts (Geinisman, 1993; Sulzer and Pothos, 2000), PT-type terminals are likely to have a higher probability of transmitter release and may more effectively activate their target indirect pathway neurons than do IT-type terminals their target direct pathway neurons. This may explain, in part, why indirect pathway striatal neurons have higher basal firing rates (Mallet et al., 2006). Ding et al. (2008) also showed that that indirect pathway neurons have a lower paired-pulse ratio. Cepeda et al. (2008) reported that D2+ striatal neurons showed a higher mEPSC frequency than D1+ striatal neurons. They also found that when increased cortical firing was induced by bath application of GABAA antagonist to forebrain slices, D2+ but not D1+ neurons displayed prominent inward currents and large, long-lasting depolarization. Additionally, direct electrical activation of cortical input more readily elicited D2+ neuron responses than D1+ neuron responses at low stimulus current intensities. These various findings are consistent with and can be explained by our observation that indirect pathway neurons preferentially receive the large PT-type terminals, which would be likely to release more transmitter and thereby more readily activate indirect pathway neurons than does the IT-type input to direct pathway neurons. Our results are also consistent with the finding that activation of cortex *in vivo* tends to preferentially induce immediate early gene expression in ENK+ striatal neurons (Berretta et al., 1997; Parthasarathy and Graybiel, 1997). The differential synaptology of the cortical input, and the greater responsiveness of indirect pathway neurons to it (Kreitzer and Malenka, 2007; Gertler et al., 2008), may favor more robust indirect pathway than direct pathway responses to cortical activation.

One set of investigators has reported evidence that apparently challenged the notion that PT-type input ends preferentially on indirect pathway type striatal neurons (Ballion et al., 2008), based on two lines of electrophysiological evidence. In one approach, they characterized the conduction of velocity of IT-type axons to striatum and of the PT-type projection to brainstem. As others had previously found, they noted that the conduction velocity of the main axon of the PT neurons was about four times faster than that of IT neurons. Ballion et al., therefore, hypothesized that if PT neurons project preferentially to indirect pathway neurons and IT neurons to direct pathway neurons, cortical activation should induce spikes in ipsilateral indirect pathway striatal neurons with a four times shorter latency than in direct pathway striatal neurons. Using antidromic activation from substantia nigra to distinguish direct pathway neurons (antidromically activated from substantia nigra) from indirect pathway neurons (not antidromically activated from substantia nigra), they found that the earliest spikes in response to the second cortical pulse in a 100 ms pair were similar in latency for the two striatal projection neuron types. In a second approach, they hypothesized that striato-GPe neurons should not respond as commonly as striato-GPi/SNr neurons to contralateral cortical activation, since the IT-type input preferentially targets the latter. Again using antidromic activation from substantia nigra to distinguish direct pathway neurons from indirect pathway neurons, they found that the two striatal projection neuron types responded equally commonly to the second pulse in a 100 ms pair delivered to contralateral cortex. Since neither outcome matched their simple prediction from the differential projection of IT-type and PT-type neurons to striatum reported by Lei et al. (2004), Ballion et al. (2008) concluded that IT-type input did not preferentially end on direct pathway neurons but rather ended equally on both direct and indirect pathway neurons, and PT-type input was meager to indirect pathway neurons as well as to direct pathway neurons. Setting aside possible concerns about their strategy for distinguishing striatonigral and striato-GPe neurons and setting aside for the moment a concern about relying on the response to the second pulse in the pair to draw conclusions, the prediction being tested is overly strong given the data of Lei et al. (2004). Our study indicated that striato-GPe neurons receive significant IT-type input and that striato-GPi/SNr neurons receive significant PT-type input. Thus, while it is informative to demonstrate that both striatal projection neuron types can spike with equal latency to ipsilateral cortical activation and that striato-GPe neurons can spike in response to IT-type input, these findings are not inconsistent with the idea that IT-type input preferentially targets striato-GPi/SNr neurons and PT-type input preferentially target striato-GPe neurons. Rather, the results support our finding that all striato-GPi/SNr neurons receive some PT-type input and all striato-GPe neurons receive some ITtype input. It may also be that by assessing the response to the second pulse in a pair, Ballion et al. were studying the effects of potentiation of the contralateral IT-type input to indirect pathway neurons and potentiation of the ipsilateral PT-type input to direct pathway neurons, since the second pulse in a pair is typically potentiated. In this light, it should be noted that in a prior study in which they assessed direct and indirect pathway striatal neuron responses to the first of a 100 ms pair of stimulus pulses to ipsilateral cortex, indirect pathway striatal neurons responded significantly more rapidly than did direct pathway striatal neurons (Mallet et al., 2006).

# C. Open Questions

The work of Ballion et al. (2008) and Mallet et al. (2006), however, raises the issue of the relative abundance of the IT-type and PT-type inputs to the striatum. IT-type input arises from layer 3 and upper layer 5 of all cortical areas, suggesting the input to be substantial. PT-type input clearly arises from somatosensory and motor cortices. Based on the data of Jinnai and Matsuda (1979), it may be that about 40% of the overall cortical input to dorsolateral striatum from motor and sensory cortex is from PT-type neurons. Given their preferential input to indirect pathway neurons, a lesser PT-type input to striatum would be consistent with the sparser dendritic trees of indirect pathway type striatal projection neurons (Gertler et al., 2008). PT-type neurons projecting to the pons are present, however, throughout cortex, although the extent of their overall projection to striatum and the nature of the signal that non-motor or nonsomatosensory cortices convey to striatum is uncertain.

Finally, our work has mostly addressed cortical inputs to striatal projection neurons. However, striatal interneurons might also be differentially targeted by these two types of corticostriatal neurons. For example, parvalbumincontaining (PARV+) interneurons (see Chapter 18) receive substantial cortical input and disproportionately little thalamic input in rats and monkeys (Ichinohe et al., 2001; Rudkin and Sadikot, 1999; Sidibe and Smith, 1999). PARV+ interneurons fire repetitively when depolarized by cortical stimulation, with a shorter latency than do striatal projection neurons (Kawaguchi et al., 1995; Mallet et al., 2005). As a consequence, cortical activation of PARV+ neurons prevents or reduces the response to this same cortical activation of the striatal projection neurons to which the PARV+ neurons project (Koos and Tepper, 1999; Mallet et al., 2005; Tepper et al., 2004). The cortical input to PARV+ striatal interneurons ends on their aspiny dendrites as relatively small terminals (Kita, 1993; Kawaguchi et al., 1995; Tepper et al., 2004; Rudkin and Sadikot, 1999). It seems likely, therefore, that PARV+ striatal interneurons receive input from IT-type cortical neurons.

Genes have been identified that are uniquely expressed by either PT-type or IT-type neurons, and mice have been engineered that express green fluorescent protein (EGFP) in one or the other of these neuron types (Gong et al., 2007; Molyneaux et al., 2007). Such mice will be useful for assessing the relative abundances, as well as the targets, of the PT and IT inputs to the entire striatum.

#### **VI. FUNCTIONAL CONSIDERATIONS**

#### A. Motor Control

The finding of differential cortical input to striatal projection neurons may have implications for understanding how the cortical input contributes to the role of the direct and indirect pathway type striatal projection neurons in motor control. In the case of direct pathway striatal neurons, convergence of IT-type input from diverse cortical areas (e.g., providing information on movement planning, body position and the environment) and reward-prediction-related information from dopaminergic midbrain neurons onto individual striato-GPi/SNr neurons (Wilson, 1987; Cowan and Wilson, 1994; Zheng and Wilson, 2001) may provide the coherent activation required to activate individual direct pathway neurons so that they facilitate the movement they control. Because they are inherently less excitable and because their IT-type inputs are relatively ineffective at producing postsynaptic depolarization, more temporally correlated activation may be needed for direct pathway neurons than indirect pathway neurons and suited to the role of direct pathway neurons in motor sequence selection and initiation. Thalamic input related to attentional mechanisms may provide further excitatory drive needed to push the direct pathway neuron activation over the threshold required for motor initiation (Smith et al., 2004). The somewhat slower conduction velocity of the IT-type inputs onto striato-GPi/SNr neurons may also be suited to a role in an integrative process by which direct pathway striatal neurons play a pre-movement role in motor routine selection and facilitation.

Our findings also raise the possibility that striato-GPe neurons use an efference copy of movement commands provided by the PT-type input, which enables their role in suppressing movements that would otherwise conflict with ongoing selected movements. The more rapid conduction velocity of the PT-type axons seems suited to such a role. The possibility of preferential PT-type input to striato-GPe neurons might also explain why movement-related activity exhibited by striatal projection neurons typically occurs during but not before movement (Jaeger et al., 1995; Mink, 1996) - most of the active striatal neurons are indirect pathway type neurons that are responding to collaterals of cortical pyramidal neuron axons, at the same time that the main PT axon produces a movement by activation of brainstem premotor and/or spinal cord motor centers. Nonetheless, the PT signal will reach premotor and motor neurons before the PT-engendered feedback signal reaches motor cortex via the striato-GPe-STN-GPi-motor thalamus loop, and thus be too late to prevent movements conflicting with the by-then already initiated movement. This implies that the movement suppression engendered by the PT signal to striato-GPe neurons may serve to suppress movements that would conflict with the next desired movement in the action sequence. The topographic organization of the PT-type input from somatosensory and somatomotor cortex to dorsolateral striatum may be such as to facilitate this role. Graybiel (2005) has also suggested the possibility that the PT-type input to ENK+ neurons may serve to terminate the specific act in the sequence initiated by SP+ neurons.

# **B.** Motor Learning, Corticostriatal Plasticity and the Differential Cortical Input to Striatum

Motor learning is a key part of the role of the basal ganglia in motor function (Graybiel, 2005). Considerable evidence supports the view that dopamine released from the intrastriatal terminals of substantia nigra both acts as a reward signal that sculpts the activity of striatal neurons during motor learning (Graybiel, 2005; Schultz et al., 2003), and instructs striatal neurons on the likelihood that a given circumstance can lead to reward (and thereby act as an incentive that is part of the go signal) (Ljungberg et al., 1992; Satoh et al., 2003; Morris et al., 2004; Tobler et al., 2005) (see also Chapter 31). The means by which motor learning occurs appears to be, in large part, changes in the efficacy of cortical synapses on striatal projection neurons. The adaptive cortical control of striatal neuron activity, as it relates to the differential cortical input to striatum, is discussed in this section.

Because of the low membrane excitability of striatal projection neurons, only temporally correlated excitatory input from a sufficiently large number of convergent cortical inputs can depolarize neurons to firing threshold (Kawaguchi et al., 1989; Wilson, 1992, 1995; Nisenbaum and Wilson, 1995). Given this corticostriatal physiology, modulation of the synaptic efficacy of the cortical input to striatal projection neurons can mediate the role of the basal ganglia in motor learning. To explore how the efficacy of corticostriatal synapses is modulated, numerous investigators have studied the long-term potentiation (LTP) or longterm depression (LTD) in the efficacy of synaptic inputs to striatal projection neurons (Walsh, 1993; Mahon et al., 2004; Fino et al., 2005; Kreitzer and Malenka, 2008) (see Chapter 12). Given that direct pathway and indirect pathway neurons play opposing roles in motor control, for any given behavior and any given region of striatum they must behave oppositely with respect to corticostriatal plasticity. For example, to facilitate the onset of a specific motor routine, the efficacy of the cortical input to direct pathway neurons controlling that onset must be increased while the efficacy of the cortical input to the indirect pathway neurons suppressing that same routine must be reduced. Similarly, for those movements potentially conflicting with the desired routine, the efficacy of the cortical input to direct pathway neurons controlling the onset of such competing routines must be decreased, while the efficacy of the cortical input to the indirect pathway neurons suppressing those competing routines must be enhanced. Our findings on differential cortical input to striatal projection neurons have implications for differences among striatal projection neuron types in the corticostriatal plasticity that underlies motor learning. The fact that D1-dependent LTP has been demonstrated in direct pathway neurons and D1 receptors are preferentially localized to direct pathway neurons suggests that the rewarding effects of dopamine on behavior are mediated via facilitation of IT-type inputs to direct pathway striatal neurons that control behaviors that obtain that reward (Shen et al., 2008; Kreitzer and Malenka, 2008). In this manner, the coincident activation of the convergent cortical inputs to the direct neurons mediating the rewarded behavior becomes more able to fire those neurons. This phenomenon may explain the emergence of striatal activity in response to a go cue during procedural learning (Ljungberg et al., 1992; Satoh et al., 2003; Morris et al., 2004; Tobler et al., 2005). The striatal activity in this sense reflects a motor go cue (rather than a sensory response) when the combination of exteroceptive and interoceptive circumstances are appropriate. The observation that dopamine depletion converts LTP to LTD in direct pathway neurons is consistent with the notion that absence of a dopaminergic reward signal to the IT-type inputs projecting to those striatal neurons initiating the unrewarded behaviors makes those synapses less effective and less likely to initiate the unrewarded response (Kreitzer and Malenka, 2008).

Understanding the role of plasticity in PT-type synapses on indirect pathway neurons is more problematic, since it is uncertain what role these inputs play in regulating ongoing movement. Dopamine has been reported to act on indirect pathway neurons via their D2 receptors to produce robust LTD (Kreitzer and Malenka, 2007, 2008; Shen et al., 2008), presumably to reduce cortical activation that would otherwise activate indirect pathway neurons to suppress desired movements. Conversely, the absence of a dopamine signal or its pharmacological blockade seems to favor some synaptic potentiation in these neurons, presumably at their PTtype inputs (Kreitzer and Malenka, 2007, 2008; Centonze et al., 2004). If the indirect pathway neurons function to serve as a stop feedback signal to the PT-type neurons that provide them an efference copy of their discharge to motor or premotor neurons, then plasticity at the PT-type synapse could serve to modulate the stop signal. In the case of desirable behaviors, the stop signal might be inappropriate. Reward-mediated depression of the PT-type input would then help adjust the pyramidal neuron control of the movement so its duration is better suited to achieve a rewarded outcome. It may be that the depressed PT-type terminals are among those lacking perforated PSDs, since perforated postsynaptic densities are characteristic of sites of synaptic potentiation (Geinisman, 1993; Sulzer and Pothos, 2000). Conversely, PT-type neuronal activity that brings about unrewarded behaviors would send a corollary discharge to

indirect pathway neurons that becomes strengthened by the absence of dopamine reward, leading to a heightened tendency of the indirect pathway neurons to suppress these same PT-type cortical neurons, and reduce likelihood of the occurrence of the unsuccessful behavior. These potentiated PT-type terminals may include the larger ones with prominent perforated PSDs. Because of the efficacy of this synaptic contact and the high responsivity of indirect pathway neurons to their cortical input, basal firing of the PT input that is subthreshold for movement may be adequate to maintain sufficient indirect pathway neuron output so as to keep pyramidal tract neuron firing below movement threshold. Note that unrewarded behaviors are not necessarily those that are diametrically opposed to the correct behavior. Imperfect execution of a motor sequence can fail to obtain reward as well. In this case then, learning at the PT-type synapse can serve to refine the sequence of movements constituting the procedure, facilitating the correct (by LTD at the PT-type synapse) and suppressing the incorrect (by LTP at the PT-type synapse) movement topography. This suggests that motor learning in basal ganglia may involve learning which movements to suppress and which not to suppress, and the PT-type input to striato-GPe neurons may thus be an important neural substrate by which the basal ganglia learns to refine motor sequences (by learning to suppress extraneous movement) during procedural learning.

Our results on differential cortical input to striatal projection neurons also provide clues regarding the means by which dopamine loss causes the akinesia and bradykinesia in PD. Loss of basal dopamine levels due to PD-related dopaminergic neuron degeneration results in loss of basal dopamine inhibition of indirect pathway neurons and basal dopamine excitation of direct pathway neurons (Kreitzer and Malenka, 2008). As a consequence, the basal ganglia output is abnormal and yields increased movement suppression and decreased movement initiation. The loss of striatal dopamine, however, impairs corticostriatal plasticity as well. LTD at the PT-type inputs to indirect pathway neurons may be diminished, further contributing to their excess inhibition of movement. Similarly, loss of dopamine may impair LTP at IT-type synaptic input to direct pathway neurons. This would impair the corticostriatal facilitation that underlies the learning of new motor routines. Thus, both learning of motor routines and their execution may be impaired in PD, and both are likely to contribute to akinesia and bradykinesia with the loss of dopaminergic neurons (Arbuthnott and Wickens, 2006).

Furthermore, inadequate LTD at PT-type synapses with striato-GPe neurons or exuberant LTP at IT-type synapses on striato-GPi/SNr neurons could underlie the inappropriate motor outputs characteristic of Tourette Syndrome. Similarly, L-DOPA-induced dyskinesia in PD patients is thought to stem from aberrant facilitation of corticostriatal transmission in D1-bearing striatal neurons by the pulsatile therapeutic delivery of L-DOPA (Cenci, 2007) (see Chapter 36), suggesting thus that the defect in L-DOPA-induced dyskinesia involves IT-type terminals on striato-GPi/SNr neurons.

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#### REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alloway KD, Mutic JJ, Hoover JE (1998) Divergent corticostriatal projections from a single cortical column in the somatosensory cortex of rats. Brain Res 785:341–346.
- Arbuthnott GW, Wickens J (2006) Space, time and dopamine. Trends Neurosci 30:62–69.
- Arikuni T, Kubota K (1986) The organization of prefrontocaudate projections and their laminar origin in the macaque monkey: a retrograde study using HRP-gel. J Comp Neurol 244:492–510.
- Ballion B, Mallet N, Bezard E, Lanciego JL, Gonon F (2008) Intratelencephalic corticostriatal neurons equally excite striatonigral and striatopallidal neurons and their discharge activity is selectively reduced in experimental parkinsonism. Eur J Neurosci 27:2313–2321.
- Bauswein E, Fromm C, Preuss A (1989) Corticostriatal cells in comparison with pyramidal tract neurons: contrasting properties in the behaving monkey. Brain Res 493:198–203.
- Beloozerova IN, Sirota MG, Swadlow HA, Orlovsky GN, Popova LB, Deliagina TG (2003) Activity of different classes of neurons of the motor cortex during postural corrections. J Neurosci 23:7844–7853.
- Berretta S, Parthasarathy HB, Graybiel AM (1997) Local release of GABAergic inhibition in the motor cortex induces immediateearly gene expression in indirect pathway neurons of the striatum. J Neurosci 17:4752–4763.
- Brown LL, Smith DM, Goldbloom LM (1998) Organizing principles of cortical integration in the rat neostriatum: Corticostriate map of the body surface is an ordered lattice of curved laminae and radial points. J Comp Neurol 392:468–488.
- Canteras NS, Shammah-Lagnado SJ, Silva BA, Ricarddo JA (1990) Afferent connections of the subthalamic nucleus: a combined retrograde and anterograde horseradish peroxidase study in the rat. Brain Res 513:43–59.
- Cenci MA (2007) Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. Trends Neurosci 30:236–243.

- Centonze D, Usiello A, Costa C, Picconi B, Erbs E, Bernardi G, Borrelli E, Calabresi P (2004) Chronic haloperidol promotes corticostriatal longterm potentiation by targeting dopamine D2L receptors. J Neurosci 24:8214–8222.
- Cepeda C, André VM, Yamazaki I, Wu N, Kleiman-Weiner M, Levine MS (2008) Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. Eur J Neurosci 27:671–682.
- Chung JW, Hassler R, Wagner A (1977) Degeneration of two of nine types of synapses in putamen after centre median coagulation in the cat. Exp Brain Res 28:345–361.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- DeLong M (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Deng YD, Lei WL, Reiner A (2006) Differential localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. J Chem Neuroanat 32:101–116.
- Ding J, Peterson JD, Surmeier DJ (2008) Corticostriatal and thalamostriatal synapses have distinctive properties. J Neurosci 28:6483–6492.
- Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 12:397–403.
- Donoghue JP, Kitai ST (1981) A collateral pathway to the neostriatum from corticofugal neurons of the rat sensory-motor cortex: an intracellular HRP study. J Comp Neurol 210:1–13.
- Fino E, Glowinski J, Venance L (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 25:11279–11287.
- Flaherty AW, Graybiel AM (1993) Two input systems for body representations in the primate striatal matrix: experimental evidence in the squirrel monkey. J Neurosci 13:1120–1137.
- Geinisman Y (1993) Perforated axospinous synapses with multiple, completely partitioned transmission zones: probable structural intermediates in synaptic plasticity. Hippocampus 3:417–433.
- Gerfen CR (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. Nature 311: 461–464.
- Gerfen CR (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science 246:385–388.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 15:133–139.
- Gerfen CR, Wilson CJ (1996) The basal ganglia. In: Handbook of Chemical Anatomy. Integrated system of the CNS. Part III (Swanson LW, Bjorklund A, Hokfelt T, Eds), pp. 360–466. Amsterdam: Elsevier.
- Gertler TS, Chan CS, Surmeier DJ (2008) Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci 28:10814–10824.
- Goldman-Rakic P, Selemon LD (1986). Topography of corticostriatal projections in nonhuman primates and implications for functional parcellation of the neostriatum. In Cerebral Cortex vol. 5, (Jones EG, Peters A, Eds.), pp. 447–466: Plenum Press. New York.
- Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR (2007) Targeting cre-recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci 2:9817–9823.
- Graybiel AM (2005) The basal ganglia: learning new tricks and loving it. Curr Opin Neurobiol 15:638–644.

- Hedreen JC (1977) Corticostriatal cells identified by the peroxidase method. Neurosci Lett 4:1–7.
- Hedreen JC, McGrath S (1977) Observations on labeling of neuronal cell bodies, axons and terminals after injection of horseradish peroxidase into rat brain. J Comp Neurol 176:225–246.
- Hoffer ZS, Alloway KD (2001) Organization of corticostriatal projections from the vibrissal representations in the primary motor and somatosensory cortical areas of rodents. J Comp Neurol 439:87–103.
- Ichinohe N, Iwatsuki H, Shoumura K (2001) Intrastriatal targets of projection fibers from the central lateral nucleus of the rat thalamus. Neurosci Lett 302:105–108.
- Jaeger DJ, Gilman S, Aldridge JW (1995) Neuronal activity in the striatum and pallidum of primates related to the execution of externally cued reaching movements. Brain Res 694:111–127.
- Jinnai K, Matsuda Y (1979) Neurons of the motor cortex projecting commonly on the caudate nucleus and the lower brainstem in the cat. Neurosci Lett 13:121–126.
- Jones EG, Wise SP (1977) Size, laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. J Comp Neurol 175:391–438.
- Jones EG, Coulter JD, Burton H, Porter R (1977) Cells of origin and terminal distribution of corticostriatal fibers arising in the sensory-motor cortex of monkeys. J Comp Neurol 173:53–80.
- Kawaguchi Y, Wilson CJ, Emson PC (1989) Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. J Neurophysiol 62:1052–1068.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 10:3421–3438.
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurons: chemical, physiological and morphological characterization. Trends Neurosci 18:527–535.
- Kemp JM, Powell TPS (1970) The cortico-striate projection in the monkey. Brain 93:525–546.
- Kincaid AE, Wilson CJ (1996) Corticostriatal innervation of the patch and matrix in the rat neostriatum. J Comp Neurol 374:578–592.
- Kita H (1993) GABAergic circuits of the striatum. In: Chemical Signalling in the Basal Ganglia. (Arbuthnott GW, Emson PC, eds) Prog Brain Res 99:51-72.
- Kitai ST, Kocsis JD, Wood J (1976) Origin and characteristics of the cortico-caudate afferents: an anatomical and electrophysiological study. Brain Res 118:137–141.
- Koos T, Tepper JM (1999) Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nature Neurosci 2:467–472.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543–554.
- Landry P, Wilson CJ, Kitai ST (1984) Morphological and electrophysiological characteristics of pyramidal tract neurons in the rat. Exp Brain Res 57:177–190.
- Lei WL, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.
- Levesque M, Parent A (1998) Axonal arborization of corticostriatal and corticothalamic fibers arising from prelimbic cortex in the rat. Cerebral Cortex 8:602–613.

- Levesque M, Charara A, Gagnon S, Parent A, Descenes M (1996a) Corticostriatal projections from layer V cells in rat are collaterals of long-range corticofugal axons. Brain Res 709:311–315.
- Levesque M, Gagnon S, Parent A, Descenes M (1996b) Axonal arborizations of corticostriatal and corticothalamic fibers arising from the second somatosensory area in the rat. Cerebral Cortex 6:759–770.
- Ljungberg T, Apicella P, Schultz W (1992) Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol 67:145–163.
- Mahon S, Deniau JM, Charpier S (2004) Corticostriatal plasticity: life after the depression. Trends Neurosci 27:460–467.
- Mallet N, Le Moine C, Charpier S, Gonon F (2005) Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum *in vivo*. J Neurosci 25:3857–3869.
- Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of Parkinsonian rats. J Neurosci 26:3875–3884.
- McGeorge AJ, Faull RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- Mink JW (1996) The basal ganglia: Focussed selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.
- Molnar Z, Cheung AFP (2006) Towards the classification of subpopulations of layer V pyramidal projection neurons. Neurosci Res 55:105–115.
- Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD (2007) Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci 8:427–437.
- Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H (2004) Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron 43:133–143.
- Nisenbaum ES, Wilson CJ (1995) Potassium current responsible for inward and outward rectification in rat neostriatal spiny projection neurons. J Neurosci 15:4449–4463.
- Oka H (1980) Organization of the cortico-caudate projections: a horseradish peroxidase study in the cat. Exp Brain Res 40:203–208.
- Parent M, Parent A (2006) Single-axon tracing study of corticostriatal projections arising from primary motor cortex in primates. J Comp Neurol 496:202–213.
- Parthasarathy HB, Graybiel AM (1997) Cortically driven immediate early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. J Neurosci 17:2477–2491.
- Ramon y Cajal S (1911) Histologie du Systeme Nerveux de l'Homme et des Vertebres. Paris: Maloine.
- Reiner A, Jiao Y, Del Mar N, Laverghetta AV, Lei WL (2003) Differential morphology of pyramidal-tract type and intratelencephalicallyprojecting type corticostriatal neurons and their intrastriatal terminals in rats. J Comp Neurol 457:420–440.
- Royce GJ (1982) Laminar origin of cortical neurons which project upon the caudate nucleus: a horseradish peroxidase investigation in the cat. J Comp Neurol 205:8–29.
- Rudkin TM, Sadikot AF (1999) Thalamic input to parvalbuminimmunoreactive GABAergic interneurons: organization in normal striatum and effect of neonatal decortication. Neuroscience 88: 1165–1175.
- Satoh T, Nakai S, Sato T, Kimura M (2003) Correlated coding of motivation and outcome of decision by dopamine neurons. J Neurosci 23:9913–9923.

- Schultz W, Tremblay L, Hollerman JR (2003) Changes in behavior-related neuronal activity in the striatum during learning. Trends Neurosci 26:321–328.
- Schwab M, Agid Y, Glowinski L, Thoenen H (1977) Retrograde axonal transport of I-tetanus toxin as a tool for tracing fiber connections in the central nervous system: connections of the rostral part of the rat neostriatum. Brain Res 126:211–244.
- Selemon LD, Goldman-Rakic PS (1985) Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. J Neurosci 5:776–794.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotamous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Sidibe M, Smith Y (1999) Thalamic inputs to striatal interneurons in monkeys: synaptic organization and co-localization of calcium binding proteins. Neuroscience 89:1189–1208.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Sulzer D, Pothos EN (2000) Regulation of quantal size by presynaptic mechanisms. Rev Neurosci 11:159–212.
- Tanaka D, Gorska T, Dutkiewicz K (1981) Corticostriate projections from the primary motor cortex in the dog. Brain Res 209:287–303.
- Tanaka D (1987) Differential laminar distribution of corticostriatal neurons in the prefrontal and pericruciate gyri of the dog. J Neurosci 7:4019–4095.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307:1642–1645.
- Turner RS, DeLong MR (2000) Corticostriatal activity in primary motor cortex of the macaque. J Neurosci 20:7096–7108.
- Uhl GR, Navia B, Douglas J (1988) Differential expression of preproenkephalin and preprodynorphin mRNAs in striatal neurons: high levels of preproenkephalin expression depend on cerebral cortical afferents. J Neurosci 8:4755–4764.
- Veening JG, Cornelissen FM, Lieven PAJM (1980) The topical organization of the afferent to the caudatoputamen of the rat. A horseradish peroxidase study. Neuroscience 5:5253–5268.
- Walsh JP (1993) Depression of excitatory synaptic input in rat striatal neurons. Brain Res 608:123–128.

- Wang H, Pickel VM (1998) Dendritic spines containing mu-opioid receptors in rat striatal patches receive asymmetric synapses from prefrontal corticostriatal afferents. J Comp Neurol 396:223–237.
- Wang HB, Laverghetta AV, Foehring R, Deng YP, Sun Z, Yamamoto K, Lei WL, Jiao Y, Reiner A (2006) Single-cell RT-PCR, in situ hybridization histochemical, and immunohistochemical studies of substance P and enkephalin co-occurrence in striatal projection neurons in rats. J Chem Neuroanat 31:178–199.
- Wilson CJ (1986) Postsynaptic potentials evoked in spiny neostriatal projection neurons by stimulation of ipsilateral and contralateral neocortex. Brain Res 367:201–213.
- Wilson CJ (1987) Morphology and synaptic connections of crossed corticostriatal neurons in the rat. J Comp Neurol 263:567–580.
- Wilson CJ (1992) Dendritic morphology, inward rectification and the functional properties of neostriatal neurons. In: Single Neuron Computation (McKenna T, David J, Zornetzer SF, eds), pp. 141–171. St Louis, MO: Academic Press.
- Wilson CJ (1995) The contribution of cortical neurons to the firing pattern of striatal spiny neurons. In: Models of Information Processing in the Basal Ganglia (Houk JC, David JL, Beiser DG, eds), pp. 29–50. Cambridge: MIT Press.
- Wilson CJ, Chang HT, Kitai ST (1982) Origins of postsynaptic potentials evoked in identified neostriatal neurons by stimulation in substantia nigra. Exp Brain Res 45:157–167.
- Wise SP, Jones EG (1977) Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. J Comp Neurol 175:129–158.
- Wright AK, Norrie L, Ingham CA, Hutton AM, Arbuthnott GW (1999) Double anterograde tracing of the outputs from adjacent "barrel columns" of rat somatosensory cortex neostriatal projection patterns and terminal ultrastructure. Neuroscience 88:119–133.
- Wright AK, Ramanthan S, Arbuthnott GW (2001) Identification of the source of the bilateral projection from cortex to somatosensory neostriatum and an exploration of its physiological actions. Neuroscience 103:87–96.
- Wu Y, Richard S, Parent A (2000) The organization of the striatal output system: a single-cell juxtacellular labeling study in the rat. Neurosci Res 38:49–62.
- Zheng T, Wilson CJ (2001) Corticostriatal combinatorics: The implications of corticostriatal axonal arborizations. J Neurophysiol 87:1007–1017.

# Gating of Cortical Input to the Striatum

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#### I. INTRODUCTION

The corticostriatal projection is both a major output of the cerebral cortex and the major input to the basal ganglia. In this chapter we consider this projection from two perspectives. First, the anatomy of the corticostriatal projection limits the possible connectivity of individual spiny neurons with its cortical inputs. Second, we discuss the dopaminer-gic regulation of synaptic plasticity. Progress in these areas over the past decade has led to important new insights into the possible operation of the striatum.

After outlining the macroscopic organization of the projection we review studies of the axonal arborization of individual corticostriatal neurons. Two distinct types of projection neurons with distinctive axonal arborization probably represent functionally distinct systems (Cowan and Wilson, 1994). More generally, quantitative neuro-anatomical studies of the arborization of single neurons led to new insights based on the statistics of connections (Levesque et al., 1996a; Kincaid et al., 1998; Wright et al., 1999; Reiner et al., 2003). These emphasize the sparse connectivity and selectivity of the input to individual cells. The anatomical connectivity has important implications

for the functional significance of synaptic plasticity in this pathway. We review studies of dopamine-dependent synaptic plasticity in the corticostriatal pathway and consider how this might operate within the sparse connectivity scheme derived above. Finally, we consider what our current understanding tells us about corticostriatal interplay in ongoing behavior.

Most of the work cited in this review comes from the dorsal striatum. Segregation of the striatum into distinct dorsal and ventral zones has been proposed on functional grounds, although there is no sharp line of demarcation between these regions anatomically. Here we include in the striatum not only the dorsal region, that encompasses the caudate nucleus and putamen, but also the ventral region that includes the core and shell of the nucleus accumbens (Heimer, 2003). Subtle differences in cell morphology and intrinsic connectivity exist. For example, differences in the degree of spinyness of projection neurons in different accumbal areas have been reported (Meredith et al., 1992; O'Donnell and Grace, 1993), although the differences are quantitative rather than qualitative (Chang and Kitai, 1985; Meredith, 1999). The convergence of cortical inputs with dopamine inputs in dorsal striatum (Smith et al., 1994) - which is crucial for the

reinforcement learning mechanism – is similar in the ventral striatum, where dopamine inputs to spiny neurons converge with hippocampal inputs (Totterdell and Smith, 1989), often on the same dendritic spine (Sesack and Pickel, 1990). There are similar patterns for prefrontal (Sesack and Pickel, 1992) and amygdalar (Johnson et al., 1994) inputs. However, it should be noted that the generalisation of conclusions based on dorsal striatum to the ventral striatum relies on argument by extrapolation, because the same kind of data is not available for the ventral striatum.

# II. ANATOMY OF CORTICOSTRIATAL INPUT PATHWAYS

The corticostriatal projection is a major output of the cortex and provides at least half of the excitatory input to the striatum (see Chapter 1). It is widespread in origin, powerfully excitatory, and spatially ordered. The neocortical afferents form most of their contacts with spines on striatal spiny projection neurons, where they make excitatory synapses (Wilson and Groves, 1980; Somogyi et al., 1981). Each spiny projection neuron receives around 5000 synapses of cortical origin (Kincaid et al., 1998). However, firing the spiny projection neuron requires a significant excitatory synaptic input to overcome the braking effect of potassium currents activated by depolarization. Understanding the interplay of corticostriatal connectivity and the spiny neuron excitability is likely to provide important insights into corticostriatal interplay.

#### **III. CORTICOSTRIATAL MAPPING**

The organization of the projection requires description on different scales, ranging from larger cortical regions, to smaller somatotopically defined areas, to the projections of individual corticostriatal cells. Within these levels we can ask how the neighboring regions, areas, or cells in the cortex are rearranged in the striatum.

The neocortical afferents to the neostriatum originate from all major regions of both sides of the cortex (McGeorge and Faull, 1989). A precise, mathematical formulation of the transformation of the cortical map to the three-dimensional volume of the striatum has not yet been achieved, either empirically or conceptually. Existing data indicate a complex representation of the cortex within the striatal volume (see also Chapters 18 and 20). It is not, as once thought, a smooth somatotopic representation of the body like that proposed for the sensorimotor cortex, nor is it like the fractured somatotopy of the cerebellum.

Individual locations in the cortex give rise to multiple separate foci of innervation in the striatum. An area of cortex representing a single body part sends fibres that diverge to innervate multiple regions in the striatum. For example, in the primate somatosensory cortical regions representing hand, mouth and foot send globally somatotopic, nonoverlapping projections to the putamen, but regions with closely related representations (thumb and 5th finger in area 3b) send convergent, overlapping corticostriatal projections (Flaherty and Graybiel, 1991). Conversely, projections from different somatosensory areas, representing different sensory modalities for the same bodily parts, send projections that converge on same striatal regions. Projections from functionally related cortical regions (e.g. finger area of primary motor and somatosensory cortex) converge on common focal innervation zones. In the cat, Malach and Graybiel (1988) have described a similar scheme. In the rat, extensive and patchy arrangements of metabolic activity indicating corticostriatal activity occurred in response to sensory stimulation, suggesting integrative, combinational networks (Brown and Sharp, 1995). Thus, information from different parts of the body is kept separate (in a somatotopic framework), while there is convergence of information concerning different modalities, but the same bodily parts.

### **IV. CORTICAL CELLS OF ORIGIN**

Two types of cortical pyramidal neurons project to the striatum (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996b; Levesque and Parent, 1998; Wright et al., 1999; Wright et al., 2001; Reiner et al., 2003) (see also Chapter 18). Firstly, corticocortical neurons that project bilaterally to the striatum but not outside the telencephalon have been called intratelencephalically projecting (IT) type neurons. Secondly, long-range corticofugal neurons that project ipsilaterally to the brainstem or spinal cord, from which a striatal projection arises as a collateral from the descending axon, are called pyramidal tract neurons (PT). It is currently thought that there are no exclusively corticostriatal cells, but that the cortical afferents are collaterals of other projections (i.e., IT and PT neurons).

The cell bodies of the PT type of neuron are located in upper layer V and layer III (Reiner et al., 2003). Intracortical axon collaterals from these cells ramify in layers V and VI,



**FIGURE 19.1** Illustration of the combinatorial principle, showing location of activation related to body region at three anteroposterior levels in the striatum. At different levels, hindlimb, trunk and forelimb were juxtaposed in different combinations. Ovals represent 400 µm diameter zones of potential interaction among spiny projection neurons. Small circles within ovals indicate contralateral activation; rectangles indicate ipsilateral activation. Arrow pairs show examples of different juxtapositions of forelimb points with other points. F, forelimb; H, hindlimb; T, trunk. From Brown (1992), with permission of the author.

and also project to the upper layers of cortex near the apical dendrite tufts (Donoghue and Kitai, 1981). This system is the one responsible for the topographic arrangement of terminals from individual rows of barrels in S1 cortex in rodents (Alloway et al., 1999; Wright et al., 1999; Alloway et al., 2000; Wright et al., 2000; Wright et al., 2000; Wright et al., 2000; Wright et al., 2001). The other corticostriatal system arises from between the barrels and projects widely to both striata. The IT system of Reiner (Reiner et al., 2003) is also visible in the detailed tracing from S1 (Alloway et al., 1999; Wright et al., 1999; Alloway et al., 2000; Wright et al., 2000; Wrigh

## V. TERMINAL DISTRIBUTION OF CORTICOSTRIATAL AXONS

Studies of the axonal arborization of individual corticostriatal cells have shown two distinguishable patterns of arborization, which have been termed "extended" and "focal" (Kincaid et al., 1998). These have been described in detailed axonal tracing from motor (medial agranular or anterior cingulate) cortex (Zheng and Wilson, 2002) and sensory (S1) cortex (Wright et al., 1999; Wright et al., 2001). Extended axonal arborizations innervate very large regions of the neostriatum. In the extreme case a single axon of this type occupied as much as 14% of the total volume of the striatum. This is the upper extreme of the distribution which on average occupies 4% (Zheng and Wilson, 2002). The focal type of arborization makes more focal and discontinuous arborizations that occupy small striatal volumes but innervate them very sparsely (Kincaid et al., 1998).

Two distinct types of corticostriatal pathways also arise from the barrel cortex, analagous to those described above (Wright et al., 1999; Wright et al., 2001). There is a contralateral nontopographic projection with a diffuse arborization that resembles the extended projection. This arises from neurons located under the septa between barrel columns. There is also a topographically ordered projection with a more focal arborization that arises from the cells under the barrels, via corticofugal axons descending through the striatum and with branches projecting to the ipsilateral striatum.

The activity of corticostriatal neurons has been studied in awake, behaving primates (Bauswein et al., 1989; Turner and DeLong, 2000). In these studies the IT neurons differ from PT neurons in several ways. In contrast to the PT neurons, the IT neurons have slowly-conducting axons, and low spontaneous activity. Their firing in relation to movement is small in magnitude but strongly directional, and not related to load effects. Although they were initially described as discharging later in relation to active movement (Bauswein et al., 1989), a subsequent study has found perimovement activity in most IT neurons that began before movement onset (Turner and DeLong, 2000). The activity was more selective than that of PT cells in that the sensory responses and perimovement activities were often directionally specific, and IT cells were often activated exclusively by one aspect of the movement and a substantial fraction of IT cells was unresponsive to any task or manipulation. The IT cells activity is suggestive of a sparse code which transmits very specific aspects of sensory stimuli and motor intentions to striatum (Bauswein et al., 1989; Turner and DeLong, 2000).

Individual spiny projection neurons (see Chapter 5) receive input from both IT and PT systems. It may be that the two systems are differentially distributed between the D1 receptor- and D2 receptor-containing neurons in striatum (Lei et al., 2004). However, there is some evidence that IT neurons provide the main excitatory input to both striatal populations and the corticostriatal PT input is weaker (Ballion et al., 2008).

# VI. SIGNIFICANCE OF CORTICOSTRIATAL STATISTICS

In earlier models of the striatum, spiny striatal neurons were envisaged to form an array of pattern detectors, acting like the "Perceptrons" proposed in early artificial intelligence studies (Rosenblatt, 1962; Minsky and Papert, 1969). The key elements of this type of pattern detector include a threshold and a set of weighted inputs. Any input pattern can be stored for later recognition by strengthening the inputs that are active when the pattern is presented.

The concept of the striatum as an array of pattern detectors assumes that a striatal spiny neuron fires when excited by a certain combination of cortical inputs. The corticostriatal synapses are a template for recognition of a matching set of corticostriatal neurons. These neurons may be activated as part of a cortical cell assembly (Braitenberg, 1978) or synfire chain (Abeles, 1991; Abeles et al., 1993; Prut et al., 1998), which represents a particular situation, action, or combination of situation and action. By acting as detectors of particular cortical cell assemblies, striatal cells can encode specific patterns of cortical activity corresponding to situations and actions.

Whether a striatal cell fires in response to a given pattern of cortical activity is determined by the matrix (in the mathematical sense) of connections between the cortex and striatum. This matrix is the product of anatomical connectivity established during growth and development, and plasticity of established connections. For the effective operation of such a pattern recognition device, it is typically assumed that the inputs to a network of cells diverge and converge so that each cell can learn to recognise any arbitrary pattern of activity (Fig. 19.2A).

In reality, however, there are physical constraints on the connectivity of spiny neurons. One of the important advances in the last decade has been the determination of several key facts from which the possible connectivity can be estimated. These facts include the density of cells in the striatum (Oorschot, 1996), the density of synapses (Ingham et al., 1997; Ingham et al., 1998), and the frequency of synaptic contacts along a corticostriatal axon (Kincaid et al., 1998). These led unavoidably to the conclusion that the connectivity scheme of Fig. 19.2A is unrealistic, as will now be reviewed.

The cells of origin of the corticostriatal projection are small- to medium-sized pyramidal cells, evenly spread with gaps up to several hundred microns between individual cells (Jones et al., 1977). Early studies (DiFiglia et al.,



**FIGURE 19.2** Topology of the corticostriatal connection at the cellular level. A. Hypothetical connectivity assuming overlap in striatal input to allow arbitrary input-output mappings. B. A more realistic connectivity highlighting the limited access of cortical cells to striatal output neurons, prohibiting arbitrary input-output mappings.

1978) described axons in Golgi preparations that were of probable cortical origin as being thin, with side twigs running longitudinally throughout the striatum. Single axons of this type traversed distances of up to 900  $\mu$ m with 10–15 terminal bulbs per 100  $\mu$ m of axon length. These earlier observations have been confirmed in reconstructions of the arborizations of single neurons, which show that the interval between synaptic boutons along a corticostriatal axon has an approximately Poisson distribution with a mean of about 10  $\mu$ m (Kincaid et al., 1998).

The statistics of corticostriatal connections were reviewed by Wilson (2000) who argued as follows. Assuming a spiny neuron's dendritic tree occupies a spherical space, radius 200 µm, the maximum number of corticostriatal synapses formed by a single corticostriatal axon traversing the volume is 40 (Kincaid et al., 1998). A total of 15,250,000 corticostriatal synapses exist in such a volume, based on a density of 0.91 asymmetrical synapses per cubic µm (Ingham et al., 1997; Ingham et al., 1998) and assuming 50% of these are corticostriatal. Dividing the total number of synapses by the number formed by a single axon implies in such a volume on the order of 380,000 axons participate. In the same volume there are a total number of 2845 striatal neurons each of which receives about 5360 synapses. From these numbers, assuming that n = 5000 connections are formed at random between the N = 380,000 cortical cells and a given spiny neuron, the probability of a given degree of overlap in input with a second spiny neuron can be calculated from the binomial distribution. These methods were used to calculate the probability of varied degrees of overlap in the corticostriatal inputs to adjacent neurons. The cumulative



**FIGURE 19.3** Probability of a given number of inputs in common. A. Assuming 380,000 corticostriatal afferents, each making 40 synapses in the volume of one spiny neuron, and a total of 5000 corticostriatal synapses per cell. Using the cumulative binomial distribution, the probability of randomly obtaining more than 100 inputs in common is close to zero. B. The cumulative probability distribution of number of inputs to one cell given the activation of 100 inputs to another cell in the same volume of tissue. There is negligible probability of 10 active inputs to the second cell from the 100 inputs activating the first cell. Recalculated from Wilson (2000).

probability distribution is reproduced in Figure 19.3. Note that the probability of a given degree of overlap decreases very steeply around 75.

The probability distribution has the implication that of the 5000 or so cortical inputs received by a given spiny neuron, in general no more than 100 of these are shared with any other spiny neuron. This suggests that if the firing threshold was made above 100 inputs, then any arbitrary selection of 100 inputs to a given cell could fire the cell in question, but no other cell, since the probability of even as few as 10 inputs to another cell is exceedingly small (Fig. 19.3B). In this threshold range – or any higher value - the spiny neuron could function as a virtually noise-free detector of its sufficient stimulus, in that the activation of 100 inputs that fired one spiny neuron would be far below threshold for any other. This allows for exceptional selectivity purely on a combinatorial basis, without need for lateral inhibition, permitting the cortex to address a striatal cell uniquely (Wilson, 2000).

The small amount of overlap in the input to different spiny neurons means that each cell is unique and shares remarkably little of its input with its neighbors (Kincaid et al., 1998; Zheng and Wilson, 2002). This is consistent with electrophysiological studies that show although the striatal neurons responding to movement of a given body part are located near to each other (Alexander and DeLong, 1985a,b), adjacent neurons in such a location can have very different properties (Alexander and Crutcher, 1990; Crutcher and Alexander, 1990), and little correlation in action potential firing is seen in simultaneously recorded pairs of striatal neurons (Jaeger et al., 1995), although subthreshold activity may be well correlated (Stern et al., 1997; Stern et al., 1998).

The foregoing argument shows that the model depicted in Figure 19.2A is not realistic, and must be rejected in favor of the alternative model shown in Figure 19.2B. This is a very important result that has profound implications for the way we think about how the striatum works. We will call the model of Figure 19.2B the combinatorial selection model, because the effective combination of inputs is highly selective for a particular cell. The advantage of such selectively, however, is bought at the price of reduced flexibility in output, and broad tuning within the effective input set.

#### A. Lack of Output Flexibility

A drawback of the combinatorial selection principle is that there is no possibility of changing the output neuron by changing the strengths of existing inputs, since by the argument given above the cortical input set of one spiny neuron does not have access to any other spiny neuron. This would only be possible if there were some way to move synapses from one cell to another. Changes in strength of existing synapses logically will not permit learning of new outputs. The input combinations have access to only one spiny neuron. If it represented the wrong choice of action in a situation, there is little that can be done. In other words, arbitrary stimulus-response associations cannot be learned if the output code of the striatum is a mapping from spiny neurons onto responses. For this system to be flexible, we must reinterpret the output cell activity as a code, so that the mapping is based on a group of cells, i.e., that the output can be changed by activating different partners.

### B. Broad Tuning

A second drawback is that the tuning of an individual spiny neuron within its set of 5000 inputs is inherently broad if the threshold is much lower than 5000, because there is an astronomical number of combinations in which a suprathreshold number of the 5000 inputs to the cell might be activated. The selectivity within the set of inputs is poor and needs to be combined with some form of competition. Synaptic plasticity to strengthen selected synapses and weaken others provides a potential mechanism for sharpening the tuning.

# VII. SYNAPTIC PLASTICITY IN THE CORTICOSTRIATAL PATHWAY

Activity-dependent synaptic plasticity is a long-lasting change in the functional efficacy of synaptic connections that is induced by certain patterns of brain stimulation. The associative properties and persistence of certain forms of plasticity have led to its widespread use as an experimental model for learning and memory mechanisms of the brain (Bliss and Collingridge, 1993). In view of the role of the basal ganglia in learning, several authors have speculated on possible synaptic plasticity mechanisms in the corticostriatal pathway (Beninger, 1983; Groves, 1983; Miller, 1988; Wickens, 1990; Wickens and Kotter, 1995).

Plasticity of the corticostriatal pathway is evident in extracellular recordings from neostriatal neurons in awake, behaving animals, in the form of long-lasting changes in activity patterns related to the acquisition and performance of learnt behavior. New responses to task-related stimuli are acquired during learning (Kawagoe et al., 1998; Shimo and Hikosaka, 2001; Lauwereyns et al., 2002; Takikawa et al., 2002) and such acquired responses persist as long as performance is maintained (Aosaki et al., 1994).

Dendritic spines, in many parts of the brain, are associated with synaptic plasticity. The corticostriatal synapse is, moreover, in close spatial association with dopaminergic afferents from the midbrain (see also Chapter 6). Dopaminergic synapses commonly occur on the necks of spines that also receive an asymmetrical synapse on the head (Freund et al., 1984; Groves et al., 1994). This anatomical arrangement provides a potential site of interaction of a specific glutamatergic system with dopamine release triggered by positive reinforcement during learning of behavioral actions (Schultz et al., 1993; Mirenowicz and Schultz, 1996). Although the association of a dopaminergic synapse and an excitatory corticostriatal synapse on the same dendritic spine occurs at only a minority of corticostriatal synapses, volume transmission of dopamine from nearby synapses is a major aspect of dopaminergic signalling in the striatum (Rice, 2000; Venton et al., 2003; Wickens and Arbuthnott, 2005; Arbuthnott and Wickens, 2007; Moss and Bolam, 2008).

Dopaminergic modulation of activity-dependent plasticity in the corticostriatal pathway has recently been reviewed (Reynolds and Wickens, 2002; Mahon et al., 2004; Calabresi et al., 2007; Wickens, 2008). A conjunction of presynaptic and postsynaptic activity, which in the hippocampus and cerebral cortex would normally lead to long-term potentiation (LTP), typically leads to long-term depression (LTD) in the striatum (Walsh, 1991; Calabresi et al., 1992b; Calabresi et al., 1992c; Calabresi et al., 1992a; Calabresi et al., 1993; Lovinger et al., 1993; Walsh, 1993; Walsh and Dunia, 1993; Calabresi et al., 1994; Kombian and Malenka, 1994) (see also Chapters 9 and 12). It is a depolarization-dependent process that requires activation of voltage-sensitive calcium channels and an increase in intracellular  $Ca^{2+}$  in the postsynaptic cell (Calabresi et al., 1992c; Calabresi et al., 1994). Action potential firing is not necessary for LTD, but cells must be depolarized near to threshold (Calabresi et al., 1992c).

In view of the evidence linking dopamine neuron firing to reinforcement learning, a number of authors have proposed that dopamine might govern plasticity at corticostriatal synapses (Miller, 1981; Miller and Wickens, 1989; Wickens and Kotter, 1995). In brain slice experiments, we tested the effects of pulsatile application of dopamine timed to coincide with a conjunction of presynaptic cortical and postsynaptic striatal activity. We found that highfrequency stimulation that would normally induce LTD led to LTP when paired with pulsatile application of dopamine (Wickens et al., 1996; Wickens, 2000).

Dopamine-dependent LTP appears to be mediated by D1 dopamine-like receptors since it is blocked by selective D1 receptor antagonists (Kerr and Wickens, 2001). Using a spike-timing dependent plasticity induction protocol, Pawlak and Kerr (2008) also showed that blocking D1-like receptors prevented both LTD and LTP induction. In contrast, blocking D2 receptors delayed, but did not prevent, LTD and sped induction of LTP. Thus, the dopaminergic reinforcement signal is potentially able to interact with timing of postsynaptic action potentials relative to cortical inputs to produce dopamine-dependent plasticity.

Recent use of transgenic mice in which the expression of D1 or D2 cells was marked by expression of green fluorescent protein has allowed definitive identification of spiny neuron subtypes (Surmeier et al., 2007; Shen et al., 2008). Different requirements for induction of plasticity have been found in different subtypes (Shen et al., 2008) (see also Chapter 6). A complex picture is developing that involves interaction of dopamine, adenosine and endocannabinoid receptors in determining the direction of synaptic plasticity in the corticostriatal pathway.

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Studies of synaptic plasticity at the cellular level in brain slices may seem far removed from behavioral reinforcement. Using in vivo intracellular recording, we found that HFS of the cerebral cortex induces LTD of the corticostriatal pathway, as in slices. When stimulation of the substantia nigra pars compacta with 20Hz trains is paired with cortical HFS, a short-lasting potentiation is induced (Reynolds and Wickens, 2000). This short-lasting potentiation is blocked by dopamine depletion. Thus, the phasic activation of dopaminergic afferents induced potentiation in vivo, although this was less enduring than the effect of pulsatile application of dopamine seen in vitro (Wickens et al., 1996).

Experiments using extracellular single unit recordings of nucleus accumbens neurons in combination with chronoamperometric measures of dopamine efflux lead to a similar conclusion. Potentiation of hippocampal-evoked response is induced in nucleus accumbens cells by HFS of the fimbria. This potentiation was blocked by SCH23390 or an NMDA antagonist (Floresco et al., 2001), and is associated with a transient increase in dopamine concentration in the nucleus accumbens. Thus, as in the dorsal striatum, a transient increase in dopamine concentration which is time-locked to the HFS-induced depolarisation of nucleus accumbens neurons, is sufficient to facilitate subsequent hippocampalevoked activity (see Fig. 19.4A). The subsequent release of dopamine after induction of this facilitation does not appear to play a role (Floresco et al., 2001).

To address the possible role of synaptic plasticity in reward-related learning Reynolds et al. (2001) investigated whether behaviorally rewarding stimulation might also modulate synaptic plasticity. Intracranial self-stimulation (ICSS) was used as a model for reward-related learning, in which rats learn to press a lever repeatedly to electrically stimulate their own dopamine neurons in the substantia nigra. Using the same animals in which ICSS responding had been measured, we later made in vivo intracellular recordings from striatal neurons, and measured responses to cortical afferents before and after ICSS-like stimulation of the substantia nigra dopamine neurons (see Fig. 19.4B). Stimulation of the substantia nigra with behaviorallyreinforcing parameters induced potentiation of corticostriatal synapses. In addition, the degree of potentiation up to 10 minutes after the stimulus trains was correlated with the



**FIGURE 19.4** Synaptic plasticity in vivo. A. Potentiation of hippocampal-evoked response is induced in accumbens cells by HFS of the fimbria. This potentiation was blocked by SCH23390. Reproduced from Floresco et al. (2001). B. In the dorsal striatum, when electrical stimulation that was effective in inducing ICSS behavior was paired with spontaneous cortical activity, it produced dopamine D1 receptor-dependent LTP. Reproduced from Reynolds et al. (2001).

rate of learning of ICSS. Animals showing a greater degree of potentiation were correspondingly faster to reach criteria for ICSS, and vice versa. Potentiation was blocked in control animals administered a D1-like receptor antagonist (Reynolds et al., 2001). These findings suggest that stimulation of the substantia nigra may positively reinforce behavior by D1 receptor-dependent potentiation of cortical inputs to the striatum. Importantly, they show an association between the rate of learning and the degree of synaptic change induced by the rewarding electrical stimulation.

In summary dopamine-dependent synaptic plasticity is a potential cellular mechanism for reward-related learning in the striatum. Dopamine pulses produced by pressureejection or substantia nigra stimulation may mimic the effects of natural reward. The correlation of degree of synaptic change with rate of learning with ICSS is highly suggestive of a relationship between reward-related learning and dopamine-dependent synaptic plasticity in the striatum.

#### VIII. SYNTHESIS AND CONCLUSIONS

Synaptic plasticity operating on the corticostriatal projection can strengthen connections that are active in association with dopamine release, and weaken connections that are active when there is no dopamine release. Previously, it has been suggested that such strengthening and weakening of connections between the cortex and striatum might be involved in the learning of stimulus-response associations. However, the anatomical evidence reviewed above shows that arbitrary stimulus-response mappings cannot be learnt, because a given pattern of cortical activity has only very limited access to output neurons.

On the other hand, the combinatorial selection scheme implied by the anatomy does allow exceptionally specific responses to the cortical input patterns to be acquired. The activity of a cortical cell assembly may be detected by one, or a number of, striatal cells, and this detection can be accomplished with great accuracy. Dopamine-dependent synaptic plasticity may reinforce the detection of cortical assemblies associated with positive outcomes. Such assemblies may represent situations, actions, or situations plus actions. The convergence of different modalities particularly favors the detection of assemblies with both motor and sensory components, which might represent a combination of stimuli and responses, or more abstractly, actions, intentions, and strategies.

Emerging from this evidence is a view of the striatum as an evaluator of the cortical state, computing a value function based on reinforcement history. The output from the striatum would then be interpreted as a valuation of the current cortical state, formed from the sum of activity across many spiny neurons, representing the strengthened synapses associated with that state. Feedback of this valuation to the cortex would serve to amplify cortical states that had been reinforced in previous experience. The convergence in the striato-pallidal and striatonigral projection (on the order of 30:1 in the rat, and 100:1 in the human) is consistent with this view. Since the outputs are combined into low-dimensional output representing the value of the cortical state, the inability to make arbitrary corticostriatal mappings is not a problem: the stimulus-response mappings are in the cortex.

In conclusion, consideration of the anatomy and plasticity of the corticostriatal pathway suggests the spiny cell network may compute a value function with which to evaluate cortical propositions, and select the ones associated with reinforcement. Figuratively, one may now think of the striatum in the role of a navigator, looking up at the cortical cell activity as one might look at the stars in the night sky, and charting a course towards distant rewards.

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#### REFERENCES

- Abeles M (1991) Corticonics. Neural circuits in the cerebral cortex. Cambridge: Cambridge University Press.
- Abeles M, Bergman H, Margalit E, Vaadia E (1993) Spatiotemporal firing patterns in the frontal cortex of behaving monkeys. J Neurophysiol 70:1629–1638.
- Alexander GE, DeLong MR (1985a) Microstimulation of the primate neostriatum: II Somatotopic organization of striatal microexcitable zones and their relation to neuronal response properties. J Neurophysiol 53:1417–1430.
- Alexander GE, DeLong MR (1985b) Microstimulation of the primate neostriatum: I Physiological properties of striatal microexcitable zones. J Neurophysiol 53:1401–1416.
- Alexander GE, Crutcher MD (1990) Preparation for movement: Neural representation of intended direction in three motor areas of the monkey. J Neurophysiol 64:133–150.
- Alloway KD, Crist J, Mutic JJ, Roy SA (1999) Corticostriatal projections from rat barrel cortex have an anisotropic organization that correlates with vibrissal whisking behavior. J Neurosci 19:10908–10922.
- Alloway KD, Mutic JJ, Hoffer ZS, Hoover JE (2000) Overlapping corticostriatal projections from the rodent vibrissal representations in primary and secondary somatosensory cortex. J Comp Neurol 426:51–67.
- Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M (1994) Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. J Neurosci 14:3969–3984.
- Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30:62–69.
- Ballion B, Mallet N, Bezard E, Lanciego JL, Gonon F (2008) Intratelencephalic corticostriatal neurons equally excite striatonigral and striatopallidal neurons and their discharge activity is selectively reduced in experimental parkinsonism. Eur J Neurosci 27:2313–2321.
- Bauswein E, Fromm C, Preuss A (1989) Corticostriatal cells in comparison with pyramidal tract neurons: contrasting properties in the behaving monkey. Brain Res 493:198–203.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. Brain Res 287:173–196.

- Bliss TVP, Collingridge GL (1993) A synaptic model of memory: Longterm potentiation in the hippocampus. Nature 361:31–39.
- Braitenberg V (1978) Cell assemblies in the cerebral cortex. In: Theoretical Approaches to Complex Systems (Heim R, Palm G, eds), pp. 171–188. Berlin: Springer.
- Brown L (1992) Somatotopic organization in rat striatum: Evidence for a combinatorial map. Proc Natl Acad Sci USA 89:7403–7407.
- Brown LL, Sharp FR (1995) Metabolic mapping of rat striatum: somatotopic organization of sensorimotor activity. Brain Res 686:207–222.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1992a) Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. Eur J Neurosci 4:929–935.
- Calabresi P, Maj R, Mercuri NB, Bernardi G (1992b) Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. Neurosci Lett 142:95–99.
- Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992c) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:4224–4233.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1993) Lithium treatment blocks long-term synaptic depression in the striatum. Neuron 10:955–962.
- Calabresi P, Pisani A, Mercuri NB, Bernardi G (1994) Post-receptor mechanisms underlying striatal long-term depression. J Neurosci 14:4871–4881.
- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopaminemediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Chang HT, Kitai ST (1985) Projection neurons of the nucleus accumbens: an intracellular labeling study. Brain Res 347:112–116.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- Crutcher MD, Alexander GE (1990) Movement-related neuronal activity selectively coding either direction or muscle pattern in three motor areas of the monkey. J Neurophysiol 64:151–163.
- DiFiglia M, Pasik T, Pasik P (1978) A Golgi study of afferent fibres in the neostriatum of monkeys. Brain Res 152:341–347.
- Donoghue JP, Kitai ST (1981) A collateral pathway to the neostriatum from corticofugal neurons of the rat sensory-motor cortex: An intracellular HRP study. J Comp Neurol 210:1–13.
- Flaherty AW, Graybiel AM (1991) Corticostriatal transformations in the primate somatosensory system. Projections from physiologically mapped body-part representations. J Neurophysiol 66:1249–1263.
- Floresco SB, Blaha CD, Yang CR, Phillips AG (2001) Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. J Neurosci 21:2851–2860.
- Freund TF, Powell JF, Smith AD (1984) Tyrosine hydroxylaseimmunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 13:1189–1215.
- Groves PM (1983) A theory of the functional organisation of the neostriatum and the neostriatal control of voluntary movement. Brain Res Rev 5:109–132.
- Groves PM, Linder JC, Young SJ (1994) 5-hydroxydopamine-labeled dopaminergic axons: three-dimensional reconstructions of axons,

synapses and postsynaptic targets in rat neostriatum. Neuroscience 58:593-604.

- Heimer L (2003) A new anatomical framework for neuropsychiatric disorders and drug abuse. Am J Psychiatry 160:1726–1739.
- Ingham CA, Hood SH, Taggart P, Arbuthnott GW (1998) Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18:4732–4743.
- Ingham CA, Hood SH, Mijnster MJ, Baldock RA, Arbuthnott GW (1997) Plasticity of striatopallidal terminals following unilateral lesion of the dopaminergic nigrostriatal pathway: a morphological study. Exp Brain Res 116:39–49.
- Jaeger D, Gilman S, Aldridge JW (1995) Neuronal activity in the striatum and pallidum of primates related to the execution of externally cued reaching movements. Brain Res 694:111–127.
- Johnson LR, Aylward RL, Hussain Z, Totterdell S (1994) Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. Neuroscience 61:851–865.
- Jones EG, Coulter JD, Burton H, Porter R (1977) Cells of origin and terminal distribution of corticostriatal fibres arising in the sensory-motor cortex of monkeys. J Comp Neurol 173:53–80.
- Kawagoe R, Takikawa Y, Hikosaka O (1998) Expectation of reward modulates cognitive signals in the basal ganglia. Nat Neurosci 1:411–416.
- Kerr JN, Wickens JR (2001) Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J Neurophysiol 85:117–124.
- Kincaid AE, Zheng T, Wilson CJ (1998) Connectivity and convergence of single corticostriatal axons. J Neurosci 18:4722–4731.
- Kombian SB, Malenka RC (1994) Simultaneous LTP of non-NMDA- and LTD of NMDA-receptor-mediated responses in the nucleus accumbens. Nature 368:242–246.
- Koos T, Tepper JM, Wilson CJ (2004) Comparison of IPSCs evoked by spiny and fast-spiking neurons in the neostriatum. J Neurosci 24:7916–7922.
- Lauwereyns J, Watanabe K, Coe B, Hikosaka O (2002) A neural correlate of response bias in monkey caudate nucleus. Nature 418:413–417.
- Lei W, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.
- Levesque M, Parent A (1998) Axonal arborization of corticostriatal and corticothalamic fibers arising from prelimbic cortex in the rat. Cereb Cortex 8:602–613.
- Levesque M, Gagnon S, Parent A, Deschenes (1996a) Axonal arborizations of corticostriatal and corticothalamic fibers arising from the second somatosensory area in the rat. Cereb Cortex 6:759–770.
- Levesque M, Charara A, Gagnon S, Parent A, Deschenes M (1996b) Corticostriatal projections from layer V cells in rat are collaterals of long-range corticofugal axons. Brain Res 709:311–315.
- Lovinger DM, Tyler EC, Marritt A (1993) Short- and long-term depression in the rat neostriatum. J Neurophysiol 70:1937–1949.
- Mahon S, Deniau JM, Charpier S (2004) Corticostriatal plasticity: life after the depression. Trends Neurosci 27:460–467.
- Malach R, Graybiel AM (1988) Mosaic architecture of the somatic sensory-recipient sector of the cat's striatum. J Neurosci 6:3436–3458.
- McGeorge AJ, Faull RL (1989) The organisation of the projections from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- Meredith GE (1999) The synaptic framework for chemical signaling in nucleus accumbens. Ann N Y Acad Sci 877:140–156.

- Miller R (1981) Meaning and Purpose in the Intact Brain. Oxford: Oxford University Press.
- Miller R (1988) Cortico-striatal and cortico-limbic circuits: A two tiered model of learning and memory function. In: Information Processing by the Brain: Views and Hypotheses from a Cognitive-Physiological Perspective (Markowitsch H, ed), pp. 179–198. Bern: Hans Huber Press.
- Miller R, Wickens JR (1989) Reward as fulfillment of motor intentions: a unifying concept for the function of the mammalian striatum. Int J Neurosci 46:23–24.
- Minsky M, Papert S (1969) Perceptrons. Cambridge, MA: MIT Press.
- Mirenowicz J, Schultz W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379:449–451.
- Moss J, Bolam JP (2008) A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. J Neurosci 28:11221–11230.
- O'Donnell P, Grace AA (1993) Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. Synapse 13:135–160.
- Oorschot DE (1996) Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: A stereological study using the Cavalieri and optical dissector methods. J Comp Neurol 366:580–599.
- Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 28:2435–2446.
- Prut Y, Vaadia E, Bergman H, Haalman I, Slovin H, Abeles M (1998) Spatiotemporal structure of cortical activity: properties and behavioral relevance. J Neurophysiol 79:2857–2874.
- Reiner A, Jiao Y, Del Mar N, Laverghetta AV, Lei WL (2003) Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. J Comp Neurol 457:420–440.
- Reynolds JN, Wickens JR (2002) Dopamine-dependent plasticity of corticostriatal synapses. Neural Netw 15:507–521.
- Reynolds JNJ, Wickens JR (2000) Substantia nigra dopamine regulates synaptic plasticity and membrane potential fluctuations in the rat neostriatum, in vivo. Neuroscience 99:199–203.
- Reynolds JNJ, Hyland BI, Wickens JR (2001) A cellular mechanism of reward-related learning. Nature 413:67–70.
- Rice ME (2000) Distinct regional differences in dopamine-mediated volume transmission. Prog Brain Res 125:277–290.
- Rosenblatt F (1962) Principles of Neurodynamics: Perceptrons and the Theory of Brain Mechanism. New York: Spartan.
- Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci 13:900–913.
- Sesack SR, Pickel VM (1990) In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. Brain Res 527:266–279.
- Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 320:145–160.

- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Shimo Y, Hikosaka O (2001) Role of tonically active neurons in primate caudate in reward-oriented saccadic eye movement. J Neurosci 21:7804–7814.
- Smith Y, Bennett BD, Bolam JP, Parent A, Sadikot AF (1994) Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. J Comp Neurol 344:1–19.
- Somogyi JP, Bolam JP, Smith AD (1981) Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscope study using the Golgi-peroxidase transport degeneration procedure. J Comp Neurol 195:567–584.
- Stern EA, Kincaid AE, Wilson CJ (1997) Spontaneous subthreshold membrane potential fluctuations and action potential variability of rat corticostriatal and striatal neurons in vivo. J Neurophysiol 77:1697–1715.
- Stern EA, Jaeger D, Wilson CJ (1998) Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo. Nature 394:475–478.
- Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30:228–235.
- Takikawa Y, Kawagoe R, Hikosaka O (2002) Reward-dependent spatial selectivity of anticipatory activity in monkey caudate neurons. J Neurophysiol 87:508–515.
- Taverna S, van Dongen YC, Groenewegen HJ, Pennartz CM (2004) Direct physiological evidence for synaptic connectivity between mediumsized spiny neurons in rat nucleus accumbens in situ. J Neurophysiol 91:1111–1121.
- Totterdell S, Smith AD (1989) Convergence of hippocampal and dopaminergic input onto identified neurons in the nucleus accumbens of the rat. J Chem Neuroanat 2:285–298.
- Tunstall MJ, Oorschot DE, Kean A, Wickens JR (2002) Inhibitory interactions between spiny projection neurons in the rat striatum. J Neurophysiol 88:1263–1269.
- Turner RS, DeLong MR (2000) Corticostriatal activity in primary motor cortex of the macaque. J Neurosci 20:7096–7108.
- Venance L, Glowinski J, Giaume C (2004) Electrical and chemical transmission between striatal GABAergic output neurones in rat brain slices. J Physiol 559:215–230.
- Venton BJ, Zhang H, Garris PA, Phillips PE, Sulzer D, Wightman RM (2003) Real-time decoding of dopamine concentration changes in the caudateputamen during tonic and phasic firing. J Neurochem 87:1284–1295.
- Walsh JP (1991) Long-term potentiation (LTP) of the excitatory synaptic input to medium spiny neurons of the rat striatum. Society for Neuroscience Abstracts 17:852.
- Walsh JP (1993) Depression of excitatory synaptic input in rat striatal neurons. Brain Res 608:123–128.
- Walsh JP, Dunia R (1993) Synaptic activation of N-methyl-D-aspartate receptors induces short-term potentiation at excitatory synapses in the striatum of the rat. Neuroscience 57:241–248.
- Wickens JR (1990) Striatal dopamine in motor activation and rewardmediated learning. Steps towards a unifying model. J Neural Transmission 80:9–31.
- Wickens JR (2000) Dopamine regulation of synaptic plasticity in the neostriatum: a cellular model of reinforcement. In: Brain Dynamics and the Striatal Complex (Miller R, Wickens JR, eds), pp. 65–76: Harwood Academic.

- Wickens JR (2008) Synaptic plasticity in the basal ganglia. Behav Brain Res 199:119–128.
- Wickens JR, Kotter R (1995) Cellular models of reinforcement. In: Models of Information Processing in the Basal Ganglia (Houk JC, Davis JL, Beiser DG, eds), pp. 187–214. Massachusetts: MIT Press.
- Wickens JR, Arbuthnott G (2005) Structural and functional interactions in the striatum at the receptor level. In: Handbook of Chemical Neuroanatomy (Dunnett SB, Bentivoglio M, Björklund A, Hökfelt I, eds), Vol 21. Dopamine. Elsevier, Amsterdam.
- Wickens JR, Begg AJ, Arbuthnott GW (1996) Dopamine reverses the depression of rat cortico-striatal synapses which normally follows high frequency stimulation of cortex in vitro. Neuroscience 70:1–5.
- Wilson CJ (1987) Morphology and synaptic connections of crossed corticostriatal neurons in the rat. J Comp Neurol 263:567–580.
- Wilson CJ (2000) Striatal circuitry: Categorically selective, or selectively categorical? In: Brain dynamics and the striatal complex (Miller R, Wickens J, eds), pp 289–305.

- Wilson CJ, Groves PM (1980) Fine structure and synaptic connection of the common spiny neuron of the rat neostriatum: A study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615.
- Wright AK, Norrie L, Arbuthnott GW (2000) Corticofugal axons from adjacent 'barrel' columns of rat somatosensory cortex: cortical and thalamic terminal patterns. J Anat 196(Pt 3):379–390.
- Wright AK, Ramanathan S, Arbuthnott GW (2001) Identification of the source of the bilateral projection system from cortex to somatosensory neostriatum and an exploration of its physiological actions. Neuroscience 103:87–96.
- Wright AK, Norrie L, Ingham CA, Hutton EAM, Arbuthnott GW (1999) Double anterograde tracing of outputs from adjacent "barrel columns" of rat somatosensory cortex. Neostriatal projection patterns and terminal ultrastructure. Neuroscience 88:119–133.
- Zheng T, Wilson CJ (2002) Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 18:4722–4731.

# Organization of Prefrontal-Striatal Connections

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Introduction: Prefrontal Cortex-IV. Relationships of the Prefrontal-VI. Relationships of the Prefrontal-I. Striatal Projections with the Striatal Topography with Other **Basal Ganglia Circuits II. Prefrontal Cortex and Striatum Compartmental Structure of the Striatal Inputs III.** Topographical Organization Striatum A. Triadic Relationships of of Prefrontal-Striatal A. Striatal Compartments: the Thalamic and Limbic Projections Patch-Matrix and Shell-Core Projections with the A. Medial Prefrontal and B. Prefrontal Cortical Prefrontal-Striatal System Agranular Insular Projections Lamination and Striatal VII. Medium-Sized Spiny Projection to the Striatum Compartments **Neurons: Integrators of Striatal** B. Orbital-Prefrontal Projections V. Cortico-Cortical and Inputs to the Striatum **Corticostriatal Relationships** References

# I. INTRODUCTION: PREFRONTAL CORTEX-BASAL GANGLIA CIRCUITS

The cerebral cortex and the basal ganglia maintain intricate anatomical and functional relationships. The basal ganglia, composed of the striatum, pallidum, subthalamic nucleus and substantia nigra, receive inputs from virtually all parts of the cerebral cortex and these subcortical structures project via the thalamus back to extensive parts of the cortical hemisphere, in particular the frontal lobe (see Chapter 1). The main, although not exclusive, target of cortical projections within the complex of basal ganglia structures is the striatum which consequently can be considered the input structure of the basal ganglia (Alexander et al., 1986, 1990; Parent and Hazrati, 1995; Gerfen and Wilson, 1996; Wise et al., 1996) (for more details on corticostriatal projections, see Chapters 18 and 19). The input character of the striatum is strengthened by the fact that significant projections are derived from limbic structures, like the amygdala and hippocampus, as well as from the midline, intralaminar and ventral thalamic nuclei (Groenewegen et al., 1987, 1990, 1996). Further inputs to the striatum originate in brainstem nuclei, that is, dopaminergic inputs from the substantia nigra pars compacta and serotonergic inputs from the mesencephalic raphe nuclei. The inputs from the cortical and subcortical structures are in general topographically ordered, thus, forming the anatomical basis for various functional (i.e., sensorimotor, associative and limbic) striatal domains (Parent and Hazrati, 1995). In particular, the topographical organization of the corticostriatal projections provides the basis for this functional subdivision of the striatum, globally shown in Figure 20.1. Within the functionally distinct domains of the striatum, there is extensive convergence of inputs from functionally related cortical, limbic and thalamic inputs at both the regional and cellular striatal level. In that respect, the striatum forms, par excellence, the site of convergence and integration of different streams of cortical and subcortical inputs.



**FIGURE 20.1** Schematic representation of the topographical organization of the projections from functionally different cortical areas to the striatum. Note that the functional subdivision of the striatum, related to the corticostriatal topography, does not follow the boundaries between caudate nucleus and putamen: there exists a ventromedial-to-dorsolateral gradient rather than a functional division between the caudate nucleus and the putamen. Note also that the boundaries between the different functional areas are not sharply defined but merely consist of transition zones. Abbreviations: Acb, nucleus accumbens; Caud, caudate nucleus; Put, putamen.

These information streams, in turn, are modulated by the dopaminergic and serotonergic brainstem projections. This integrated information is guided via striatal efferent projections within the intrinsic circuitry of the basal ganglia to their output structures, that is, the internal segment of the globus pallidus, the reticular part of the substantia nigra and the ventral pallidum, to both the medial and ventral thalamus and regions in the brainstem, such as the superior colliculus and specific parts of the reticular formation. Finally, the thalamic nuclei targeted by the basal ganglia, are in reciprocal connection with the premotor and prefrontal cortical areas. Thus, the basal ganglia exert their influence on those parts of the cortical hemisphere that are involved in the preparation of movements and the organization of cognitive processes and complex, flexible behaviors, as well as via brainstem projections on more basic, innate motor and behavioral aspects (Wise et al., 1996; Mink, 1996; Redgrave et al., 1999). The organization of the projections from the cerebral cortex, limbic and thalamic structures through the striatum and other nuclei of the basal ganglia back to both the cerebral cortex as well as to the brainstem, is characterized by parallel as well as integrative aspects (Redgrave et al. 1999; Haber, 2003; Haber et al., 2000; McFarland and Haber, 2002). Both aspects are important for our understanding of the role of the interactions between the cerebral cortex and the basal ganglia in the organization of sensorimotor, cognitive and emotional-motivational and behavioral processes. The intricate functional-anatomical relationships between the prefrontal cortex and striatum become apparent when considering the results of neurophysiological and behavioral studies. Likewise, the results of human brain imaging support the strong interactions between the frontal cortex and the basal ganglia and their functional-anatomical unity (e.g., Everitt and Robbins, 2005; Chudasama and Robbins, 2006; Remijnse et al., 2006; Seger, 2008).

In this chapter, we will review the organization of the prefrontal corticostriatal projections and the relationship with other cortical and subcortical striatal inputs, with emphasis on rodents. The distinction of different functional striatal domains will be contrasted with the integrative aspects of both the intrinsic and extrinsic striatal connections.

#### **II. PREFRONTAL CORTEX AND STRIATUM**

There is a longstanding appreciation of the close functional-anatomical relationships between the frontal cortex and the basal ganglia, in particular the striatum. Lesions of parts of the frontal or prefrontal cortex have been demonstrated to lead to similar behavioral or cognitive deficits as lesions of their connectionally-related striatal parts. Such early conclusions, based on incidental findings in humans, and experimentally substantiated in both primates and rodents, are nowadays generally accepted. Based on an extensive recent body of literature, including functional-anatomical marker studies in experimental animals, behavioral lesion- and pharmacological experiments, and functional human brain imaging, it has become clear that the (pre)frontal-striatal system can be subdivided into multiple subsystems, each concerned with a different aspect of the broad range of functions in which the frontal corticalstriatal system is involved, that is, sensorimotor, cognitive and emotional-motivational behavior (e.g., Masterman and Cummings, 1997; Cardinal et al., 2002; Zgaljardic et al., 2003; Chudasama and Robbins, 2006; Voorn et al., 2004; Robbins, 2007; Dalley et al., 2008). The structural basis for the involvement in diverse functions, at least in part,

lies in the topographical organization of the cortico-striatal projections, their mutual segregation and convergence, and the interrelationships with other striatal inputs from thalamus, limbic structures and brainstem. Below, we will first provide a description of the organization of the prefrontalstriatal projections, with emphasis on the situation in rats. Thereafter, we will discuss the patterns of convergence and segregation of prefrontal-striatal projections with inputs from other brain areas within the functionally different striatal sectors.

## III. TOPOGRAPHICAL ORGANIZATION OF PREFRONTAL-STRIATAL PROJECTIONS

The prefrontal cortex of rodents can be divided into medial, lateral and orbital parts, each consisting of different subareas (Uylings and Van Eden, 1990; Uylings et al., 2003; Van de Werd and Uylings, 2008). If we consider cortical areas with the relative heaviest interconnections with the mediodorsal thalamic nucleus as "prefrontal", the following frontal areas in the medial wall of the hemisphere of the rat can be considered prefrontal: the infralimbic, prelimbic, anterior cingulate and Fr2 (medial agranular) areas (Groenewegen, 1988; Van Eden et al., 1990). Areas in the lateral part of the frontal lobe considered to belong to the prefrontal cortex are the dorsal and ventral agranular insular areas. Orbital areas that have their densest interconnections with the mediodorsal thalamic nucleus include the medial and ventral orbital areas, as well as the lateral orbital area. The ventrolateral orbital area, although having intensive connections with the mediodorsal thalamic nucleus, is most strongly interconnected with the thalamic nucleus submedius while the dorsolateral orbital cortex has the ventromedial nucleus as main thalamic target (review: Groenewegen and Witter, 2004).

The striatal projections from the orbital areas in the rat have long been relatively ignored. In papers describing the corticostriatal projections in rats, most emphasis has been placed on the medial prefrontal areas, followed by the above-mentioned projections from the agranular insular areas (Beckstead, 1979; Berendse et al., 1992; Reep et al., 2003). In fact, the relative lack of attention for the orbital areas, not only in rodents, has also been the case for the functional and dysfunctional aspects of this part of the frontal lobe (Murray et al., 2007). In the last decade there has been a strong growth in the number of studies of the orbital prefrontal cortex and related parts of the striatum, including both rodent and primate research. In this context,

it is important that the projections of the orbital areas to the striatum have been restudied in both primates (Haber et al., 1995; Haber, 2003) and rats (Schilman et al., 2008) with modern neuroanatomical tracer methods.

Before describing in global terms the topographical organization of the prefrontal-striatal projections, it is important to note that all prefrontal cortical areas project to a primary striatal target area and, in addition, have more or less extensive, but less dense projection areas that overlap with the primary striatal target areas of other, mostly closely adjacent and interconnected cortical areas (e.g., Reep et al., 2003; Calzavara et al., 2007; Haber et al., 2007).

# A. Medial Prefrontal and Agranular Insular Projections to the Striatum

The general organizational pattern in the medial prefrontal-striatal projections consists of a ventromedial-todorsolateral topography (Berendse et al., 1992; Voorn et al., 2004). As argued by Voorn et al. (2004), this orientation in the prefrontal-striatal system adds an extra dimension to the generally accepted functional organization of the striatum in a dorsal, sensorimotor-related and a ventral, associational- and limbic-related striatum. Thus, as illustrated in Figure 20.2C and D, the most ventromedial areas like the infralimbic cortex project to the most ventral and medial parts of the striatum, including the medial shell of the nucleus accumbens and the medially and ventrally adjacent parts of the olfactory tubercle, whereas gradually more dorsally located areas in the medial wall of the hemisphere project to successively more lateral and dorsal striatal areas. As can be appreciated from this figure, the main projection area of a particular medial prefrontal cortical area is represented as a "slab-like" region oriented in general from dorsal to ventral-ventrolateral in the rostral half of the striatum. In the rostral half of the striatum, the ventrally located infra- and prelimbic areas occupy the most extensive striatal area. At more caudal striatal levels, the dorsomedially located anterior cingulate and FR2 areas occupy a larger striatal territory (Fig. 20.2D), but the general ventromedial-to-dorsolateral topography is maintained. As indicated above, the striatal projections of these medial prefrontal cortical areas may overlap rather extensively with primary projection area of adjacent cortical areas.

The striatal projections from the laterally located agranular insular areas are fairly complementary to those of the medial prefrontal cortex. The ventral agranular insular area projects to the lateral part of the shell of the



FIGURE 20.2 Schematic drawing summarizing the topographical arrangement of the cortico-striatal projections originating in the orbital prefrontal cortex (A, C, D) and the medial and lateral prefrontal cortices (A, B, E, F). The prefrontal cortical areas and their connectionally related striatal targets are coded in the same color. Since there is a considerable overlap between the orbital projections on the one hand (C, D) and the medial and lateral prefrontal projections on the other hand (E, F), these projections are represented in two different sets of a rostral and a caudal striatal level. As shown in E and F, the dorsolateral striatum receives somatotopically organized inputs from the sensorimotor cortices (light blue), the most ventromedial part of the striatum collects inputs from the infralimbic and ventral prelimbic areas (red and purple). Striatal areas intermediate between these extremes receive projections from the dorsal prelimbic, anterior cingulate and Fr2 areas. The ventral agranular insular area projects to the lateral shell and adjacent olfactory tubercle while the dorsal agranular insular area sends fibers to the core (E) and a broad mediolateral zone in the ventral caudate-putamen more caudally (F). Although the global relationships between the projection areas from different medial and lateral prefrontal cortices are maintained from rostral to caudal, the relative space occupied by the projections from various cortical areas changes from rostral to caudal (compare E and F). The orbital prefrontal projection areas in the striatum show a medial-to-lateral topographical organization (C, D). The medial and ventral orbital areas overlap considerably in the medial part of the striatum while the lateral and dorsolateral orbital areas overlap quite extensively in the lateral part of the striatum (stippled lines). In an intermediate position, in the central part of the caudate-putamen, lies the projection area of the ventrolateral orbital area. To show the overlap of the ventrolateral orbital projection with the projections of the medial and lateral prefrontal areas, the ventrolateral projection area is represented in E and F with a stippled line. The ventrolateral and lateral orbital areas both project quite strongly to the most lateral part of the shell of the nucleus accumbens. In C and E, shell and core are delineated with stippled lines (black in C and white in E). Abbreviations: ac, anterior commissure; ACd, dorsal anterior cingulate cortex; AId, dorsal agranular insular cortex; AIv, ventral agranular insular cortex; DLO, dorsolateral orbital cortex; Fr2, frontal area 2; IL, infralimbic cortex; LO, lateral orbital cortex; MO, medial orbital cortex; PFC, prefrontal cortex; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; VLO, ventrolateral orbital cortex; VO, ventral orbital cortex. (see Color Plate Section to view the color version of this figure)

nucleus accumbens and its projection area extends into the lateral part of the olfactory tubercle. In the shell, the ventral agranular insular projections are rather complementary to those of the infralimbic cortex, although there is overlap in the intermediate shell (Berendse et al., 1992). The dorsal agranular insular area projects more dorsally and medially, and primarily targets the core of the nucleus accumbens, extending into a broad mediolaterally oriented ventral part of the caudate-putamen at more caudal levels (Fig. 20.2D). Overlap exists with the dorsal-medial

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prefrontal areas, in particular the dorsal prelimbic and anterior cingulate areas (Berendse et al., 1992). It should be noted that ventral prelimbic and dorsal agranular insular areas maintain relatively strong reciprocal cortico-cortical connections (Gabbott et al., 2003; Vertes, 2004; Hoover and Vertes, 2007).

# **B.** Orbital-Prefrontal Projections to the Striatum

The projections from the orbital areas to the striatum in the rat show a strong overlap with those from the medial prefrontal areas, while, in contrast, the orbital-striatal projections are largely complementary with those from the dorsal and ventral agranular insular areas. The dorsolateral and lateral orbital areas may be considered as the rostral continuations of the dorsal and ventral agranular insular areas, respectively. Whereas the dorsal and ventral agranular insular areas have the core and shell of the nucleus accumbens as their main striatal target, the orbital areas in the rat largely avoid the nucleus accumbens and project in a medial-to-lateral topographical fashion to the central parts of the caudate-putamen complex, together occupying the entire width of the striatum (Berendse et al., 1992; Schilman et al., 2008). Exceptions are small but distinct projections to the most lateral part of the accumbens shell that stem from the ventrolateral and lateral orbital areas. Projections from ventral orbital area extend into the dorsal part of the core of the nucleus accumbens (Fig. 20.2A) (Schilman et al., 2008). In addition, medially located orbital areas tend to project more rostrally in the striatum than the more lateral areas. However, terminal fields from the different orbitofrontal areas show a considerable overlap.

In more detail, the medial orbital area has a relatively small striatal projection area in the most medial part of the caudate-putamen complex. This projection area lies completely within the main striatal target area of the prelimbic cortex and also strongly overlaps with the projections from the ventral orbital area (Fig. 20.2A,B). The ventral orbital area has a main striatal projection area that extends more lateral, dorsal and ventral than the medial orbital projection area. Some ventral orbital fibers extend into the dorsal core of the nucleus accumbens (Schilman et al., 2008). The projections from the ventrolateral orbital area are strongest and occupy a "central" position in the caudateputamen. Fibers from the ventrolateral orbital area do not extend into the nucleus accumbens core and also leave the dorsal aspects of the caudate-putamen free of projections. As shown in Figure 20.2C and D, there is some overlap of ventrolateral orbital projections with the main striatal target area of the dorsal prelimbic and dorsal agranular insular areas and an extensive overlap with the main projection areas of the anterior cingulate and FR2 prefrontal areas (Fig. 20.2C,D). The lateral and dorsolateral orbital areas project even further laterally in the caudate-putamen and together "fill" the ventrolateral part of the caudate-putamen that is not projected upon by the ventrolateral orbital area. There is extensive overlap between the main striatal targets of the lateral and ventrolateral orbital areas, but the dorsolateral orbital area projects more laterally and ventrally than the lateral orbital area (Fig. 20.2A,B).

# IV. RELATIONSHIPS OF THE PREFRONTAL-STRIATAL PROJECTIONS WITH THE COMPARTMENTAL STRUCTURE OF THE STRIATUM

# A. Striatal Compartments: Patch-Matrix and Shell-Core

The above description of the organization of the prefrontal cortical-striatal projections emphasizes the global topographical arrangement of these projections. However, the striatum is not a homogeneous structure and at the regional striatal level a further differentiation in the organization of the prefrontal cortical-striatal projections exists. Classically, the striatum can be subdivided in a dorsal and a ventral striatum. A border between these two parts is far from sharp and has been variably defined depending upon the criteria used. As argued by Voorn et al. (2004), a distinction between dorsal and ventral striatum may not be very useful in functional terms and a ventromedialto-dorsolateral gradient based on the topographical organization of the prefrontal cortical input (see Fig. 20.2C,D) may be functionally much more relevant. Likewise, on the basis of similar considerations, a distinction between nucleus accumbens and caudate-putamen complex appears to be less relevant since borders between these two macrostructures are hard to define, and afferent and efferent connections do not obey such presumed borders (Voorn et al., 2004). Nevertheless, at the regional striatal level different tissue compartments exist that have a specific neurochemical composition and distinct afferent and efferent fiber connections, that is, the patch-matrix compartments in



**FIGURE 20.3** Photomicrographs of a transverse section of the rat striatum to illustrate the compartmental structure. In this section,  $\mu$ -opioid receptors (A; MOR) and calbindin D<sub>28K</sub> (B; CaB) were visualized using specific primary antibodies against MOR and CaB, and secondary antibodies coupled to different fluorophores. Within their specific projection area the deep layers of the medial and lateral prefrontal cortical areas terminate in patches while the more superficial layers of these cortical areas terminate in the matrix (see also text). Courtesy of Dr. Floris Wouterlood. (see Color Plate Section to view the color version of this figure)

the dorsal parts of the striatum and the shell-core subdivision in the nucleus accumbens (Fig. 20.3). The subdivision of striatal tissue into patch-matrix (rats) or striosome-matrix compartments (primates, cats) is originally based upon the differential neuro- and immunochemical staining characteristics of these compartments (Graybiel and Ragsdale, 1978; Graybiel, 1990; Gerfen 1992). Likewise, the subdivision of the nucleus accumbens into a shell and core subregion is primarily based upon differential neuro- and immunochemical characteristics of the medial and ventral part of the nucleus (shell) versus its central part (core) (e.g., Záborszky et al., 1985; Zahm and Brog, 1992; Groenewegen et al., 1999). The patch-matrix structure is not restricted to the caudate-putamen complex, but also extends into the dorsal part of the core of the nucleus accumbens, illustrating the difficulty in defining sharp boundaries between different striatal regions.

As argued above, to a large degree based on the organization of the prefrontal cortical-striatal projections, a ventromedial-to-dorsolateral orientation may be taken as the functional-anatomical organizational principle within the striatum. Along this orientation axis, in the extreme ventromedial part of the striatum the shell of the nucleus accumbens is encountered. Strikingly, there is no marker that fully distinguishes the shell from the core of the nucleus accumbens. Most frequently the absence of immunoreactivity for the calcium-binding protein calbindin  $D_{28K}$  is taken as a marker for the shell, contrasting with the relatively strong staining of the core and adjacent caudate-putamen. Within the shell itself various different compartments can be recognized that are unique as compared to the striatal patch-matrix compartments. Moving up in dorsolateral direction, the core of the nucleus accumbens appears; in the most medial part of the core patches are not yet present. Moving further in dorsolateral direction, the dorsal core and ventral caudate-putamen express the classical patchmatrix distinction (Fig. 20.3). Sections immunostained for calbindin D<sub>28K</sub> show a relatively darkly stained matrix in which immunonegative patches can be recognized. Patch and matrix have different characteristics for a number of other neurotransmitter or neurochemical substances and/or for neurotransmitter receptors. A prominent example is the strong expression of µ-opioid receptors in patches contrasting with its low expression in the matrix. In the dorsolateral sector of the striatum, patch-matrix compartments are still present but their characteristics are different from those located more ventrally and medially. In this part of the striatum the expression of calbindin D<sub>28K</sub> is low in both patch and matrix. However, µ-opioid receptor positive patches can still be recognized here (Fig. 20.3A).

The conclusion based on these data must be that throughout the striatum compartmental heterogeneities exist in the composition of the striatal tissue and that these compartments exhibit different characteristics along a ventromedial-to-dorsolateral axis within the striatum.

# **B.** Prefrontal Cortical Lamination and Striatal Compartments

Medial and lateral prefrontal cortical areas in rats have a clear relationship with the compartmental structure of the striatum. Thus, the deeper laminae of layer V project predominantly to the striatal patches while the more superficial laminae of layer V and layers II and III send

their fibers primarily to the striatal matrix (Gerfen, 1989; Berendse et al., 1992). In particular the prelimbic and dorsal agranular insular areas show this particular pattern and, consequently, these cortical laminar and striatal compartmental relationships hold in principle for the striatal areas to which these prefrontal cortices project (Fig. 20.2C,D). The medially located infralimbic area, together with the ventral part of the prelimbic area, projects to the medial shell of the nucleus accumbens, distributing their fibers over different compartments within the shell (review: Groenewegen et al., 1996). Likewise, the ventral agranular insular area projects to only the lateral part of the shell of the nucleus accumbens. Prefrontal cortical areas in the dorsomedial wall of the hemisphere, like the anterior cingulate and Fr2 areas, gradually express a less distinct laminarcompartmental organization as apparent for the prelimbic and dorsal agranular insular areas. In our recent study on the organisation of the orbital-striatal projections, we were not able to distinguish apparent relationships between the projections from deep or superficial layers with the striatal compartments as distinguished with immunoreactivity for calbindin D<sub>28K</sub> (Schilman et al., 2008).

The functional differentiation between the shell and the core has been strongly substantiated in the past 10-15 years (reviews: e.g., Groenewegen et al., 1999; Zahm, 2000; Di Chiara, 2002; Cardinal et al., 2002; Meredith et al., 2008). The deep layers of the ventral prelimbic area and the infralimbic, medial orbital and ventral agranular insular areas have strong projections to different parts of the shell, terminating in both overlapping and complementary fashion. Projections from the prelimbic, anterior cingulate and dorsal agranular insular areas target the core of the nucleus accumbens and these projections extend dorsally and caudally into the caudate-putamen (Fig. 20.2C,D). The functional significance of the differential projections of these cortical areas to patch and matrix compartments within this intermediate striatal zone is still poorly understood. Inputs to patches arise in the paraventricular thalamic nucleus, and specific parts of the basal amygdaloid nucleus and, as discussed above, the prefrontal cortex, all interpreted as limbicrelated structures. Based on the results of tracing studies, striatal patches appear to be in reciprocal connection with a specific set of dopaminergic neurons in the substantia nigra pars compacta, that is, the ventral tier of dopaminergic cells (Gerfen, 1992). The deep layers of the mentioned prefrontal cortical areas might thus have an influence on these striatal dopaminergic interactions. In which way the striatal patches have an influence on behavioral or cognitive functions is still largely undisclosed. It has been hypothesized that, mediated by the specific dopaminergic input to the patches, there is an influence on the surrounding striatal matrix, providing a teaching signal (Houk et al., 1995; Groenewegen et al., 2003; Canales, 2005).

Prefrontal cortical projections to the striatal matrix primarily target the medium-sized spiny projection neurons that project directly or indirectly to pallidal and nigral neurons that, in turn, form the output neurons of the basal ganglia. In this way, the prefrontal cortical-striatal system forms the first link in a number of parallel, functionally segregated and closed basal ganglia-thalamocortical circuits (Fig. 20.4).

## V. CORTICO-CORTICAL AND CORTICOSTRIATAL RELATIONSHIPS

It has been first postulated by Yeterian and Van Hoesen (1978) on the basis of observations in primates that convergence of cortical projections in the striatum is most prevalent between functionally related cortical areas that are interconnected via cortico-cortical connections. This principle has been shown also in rats for various cortical areas, for example, for those that are involved in multimodal spatial orientation and attention (Reep et al., 2003). Thus, the Fr2 area in the dorsomedial part of the medial prefrontal cortex [medial agranular cortex (AGm) in their nomenclature] projects to a dorsocentral part of the caudate-putamen to which also the ventrolateral and lateral orbital areas project, in addition to specific parts of the posterior parietal and visual association cortices. These cortical areas all maintain cortico-cortical projections, forming together with their converging striatal projections a distributed network for spatial orientation and directed attention. As further indicated by Reep et al. (2003), the relationships between the main striatal terminal field of AGm with the terminal areas of the other cortical areas in the striatum is more complex than simple convergence. The lateral agranular (AGl or Fr1), orbital, posterior parietal and visual association cortices all have their main striatal target field in the periphery of the main AGm projection area, while sending less dense projections into the "core" of the AGm target area.

Dense and diffuse corticostriatal projections have also been described in other studies. For example, projections from the barrel cortex in rats can be distinguished into distinct, strictly topographically organized projections, as well as a much more diffuse one covering a much wider area of the striatal barrel area (Wright et al., 1999) (see Chapter 19).





FIGURE 20.4 Schematic representation of the convergence of prefrontal cortical, thalamic and amygdaloid inputs at the striatal level. (A) At the macrocircuit level the convergence of the projections from the prefrontal cortex, midline/intralaminar thalamus and basal amygdala is shown on a striatal projection neuron that forms the striatal link in a closed basal ganglia-thalamocortical circuit (thick lines). Projections from the midline/intralaminar thalamic nuclei and the basal amygdala reach both the prefrontal cortical and striatal way stations in such a basal ganglia-thalamocortical circuit, forming 'triadic' relationships (thinner lines). (B) At the microcircuit level the excitatory inputs from the cerebral cortex, thalamus and amygdala terminate on the spines of the medium-sized densely spiny projection neurons. Dopaminergic terminals are present on the necks of spines and dendritic shafts. Terminals of cholinergic and GABAergic interneurons, as well as collaterals from other medium-sized spiny neurons contact the dendrites more proximally. Abbreviations: Acb, nucleus accumbens; Caud, caudate nucleus; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; MD, mediodorsal nucleus; ML/IL, midline and intralaminar nuclei; MSN, medium sized spiny neuron; PFC, prefrontal cortex; Put, putamen; SNC, pars compacta of the substantia nigra; SNR, pars reticulata of the substantia nigra; STN, subthalamic nucleus; VA, ventral anterior nucleus; VL ventral lateral nucleus; VTA, ventral tegmental area.

The two types of corticostriatal projections appear to originate from different cortical layers and follow a different trajectory into the striatum. Tracing studies in primates have also indicated different patterns of corticostriatal terminations originating from limbic and association cortical areas, that is, discrete and relatively dense versus more widespread and diffuse (Calzavara et al., 2007; Haber et al., 2007). As indicated above, our schemes in Figure 20.2 represent the main terminal projections of specific prefrontal cortical areas, suggesting a relatively strict segregation of these projections, in particular when it concerns the medial prefrontal and agranular insular areas (Fig. 20.2C and D). However, most if not all these prefrontal areas have additional, smaller and in most cases more diffuse projections that terminate in the adjacent main striatal terminal field of a neighbouring cortical area (e.g., consult original figures of Berendse et al., 1992). Distinct medial prefrontal areas like the infralimbic, prelimbic and anterior cingulate areas have rather strong interconnections via cortico-cortical projections (e.g., Vertes, 2004).

While the projections from the medial prefrontal cortex (Fr2, anterior cingulate, prelimbic and infralimbic areas) extensively overlap with those from the orbital areas (MO, VO, VLO and LO) (as can be appreciated by comparing Figures 20.2A,B with 20.2C,D), it is remarkable that there appear to be only sparse interconnections between these two parts of the rat prefrontal cortex (e.g., Vertes, 2004). The functional relevance of this observation needs to be further investigated. While connectionally and functionally related cortical areas, converging onto a particular striatal region, may form part of a distributed neuronal network that is dedicated to a particular cognitive or behavioral function, the orbital areas may contribute information with another functional content at the striatal level. In that respect it is important to consider the afferents of the orbital areas. For example, the ventrolateral orbital area is known to receive thalamic input from the nucleus submedius which is a distinct relay station in ascending pain and other sensory pathways (Craig et al., 1982; Reep et al., 2003; Jasmin et al., 2004). Relay of such information via the ventrolateral orbital area to the central caudateputamen may have an important influence on the distributed network of cortico-cortical and corticostriatal connections involving the prelimbic and anterior cingulate and their related cortical areas that converge on the same central striatal region.

It must be emphasized that the precise organization of the cortico-cortical and the associated corticostriatal connections in rats still needs to be studied in a comprehensive manner, before more definite conclusions can be drawn with respect to the "rules" that govern their interrelationships.

# VI. RELATIONSHIPS OF THE PREFRONTAL-STRIATAL TOPOGRAPHY WITH OTHER STRIATAL INPUTS

As indicated above, the topography of the cortical-striatal projections forms the basis for a global functional subdivision of the striatum, that is, a sensorimotor, an associative or cognitive and a limbic striatum (see Fig. 20.1). In rats, like in primates (Haber, 2003), the prefrontal cortex-associated part of the striatum occupies the ventromedial and intermediate parts of the striatum, including the nucleus accumbens, while the sensorimotor part occupies the dorsolateral part of the striatum, in particular of the caudateputamen (Fig. 20.2). Within the various functional sectors of the striatum, prefrontal cortical inputs converge with inputs from the thalamus, most prominently the midline and intralaminar thalamic nuclei, limbic structures such as the hippocampus and amygdala and, as briefly discussed above, inputs from parietal, occipital and temporal cortices. All mentioned striatal inputs employ excitatory neurotransmitters. GABAergic inputs arise from the pallidum and are also organized in a roughly topographical manner (Bolam et al., 2000) (see Chapter 14). Furthermore, dopaminergic and serotonergic inputs reach the striatum from the substantia nigra/ventral tegmental area (VTA) complex and the mesencephalic raphe nuclei, respectively. Dopaminergic fibers from the medially located VTA primarily target the ventromedial, limbic striatum, while successively more lateral parts of the nigral complex reach progressively more lateral and dorsolateral parts of the striatum (Haber et al., 2000). The topographical arrangement within the ascending dopaminergic system is much less strict than the corticostriatal organization. Serotonergic fibers reach primarily the ventromedial parts of the striatum, in particular the shell and core of the nucleus accumbens and the ventromedial part of the caudate-putamen complex. Finally, a small but distinct noradrenergic input from the locus coeruleus targets the medial shell of the nucleus accumbens (Berridge et al., 1997). As a consequence, the infralimbic and ventral prelimbic corticostriatal projection areas in the most ventromedial part of the striatum, that is, the medial shell of the nucleus accumbens, receives the densest and most diverse catecholaminergic modulatory inputs. Moving dorsolaterally along the ventromedial-to-dorsolateral axis serotonin and dopamine are present in the intermediate striatal part, while the dorsolateral striatal area is primarily innervated by dopamine.

# A. Triadic Relationships of the Thalamic and Limbic Projections with the Prefrontal-Striatal System

An interesting topographical arrangement exists with respect to projections from various midline and intralaminar thalamic nuclei (see Chapter 22), as well as different subnuclei of the amygdaloid complex and the ventral hippocampus, to specific regions of the prefrontal cortex and their striatal target areas. Distinct nuclei of the midline and intralaminar thalamic complex target rather restricted areas of the prefrontal cortex and, in addition, project to relatively confined regions of the striatum that receive corticostriatal projections from that targeted prefrontal cortical area (Berendse and Groenewegen, 1991; Berendse et al., 1992; Groenewegen and Berendse, 1994). For example, the midline paraventricular nucleus heavily projects to the infralimbic and ventral prelimbic areas, as well as to the medial shell and adjacent medial core of the nucleus accumbens that are, in turn, the recipient of cortical fibers from the ventral medial prefrontal areas. Likewise, the anterior cingulate and dorsal prelimbic areas receive a strong input from the central medial thalamic nucleus. This thalamic nucleus projects to a dorsomedially-to-ventrolaterally oriented striatal zone that is also targeted by the dorsal prelimbic and anterior cingulate areas. These are just two examples of "triadic" relationship between individual prefrontal cortical areas, specific midline or intralaminar thalamic nuclei and their mutual striatal target areas (Fig. 20.4).

Similar triadic relationships exist between different subnuclei of the basal amygdaloid complex, specific prefrontal areas and their projection area in the ventral striatum (McDonald, 1991). For example, the caudal part of the parvicellular basal amygdaloid nucleus projects to the deeper layers of the infralimbic area and the rostrally adjacent medial orbital area of the medial prefrontal cortex, as well as to the medial part of the shell of the nucleus accumbens (Fig. 20.5A-C). As indicated in Figure 20.2, the infralimbic area is strongly associated with the medial shell of the nucleus accumbens. Likewise, the magnocellular basal amygdaloid nucleus strongly projects to the deep layers of the dorsal and ventral agranular insular areas as well as to the patches of the core and the lateral part of the shell of the nucleus accumbens (Fig. 20.5D-F). In turn, these areas of the nucleus accumbens receive inputs from the dorsal and ventral agranular insular areas, respectively.

The hippocampal formation, in particular the subiculum and directly adjacent CA1 field, projects in a topographical manner to the ventral striatum, primarily targeting the shell of the nucleus accumbens and adjacent striatal elements of the olfactory tubercle (Groenewegen et al., 1987). The ventral subiculum and CA1 field also project to the medial prefrontal cortex, most densely the prelimbic and infralimbic areas (Jay and Witter, 1991; Cenquizca and Swanson,



FIGURE 20.5 Triadic relationships between basal amygdaloid nuclei, the prefrontal cortex and the ventral striatum. (A-C) An injection of an anterograde tracer in the caudal part of the parvicellular part of the basal amygdaloid nucleus (C) results in labeling of fibers and terminals in the infralimbic area of the medial prefrontal cortex (A) and the medial shell of the nucleus accumbens (B). An injection of an anterograde tracer in the rostral part of the magnocellular basal amygdaloid nucleus (F) results in labeling of fibers and terminals in the dorsal and ventral agranular insular areas of lateral prefrontal cortex (D) and the lateral shell and the lateral core of the nucleus accumbens (E). Abbreviations: ac, anterior commissure; Acb, nucleus accumbens; AId, dorsal agranular insular cortex; ABmg, magnocellular part of the accessory basal nucleus; ABpc, parvicellular part of the accessory basal nucleus; AON, accessory olfactory nucleus; Bmg, magnocellular part of the basal nucleus; Bpc, parvicellular part of the basal nucleus; CP, caudate-putamen complex; IL, infralimbic cortex; lot, lateral olfactory tract; MO, medial orbital area; OT, olfactory tubercle; PL, prelimbic area; Se, septum.

2007) which, in turn, target the medial shell and olfactory tubercle (Fig. 20.2). Thus, for the ventral part of the hippocampal formation, the ventral part of the medial prefrontal cortex and the medial part of the ventral striatum a triadic relationship also exists.

As indicated above, the prefrontal cortical-striatal projections form the first link in the circuits that subsequently
involve direct and indirect intrinsic basal ganglia connections and basal ganglia outputs to the thalamocortical system as well as to the brainstem. Topographical ordering in the various links within the connections between the prefrontal cortex, basal ganglia and thalamocortical system forms the basis for the existence of the well-established parallel, functionally segregated basal ganglia-thalamocortical loops, as well as outputs to the hypothalamus and brainstem. The specific input from individual midline and intralaminar thalamic nuclei, basal amygdaloid nuclei and restricted parts of the hippocampal formation to both the cortical and striatal "nodes" in these circuits places these nuclei in a position to exert a relatively strong influence on individual basal ganglia thalamocortical circuits or basal ganglia output channels to other brain regions, including the hypothalamus and brainstem.

## VII. MEDIUM-SIZED SPINY PROJECTION NEURONS: INTEGRATORS OF STRIATAL INPUTS

The cellular structure of the striatum also reflects its integrative role. The principal striatal neurons are the medium-sized densely spiny neurons that project directly or indirectly to the basal ganglia output structures, that is, the internal segment of the globus pallidus and the reticular part of the substantia nigra. These neurons form by far the largest fraction (95-97%) of the total striatal population, while the neurochemically and morphologically heterogeneous group of interneurons represents the remaining 3–5% of the neurons (Gerfen, 2004). The excitatory inputs from the prefrontal cortex, as well as from other cortical areas, limbic structures and the thalamus, arrive at the heads of the dendritic spines (Smith and Bolam, 1990; Gerfen and Wilson, 1996). The throughput of the excitatory information from these cortical and subcortical sources to the cell body and ultimately the axon of the medium-sized neuron is modulated in various ways. Dopaminergic terminals tend to terminate on the spine necks and are therefore in a position to influence the transfer of information from the spine head to the shaft of the dendrite (Smith and Bolam, 1990). Cholinergic and GABAergic terminals from striatal interneurons and neighboring medium-sized spiny projection neurons terminate on the shafts of the dendrites and are likewise in a position to modulate the information flow from more distal parts of the dendrite to the cell body (Gerfen and Wilson, 1996; Bolam et al., 2000) (Fig. 20.4). Medium-sized spiny neurons thus integrate the inputs from the various striatal afferents and the intrinsic striatal neurons and they formulate the striatal output.

To understand the significance of the convergence of various excitatory and inhibitory inputs on the mediumsized densely spiny striatal projection neurons, it is important to briefly mention some of their electrophysiological characteristics (see also Chapter 5). Thus, electrophysiologically these striatal neurons can be characterized by two states: a down-state with a low resting membrane potential (-80/-90 mV) and a so-called up-state with a membrane potential close to the firing threshold (-55 mV). In the down-state the medium-sized spiny neurons are "silent". On the basis of coincident excitatory afferent activity converging on the dendrites of these neurons, for example, from different cortical, limbic and thalamic sources (Fig. 20.4), they may be brought to the up-state and more easily be elicited to firing (Gerfen and Wilson, 1996; Wilson, 2009). Based on these intrinsic electrophysiological properties and the specific arrangement of afferent fibers on their dendrites, the medium-sized spiny projection neurons may be characterized as "coincidence detectors" (Houk, 1995). In line with this, it has been shown that particular inputs only lead to an output of striatal projection neurons when a specific second input is active at the same time. Thus, with respect to the prefrontal striatal projections, there is evidence that the throughput of signals of this pathway is "gated" by other excitatory inputs, in particular originating from the hippocampus (O'Donnell and Grace, 1995) (see Chapter 21). It has been hypothesized that such a phenomenon may be very relevant for the understanding of the pathophysiological mechanisms of diseases such as schizophrenia (Grace et al., 2007).

#### REFERENCES

- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Ann Rev Neurosci 9:357–381.
- Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, 'prefrontal' and 'limbic' functions. Prog Brain Res 85:119–146.
- Beckstead RM (1979) An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J Comp Neurol 184:43–62.
- Berendse HW, Groenewegen HJ (1991) Restricted cortical terminal fields of the midline and intralaminar thalamic nuclei in the rat. Neuroscience 42:73–102.
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314–347.

- Berridge CW, Stratford TL, Foote SL, Kelley AE (1997) Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. Synapse 27:230–241.
- Bolam JP, Hanley JJ, Booth PAC, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196:527–542.
- Calzavara R, Mailly P, Haber SN (2007) Relationship between the corticostriatal terminals from areas 9 and 46, and those from area 8A, dorsal and rostral premotor cortex and area 24c: an anatomical substrate for cognition to action. Eur J Neurosci 26:2005–2024.
- Canales JJ (2005) Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. Neurobiol Learn Mem 83:93–103.
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321–352.
- Cenquizca LA, Swanson LW (2007) Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev 56:1–26.
- Chudasama Y, Robbins TW (2006) Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol Psychol 73:19–38.
- Craig AD Jr, Wiegand SJ, Price JL (1982) The thalamo-cortical projection of the nucleus submedius in the cat. J Comp Neurol 206:28–48.
- Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Behav 90:250–260.
- Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137:75–114.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Gabbott PL, Warner TA, Jays PR, Bacon SJ (2003) Areal and synaptic interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the rat. Brain Res 993:59–71.
- Gerfen CR (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science 246:385–388.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Ann Rev Neurosci 15:285–320.
- Gerfen CR (2004) Basal ganglia. In: The Rat Nervous System, 3rd edn (Paxinos G, ed), pp. 455–508. Amsterdam: Elsevier.
- Gerfen CR, Wilson CJ (1996). The basal ganglia. In: Handbook of Chemical Neuroanatomy. Vol. 12. Integrated systems of the CNS. Part III (Swanson LW, Björklund A, Hökfelt T, eds), pp. 371–468. Amsterdam: Elsevier.
- Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220–227.
- Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254.
- Graybiel AM, Ragsdale CW Jr (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci USA 75:5723–5726.
- Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsalprefrontal topography. Neuroscience 24:379–431.
- Groenewegen HJ, Berendse HW (1994) The specificity of the non-specific midline and intralaminar thalamic nuclei. Trends Neurosci 17:52–57.

- Groenewegen HJ, Witter MP (2004) Thalamus. In: The Rat Nervous System, 3rd edn (Paxinos G, ed), pp. 407–453. Amsterdam: Elsevier.
- Groenewegen HJ, Berendse HW, Wolters JG, Lohman AHM (1990) The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. Progr Brain Res 85:95–118.
- Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP (1987) Organization of the projections from the subiculum to the ventral striatum in the rat: A study using anterograde transport of Phaseolus vulgaris-leucoagglutinin. Neuroscience 23:103–120.
- Groenewegen HJ, Wright CI, Beijer AVJ (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system?. Prog Brain Res 107:485–511.
- Groenewegen HJ, Wright CI, Beijer AV, Voorn P (1999) Convergence and segregation of ventral striatal inputs and outputs. Ann NY Acad Sci 877:49–63.
- Haber SN (2003) The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.
- Haber SN, Kunishio K, Mizobuchi M, Lynd-Balta E (1995) The orbital and medial prefrontal circuit through the primate basal ganglia. J Neurosci 15:4851–4867.
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Haber SN, Kim KS, Mailly P, Calzavara R (2007) Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentivebased learning. Eur J Neurosci 26:2005–2024.
- Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179.
- Houk JC (1995) Information processing in modular circuits linking basal ganglia and cerebral cortex. In: Models of Information Processing in the Basal Ganglia (Houk JC, Davis JL, Beiser DG, eds), pp. 3–9. Cambridge, MA: MIT Press.
- Jasmin L, Burkey AR, Granato A, Ohara PT (2004) Rostral agranular insular cortex and pain areas of the central nervous system: a tracttracing study in the rat. J Comp Neurol 468:425–440.
- Jay TM, Witter MP (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of *Phaseolus vulgaris*-leucoagglutinin. J Comp Neurol 313:574–586.
- Masterman DL, Cummings JL (1997) Frontal-cortical circuits: the anatomic basis of executive, social and motivated behaviors. J Psychopharmacol 11:107–114.
- McDonald AJ (1991) Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience 44:1–14.
- McFarland NR, Haber SN (2002) Thalamic relay nuclei of the basal ganglia form both reciprocal and nonreciprocal cortical connections, linking multiple frontal cortical areas. J Neurosci 22:8117–8132.
- Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE (2008) The structural basis for mapping behavior onto the ventral striatum and its subdivisions. Brain Struct Funct 213:17–27.
- Mink JW (1996) The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.
- Murray EA, O'Doherty JP, Schoenbaum G (2007) What we know and do not know about the functions of the orbitofrontal cortex after 20 years of cross-species studies. J Neurosci 27:8166–8169.

- O'Donnell P, Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. J Neurosci 15:3622–3639.
- Parent A, Hazrati L-N (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Rev 20:91–127.
- Redgrave P, Prescott TJ, Gurney K (1999) The basal ganglia: a vertebrate solution to the selection problem? Neuroscience 89:1009–1023.
- Reep RL, Cheatwood JL, Corwin JV (2003) The associative striatum: organization of cortical projections to the dorsocentral striatum in rats. J Comp Neurol 467:271–292.
- Remijnse PL, Nielen MM, van Balkom AJ, Cath DC, van Oppen P, Uylings HB, Veltman DJ (2006) Reduced orbitofrontal-striatal activity on a reversal learning task in obsessive-compulsive disorder. Arch Gen Psychiatry 63:1225–1236.
- Robbins TW (2007) Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications.. Philos Trans R Soc Lond B Biol Sci 362:917–932.
- Seger CA (2008) How do the basal ganglia contribute to categorization? Their roles in generalization, response selection, and learning via feedback. Neurosci Biobehav Rev 32:265–278.
- Schilman EA, Uylings HBM, Galis-de Graaf Y, Joel D, Groenewegen HJ (2008) The orbital cortex in rats topographically projects to central parts of the caudate-putamen complex. Neurosci Lett 432:40–45.
- Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13:259–265.
- Uylings HB, van Eden CG (1990) Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. Prog Brain Res 85:31–62.
- Uylings HBM, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behav Brain Res 146:3–17.
- Van de Werd HJJM, Uylings HBM (2008) The rat orbital and agranular insular prefrontal cortical areas: a cytoarchitectonic and chemoarchitectonic study. Brain Struct Funct 212:387–401.

- Van Eden CG, Kros JM, Uylings HB (1990) The development of the rat prefrontal cortex. Its size and development of connections with thalamus, spinal cord and other cortical areas. Prog Brain Res 85:169–183.
- Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32–58.
- Voorn P, Vanderschuren LJMJ, Groenewegen HJ, Robbins TW, Pennartz CMA (2004) Putting a spin on the dorsal–ventral divide of the striatum. Trends Neurosci 27:468–474.
- Wilson CJ (2009) What controls the timing of striatal spiny cell action potentials in the up state? pp. xx-xx. In: Basal Ganglia IX (Groenewegen HJ, Berendse HW, Mulder AB, Voorn P, Cools AR, eds). New York: Springer.
- Wise SP, Murray EA, Gerfen CR (1996) The frontal cortex-basal ganglia system in primates. Crit Rev Neurobiol 10:317–356.
- Wright AK, Norrie L, Ingham CA, Hutton EA, Arbuthnott GW (1999) Double anterograde tracing of outputs from adjacent "barrel columns" of rat somatosensory cortex. Neostriatal projection patterns and terminal ultrastructure. Neuroscience 88:119–133.
- Yeterian EH, Van Hoesen GW (1978) Cortico-striate projections in the rhesus monkey: the organization of certain cortico-caudate connections. Brain Res 139:43–63.
- Záborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M (1985) Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. Neuroscience 14:427–453.
- Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neurosci Biobehav Rev 24:85–105.
- Zahm DS, Brog JS (1992) On the significance of subterritories in the "accumbens" part of the rat ventral striatum. Neuroscience 50:751–767.
- Zgaljardic DJ, Borod JC, Foldi NS, Mattis P (2003) A review of the cognitive and behavioral sequelae of Parkinson's disease: relationship to frontostriatal circuitry. Cogn Behav Neurol 16:193–210.

# Gating of Limbic Input to the Ventral Striatum

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### I. INTRODUCTION

The nucleus accumbens (NAc) is a hub for information related to reward, motivation, and decision-making. As a striatal region, it receives cortical inputs and sends information back to the cortex and subcortical structures via disinhibitory projections through the ventral pallidum, but what distinguishes this division of the ventral striatum is the integration of inputs originated in areas as diverse as the prefrontal cortex (PFC), amygdala and hippocampus. Several years ago, Anthony Grace and I proposed that the ventral hippocampus in particular provides a gating mechanism by which other inputs would cause NAc cell firing only if they were coincident with hippocampal inputs (O'Donnell and Grace, 1995, 1998). This concept helped understand some behavioral observations and drove further research on these circuits. Several models of the behavioral role of the NAc and its involvement in psychiatric disorders have indeed been built with this gating

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. mechanism as a critical component (O'Donnell and Grace, 1998; Chambers et al., 2001; Schmajuk, 2001; Schmajuk et al., 2001; Everitt and Wolf, 2002; Horvitz, 2002; Levy, 2004; Depue and Morrone-Strupinsky, 2005; White et al., 2006; Zhang et al., 2006; Day and Carelli, 2007; Floresco, 2007; Goto and Grace, 2008). However, the gating hypothesis was developed based on data obtained from anesthetized rats; it is likely that NAc information processing in a behaving animal is more complex than one set of inputs gating everything else. Here, I will review the observations that led to the limbic gating hypothesis, its limitations, as well as recent and ongoing work that may help us re-conceptualize the gating idea, proposing that afferent interactions in the NAc are dynamic. In short, emerging data from awake animals indicate that the NAc is driven by the dominant cortical region during any given behavior, and this drive is reflected in transiently correlated activity. Furthermore, local processes such as inhibitory interneurons can contribute to determining the afferent system NAc neurons synchronize with by providing shunting inhibition. Thus, the NAc can be envisioned as a behavioral switchboard in which the impact of afferent inputs and local mechanisms can determine the ensemble of active neurons, thereby contributing to decision-making and selecting the most appropriate behavioral repertoire for a given set of goals and context.

#### II. THE NUCLEUS ACCUMBENS: A FOREBRAIN GATEWAY

The ventral striatum has been repeatedly referred to as a gateway where information from limbic cortical circuits is integrated with information from the PFC. The NAc, a component of the ventral striatum, was described as the "limbic-to-motor interface" (Mogenson et al., 1980), because it was perceived as a means to convey hippocampal and amygdala influences into basal ganglia loops that control motor activity. This concept evolved over the years, becoming neglected for some time but later reinvigorated with the description of the basal ganglia as forming a spiral in which information is transferred from "more limbic" circuits to "more motor" circuits (Haber et al., 2000; Haber, 2003) (see Chapter 24). Anatomical studies reveal a heterogeneous distribution of inputs to the striatum, with limbic inputs dominant in medial and ventral regions. Indeed, the density of hippocampal afferents to the NAc and the medial part of the caudate-putamen is the highest in any striatal region in rodents (Voorn et al., 2004) and underscores the impact these inputs may have on integration of information in this region. Furthermore, while practically all frontal cortical terminals (see Chapter 20) contact distal processes of NAc medium spiny neurons (MSNs), a relatively large proportion of hippocampal afferents (10%) do so at the soma or primary dendrites (Meredith et al., 1990). Such arrangement would provide the hippocampus with a stronger control over somatic membrane potential and action potential firing in NAc neurons, as their postsynaptic responses would be more efficient in driving depolarizations at the axon initial segment. Action potential firing in NAc MSNs inhibits target neurons in the ventral pallidum, disinhibiting thalamocortical projections (Chevalier and Deniau, 1990). The NAc also projects to basal forebrain cholinergic neurons (Neigh-McCandless et al., 2002), providing control over widely distributed attentional processes (Sarter and Bruno, 2002). Thus, the integration of limbic and prefrontal information in the NAc is likely to have a

strong impact not only on motor planning but also on a wide array of higher order cognitive functions.

An anatomical aspect that shapes such integration is the high degree of convergence among diverse inputs onto single NAc MSNs. For some time, tract-tracing studies highlighted that afferent fibers tend to cluster into territories (Berendse et al., 1992), suggesting that separate channels of information coexist in the NAc. As MSN dendritic trees extend for several hundred micrometers (O'Donnell and Grace, 1993b), it is likely that they cover several of these territories, allowing individual neurons to integrate information from different sources. In addition, MSNs communicate via gap junctions (O'Donnell and Grace, 1993a), which are modulated by dopamine. The level of synchronization that gap junctions may offer would certainly help determining an ensemble of NAc neurons that can be affected by information arriving from several different sources. In vivo intracellular recordings reveal a remarkable degree of convergence onto single NAc MSNs. Most MSNs recorded exhibit monosynaptic excitatory post-synaptic potentials (EPSPs) in response to stimulation of several different afferents including the medial PFC, ventral hippocampus, basolateral amygdala, and paraventricular nucleus of the thalamus (Fig. 21.1) (O'Donnell and Grace, 1995; Goto and O'Donnell, 2002b). Extracellular recordings also provided data consistent with a high degree of convergence among these inputs (Finch, 1996). The anatomical correlate of such convergence was later demonstrated with elegant and painstaking single neuron reconstruction and tract tracing at the electron-microscopy level in which afferents from different regions were observed to contact the same MSN (French and Totterdell, 2002, 2003). Thus, individual NAc MSNs are able to integrate limbic and frontal cortical information, and action potential firing in these neurons is likely to depend on summated activity driven by several different afferent systems.

## III. ELECTROPHYSIOLOGICAL PROPERTIES OF MSNs THAT SHAPE INPUT INTEGRATION

### A. Up and Down Membrane Potential States and Ensemble Coding in the NAc

The diverse intrinsic ion channels and ionotropic receptors present in MSNs from both dorsal and ventral striatal regions allow these neurons a distinct membrane potential pattern and can impel them into persistent depolarizations



**FIGURE 21.1** Nucleus accumbens neurons typically exhibited evoked responses to stimulation of each of the afferent sites examined. A, Diagram illustrating the sources of most glutamatergic afferents to ventral striatal medium spiny neurons. B, Stimulation of the BL nucleus of the amygdala evoked an EPSP that exhibited long onset latency. C, The same accumbens neuron responded to PFC stimulation with a short-latency EPSP. D, Stimulation of the fornix also evoked a short-latency EPSP in this same neuron. E, Overlay of the three responses (resting membrane potential: -77 mV). From (O'Donnell and Grace, 1995).



**FIGURE 21.2** Nucleus accumbens medium spiny neurons exhibit a membrane potential with Up and Down states, which can be critical for ensemble coding. A, Representative trace of a neuron with alternating Down (-78 mV; *bottom green line*) and Up (-59 mV; *top green line*) membrane potential states. Calibration bar: 20 mV; 200 ms. B, Neurobiotin staining revealed morphology typical of medium spiny neurons. Calibration bar: 50 µm. C, Up–Down activity in a hypothetical set of 12 NA MSNs. During slow-wave sleep or in anesthetized animals, all neurons alternate between Up and Down states in synchrony. In wake and resting conditions, the strong excitatory input required to drive neurons into the Up state may be present for only small clusters of cells rather than for all neurons. Gray circles represent MSNs set in a persistent depolarization by a combination of afferent and local activity. Representative traces are displayed next to the circle. Open circles represent neurons with insufficient inputs, which will remain at a negative membrane potential. D, When salient stimuli demand the animal's attention, the relevant population of a distributed set of NAc neurons in a depolarized state, encoding information relevant to a given context (context 1; white cage). Right: placing the animal in a different context (context 2; gray cage) will result in a different neural ensemble being activated by the pattern of afferent activity. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

(see Chapters 5 and 6). Ventral and dorsal MSNs alternate between a negative resting membrane potential (Down state) and a relatively stable depolarization that lasts several hundred milliseconds (Up states; Fig. 21.2) (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996; Stern et al., 1998). The alternation between Up and Down states is not unique to MSNs, as it can be observed in cortical pyramidal neurons and other cell types (Steriade et al., 1993; Branchereau et al., 1996; Lewis and O'Donnell, 2000). Regular, global transitions between Up and Down states are typically observed in vivo in anesthetized preparations or during slow wave sleep (Timofeev et al., 2001; Mahon et al., 2006). These observations have allowed postulating that persistent depolarizations allow plasticity mechanisms (by partially removing Mg<sup>++</sup> blockade of NMDA receptors) and may facilitate learning during sleep. In awake animals, the membrane potential of MSNs or cortical pyramidal neurons cannot be described as exhibiting

oscillatory Up and Down state alternation; however, in cortical pyramidal neurons it does fluctuate between those values albeit in a more irregular fashion (Petersen et al., 2003; Poulet and Petersen, 2008). In a very elegant series of studies, the group of Carl Petersen has identified that at least in somatosensory cortex, activation of natural sensory stimuli via vibrissae deflection can cause persistent depolarizations similar to Up states, but only if the vibrissa deflected is in the receptive field of the neuron recorded (Petersen et al., 2003). Thus, persistent depolarizations present in an orderly repetitive manner during sleep or anesthesia, but they can also be recruited by sufficient input activation in behaving animals. In the NAc, field potential recordings have revealed slow oscillations in awake animals during quiet rest or during grooming (Leung and Yim, 1993), keeping open the possibility that membrane potential oscillations exist during non-attentive states. In any event, the regular alternation between Up and Down states in MSNs during anesthesia may not bear relevance to the information processing occurring in behaving circuits, as it is a reflection of global cortical oscillations (Kasanetz et al., 2006); however, it is possible that in behaving animals only neurons activated during a given behavioral condition are in a persistent depolarization similar to the Up state even if they are spatially distributed, allowing them to fire action potentials within a discrete time window. Other neurons in the vicinity not receiving sufficient glutamatergic inputs at that time (and therefore not encoding information relevant to the ongoing behavior) would not engage in such persistent depolarization (Fig. 21.2C). Information processing in the NAc has been proposed to involve activation of distributed sets of neurons (Pennartz et al., 1994; O'Donnell, 2003), and recording the activity of multiple NAc neurons simultaneously in awake animals provided data supporting this idea (Pennartz et al., 2004; Lansink et al., 2008; Nordquist et al., 2008). Thus, ensembles of neurons can be defined by the state of their inputs, and a distributed array of neurons could encode information relevant to the ongoing behavior if they are set into persistent depolarizations in the awake and attentive animal.

The concept of functionally relevant ensemble coding in the NAc has been recently supported by studies using immediate early genes. Ensembles of NAc neurons activated in two different environments were revealed with two immediate early genes with different, non-overlapping temporal courses. Recent neuronal activation in the NAc and caudateputamen was assessed with c-fos in situ hybridization, and previously activated neurons were revealed with FosB immunohistochemistry. Striatal ensembles were contextspecific, as the populations overlapped if the context tested were similar but did not if the contexts were different (Mattson et al., 2008). Although it is difficult to assign electrophysiological patterns to immediate early gene activity, the data suggest that distributed sets of neurons can be activated at a given time in the NAc, and these sets can change over time (Fig. 21.2D).

# B. Up States Depend on Glutamatergic Inputs

Persistent depolarizations such as Up states could be sustained by continued glutamatergic inputs or by voltagegated conductances that may become activated by an initial AMPA receptor activation. This is a difficult question to assess experimentally, as Up states can only be observed in intact preparations, not amenable to manipulations involving voltage-gated conductances in individual neurons. Although ventral striatal MSNs exhibit ionic conductances that can contribute to stabilizing the membrane potential in the Up and Down states (Uchimura et al., 1989; Wolf et al., 2005; Perez et al., 2006), the data available do not conclusively solve the issue; it is however possible that both scenarios are correct, depending on the state of the system and whether dopamine or other modulators are present. On one hand, NAc MSNs do exhibit NMDA and other voltage-gated currents (Pennartz et al., 1991; O'Donnell and Grace, 1993b; Cooper and White, 2000), which could allow sustained depolarizations once glutamatergic inputs have stopped. In organotypic corticostriatal co-cultures, NMDA receptors contribute a slow, persistent component in corticostriatal EPSPs evoked with single-pulse stimulation (Tseng et al., 2007). These data support the possibility of intrinsic mechanisms sustaining Up states. On the other hand, in vivo data suggest that the sustained depolarization characteristic of Up states in anesthetized animals requires continued glutamatergic drive. First, injecting depolarizing or hyperpolarizing current (a procedure that would affect voltage-gated currents) does not alter Up-Down transitions in NAc MSNs (O'Donnell and Grace, 1995). Second, striatal Up states are tightly correlated with ongoing cortical activity, with the termination of MSN Up states linked to termination of active cortical events (Kasanetz et al., 2006). In addition, a computer model has been used to test the role of NMDA and intrinsic currents in NAc Up states. A multi-compartment model of a NAc MSN was built using NEURON by entering conductance values for all known ion currents based on actual recordings in brain slices from adult rats (Wolf et al., 2005). Persistent synaptic



**FIGURE 21.3** VTA stimulation evoked persistent depolarizations in NAc neurons. A, Overlay of five intracellular recordings showing a prolonged depolarization resembling the Up state in response to VTA stimulation (*vertical lines*) before (*top*) and after (*bottom*) applying the dopamine  $D_1$  antagonist SCH23390 (0.5 mg/kg). B, Traces from another neuron showing a similar response in control conditions (*top*), with the  $D_2$  antagonist sulpiride (40 mg/kg) (*middle*), and with the  $D_1$  and  $D_2$  antagonists combined (*bottom*). C, Bar graphs showing the duration and amplitude of VTA evoked depolarization obtained in the presence of the drugs as a proportion of the baseline responses. A combined administration of  $D_1$  and  $D_2$  antagonists reduced the duration of VTA-evoked responses. Numbers in parentheses indicate the number of samples. From (Goto and O'Donnell, 2001b).

inputs were required to maintain the modeled neurons in the Up state, suggesting that MSN Up states do require continued inputs. Although increasing NMDA currents did not confer bistability per se, it enhanced the ability of MSNs to respond to glutamatergic inputs with action potential firing and the ability of the neuron to entrain to oscillations in afferent inputs. A recent in vivo study reported that intra-striatal administration of NMDA antagonists prevented MSN firing but not Up states (Pomata et al., 2008), demonstrating that NMDA receptors are critical for the membrane potential of MSNs to reach action potential threshold, but not necessarily for sustaining Up states. Other models did reveal a role of NMDA receptors in the onset of Up states (Kepecs and Raghavachari, 2007), but not in sustaining the depolarization. Thus, voltage-gated conductances such as NMDA and calcium currents may not be necessary for sustained depolarizations in resting conditions (see below for a different scenario in presence of phasic dopamine release), but nonetheless play a role in shaping the response of MSNs to sustained inputs. It is likely that synchronous, massive glutamatergic inputs impel MSNs to the Up state, and NMDA activation can contribute to allowing neurons in the Up state to fire action potentials.

#### C. Dopamine Modulation of up States

Up states are modulated by dopamine in striatal MSNs (see also Chapter 6). Delivering  $D_1$  or  $D_2$  antagonists locally via reverse dialysis affects MSN membrane potential states, with  $D_1$  blockade reducing the amplitude of Up states (West and Grace, 2002). These data suggest that tonic dopamine levels in the NAc contribute to the depolarization of Up states. Experiments using endogenously released dopamine support the notion that dopamine can contribute to sustaining Up state depolarizations. Electrical stimulation of the ventral tegmental area (VTA) with a train of stimuli mimicking dopamine cell burst firing (five pulses at 20Hz) yields a persistent depolarization similar to Up states in NAc MSNs, and this depolarization can be shortened by co-administration of  $D_1$  and  $D_2$  antagonists (Goto and O'Donnell, 2001b) (Fig. 21.3). These findings suggest that although the onset of the persistent depolarization driven by VTA stimulation does not involve dopamine receptors (full receptor blockade reduces the duration of the response, but does not eliminate it), endogenously released dopamine contributes to sustain the depolarization. Furthermore, computer modeling has suggested



**FIGURE 21.4.** Spontaneous and cortically evoked synaptic activity of striatal neurons recorded from organotypic co-cultures. A, Traces (left) of spontaneous depolarizations recorded in cortico–striatal–substantia nigra/ventral tegmental area (Cx–Str–SN/VTA), cortico–striatal (Cx–Str), and cortico–striatal–cortical (Cx–Str–Cx) cocultures. Only neurons recorded from Cx–Str–SN/VTA cocultures displayed recurrent plateau depolarizations resembling in vivo Up states. Insets show representative spontaneous depolarizations at faster time scales. Right: membrane potential distribution histogram from the traces shown on the left, revealing a bimodal distribution in the cocultures containing dopamine innervation. B, Traces illustrating typical striatal responses to electrical cortical stimulation in Cx–Str–SN/VTA, Cx–Str, and Cx–Str–Cx cocultures. Plateau depolarizations were evoked only in cultures containing SN/VTA neurons. Only brief depolarizing postsynaptic potentials were observed in Cx–Str–Cx cocultures. Several repetitions were overlaid in each example and vertical arrowheads below the traces point to the time of electrical stimulation. From (Tseng et al., 2007).

that MSNs can develop hysteresis (i.e., bistability in their membrane potential) in presence of dopamine (Gruber et al., 2006). These data and the model suggest that intrinsic conductances able to support Up states can be activated in presence of sufficient dopamine levels.

The problem of characterizing cellular mechanisms involved in onset and maintenance of Up states is the absence of such events in most in vitro preparations, which are more amenable to cellular pharmacology than in vivo preparations. There have been some reports, however, of striatal MSNs exhibiting repeated persistent depolarizations in brain slices. In dorsal striatal slices, cortical stimulation concurrent with D<sub>1</sub> receptor activation yields membrane potential oscillations similar to Up states (Vergara et al., 2003). In NAc slices, afferent stimulation with bursts can induce a prolonged depolarization that is dependent on continued presence of glutamate (Lape and Dani, 2004). In PFC pyramidal neurons, D<sub>1</sub>-NMDA coactivation also yields Up states, but only in slices obtained from adult rats (Tseng and O'Donnell, 2005). Another preparation in which the dopamine contribution to Up states can be studied is organotypic corticostriatal co-cultures. Placing a cortical piece next to a striatal piece allows cortical axons to enter the striatum and innervate MSNs. Stimulating the cortical piece in that preparation evokes synaptic responses in striatal MSNs (Tseng et al., 2007), but no Up states could be observed, neither spontaneous nor evoked. If a piece containing dopamine neurons is added to the preparation, tyrosine hydroxylase-positive fibers enter the striatum and spontaneous persistent depolarizations as well as persistent cortically-evoked depolarizations resembling Up states can be observed (Plenz and Kitai, 1998; Tseng et al., 2007) (Fig. 21.4). As a general mechanism, it is likely that Up states are driven by excitatory afferents via both AMPA and NMDA

receptors; a dopamine upregulation of their responses by D<sub>1</sub> receptors may be critical for sustained depolarizations and Up states. NMDA receptors would be activated only in the presence of strong glutamatergic inputs able to drive MSNs away from a very negative resting membrane potential dominated by  $K^+$  conductances. In anesthetized, tonic dopamine conditions, Up states are tightly driven by persistent glutamate release by afferents. Only in the presence of phasic dopamine, AMPA receptor activation may depolarize MSNs allowing an NMDA component and voltage-gated conductances to sustain depolarizations beyond the termination of afferent activity. Indeed, L-type calcium channels and NMDA receptors are upregulated by D<sub>1</sub> dopamine receptors in the NAc and dorsal striatum (Surmeier et al., 1995; Cepeda et al., 1998; Flores-Hernandez et al., 2002; Anderson et al., 2008). Thus, at rest MSNs are locked to the activity of the cortical region they receive inputs from, but during epochs of high dopamine cell firing (i.e., in presence of unexpected reward or reward-predicting stimuli (Schultz, 1997)) activation of D<sub>1</sub> receptors could sustain depolarizations in strongly activated neurons.

### IV. HIPPOCAMPAL GATING OF PREFRONTOCORTICAL THROUGHPUT

Which afferent inputs drive Up states in NAc MSNs? Several brain regions provide glutamatergic afferents to the NAc, so there are many candidates. In vivo intracellular recordings from NAc MSNs revealed that activation of hippocampal inputs with electrical stimulation of the fimbria-fornix can evoke persistent depolarizations resembling Up states, whether stimulation was carried out with single pulses or trains (O'Donnell and Grace, 1995). Furthermore, interrupting hippocampal afferents to the NAc with a fornix transection eliminated spontaneous Up states (O'Donnell and Grace, 1995), suggesting hippocampal afferents are necessary to achieve a critical level of depolarization in NAc neurons. The dependence of Up states on hippocampal afferents was further supported by injections of the local anesthetic lidocaine into the fornix transiently suppressing Up states (O'Donnell and Grace, 1995). On the other hand, single-pulse PFC stimulation failed to elicit transitions to the Up state, but was effective in driving action potential firing. However, PFC stimulation evoked action potentials only if it occurred during an Up state in the NAc neuron, either spontaneous or driven by hippocampal stimulation (O'Donnell and Grace, 1995) (Fig. 21.5). Thus, we proposed the findings represented a



FIGURE 21.5 Synaptic responses evoked in accumbens neurons by PFC stimulation are facilitated when preceded by fornix stimulation induced depolarized state. A, Stimulation of the fornix with a train of pulses (stimulus artifacts retained) induces a long-lasting depolarization of the accumbens cell membrane that resembles the Up state. B, In the same neuron, PFC stimulation when the cell is in the Down state fails to evoke action potential firing (first arrow). However, when the cell shows a spontaneous transition to the Up state, the same amplitude of PFC stimulation (second arrow) evokes an action potential. C, Stimulation of the fornix with trains evokes a prolonged transition of the membrane to the Up state. During this depolarization, stimulation of the PFC at an intensity that was subthreshold for spike triggering in the Down state (i.e., at the same amplitude used in B) readily evokes a spike. The stimulus intensity and evoked spike latency were identical to that in B (RMP, -79 mV and -69 mV). D, Cartoon illustrating the hippocampal gating model. Information in the form of action potential firing from PFC neurons (circles) provides inputs to NAc neurons. Only those cells in the Up state (gate open) as a consequence of hippocampal input (thick arrow) will fire action potentials in response to cortical afferent stimulation, thereby activating projections to the ventral pallidum (VP) and passing information onto the thalamus. From (O'Donnell and Grace, 1995) and (O'Donnell and Grace, 1998).

gating mechanism by which hippocampal afferents would set an ensemble of active neurons by setting them in the Up state, and PFC information would thereby be allowed to elicit action potential firing only in those units that are part of the active ensemble (Fig. 21.5D).

Several subsequent studies reinforced the hippocampal gating model. Simultaneous NAc intracellular recordings with hippocampal field potentials revealed a higher degree of synchrony between NAc Up states and ventral hippocampal field oscillations than with PFC oscillations (Goto and O'Donnell, 2001a), suggesting that in anesthetized rats hippocampal inputs may have a stronger impact than PFC afferents. Consistent with this idea, PFC-evoked responses are potentiated if they follow hippocampal or amygdala afferent stimulation, but PFC stimulation dampens subsequent responses to the ventral hippocampus (Goto and O'Donnell, 2002b). The prolonged depolarizations evoked by VTA stimulation cannot be observed when the ventral hippocampus is taken offline with a lesion (Goto and O'Donnell, 2002a). This observation suggests that the onset of VTA-evoked Up states depends on intact set of glutamatergic fibers from the hippocampus, and this may involve either an activation of hippocampal-PFC fibers by the VTA stimulation or a local interaction within the NAc. Recordings from awake freely moving animals also support the notion that electrical activity of NAc neurons is strongly linked to hippocampal activity. Cue-related ventral striatal neuron firing changes as the animals learn the significance of cues (Setlow et al., 2003), suggesting that contextual signals (likely of hippocampal origin) strongly impact NAc cell firing. Furthermore, simultaneous recordings of single unit activity or field potentials in both regions reveal a high level of synchronization and coherent activity (Tabuchi et al., 2000; Berke et al., 2004). This synchronization is entrained to hippocampal theta rhythms (Berke et al., 2004), but changes with behavior and spatial position (Tabuchi et al., 2000; Gruber et al., 2009b). These findings could be explained by neural activity in the hippocampus driving selected units in the NAc, but the units driven change with variations in hippocampal activity. In other words, the composition of NAc neural ensembles may reflect the composition of hippocampal ensembles (Pennartz et al., 2004; Lansink et al., 2008), at least during brief epochs, and this interaction may be important for learning mechanisms. Indeed, NAc ensemble activity patterns are reactivated during sleep following learning periods (Pennartz et al., 2004). Based on the considerations shown above, it is proposed that such interaction is possible only during brief epochs of phasic dopamine cell activity. The hippocampus has a clear strong influence over NAc cell activity, but this influence is dynamic allowing for changes according to the behavioral condition.

Not all behavioral observations, however, are consistent with a hippocampal gating of information in the NAc. For example, exposure to cocaine-conditioned stimuli increases zif268 expression in the NAc, amygdala, and PFC, but not in the hippocampus (Thomas et al., 2003), indicating that NAc activity can be associated with activation of other sources of inputs that spares the hippocampus. Also, dopamine in the medial PFC, but not the hippocampus, is important for reinstatement of drug seeking behavior, a pattern that requires the NAc and ventral pallidum (McFarland and Kalivas, 2001). In fact, the NAc can be driven by other inputs including the basolateral amygdala (Goto and O'Donnell, 2002b), which are also modulated by dopamine (Floresco et al., 2001). There is also evidence that an intact PFC is necessary for evoked NAc responses to ventral hippocampal stimulation (Belujon and Grace, 2008) and PFC inactivation suppresses NAc activity in awake animals (Ishikawa et al., 2008), suggesting that the PFC may also play at least a permissive role on NAc information processing. NAc MSNs show summation of responses to diverse inputs, and these responses can summate sub- or supra-linearly depending on the afferents activated (Carter et al., 2007). In awake rats, stimulation of PFC and the hippocampus reveal a sublinear summation of responses in the NAc, assessed as probability of firing action potentials (Wolf et al., 2009). Thus, although a facilitation of responses to PFC stimulation by hippocampal activation is clearly seen in anesthetized preparations (O'Donnell and Grace, 1995; Goto and O'Donnell, 2001a), the actual interactions among inputs in the NAc in awake animals seem more complex, being affected by the pattern and timing of inputs, and the behavioral condition.

## V. OTHER INPUTS CAN ALSO DRIVE UP STATES AND COMMAND NEURONAL ACTIVITY IN THE NUCLEUS ACCUMBENS

The hippocampal gating hypothesis was based on studies assessing the impact of hippocampal stimulation on MSN membrane potential, but other afferents could have a similar effect if they are sufficiently activated. PFC neurons, for example, can fire brief and rapid bursts of action potentials during PFC-dependent behaviors (Peters et al., 2005). Although analyzing the correlation between NAc Up states and PFC field potential oscillations over several seconds of recording revealed a weak correlation (Goto and O'Donnell, 2001a), analyzing the time-dependence of NAc-PFC correlations using a multi-taper sliding window tool reveals that NAc Up states can occasionally show high coherence with PFC activity (Gruber et al., 2009b). The data suggest that NAc neurons can be driven by epochs of high activity in the PFC. This possibility was directly tested with electrical stimulation of the medial PFC using trains of pulses mimicking the bursty pattern of firing this region can exhibit. Unlike single-pulse stimulation, burst PFC stimulation elicited persistent depolarizations in the range of Up states in NAc MSNs (Gruber and O'Donnell, 2009) (Fig. 21.6). Thus, behaviorally appropriate neural ensembles can be driven by the hippocampus when contextual and spatial information are critical for decisionmaking, but other sources of glutamatergic afferents to the



FIGURE 21.6 PFC stimulation can evoke Up states in NAc neurons, and individual neurons can show different responses to two adjacent cortical sites. A, Membrane potential trace from a representative MSN recorded in vivo showing spontaneous fluctuations between Up and Down states. B, Overlay of traces showing the response of a representative MSN to multiple trials of single-pulse electrical PFC stimulation. C, Overlay of traces showing the response of the same MSN as in B to multiple trials of PFC stimulation with a 10-pulse train. Arrows indicate times of stimulation pulses. D, Evoked response of an MSN showing a suppression of firing for stimulation in one PFC location. E, The same neuron shows an enhancement of firing for stimulation in a different PFC location. Black traces were recorded without current injection; gray traces were recorded with constant intracellular current injection that caused spontaneous firing. From (Gruber and O'Donnell, 2009) and (Gruber et al., 2009a).

NAc can determine active neural ensembles during other behavioral conditions.

## VI. THE NUCLEUS ACCUMBENS, A BEHAVIORAL SWITCHBOARD

The integration of information in the NAc changes second to second depending on the state of its inputs. In the regularly oscillating condition of anesthetized preparations, NAc Up states are strongly synchronized with the hippocampus but weakly with the PFC (Goto and O'Donnell, 2001a). However, even in anesthetized rats Up state transitions can occasionally synchronize with PFC neural activity (Gruber et al., 2009b), suggesting a dynamic aspect of integration of inputs in the NAc. In awake animals, the hippocampal-NAc synchronization is strong during spatial exploration, but weakens in conditions that activate the medial PFC (Gruber et al., 2009b). Multichannel recordings in rats with chronically implanted electrodes in the NAc, PFC and ventral hippocampus reveal dominant theta rhythms in all these areas when the rats are exploring the environment, with hippocampal theta activity driving similar oscillations in the other structures (Gruber et al., 2009b). When in the same session the rats engage in an operant task, the NAc does not present significant theta activity despite persisting dominant theta rhythms in the hippocampus. Instead, the dominant frequency in the NAc was a slower component, in the delta range, synchronized with similar activity in the PFC (Gruber et al., 2009b) (Fig. 21.7). The data suggest that during epochs of high PFC activity, the NAc can disconnect from the hippocampus, one of its primary afferent sources, and be driven by the PFC.

Which synaptic mechanisms could support such activity-dependent disconnection in the NAc? A strong candidate is shunting inhibition. Indeed, PFC stimulation can induce shunting inhibition in the striatum and NA. In vivo intracellular recordings reveal that burst stimulation of the PFC yields a persistent depolarization in NAc MSNs that reverses at relatively negative membrane potentials and is accompanied by a pause in action potential firing (Gruber et al., 2009a) (Fig. 21.6). These results suggest that bursts of PFC activity can recruit local inhibitory mechanisms in the NAc. This possibility has in fact been shown in the dorsal striatum, where fast-spiking interneurons (FSI) are driven by cortical stimulation with latencies shorter than those seen in MSNs (Mallet et al., 2005). In the NAc, in vivo intracellular recordings from FSI show strong activation by repeated PFC stimulation (Gruber et al., 2009a)



FIGURE 21.7 Dominant frequencies in the ventral hippocampus (VH), NAc and PFC field potentials differ between exploration and goal-directed behavior. A, Normalized spectral densities in the NAc shell, NAc core, PFC and VH obtained from simultaneously recorded epochs (4 seconds) in which the animals were exploring the cage (red line). The epochs were selected to match the location and body orientation of the operant task. The blue line represents the normalized spectral densities for the same four locations but during 4 second epochs in which the rats were lever-pressing for sucrose (2 seconds prior and after the lever press). The graphs were constructed with data from six sessions in five rats for the NAc core, and two sessions in two rats for the NAc shell (all of them with simultaneous recordings in the PFC and VH). Strong theta peaks are evident in all regions during exploration (green arrows), but they are lost in the NAc core and PFC during the instrumental behavior. An increase in delta activity can be observed instead. B, Pseudocolor plots of relative spectral power in the NAc shell, NAc core, PFC and VH during a 5-second epoch in which rats were exploring (top) and during a 5-second epoch centered on the lever press when the animals were engaged in instrumental behavior (bottom). The LFP traces of one of the epochs included in the analyses are shown above each box. Event-triggered and exploration spectrograms were constructed from one session from each animal and the display is the averaged data of all animals, revealing a strong theta oscillation during exploration, which weakens in the NAc core and PFC (but not in the NAc shell and VH) during lever-pressing. The NAc core and PFC show instead strong activity in the delta range (arrows), which are driven by slow deflections that can be observed in the traces above. D, Cross-spectral densities were calculated to determine coherence between similar frequency peaks in LFP obtained simultaneously from different brain regions during exploration and instrumental behavior. The two leftward panels illustrate representative pairings of PFC and NAc core, and VH and NAc core while the rat was exploring (red line), revealing a high coherence in the theta range between VH and NAc core (arrow in second panel from left). The blue line in both panels are cross-spectral densities in the same pairs when the rat was bar pressing for sucrose in the same session, showing a peak in the delta range between NAc core and PFC (arrow in left panel). The two rightward panels illustrate cross-spectral densities between the NAc shell and PFC and VH in the same rat and session. A strong theta peak is present in the shell-VH cross-spectrum independently of the behavioral condition. From (Gruber et al., 2009b). To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

(Fig. 21.8), and double-labeling of c-fos and parvalbumin reveal that a large number of NAc neurons activated by burst PFC stimulation are FSI (Gruber et al., 2009a) (Fig. 21.8). Overall, these experiments indicate that strong cortical activity can drive feed-forward inhibition mechanisms and shunting inhibition in the NAc.

The appropriate set of neural ensembles in the NAc can be activated by the hippocampus during



FIGURE 21.8 Activation of NAc interneurons by PFC train stimulation in vivo. A, Parvalbumin (red) and Neurobiotin (blue) co-labeling of a fast spiking interneuron recorded intracellularly in vivo in the NAc. B, recording from the neuron in a (dark trace) and a simultaneously recorded PFC field potential (PFC FP, gray trace - the polarity was inverted to make it easier to visualize the synchronization) showing spontaneous fluctuations. Inset shows the action potential waveform. C, overlay of traces from the same neuron in response to PFC stimulation. Arrows show timing of the electrical pulses, and stimulus artifacts have been removed for clarity. D, confocal image of a NAc section labeled for c-Fos (green) and parvalbumin (red) following in vivo train stimulation (left). The presence of co-labeling (arrows) indicates that PFC train stimulation can activate NAc interneurons. Control section from an unstimulated brain (right) shows very little c-Fos immunoreactivity. Scale is the same in both panels. From (Gruber et al., 2009a). To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

hippocampal-driven behavioral conditions (e.g., spatial exploration) and by the PFC during PFC-dependent behaviors (e.g., decision-making instances). Although the dominant set of inputs may act as a gate, this process is short-lived and dynamic. Strong dopamine activation may sustain the depolarization in those neurons that are driven by a high level of glutamate afferents, and therefore relevant for the ongoing behavior (O'Donnell, 2003). Strong PFC activity can drive local inhibitory mechanisms. It remains to be determined whether other glutamatergic afferents to the NAc can also effectively drive shunting inhibition. In summary, the NAc is a switchboard in which the combination of afferent activity patterns along with local inhibitory processes and monoamine modulators select the appropriate neural ensemble to reinforce ongoing behavior if it is associated with reward or reward expectancy by the dopamine signal.

#### REFERENCES

- Anderson SM, Famous KR, Sadri-Vakili G, Kumaresan V, Schmidt HD, Bass CE, Terwilliger EF, Cha JH, Pierce RC (2008) CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci 11:344–353.
- Belujon P, Grace AA (2008) Critical role of the prefrontal cortex in the regulation of hippocampus-accumbens information flow. J Neurosci 28:9797–9805.
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314–347.
- Berke JD, Okatan M, Skurski J, Eichenbaum HB (2004) Oscillatory entrainment of striatal neurons in freely moving rats.. Neuron 43:883–896.
- Branchereau P, van Bockstaele EJ, Chan J, Pickel VM (1996) Pyramidal neurons in rat prefrontal cortex show a complex synaptic response to single electrical stimulation of the locus coeruleus region: evidence for antidromic activation and GABAergic inhibition using in vivo intracellular recording and electron microscopy. Synapse 22:313–331.
- Carter AG, Soler-Llavina GJ, Sabatini BL (2007) Timing and location of synaptic inputs determine modes of subthreshold integration in striatal medium spiny neurons. J Neurosci 27:8967–8977.
- Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS (1998) Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. J Neurophysiol 79:82–94.
- Chambers RA, Krystal JH, Self DW (2001) A neurobiological basis for substance abuse comorbidity in schizophrenia. Biol Psychiatry 50:71–83.
- Chevalier G, Deniau JM (1990) Disinhibition as a basic process in the expression of striatal functions. Trends Neurosci 13:277–280.
- Cooper DC, White FJ (2000) L-type calcium channels modulate glutamate-driven bursting activity in the nucleus accumbens in vivo. Brain Res 880:212–218.
- Day JJ, Carelli RM (2007) The nucleus accumbens and Pavlovian reward learning.. Neuroscientist 13:148–159.
- Depue RA, Morrone-Strupinsky JV (2005) A neurobehavioral model of affiliative bonding: implications for conceptualizing a human trait of affiliation. Behav Brain Sci 28:313–350 discussion 350-395.
- Everitt BJ, Wolf ME (2002) Psychomotor stimulant addiction: a neural systems perspective. J Neurosci 22:3312–3320.
- Finch DM (1996) Neurophysiology of converging synaptic inputs from the rat prefrontal cortex, amygdala, midline thalamus, and hippocampal formation onto single neurons of the caudate/putamen and nucleus accumbens. Hippocampus 6:495–512.
- Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, et al. ( 2002) Dopamine enhancement of NMDA currents in dissociated

medium-sized striatal neurons: role of D1 receptors and DARPP-32. J Neurophysiol 88:3010–3020.

- Floresco SB (2007) Dopaminergic regulation of limbic-striatal interplay. J Psychiatry Neurosci 32:400–411.
- Floresco SB, Blaha CD, Yang CR, Phillips AG (2001) Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. J Neurosci 21:2851–2860.
- French SJ, Totterdell S (2002) Hippocampal and prefrontal cortical inputs monosynaptically converge with individual projection neurons of the nucleus accumbens. J Comp Neurol 446:151–165.
- French SJ, Totterdell S (2003) Individual nucleus accumbens-projection neurons receive both basolateral amygdala and ventral subicular afferents in rats. Neuroscience 119:19–31.
- Goto Y, O'Donnell P (2001a) Synchronous activity in the hippocampus and nucleus accumbens in vivo. J Neurosci 21:RC131.
- Goto Y, O'Donnell P (2001b) Network synchrony in the nucleus accumbens in vivo. J Neurosci 21:4498–4504.
- Goto Y, O'Donnell P (2002a) Delayed mesolimbic system alteration in a developmental animal model of schizophrenia. J Neurosci 22:9070–9077.
- Goto Y, O'Donnell P (2002b) Timing-dependent limbic-motor synaptic integration in the nucleus accumbens. Proc Natl Acad Sci USA 99:13189–13193.
- Goto Y, Grace AA (2008) Limbic and cortical information processing in the nucleus accumbens. Trends Neurosci 31:552–558.
- Gruber AJ, O'Donnell P (2009) Bursting activation of prefrontal cortex drives sustained Up states in nucleus accumbens spiny neurons in vivo. Synapse 63:173–180.
- Gruber AJ, Powell EM, O'Donnell P (2009a) Cortically activated interneurons shape spatial aspects of cortico-accumbens processing. J Neurophysiol 101:1876–1882.
- Gruber AJ, Hussain RJ, O'Donnell P (2009b) The rat nucleus accumbens: a switchboard for goal-directed behaviors. PLoS ONE 2009 4:e5062.
- Gruber AJ, Dayan P, Gutkin BS, Solla SA (2006) Dopamine modulation in the basal ganglia locks the gate to working memory. J Comput Neurosci 20:153–166.
- Haber SN (2003) The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Horvitz JC (2002) Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. Behav Brain Res 137:65–74.
- Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008) Dorsomedial prefrontal cortex contribution to behavioral and nucleus accumbens neuronal responses to incentive cues. J Neurosci 28:5088–5098.
- Kasanetz F, Riquelme LA, O'Donnell P, Murer MG (2006) Turning off cortical ensembles stops striatal Up states and elicits phase perturbations in cortical and striatal slow oscillations in rat in vivo. J Physiol 577:97–113.
- Kepecs A, Raghavachari S (2007) Gating information by two-state membrane potential fluctuations. J Neurophysiol 97:3015–3023.
- Lansink CS, Goltstein PM, Lankelma JV, Joosten RN, McNaughton BL, Pennartz CM (2008) Preferential reactivation of motivationally relevant information in the ventral striatum. J Neurosci 28: 6372–6382.

- Lape R, Dani JA (2004) Complex response to afferent excitatory bursts by nucleus accumbens medium spiny projection neurons. J Neurophysiol 92:1276–1284.
- Leung LS, Yim CY (1993) Rhythmic delta-frequency activities in the nucleus accumbens of anesthetized and freely moving rats. Can J Physiol Pharmacol 71:311–320.
- Levy F (2004) Synaptic gating and ADHD: a biological theory of comorbidity of ADHD and anxiety. Neuropsychopharmacology 29: 1589–1596.
- Lewis BL, O'Donnell P (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D<sub>1</sub> dopamine receptors. Cerebral Cortex 10: 1168–1175.
- Mahon S, Vautrelle N, Pezard L, Slaght SJ, Deniau JM, Chouvet G, Charpier S (2006) Distinct patters of striatal medium spiny neuron activity during the natural sleep-wake cycle. J Neurosci 26:12587–12595.
- Mallet N, Le Moine C, Charpier S, Gonon F (2005) Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo. J Neurosci 25:3857–3869.
- Mattson BJ, Koya E, Simmons DE, Mitchell TB, Berkow A, Crombag HS, Hope BT (2008) Context-specific sensitization of cocaineinduced locomotor activity and associated neuronal ensembles in rat nucleus accumbens. Eur J Neurosci 27:202–212.
- McFarland K, Kalivas PW (2001) The circuitry mediating cocaineinduced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.
- Meredith GE, Wouterlood FG, Pattiselano A (1990) Hippocampal fibers make synaptic contact with glutamate decarboxilase-immunoreactive neurons in the rat nucleus accumbens. Brain Res 513:329–334.
- Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between limbic system and the motor system. Progr Neurobiol 14:69–97.
- Neigh-McCandless G, Kravitz BA, Sarter M, Bruno JP (2002) Stimulation of cortical acetylcholine release following blockade of ionotropic glutamate receptors in nucleus accumbens. Eur J Neurosci 16:1259–1266.
- Nordquist RE, Vanderschuren LJ, Jonker AJ, Bergsma M, de Vries TJ, Pennartz CM, Voorn P (2008) Expression of amphetamine sensitization is associated with recruitment of a reactive neuronal population in the nucleus accumbens core.. Psychopharmacology (Berl) 198:113–126.
- O'Donnell P (2003) Dopamine gating of forebrain neural ensembles. Eur J Neurosci 17:429–435.
- O'Donnell P, Grace AA (1993a) Dopaminergic modulation of dye coupling between neurons in the core and shell regions of the nucleus accumbens. J Neurosci 13:3456–3471.
- O'Donnell P, Grace AA (1993b) Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. Synapse 13:135–160.
- O'Donnell P, Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. J Neurosci 15:3622–3639.
- O'Donnell P, Grace AA (1998) Dysfunctions in multiple interrelated systems as the neurobiological bases of schizophrenic symptom clusters. Schiz Bull 24:267–283.
- Pennartz CM, Groenewegen HJ, Lopes da Silva FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles:

an integration of behavioural, electrophysiological and anatomical data. Prog Neurobiol 42:719–761.

- Pennartz CM, Boeijinga PH, Kitai ST, Lopes da Silva FH (1991) Contribution of NMDA receptors to postsynaptic potentials and paired-pulse facilitation in identified neurons of the rat nucleus accumbens in vitro. Exp Brain Res 86:190–198.
- Pennartz CM, Lee E, Verheul J, Lipa P, Barnes CA, McNaughton BL (2004) The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. J Neurosci 24:6446–6456.
- Perez MF, White FJ, Hu XT (2006) Dopamine D<sub>2</sub> receptor modulation of K<sup>+</sup> channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. J Neurophysiol 96:2217–2228.
- Peters YM, O'Donnell P, Carelli RM (2005) Prefrontal cortical cell firing during maintenance, extinction, and reinstatement of goal-directed behavior for natural reward.. Synapse 56:74–83.
- Petersen CC, Hahn TT, Mehta M, Grinvald A, Sakmann B (2003) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc Natl Acad Sci USA 100:13638–13643.
- Plenz D, Kitai ST (1998) Up and Down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fastspiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. J Neurosci 18:266–283.
- Pomata PE, Belluscio MA, Riquelme LA, Murer MG (2008) NMDA receptor gating of information flow through the striatum in vivo. J Neurosci 28:13384–13389.
- Poulet JF, Petersen CC (2008) Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. Nature 454:881–885.
- Sarter M, Bruno JP (2002) The neglected constituent of the basal forebrain corticopetal projection system: GABAergic projections. Eur J Neurosci 15:1867–1873.
- Schmajuk NA (2001) Hippocampal dysfunction in schizophrenia. Hippocampus 11:599–613.
- Schmajuk NA, Cox L, Gray JA (2001) Nucleus accumbens, entorhinal cortex and latent inhibition: a neural network model. Behav Brain Res 118:123–141.
- Schultz W (1997) Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 7:191–197.
- Setlow B, Schoenbaum G, Gallagher M (2003) Neural encoding in ventral striatum during olfactory discrimination learning. neuron 38:625–636.
- Steriade M, Nunez A, Amzica F (1993) Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13:3266–3283.
- Stern EA, Jaeger D, Wilson CJ (1998) Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo. Nature 394:475–478.
- Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P (1995) Modulation of calcium currents by a D1 dopaminergic protein kinase/ phosphatase cascade in rat neostriatal neurons. Neuron 14:385–397.

- Tabuchi ET, Mulder AB, Wiener SI (2000) Position and behavioral modulation of synchronization of hippocampal and accumbens neuronal discharges in freely moving rats. Hippocampus 10:717–728.
- Thomas KL, Arroyo M, Everitt BJ (2003) Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. Eur J Neurosci 17:1964–1972.
- Timofeev I, Grenier F, Steriade M (2001) Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci USA 98:1924–1929.
- Tseng KY, O'Donnell P (2005) Post-pubertal emergence of prefrontal cortical Up states induced by D<sub>1</sub>-NMDA co-activation. Cereb Cortex 15:49–57.
- Tseng KY, Snyder-Keller A, O'Donnell P (2007) Dopaminergic modulation of striatal plateau depolarizations in corticostriatal organotypic cocultures. Psychopharmacology (Berl) 191:627–640.
- Uchimura N, Higashi H, Nishi S (1989) Membrane properties and synaptic responses of the guinea pig nucleus accumbens neurons in vitro. J Neurophysiol 61:769–779.
- Vergara R, Rick C, Hernandez-Lopez S, Laville JA, Guzman JN, Galarraga E, Surmeier DJ, Bargas J (2003) Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. J Physiol 553:169–182.
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468–474.
- West AR, Grace AA (2002) Opposite influences of endogenous dopamine D<sub>1</sub> and D<sub>2</sub> receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci 22: 294–304.
- White IM, Whitaker C, White W (2006) Amphetamine-induced hyperlocomotion in rats: Hippocampal modulation of the nucleus accumbens. Hippocampus 16:596–603.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 16:2397–2410.
- Wolf JA, Finkel LH, Contreras D (2009) Sublinear summation of afferent inputs to the nucleus accumbens in the awake rat. J Physiol 587:1695–1704.
- Wolf JA, Moyer JT, Lazarewicz MT, Contreras D, Benoit-Marand M, O'Donnell P, Finkel LH (2005) NMDA/AMPA ratio impacts state transitions and entrainment to oscillations in a computational model of the nucleus accumbens medium spiny projection neuron. J Neurosci 25:9080–9095.
- Zhang TA, Maldve RE, Morrisett RA (2006) Coincident signaling in mesolimbic structures underlying alcohol reinforcement. Biochem Pharmacol 72:919–927.

# Anatomical and Functional Organization of the Thalamostriatal Systems

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### I. INTRODUCTION

Since its first description (Vogt and Vogt, 1941; Cowan and Powell, 1956), significant progress has been made in our understanding of the anatomical and synaptic organization of the thalamostriatal system. However, the functional role of this network in the basal ganglia circuitry remains unknown. The recent evidence that lesion or deep-brain stimulation (DBS) of the centromedian/ parafascicular (CM/Pf) complex, the main source of thalamostriatal inputs, alleviate some of the motor symptoms of Tourette's syndrome and Parkinson's disease has generated significant interest in the thalamostriatal system.

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. The cloning of vesicular glutamate transporters 1 and 2 (vGluT1 and vGluT2) has provided unique tools to differentiate thalamostriatal from corticostriatal glutamatergic terminals, thereby setting the stage for future comparative studies of the synaptology and plasticity of these two systems in normal and pathological conditions. In this chapter, we will review our current knowledge of the functional anatomy, synaptology, physiology and pathophysiology of the mammalian thalamostriatal systems. We will also discuss evidence that the thalamus may regulate basal ganglia outflow through direct interactions with extrastriatal targets. Finally, we will provide an overview of the functional properties of thalamostriatal neurons in response to attention-related stimuli and discuss the outcomes of recent neurosurgical attempts at lesioning or stimulating the CM/Pf in patients with Parkinson's disease or Tourette's syndrome. Because of space limitation, readers are referred to previous reviews for additional information and a broader coverage of early literature on the thalamo striatal projections (Groenewegen and Berendse, 1994; Parent and Hazrati, 1995; Mengual et al., 1999; Haber and McFarland, 2001; Van der Werf et al., 2002; Kimura et al., 2004; Smith et al., 2004; McHaffie et al., 2005; Benabid, 2009; Kerlerian-LeGoff et al., 2009; Haber and Calzavara, 2009; Lanciego et al., 2009; Minamimoto et al., 2009; Sadikot and Rymar, 2009; Smith et al., 2009).

# II. ANATOMY OF THE THALAMOSTRIATAL SYSTEMS

#### A. Sources of Thalamostriatal Projections

The CM/Pf is the main source of thalamostriatal projections, but significant inputs also originate from rostral intralaminar, midline and specific relay nuclei in primates and nonprimates (Smith and Parent, 1986; Berendse and Groenewegen, 1990; Fenelon et al., 1991; Francois et al.,1991; Sadikot et al., 1992a,b; Deschenes et al., 1995, 1996a,b; Mengual et al., 1999; McFarland and Haber, 2000, 2001, 2002; Elena Erro et al., 2002; Smith et al., 2004, 2009; Castle et al., 2005; McHaffie et al., 2005; Parent and Parent, 2005; Raju et al., 2006; Lacey et al., 2007). In primates, the topography of CM/Pf inputs to the striatum has been studied in great detail. Based on the anatomical relationships between sections of the CM/Pf and specific functional territories of the striatum, the primate CM/Pf complex can be divided into five major sub-regions: (1) The rostral third of Pf innervates mainly the nucleus accumbens; (2) the caudal two thirds of Pf project to the caudate nucleus; (3) the dorsolateral extension of Pf (Pfdl) targets selectively the anterior putamen; (4) the medial two thirds of CM (CMm) innervates the post-commissural putamen; and (5) the lateral third of CM (CMI) is the source of inputs the primary motor cortex (Fig. 22.1). Through these projections, the CM/Pf gains access to all functional territories of the striatum: the rostral Pf is the main source of inputs to the limbic striatum, the Pf/Pfdl is preferentially connected with associative striatal regions, whereas the CMm is a major source of inputs to the sensorimotor striatum (Fig. 22.1) (Smith et al., 2004, 2009) (see also Chapter 20). In rodents, the lateral part of Pf is considered as the homologue of the

primate CM, whereas the medial Pf displays strong similarities with the Pf proper (Groenewegen and Berendse, 1994; Smith et al., 2004, 2009). Overall, the organization of striatal projections from the different parts of the rat Pf is consistent with the organization of the CM/Pf-striatal system in primates (Figs 22.1, 22.2).

As mentioned above, thalamostriatal projections also originate from non-intralaminar nuclei, including specific, associative and midline nuclei (Fig. 22.1). In rodents, midline thalamic nuclei project preferentially to the ventral striatum, though significant inputs to dorsal striatal regions have also been reported (Groenewegen and Berendse, 1994; Smith et al., 2004, 2009). In monkeys, several non-intralaminar thalamic nuclei, including the mediodorsal nucleus, the pulvinar, the lateral posterior nucleus and the ventral motor nuclear group have been recognized as modest sources of thalamostriatal projections to the caudate nucleus, putamen and nucleus accumbens (Smith and Parent, 1986; Gimenez-Amaya et al., 1995; Smith et al., 2004, 2009). Recent data from Haber and colleagues (McFarland and Haber, 2000; 2001) have emphasized the importance of thalamostriatal projections from the ventral motor thalamic nuclei in monkeys (see also Chapter 24). In brief, striatal inputs from the ventral anterior and ventral lateral (VA/VL) nuclei terminate in broad rostrocaudal regions of the dorsal striatum that correspond to functionally related regions of the motor cortex. Interconnected regions of the ventral motor thalamic nuclei and motor cortices send convergent inputs to the sensorimotor striatum suggesting that interactions exist between corticostriatal and thalamostriatal projections that may be of importance in motor behaviors (McFarland and Haber, 2000; Smith et al., 2004, 2009; Haber and Calzavara, 2009). The pars oralis of the VL (VLo), the main recipient of sensorimotor GPi outflow, terminates preferentially in the postcommissural putamen, whereas the magnocellular division of the VA, the principal target of SNr and associative GPi outflow, innervates the caudate nucleus (McFarland and Haber, 2000, 2001). Within striatal territories, VA/VL projections terminate in a non-homogeneous patchy manner indicating another level of complexity (Groenewegen and Berendse, 1994; McFarland and Haber, 2000, 2001; Smith et al., 2004, 2009).

Thus, although the CM/Pf complex is the main source of thalamostriatal projections, many other non-intralaminar thalamic nuclei also contribute to this system. The functional role of thalamostriatal connections remain enigmatic,



FIGURE 22.1 Schematic illustrations showing, on three rostrocaudal levels of the striatum, the pattern of distribution of thalamic inputs from caudal intralaminar thalamic nuclei in monkey (left) and from rostral intralaminar, caudal intralaminar and midline thalamic nuclei in rat (right). The color-coded striatal territories receive inputs from the corresponding color-coded thalamic nuclei (insets). The lateral centromedian (CMI) nucleus in monkey projects to the primary motor cortex, but not to the striatum. The antero-posterior stereotaxic coordinates these levels correspond to are indicated at the bottom of each section. Abbreviations: A, anterior; AC, anterior commissure; Breg, Bregma; CC, corpus callosum; CD, caudate nucleus; GP/GPe, globus pallidus external segment; GPi, globus pallidus internal segment; IC, internal capsule; LV, lateral ventricle; PUT, putamen; PV, paraventricular nucleus; STR, striatum; Th, thalamus. (See Smith and Parent, 1986; Sadikot et al., 1992a; Groenewegen and Berendse, 1994; Sidibe and Smith, 1996, for more details.) (see Color Plate Section to view the color version of this figure)



**FIGURE 22.2** Summary of results from anterograde tracing studies of thalamic projections to the rat striatum. The histogram illustrates the percentage of labeled boutons from each of the thalamic or cortical (M1) regions injected. Apart from Pf, all other thalamic nuclei and M1 give rise to terminals that contact almost exclusively dendritic spines in the rat striatum. These findings are summarized in the model of a striatal medium spiny neuron on the right. Abbreviations: AV, anteroventral nucleus; CL, centrolateral nucleus; LD, laterodorsal nucleus; MD, mediodorsal nucleus; M1, primary motor cortex; PF, parafascicular nucleus; VA/VL, ventral anterior/ventral lateral nucleus. (See Raju et al., 2006, for more details.)

but recent physiological evidence suggests that the CM/Pf neurons supply striatal neurons with information related to attentional values and likely play a key role in regulating reward-related information processing of striatal cholinergic interneurons (see below). Other thalamostriatal projections may be an important substrate for relying thalamocortical information to the striatum and provide additional routes through which ascending information from brainstem and cerebellar regions may be transmitted to the basal ganglia.

# **B.** Thalamostriatal versus Thalamocortical Systems: Segregated or Collateralized Origins

As discussed in the previous section, the thalamostriatal system has two main origins, the CM/Pf and other thalamic nuclei. Striatal projections from these different regions differ significantly in their degree of collateralization to the cerebral cortex. The CM/Pf neurons send massive and topographically organized projections to specific regions of the dorsal striatum, while providing minor inputs to the cerebral cortex (Smith and Parent, 1986; Sadikot et al., 1992b; Parent and Parent, 2005). In contrast, the rostral intralaminar, associative and relay thalamic nuclei send major inputs to specific cortical areas, while contributing a modest innervation of functionally related areas of the dorsal and ventral striatum (Deschenes et al., 1996a,b; McFarland and Haber, 2001; Smith et al., 2004, 2009; Haber and Calzavara, 2009). In monkeys, CM projections to the primary motor cortex arise mainly from the lateral part of the nucleus (Smith and Parent, 1986; Sadikot et al., 1992a; Smith et al., 2004, 2009), whereas the medial CM project to the sensorimotor putamen (Fig. 22.1). The recent use of single cell filling has further refined our understanding of the origin of the CMstriatal system in primates (Parent and Parent, 2005; Smith et al., 2009). Three major groups of CM neurons have been identified based on their extent of projections to the striatum and cerebral cortex in monkeys. More than half of all neurons innervate densely and focally the striatum without any significant input to the cerebral cortex, about one third of neurons innervate diffusely the cerebral cortex, without any significant projection to the striatum, and the remaining neurons project to both targets with a preponderance of innervation towards the dorsal striatum (Parent and Parent, 2005).

Thus, although many thalamic nuclei give rise to thalamostriatal projections, those from the CM/Pf complex significantly differ from thalamostriatal projections originating from other thalamic nuclei in their degree of collateralization to the cerebral cortex. The CM/Pf appears to have a much closer relationship with the striatum and other basal ganglia nuclei (see below) than with cortical regions, while it is the opposite for other thalamic nuclei.

# C. Afferents to Thalamostriatal Neurons: Sources of Basal Ganglia-Thalamostriatal Loops

The internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) are the two main sources of synaptic inputs to thalamostriatal neurons in CM/Pf (Sidibe et al., 2002b) (see also Chapter 1). In monkeys, this projection system mostly originates from axon collaterals of the basal ganglia-thalamocortical system that terminate in the ventrobasal nuclear group (Parent and Hazrati, 1995; Sidibe et al., 2002b; Smith et al., 2004, 2009). In turn, the CM/Pf sends highly topographic and specific inputs to different functional territories of the striatum (Groenewegen and Berendse, 1994; Sidibe and Smith, 1996; Sidibe et al., 1997; Smith et al., 2004, 2009), forming segregated basal ganglia-thalamostriatal loops that involve functionally related areas of the striatum, pallidum (and SNr) and CM/Pf (Smith et al., 2004, 2009) (Fig. 22.3).

Other subcortical loops that involve projections from the superior colliculus to the rostral or caudal intralaminar nuclei, as well as the lateral posterior nucleus and pulvinar have also been suggested as routes for transmission of subcortical sensorimotor information to the striatum that may act in concert with the basal ganglia-thalamocortical system to regulate the mechanism of action selection (McHaffie et al., 2005) (see also Chapter 23). Various brainstem, cerebellar, and spinal cord nuclei, as well as the amygdala, superior colliculus, and pretectal nuclei also contribute to the innervation of rostral and caudal intralaminar nuclei (McHaffie et al., 2005). Ascending cholinergic and monoaminergic inputs from the pedunculopontine nucleus, raphe nuclei, and locus coeruleus have also been established. Projections from the pedunculopontine nucleus are mainly directed toward Pf and display a high degree of chemical heterogeneity using acetylcholine, GABA, and glutamate as co-existing neurotransmitters (Sidibe et al., 2002a) (see Chapter 23). The reticular formation (RF) also provides massive inputs to anterior and posterior intralaminar nuclei. By virtue of these strong associations with the RF, the intralaminar nuclei are traditionally seen as part of the "reticular activating system" that regulates the mechanisms of cortical arousal and attention (Kinomura et al., 1996; Smith et al., 2004, 2009; Minamimoto et al., 2009).

Through the use of the transynaptic retrograde transport of rabies viruses, Strick and colleagues recently showed that thalamostriatal projections from VA/VL and rostral intralaminar nuclei are paths through which cerebellothalamic information from the dentate nucleus reaches the striatum (Hoshi et al., 2005), thereby providing a mechanism for cerebellar outflow to influence striatal processing of motor and non-motor information. Disruption of these connections may underlie some aspects of basal ganglia and cerebellar dysfunctions observed in dystonia (Jinnah et al., 2005; Neychev et al., 2008).



**FIGURE 22.3** Segregated basal ganglia-thalamostriatal loops through the caudal intralaminar (left) and ventral motor (right, shaded) thalamic nuclei in monkeys. The black arrows indicate GABAergic projections, white arrows indicate glutamatergic pathways. The skeletomotor (ventrolateral 2/3) GPi projects exclusively to the CM, whereas the limbic (rostromedial pole) and associative/cognitive (dorsal third) GPi innervate the rostral and the dorsolateral Pf, (Pfdl), respectively. In turn, CM/Pf neurons project back to the corresponding functional territories of the dorsal and ventral striatum. The substantia nigra pars reticulata (SNr) innervates Pf neurons that project to the caudate nucleus, which provides an additional "associative" circuit between basal ganglia and thalamostriatal neurons. A basal ganglia-ventral motor thalamostriatal loop that involves specific regions of the ventral anterior/ventral lateral (VA/VL) nuclear complex, the post-commissural putamen and the ventrolateral 2/3 of GPi has been described in monkeys. (See McFarland and Haber, 2000; Sidibe and Smith, 2002b, for more details.)

# III. SYNAPTIC ORGANIZATION OF THALAMOSTRIATAL SYSTEMS

#### A. Synaptic Organization of CM/Pf Projections to the Striatum

The microcircuitry of the thalamostriatal projection from the CM/Pf has been studied in detail in rodents and nonhuman primates. Overall, there is a consensus across these data that a majority of CM and Pf terminals target preferentially dendritic shafts of medium spiny projection neurons and interneurons in the striatum (Fig. 22.2) (Dube et al., 1988; Sadikot et al., 1992b; Sidibe and Smith, 1996; Sidibe and Smith, 1999; Smith et al., 2004, 2009; Raju et al., 2006). It is worth noting that a subset of Pf neurons in rats preferentially target dendritic spines, but the existence of such neurons remains to be established in other species (Lacey et al., 2007).

Although both "direct" and "indirect" striatal output neurons (see Chapter 1) receive CM/Pf inputs, tracing studies have suggested that CM projections innervate preferentially direct striato-GPi neurons in monkeys (Sidibe and Smith, 1996; Smith et al., 2004, 2009). However, this does not rule out the possibility of CM/Pf regulation of indirect pathway neurons. In fact, lesion of Pf in a rat model of Parkinson's disease reduces the Parkinson's disease-related abnormal increase in enkephalin mRNA in indirect pathway neurons, but has no significant effect on the decreased substance P mRNA level in direct pathway neurons (Bacci et al., 2004; Kerkerian-Le Goff et al., 2009). Whether this represents a species difference between rodents and monkeys, a differential pattern of synaptic organization of CM versus Pf inputs onto the two populations of striatofugal neurons or a genuine regulatory influence of Pf upon striatal output neurons that cannot be predicted by the mere prevalence of direct Pf inputs onto direct or indirect pathway neurons remains to be established.

In primates, all striatal interneurons (see Chapter 8), except those that express calretinin, are directly contacted by CM terminals (Sidibe and Smith, 1999; Smith et al., 2004, 2009). The cholinergic interneurons (see Chapter 7) appear to be the most densely innervated by the rat Pf or the monkey CM (Meredith and Wouterlood, 1990; Lapper and Bolam, 1992; Sidibe and Smith, 1999; Smith et al., 2004, 2009). However, despite significant monosynaptic innervation from the caudal intralaminar nuclei, CM stimulation results predominantly in reduced activity of tonically active neurons (TANs; likely corresponding to cholinergic interneurons) and decreased acetylcholine release in the rat and monkey striatum (Zackheim and Abercrombie, 2005; Nanda et al., 2009, see below). It is also worth noting that CM plays a critical role in regulating the pattern of physiological responses of TANs to reward (Minamimoto and Kimura, 2002).

# **B.** Synaptic Organization of non-CM/Pf Thalamostriatal Projections

In the rat and monkey striatum, vesicular glutamate transporters 1 and 2 (vGluT1 and vGluT2) immunoreactivity is almost completely segregated between corticostriatal and thalamostriatal terminals, respectively, thereby providing unique tools to study the synaptology of the two main glutamatergic afferents to the mammalian striatum (Lacey et al., 2005; Raju et al., 2005, 2006, 2008). Taking advantage of these new markers, recent studies have shown that as much as 50-70% of vGluT2-positive thalamic terminals in the rat and monkey striatum form asymmetric synapses with spines, while the remaining contact dendritic shafts (Lacey et al., 2005; Raju et al., 2006, 2008), a pattern strikingly different from thalamic inputs originating from CM/Pf which, for the most, form axo-dendritic synapses (Dube et al., 1988; Sadikot et al., 1992a, Raju et al., 2006). Anterograde labeling studies of thalamostriatal projections from various thalamic nuclei including the centrolateral, mediordorsal, VA/VL, lateroposterior, anteroventral and laterodorsal nuclei, indeed, demonstrated that more than 95% of these thalamic afferents target dendritic spines, a pattern similar to that found for vGluT1-containing corticostriatal afferents (Raju et al., 2006).

There is a significant difference in the ratio of axodendritic vs. axo-spinous asymmetric synapses formed by vGluT2-containing thalamostriatal terminals between the patch and matrix striatal compartments in rats (see Chapter 1); although as much as 90% vGluT2-positive boutons target spines in patches, only 50–70% do so in the matrix (Fujiyama et al., 2006; Raju et al., 2006). This difference can be explained by the fact that vGluT2-positive terminals from Pf which, for the most part, form axodendritic synapses arborize preferentially in the matrix compartment (Herkenham and Pert, 1981; Sadikot et al., 1992b; Smith et al., 2004, 2009).

In addition to a differential pattern of synaptic interactions with striatal neurons, thalamic inputs from CM/Pf and other thalamic nuclei also differ in their relationships with dopaminergic afferents, the main modulator of striatal glutamatergic transmission. Thalamic terminals from nuclei other than CM/Pf are often closely apposed to dopaminergic boutons on the surface of dendritic spines, thereby suggesting tight dopaminergic modulation of glutamatergic thalamostriatal transmission at these synapses, a pattern reminiscent of the functional relationship between dopaminergic and cortical glutamatergic inputs to the striatum (Smith and Bolam, 1990; Moss and Bolam, 2008). However, the situation may be different for axo-dendritic CM/Pf inputs because these terminals do not display any particular relationships with dopaminergic afferents on dendrites of striatal neurons (Smith et al., 1994).

Thus, the thalamostriatal systems comprise two major sets of afferents based on their pattern of synaptic interactions with striatal neurons and relationships with dopaminergic afferents: (1) The CM/Pf projections that target preferentially the dendrites of striatal projection neurons and interneurons without any specific relationships with dopaminergic afferents; and (2) The projections from other thalamic nuclei that terminate almost exclusively onto spines of striatal projection neurons and display close relationships with dopaminergic afferents, reminiscent of the synaptic interactions described between dopaminergic afferents and cortical glutamatergic inputs.

## C. Plasticity of the Synaptic Connectivity of the Thalamostriatal and Corticostriatal Systems in Parkinsonism

There is a dramatic loss of dendritic spines on striatal projection neurons in Parkinson's disease patients and dopamine-depleted animal models of parkinsonism (Ingham et al., 1988, 1989, 1998; Meshul et al., 2000; Stephens et al., 2005; Day et al., 2006; Villalba et al., 2009) (see Chapters 6 and 35). This loss of spines results in significant morphological changes consistent with increased synaptic activity of remaining glutamatergic axo-spinous synapses formed by thalamic and cortical terminals in the striatum of MPTPtreated parkinsonian monkeys (Villalba et al., 2008, 2009b). Quantitative measurements have demonstrated a significant increase in the relative abundance of vGluT1 immunoreactivity in the striatum of MPTP-treated monkeys (Raju et al., 2008) and humans with Parkinson's disease (Kashani et al., 2007), while no significant change in the prevalence of vGluT2-positive terminals was found in MPTP-treated monkeys (Raju et al., 2008). These observations are surprising in light of significant spine loss in the striatum of MPTP-treated parkinsonian monkeys (Villalba et al., 2009), 6-OHDA-treated rats (Ingham et al., 1989, 1998) and Parkinson's disease patients (Stephens et al., 2005). However, because vGluT1-positive terminals undergo major ultrastructural changes characterized by an increased volume and increased incidence of perforated synapses in parkinsonian condition (Ingham et al., 1998; Meshul et al.,

2000; Villalba et al., 2008, 2009b), the higher level of vGluT1 immunoreactivity measured in the parkinsonian striatum does not necessarily imply an increase in the total number of vGluT1-immunoreactive terminals. Unbiased stereological measurements of the total number of vGluT1immunoreactive terminals are needed to directly address this issue. Although no clear change in the relative abundance of vGluT2-positive terminals was found in the striatum of parkinsonian monkeys, the ratio of axo-spinous to axo-dendritic synapses was substantially increased in the post-commissural putamen of these animals (Raju et al., 2008). This change may result from cell loss in CM and related neurodegeneration of the CM-striatal system, a pathological feature of Parkinson's disease in humans (see below). Another striking change about vGluT2-positive terminals in the parkinsonian striatum is the increased incidence of terminals forming multiple synapses with different dendritic spines (Villalba et al., 2009b), providing a mechanism for a higher degree of divergence of thalamostriatal information from non-CM/Pf nuclei in parkinsonism.

Although the functional significance of these major changes in the microcircuitry of glutamatergic projections to the striatum in parkinsonian condition have not been related to specific functional consequences, they are most likely involved in the increased synaptic strength and changes in long-term plasticity of the corticostriatal system described in animal models of Parkinson's disease (Chen et al., 2001; Calabresi et al., 2007; Liang et al., 2008) (see also Chapters 12, 35 and 37).

## IV. PHYSIOLOGY OF CM/Pf NEURONS AND RELATED THALAMOSTRIATAL PROJECTIONS

# A. Functional Characteristics of CM/Pf Neurons

Although our knowledge of the anatomy of the thalamostriatal system has grown significantly in the past decades, much less is known about the function of this system. However, the recent work of Kimura and colleagues has started shedding light on this issue and provided some important information on the physiological properties of CM/Pf neurons in monkeys. These authors proposed that CM/Pf neurons supply the striatum with information that have attentional values, acting as detectors of behaviorally significant events occurring on the contralateral side (Minamimoto and Kimura, 2002, Kimura et al., 2004; Smith et al., 2004; Minamimoto et al., 2005, 2009). These observations are consistent with functional imaging data in humans showing increased activity of the CM/Pf complex in response to attention-demanding reaction-time tasks (Kinomura et al., 1996). Two main functional characteristics have been proposed for CM/Pf neurons in monkeys. First, neurons have multimodal properties allowing them to respond to a large variety of sensory stimuli (auditory, visual, somatosensory) presented in or outside the sensorimotor conditioning tasks. Second, CM/Pf neurons are temporally tuned, i.e., they can generate in a timely fashioned manner discrete responses to a wide variety of sensory stimuli (Minamimoto and Kimura, 2002; Minamimoto et al., 2005, 2009).

Two groups of CM/Pf neurons have been identified based of their latency and pattern of responses to sensory stimuli; the short-latency facilitatory responses (SLF) neurons are mainly found in Pf, while the long-latency facilitatory responses (LLF) neurons are particularly abundant in CM (Minamimoto and Kimura, 2002; Kimura et al., 2004; Minamimoto et al., 2005, 2009). Neither SLF nor LLF neurons respond to expected reward. In contrast, their magnitude of responses is larger when the stimulus is unpredictable or different from expectations (Minamimoto et al., 2005). For instance, many CM neurons increase their firing in response to small-reward actions when a largereward option is anticipated (Minamimoto and Kimura, 2002). This pattern is different from that of tonically active neurons (TANs; putative striatal cholinergic interneurons), one of their main targets of CM inputs to the striatum (see above), which under the same experimental conditions, respond preferentially to rewarding stimuli (Aosaki et al., 1994; Graybiel et al., 1994; Matsumoto et al., 2001; Cragg, 2006). Despite this differential response between TANs and CM/Pf neurons to rewarding stimuli, CM/Pf inputs are required for the expression of the sensory responses of TANs acquired through sensorimotor learning (Matsumoto et al., 2001; Kimura et al., 2004; Minamimoto et al., 2009). Inactivation of the CM/Pf, indeed, decreases the characteristic pause and subsequent rebound facilitation, but does not affect the early short latency facilitation, of TANs in response to sensorimotor conditioning in monkeys (Matsumoto et al., 2001; Kimura et al., 2004; Minamimoto et al., 2009). Taken into consideration the importance of the dopaminergic system in modulating striatal activity through TANs, one may suggest that the behavioral events transmitted along the thalamostriatal projection from

Dual thalamostriatal systems			
<ul> <li>Neurons have reticular dendrites</li> <li>Innervate preferentially the striatum with collaterals to cortex</li> <li>Focal highly convergent sites of termination in the striatum</li> <li>Form axo-dendritic synapses (75%)</li> <li>Do not display any relationships with dopaminergic afferents</li> </ul>	<ul> <li>Neurons have bushy-like dendrites</li> <li>Innervate preferentially the cortex with collaterals to striatum</li> <li>Diffuse less convergent innervation of the striatum</li> <li>Form axo-spinous synapses (&gt;95%)</li> <li>Converge with dopaminergic inputs onto common dendritic spinoes</li> </ul>		
• Discharge single spikes during cortical slow-wave activity	<ul> <li>Discharge low-threshold calcium bursts during cortical slow- wave activity</li> </ul>		
<ul> <li>Sensitive to attention-related multisensory information</li> <li>Provide the striatum with attention-related information from brainstem?</li> <li>Key components of sub-cortical loops with basal ganglia and brainstem (superior colliculus, PPN etc)</li> <li>Partly degenerate in Parkinson's disease</li> </ul>	<ul> <li>Respond to specific modalities (sensory, motor, limbic, etc)</li> <li>Provide the striatum with context-dependent functionally-related cortical information</li> <li>Key components of basal ganglia thalamocorticothalamic loops</li> <li>Do not degenerate in Parkinson's disease</li> </ul>		

**TABLE 22.1** Summary of key differences between the thalamostriatal systems that originate from CM/Pf versus other thalamic nuclei

CM/Pf, in coordination with the motivational value of the dopamine inputs, provide a strong basis for proper selection of actions through the basal ganglia thalamocortical/striatal circuitry (Matsumoto et al., 2001; Kimura et al., 2004). Therefore, based on their strong physiological responses to unanticipated small-reward action, CM/Pf neurons may complement decision and action bias through the thalamostriatal system and basal ganglia- thalamocortical functional loops (Kimura et al., 2004; Smith et al., 2004, 2009; Minamimoto et al., 2005, 2009; Cragg, 2006).

### **B.** Physiological Effects of CM/Pf Activation Upon Striatal Neurons

The manner in which attentional and reward-related information is relayed to the striatum by the CM/PF, and the impact of CM/PF activation on striatal physiology remain poorly understood. In early studies, carried out in anesthetized cats and rats, electrical stimulation of the intralaminar complex was found to induce short-latency excitatory responses, often followed by inhibitory postsynaptic potentials of longer latency in striatal neurons (Kitai et al., 1976; Kocsis et al., 1977; Vandermaelen and Kitai, 1980; Wilson et al., 1983, 1990) of which some were electrophysiologically characterized as spiny projection neurons (MSNs) (Wilson et al., 1983) (see also Chapter 5), or aspiny cholinergic interneurons (likely TANs) (Wilson et al., 1990) (see Chapter 7). The initial excitatory responses were considered as being monosynaptically mediated through direct activation of the thalamostriatal system, while the later inhibitory components were interpreted as polysynaptic responses.

More recently, the physiology of thalamostriatal projections has been studied in sagittal rat brain slices that preserve part of the cortical and thalamic inputs to the striatum (Smeal et al., 2007, 2008; Ding et al., 2008). Recordings in this preparation have suggested differences in short- and long-term plasticity, and probability of glutamate release between cortical and thalamic inputs to MSNs (Smeal et al., 2007; Ding et al., 2008). In addition, thalamic, but not cortical stimulation, can generate burst-pause pattern of activity in cholinergic interneurons, which differentially gates corticostriatal signaling through striatopallidal and striatonigral pathways (Ding et al., 2008). Synapse-specific differences in NMDA receptor content and pharmacology between corticostriatal and thalamostriatal synapses have also been proposed (Smeal et al., 2008). The main caveat of these in vitro studies is the limited knowledge about the exact origin(s) of thalamic inputs recruited in these stimulations.

We recently undertook a series of in vivo experiments in awake monkeys to study the electrophysiological responses of striatal MSNs and TANs to electrical stimulation of CM (Nanda et al., 2009). Various interesting features characterize striatal responses to these stimulations. Most striatal projection neurons and cholinergic interneurons respond to prolonged trains of electrical stimuli in CM, but not to single pulse stimulation of the nucleus

(Nanda et al., 2009). Striatal phasically active neurons (PANs, which likely correspond to MSNs) often present increases in firing, whereas many TANs decrease their firing in response to CM stimulation (Nanda et al., 2009). However, a large proportion of both neuronal subtypes show combinations of increases and decreases in firing following CM activation (Nanda et al., 2009). These complex response patterns are probably shaped by direct CM inputs and by secondary processing of the thalamostriatal inputs through intrastriatal GABAergic or cholinergic inputs (see above); suggesting the possibility that CM inputs may have widespread effects on striatal activity through the activation of inhibitory striatal microcircuits. Microdialysis studies have shown that chemical or electrical activation of the intralaminar nuclei, indeed, reduces striatal acetylcholine levels, probably through activation of intrastriatal GABAergic circuits (Zackheim and Abercrombie, 2005; Nanda et al., 2009).

### V. EXTRASTRIATAL BASAL GANGLIA TARGETS OF CM/PF

The CM/Pf also innervates basal ganglia nuclei other than the striatum including the subthalamic nucleus (STN) (see Chapter 15), the globus pallidus (GP) (see Chapter 13) and the substantia nigra (Sadikot et al., 1992b; Feger et al., 1994; Deschenes et al., 1996a,b; Marini et al., 1999; Vercelli et al., 2003; Lanciego et al., 2004, 2009; Smith et al., 2004; Yasukawa et al., 2004). In primates, the thalamosubthalamic projection is functionally organized, so that sensorimotor neurons in CMm terminate preferentially in the "motor-related" dorsolateral part of the STN, whereas limbic and associative neurons in Pf project almost exclusively to the medial "limbic-related" region of the STN (Sadikot et al., 1992a). A similar topographical organization was also found for the Pf-STN projection in rats (Lanciego et al., 2004). Thalamostriatal and thalamosubthalamic projections partly originate from the same Pf neurons, though the exact degree of axonal collateralization remains controversial (Feger et al., 1994; Deschenes et al., 1996a). The thalamosubthalamic input is excitatory and tonically drives the activity of STN neurons (Hirsch et al., 2000). This excitatory thalamic drive may be critical in mediating some of the pathophysiological effects of STN neurons in Parkinson's disease (Hirsch et al., 2000). In rats, the thalamopallidal projection arises predominantly from axon collaterals of thalamostriatal axons from Pf, though rostral intralaminar nuclei also provide significant pallidal inputs (Deschenes et al., 1996a,b; Yasukawa et al., 2004). This projection is highly topographic and supplies functionally segregated information to the associative, sensorimotor and limbic parts of the rat and monkey GP (Sadikot et al., 1992a, Smith et al., 2004; Yasukawa et al., 2004).

Thus, in addition to their massive thalamostriatal projection, CM/Pf neurons most likely contribute to the regulation of basal ganglia circuits via extrastriatal projections to the STN and GP. These non-striatal projections may play an important role in the dysregulation of the firing rate of STN neurons in parkinsonism.

## VI. PATHOPHYSIOLOGY OF CM/PF NEURONS IN PARKINSON'S DISEASE AND RELATED DISORDERS

There is significant degeneration of CM/Pf neurons in patients with progressive supranuclear palsy, Huntington's disease, Lewy body diseases and Parkinson's disease (Heinsen et al., 1996; Henderson et al., 2000a,b; Brooks and Halliday, 2009). In Parkinson's disease, parvalbuminimmunoreactive neurons are most affected in Pf, while non-parvalbumin/non-calbindin neurons are specifically targeted in CM (Henderson et al., 2000b). Recent imaging data reported significant changes in the shape, but not the volume, of thalami between parkinsonian patients and controls (McKeown et al., 2008). The loss of Pf neurons in rat and mice models of Parkinson's disease remains controversial (Freyaldenhoven et al., 1997; Henderson et al., 2005; Aymerich et al., 2006). In MPTP-treated monkeys, a significant reduction in the ratio of axo-dendritic vs. axo-spinous synapses was found in the putamen, thereby raising the possibility of a decreased prevalence of vGluT2-containing terminals from CM forming axo-dendritic synapses in the striatum of parkinsonian monkeys (Raju et al., 2008).

GABA levels are significantly reduced in postmortem CM/Pf tissue of parkinsonian patients (Gerlach et al., 1996). There is a significant correlation between CM/Pf neuronal activity and rest tremor or voluntary movements in parkinsonian patients, consistent with strong ascending proprioceptive inputs to CM/Pf from brainstem and spinal cord (Apkarian and Hodge, 1989). Pf firing rates are transiently decreased in anesthetized dopamine-depleted rats (Ni et al., 2000), while small changes in glucose utilization have been reported in the CM/Pf of MPTP-treated monkeys (Palombo et al., 1990).

## VII. NEUROSURGICAL CM/PF INTERVENTIONS FOR MOVEMENT DISORDERS

Although the physiologic properties of the caudal intralaminar nuclei and their projections remain poorly characterized, these nuclei have been used as targets for surgical interventions, aimed at treating pain, seizures, impairments of consciousness, or movement disorders. A complete review of these surgical approaches is beyond the scope of this chapter. We will focus our discussion on the current experience with the use of neurosurgical procedures as treatment of movement disorders, because this use is most easily linked to the connections between CM/PF and the basal ganglia.

During several decades of neurosurgical practice, two major applications for CM/Pf interventions for movement disorders have emerged: the treatment of the symptoms of Tourette's syndrome, and the treatment of some aspects of Parkinson's disease. Similar to the use of functional neurosurgery aimed at other brain targets, ablative treatments predated the current use of DBS (see also Chapter 39).

#### A. Ablative Surgeries of CM/Pf

The documentation of ablative surgical interventions at the level of the intralaminar thalamic nuclei is rudimentary. In the 1960s, Hassler and Dieckmann carried out studies of the effects of bilateral lesions of the intralaminar and medial thalamic nuclei, as well as the nucleus ventro-oralis internus (Voi) in patients with Tourette's syndrome (Hassler and Dieckmann, 1970). While methodological details are lacking, impressive reductions in tic frequency were reported. Most of these patients (uni- or bilaterally operated) experienced significant improvement in tics. Other authors who targeted the same thalamic regions as those used in these early studies also reported temporary tic improvement and some reduction of compulsive symptoms in one patient (de Divitiis et al., 1977).

The effects of CM/Pf lesions in other movement disorders (such as parkinsonism) have not been extensively studied. However, a recent study has shown that a unilateral lesion of CM does not have strong antiparkinsonian effects in MPTP-treated monkeys (Lanciego et al., 2008).

## B. CM/Pf Deep-Brain Stimulation and Tourette's Syndrome

As with most functional neurosurgical approaches to movement disorders, ablative procedure have been largely replaced by electrical stimulation approaches which offer the promise of reversibility and adjustability (see Chapter 39). Early investigations on the use of stimulation of CM/Pf against movement disorders were carried out in patients that were enrolled in pain treatment studies, but also suffered from movement disorders. Observations in such patients were communicated in short reports that described cases in whom CM/Pf stimulation eliminated both pain and various forms of hyperkinetic movement disorders (Andy, 1980; Krauss et al., 2002). Although details of the stimulation conditions and the effect duration are not provided, it seems that in all of these cases there were significant reductions in the movement disorder components of the patient's disorder.

Currently, the primary indication of CM/Pf DBS is the treatment of symptoms of Tourette's syndrome, a disorder characterized by the occurrence of motor and vocal tics, usually starting in the pre-teen and teenage years, and often combined with obsessive-compulsive symptoms. Our understanding of the pathophysiology of Tourette's syndrome remains rudimentary, although imaging studies have suggested that trans-basal ganglia pathways may be involved (Albin and Mink, 2006; Mink, 2006). Given the clinical features of the disorder, it is likely that motor- and non-motor circuits are affected. In most cases, the severity of the disorder wanes after reaching its peak in the late teenage years, and the symptoms are adequately controlled with medications such as dopamine receptor blocking agents or catecholamine depleting medications. However, in some cases, tics persist into the adult years, and are accompanied by self-injurious behaviors and severe obsessive-compulsive symptoms which often do not respond to medications alone. If other etiologies of tics and psychiatric symptoms are excluded (Mink et al., 2006), such patients may be appropriate candidates for functional neurosurgery.

Following early investigations of CM/Pf DBS in Tourette's syndrome patients (Visser-Vandewalle et al., 2003), most of the available studies have used the stereotactic CM/Pf target that had previously been used by Hassler and Dieckman (see above). In order to avoid penetration of the ventricle, the DBS lead trajectory generally followed an anterior laterodorsal-toposterior-medioventral path (i.e., it is tilted laterally from the parasagittal plane, and anteriorly from the coronal plane). The most ventral DBS contacts were placed into the lateral and central CM. An exception to this approach was the case report by Houeto et al. (2005), in which the CM/Pf lead was targeted mainly at the Pf nucleus instead.

The total number of patients treated with CM/Pf DBS remains very small. Case reports and small case series of these interventions (Visser-Vandewalle et al, 2003, 2004, 2006; Temel and Visser-Vanderwalle, 2004; Houeto et al., 2005; Ackermans et al., 2006, 2008; Bajwa et al., 2007; Maciunas et al., 2007; Riley et al., 2007; Servello et al., 2008; Shields et al., 2008) have, however, documented impressive reductions in tic frequency and severity, perhaps with greater effectiveness against motor than vocal tics (Houeto et al., 2005). In one study, unilateral and bilateral stimulations were contrasted (in patients that were bilaterally implanted). In these cases, bilateral stimulation appears to be more effective than unilateral DBS (Maciunas et al., 2007). In an attempt to counter the obvious concern that some of the reported effects are due to placebo effects, several of these studies have included trials of "sham stimu lation" (Houeto et al., 2005; Servello et al., 2008), demonstrating greater symptom severity in the sham stimulated than in the effectively stimulated patients. The time course of tic improvement seen after CM/Pf interventions differs greatly between patients. While some patients experience immediate anti-tic effects (Visser-Vandewalle et al., 2003; Maciunas et al., 2007), others described a more protracted time course (Maciunas et al., 2007; Servello et al., 2008). Immediate effects may, in part, be due to micro-lesion effects, although such differences in time course may also hint at differences in patients or electrode location.

In addition to the motor symptoms of the disorder, CM/Pf DBS also effectively treats some of the psychiatric components of Tourette's syndrome. Thus, measures of obsessive-compulsive behaviors and anxiety are significantly reduced in most of the implanted patients (Houeto et al., 2005; Mink et al., 2006; Visser-Vanderwalle et al., 2006).

Bilateral lead placement at the CM/Pf target is a relatively safe procedure. Most reports do not state surgical complications, and only one study mentions the development of a small hematoma at the DBS electrode tip, resulting in transient vertical gaze palsy (Ackermans et al., 2007). However, several studies have reported side effects of electrical stimulation at this site, with frequent reports of subjective sensations of stimulation-induced dizziness, vertigo or lack of energy (Visser-Vanderwalle et al., 2006; Servello et al., 2008), and other reports of oculomotor abnormalities (Taylor et al., 2000; Servello et al., 2008), weight loss (Houeto et al., 2005), changes in sexual functions (Temel et al., 2004), and mild dysarthria. In addition, the fact that many Tourette's syndrome patients have obsessive-compulsive symptoms may result in significant postoperative complications. There are, indeed, several reports of lead failure and wound healing problems as patients repeatedly manipulate the surgical site or pick at subcutaneous device locations (Visser-Vandewalle et al., 2003; Servello et al., 2008). Several investigators reported that close supervision and repeated programming adjustments are essential in ensuring beneficial outcomes in these patients (Maciunas et al., 2007; Servello et al., 2008).

# C. CM/Pf Deep-Brain Stimulation and Parkinson's Disease

CM/Pf DBS has also been used in a small number of patients with Parkinson's disease. Thus, in a comparative study of the experience of two French groups, it was noted that placement of thalamic DBS leads (aimed at the ventral intermediate nucleus (Vim)) in patients with severe parkinsonian tremor seemed to have additional antidyskinetic effects if the trajectory of the DBS electrode (and presumably the stimulation) involved the caudal intralaminar complex (Caparros-Lefebvre et al., 1999). In subsequent studies, Mazzone et al. combined DBS of the GPi with DBS of CM/PF and found that stimulation at the CM/PF site may be a useful treatment for freezing of gait (Stefani et al., 2006). This finding is significant, as freezing of gait is a very significant clinical problem in patients with advanced Parkinson's disease that is not satisfactorily treated with either medications or conventional DBS approaches, directed at subthalamic and pallidal targets. More recent studies of the utility of CM/PF DBS in parkinsonism have suggested that this approach may also be useful against parkinsonian tremor (Peppe et al., 2008; Stefani et al., 2009), perhaps similar to the single earlier tremor case reported by Krauss et al. (2002).

Both in patients with Parkinson's disease and in those with Tourette's syndrome, the optimal surgical target(s) and electrode configuration remain unclear. It is likely that the optimal DBS target is not the same in these disorders. For instance, Tourette's syndrome patients, suffering from motor and psychiatric symptoms, may benefit from DBS lead placement that permits stimulation of CM and Pf, so that motor (CM) and non-motor (Pf) regions of the nucleus can be reached. In contrast, patients with parkinsonism may benefit more from localized stimulation of CM, which would predominately affect the putamen (i.e., the striatal motor territory), and may limit non-motor side effects of the procedure. Summarizing the experience with functional neurosurgery at the CM/Pf target, there is relatively good empiric evidence that such interventions help patients with Tourette's syndrome, and perhaps also some individuals with specific parkinsonian symptoms (such as freezing of gait or severe tremor). The mechanism of action, and specifics of the optimal surgical approach and DBS stimulation characteristics are not clear. Furthermore, inclusion and exclusion criteria for trials of these interventions are only beginning to emerge for Tourette's syndrome patients, while no formal criteria have yet been developed for trials in patients with Parkinson's disease.

#### **VIII. CONCLUSIONS**

Although the exact functions of the thalamostriatal systems remain poorly understood, the recent knowledge gained about the anatomical and physiological organization of the relationships between the caudal intralaminar nuclei and the striatum has generated significant interest both in basic and clinical basal ganglia research. The high degree of functional specificity of basal ganglia-thalamostriatal loops that flow through the CM/Pf highlight the fact that these nuclei are an integrative part of the basal ganglia circuitry. The significant degeneration of CM/Pf neurons in parkinsonian subjects combined with recent evidence that CM/Pf deep brain stimulation has beneficial symptomatic effects in patients with Parkinson's disease or Tourette's syndrome further demonstrate the importance of the caudal intralaminar nuclear complex in the pathophysiology of basal ganglia disorders. The evidence that the thalamostriatal system does not originate solely from the CM/Pf, but also involves relay, associative and midline thalamic nuclei provides evidence for dual thalamostriatal systems which display significant differences in their anatomical and, most likely, physiological organization. A deeper understanding of the functional properties of thalamostriatal versus corticostriatal neurons in normal and pathological conditions is needed to elucidate the complementary roles these two glutamatergic systems play in the functional organization of the basal ganglia in normal and pathological conditions.

#### ABBREVIATIONS

- AC Anterior commissure
- AV Anteroventral nucleus
- CC Corpus callosum
- CD Caudate nucleus
- CL Centrolateral nucleus

CM/Pf	Centre median/parafascicular nuclei
CMl	Lateral part of the centre median nucleus
CMm	Medial part of the centre median nucleus
GABA	Gamma-aminobutyric acid
GP	Globus pallidus
GPe	Globus pallidus, external segment
GPi	Globus pallidus, internal segment
IC	Internal capsule
LD	Laterodorsal nucleus
LV	Lateral ventricle
MD	Mediodorsal nucleus
M1	Primary motor cortex
NMDA	N-methyl-D-aspartate receptor
Pfdl	Dorsolateral parafascicular nucleus
PUT	Putamen
PV	Paraventricular nucleus of the thalamus
RF	Reticular formation
SNr	Substantia nigra, pars reticulata
STN	Subthalamic nucleus
TANs	Tonically active neurons
Th	Thalamus
VA/VL	Ventral anterior/ventral lateral nuclei
VLo	Ventral lateral nucleus, pars oralis
vGluT1	Vesicular glutamate transporter 1
vGluT2	Vesicular glutamate transporter 2

#### REFERENCES

- Ackermans L, Temel Y, Cath D, et al. (2006) Deep brain stimulation in Tourette's syndrome: two targets? Mov Disord 21:709–713.
- Ackermans L, Temel Y, Bauer NJ, Visser-Vandewalle V (2007) Vertical gaze palsy after thalamic stimulation for Tourette syndrome: case report. Neurosurgery 61:E1100; discussion E1100.
- Ackermans L, Temel Y, Visser-Vandewalle V (2008) Deep brain stimulation in Tourette's Syndrome. Neurotherapeutics 5:339–344.
- Albin RL, Mink JW (2006) Recent advances in Tourette syndrome research. Trends Neurosci 29:175–182.
- Andy OJ (1980) Parafascicular-center median nuclei stimulation for intractable pain and dyskinesia (painful-dyskinesia). Appl Neurophysiol 43:133–144.
- Aosaki T, Graybiel AM, Kimura M (1994) Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. Science 265:412–415.
- Apkarian AV, Hodge CJ (1989) Primate spinothalamic pathways: III. Thalamic terminations of the dorsolateral and ventral spinothalamic pathways. J Comp Neurol 288:493–511.
- Aymerich MS, Barroso-Chinea P, Perez-Manso M, et al. (2006) Consequences of unilateral nigrostriatal denervation on the thalamostriatal pathway in rats. Eur J Neurosci 23:2099–2108.
- Bacci JJ, Kachidian P, Kerkerian-Le Goff L, Salin P (2004) Intralaminar thalamic nuclei lesions: widespread impact on dopamine denervation-

mediated cellular defects in the rat basal ganglia. J Neuropathol Exp Neurol 63:20–31.

- Bajwa RJ, de Lotbiniere AJ, King RA, Jabbari B, Quatrano S, Kunze K, Scahill L, Leckman JF (2007) Deep brain stimulation in Tourette's syndrome. Mov Disord 22:1346–1350.
- Berendse HW, Groenewegen HJ (1990) Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. J Comp Neurol 299:187–228.
- Benabid AL, Chabardes S, Mitrofanis J, Pollak P (2009) Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. Lancet Neurol 8:67–81.
- Brooks D, Halliday GM (2009) Intralaminar nuclei of the thalamus in Lewy body diseases. Brain Res Bull 78:97–104.
- Caparros-Lefebvre D, Blond S, N'Guyen JP, Pollak P, Benabid AL (1999) Chronic deep brain stimulation for movement disorders. Adv Tech Stand Neurosurg 25:61–136; discussion 136–138.
- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopaminemediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Castle M, Aymerich MS, Sanchez-Escobar C, Gonzalo N, Obeso JA, Lanciego JL (2005) Thalamic innervation of the direct and indirect basal ganglia pathways in the rat: Ipsi- and contralateral projections. J Comp Neurol 483:143–153.
- Chen MT, Morales M, Woodward DJ, Hoffer BJ, Janak PH (2001) In vivo extracellular recording of striatal neurons in the awake rat following unilateral 6-hydroxydopamine lesions. Exp Neurol 171:72–83.
- Cowan WM, Powell TP (1956) A study of thalamo-striate relations in the monkey. Brain 79:364–390.
- Cragg SJ (2006) Meaningful silences: how dopamine listens to the ACh pause. Trends Neurosci 29:125–131.
- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- de Divitiis E, D'Errico A, Cerillo A (1977) Stereotactic surgery in Gilles de la Tourette syndrome. Acta Neurochir (Wien): 73.
- Deschenes M, Bourassa J, Parent A (1995) Two different types of thalamic fibers innervate the rat striatum. Brain Res 701:288–292.
- Deschenes M, Bourassa J, Parent A (1996a) Striatal and cortical projections of single neurons from the central lateral thalamic nucleus in the rat. Neuroscience 72:679–687.
- Deschenes M, Bourassa J, Doan VD, Parent A (1996b) A single-cell study of the axonal projections arising from the posterior intralaminar thalamic nuclei in the rat. Eur J Neurosci 8:329–343.
- Ding J, Peterson JD, Surmeier DJ (2008) Corticostriatal and thalamostriatal synapses have distinctive properties. J Neurosci 28:6483–6492.
- Dube L, Smith AD, Bolam JP (1988) Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neostriatum. J Comp Neurol 267:455–471.
- Elena Erro M, Lanciego JL, Gimenez-Amaya JM (2002) Re-examination of the thalamostriatal projections in the rat with retrograde tracers. Neurosci Res 42:45–55.
- Feger J, Bevan M, Crossman AR (1994) The projections from the parafascicular thalamic nucleus to the subthalamic nucleus and the striatum arise from separate neuronal populations: a comparison with the corticostriatal and corticosubthalamic efferents in a retrograde fluorescent double-labelling study. Neuroscience 60:125–132.
- Fenelon G, Francois C, Percheron G, Yelnik J (1991) Topographic distribution of the neurons of the central complex (centre median-parafascicular

complex) and of other thalamic neurons projecting to the striatum in macaques. Neuroscience 45:495–510.

- Francois C, Percheron G, Parent A, Sadikot AF, Fenelon G, Yelnik J (1991) Topography of the projection from the central complex of the thalamus to the sensorimotor striatal territory in monkeys. J Comp Neurol 305:17–34.
- Freyaldenhoven TE, Ali SF, Schmued LC (1997) Systemic administration of MPTP induces thalamic neuronal degeneration in mice. Brain Res 759:9–17.
- Fujiyama F, Unzai T, Nakamura K, Nomura S, Kaneko T (2006) Difference in organization of corticostriatal and thalamostriatal synapses between patch and matrix compartments of rat neostriatum. Eur J Neurosci 24:2813–2824.
- Gerlach M, Gsell W, Kornhuber J, Jellinger K, Krieger V, Pantucek F, Vock R, Riederer P (1996) A post mortem study on neurochemical markers of dopaminergic, GABA-ergic and glutamatergic neurons in basal ganglia-thalamocortical circuits in Parkinson syndrome. Brain Res 741:142–152.
- Gimenez-Amaya JM, McFarland NR, de las Heras S, Haber SN (1995) Organization of thalamic projections to the ventral striatum in the primate. J Comp Neurol 354:127–149.
- Graybiel AM, Aosaki T, Flaherty AW, Kimura M (1994) The basal ganglia and adaptive motor control. Science 265:1826–1831.
- Groenewegen HJ, Berendse HW (1994) The specificity of the "nonspecific" midline and intralaminar thalamic nuclei. Trends Neurosci 17:52–57.
- Haber S, McFarland NR (2001) The place of the thalamus in frontal cortical-basal ganglia circuits. Neuroscientist 7:315–324.
- Haber SN, Calzavara R (2009) The cortico-basal ganglia integrative network: the role of the thalamus. Brain Res Bull 78:69–74.
- Hassler R, Dieckmann G (1970) Stereotactic treatment of different kinds of spasmodic torticollis. Confin Neurol 32:135–143.
- Heinsen H, Rub U, Gangnus D, et al. (1996) Nerve cell loss in the thalamic centromedian-parafascicular complex in patients with Huntington's disease. Acta Neuropathol 91:161–168.
- Henderson JM, Carpenter K, Cartwright H, Halliday GM (2000a) Loss of thalamic intralaminar nuclei in progressive supranuclear palsy and Parkinson's disease: clinical and therapeutic implications. Brain 123 (Pt 7):1410–1421.
- Henderson JM, Carpenter K, Cartwright H, Halliday GM (2000b) Degeneration of the centre median-parafascicular complex in Parkinson's disease. Ann Neurol 47:345–352.
- Henderson JM, Schleimer SB, Allbutt H, Dabholkar V, Abela D, Jovic J, Quinlivan M (2005) Behavioural effects of parafascicular thalamic lesions in an animal model of parkinsonism. Behav Brain Res 162:222–232.
- Herkenham M, Pert CB (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. Nature 291:415–418.
- Hirsch EC, Perier C, Orieux G, et al. (2000) Metabolic effects of nigrostriatal denervation in basal ganglia. Trends Neurosci 23:S78–S85.
- Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL (2005) The cerebellum communicates with the basal ganglia. Nature Neurosci 8:1491–1493.
- Houeto JL, Karachi C, Mallet L, et al. (2005) Tourette's syndrome and deep brain stimulation. J Neurol Neurosurg Psychiatry 76:992–995.
- Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res 503:334–338.

- Ingham CA, Hood SH, Taggart P, Arbuthnott GW (1998) Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18:4732–4743.
- Jinnah HA, Hess EJ, Ledoux MS, Sharma N, Baxter MG, DeLong MR (2005) Rodent models for dystonia research: characteristics, evaluation, and utility. Mov Disorders 20:283–292.
- Kashani A, Betancur C, Giros B, Hirsch E, El Mestikawy S (2007) Altered expression of vesicular glutamate transporters VGLUT1 and VGluT2 in Parkinson disease. Neurobiol Aging 28:568–578.
- Kerkerian-Le Goff L, Bacci JJ, Jouve L, Melon C, Salin P (2009) Impact of surgery targeting the caudal intralaminar thalamic nuclei on the pathophysiological functioning of basal ganglia in a rat model of Parkinson's disease. Brain Res Bull 78:80–84.
- Kimura M, Minamimoto T, Matsumoto N, Hori Y (2004) Monitoring and switching of cortico-basal ganglia loop functions by the thalamostriatal system. Neurosci Res 48:355–360.
- Kinomura S, Larsson J, Gulyas B, Roland PE (1996) Activation by attention of the human reticular formation and thalamic intralaminar nuclei. Science 271:512–515.
- Kitai ST, Kocsis JD, Preston RJ, Sugimori M (1976) Monosynaptic inputs to caudate neurons identified by intracellular injection of horseradish peroxidase. Brain Res 109:601–606.
- Kocsis JD, Sugimori M, Kitai ST (1977) Convergence of excitatory synaptic inputs to caudate spiny neurons. Brain Res 124:403–413.
- Krauss JK, Pohle T, Weigel R, Burgunder JM (2002) Deep brain stimulation of the centre median-parafascicular complex in patients with movement disorders. J Neurol Neurosurg Psychiatry 72:546–548.
- Lacey CJ, Boyes J, Gerlach O, Chen L, Magill PJ, Bolam JP (2005) GABA(B) receptors at glutamatergic synapses in the rat striatum. Neuroscience 136:1083–1095.
- Lacey CJ, Bolam JP, Magill PJ (2007) Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections. J Neurosci 27:4374–4384.
- Lanciego JL, Gonzalo N, Castle M, Sanchez-Escobar C, Aymerich MS, Obeso JA (2004) Thalamic innervation of striatal and subthalamic neurons projecting to the rat entopeduncular nucleus. Eur J Neurosci 19:1267–1277.
- Lanciego JL, Rodriguez-Oroz MC, Blesa FJ, et al. (2008) Lesion of the centromedian thalamic nucleus in MPTP-treated monkeys. Mov Disord 23:708–715.
- Lanciego JL, Lopez IP, Rico AJ, et al. (2009) The search for a role of the caudal intralaminar nuclei in the pathophysiology of Parkinson's disease. Brain Res Bull 78:55–59.
- Lapper SR, Bolam JP (1992) Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. Neuroscience 51:533–545.
- Liang L, DeLong MR, Papa SM (2008) Inversion of dopamine responses in striatal medium spiny neurons and involuntary movements. J Neurosci 28:7537–7547.
- Maciunas RJ, Maddux BN, Riley DE, et al. (2007) Prospective randomized double-blind trial of bilateral thalamic deep brain stimulation in adults with Tourette syndrome. J Neurosurg 107:1004–1014.
- Marini G, Pianca L, Tredici G (1999) Descending projections arising from the parafascicular nucleus in rats: trajectory of fibers, projection pattern and mapping of terminations. Somatosens Mot Res 16:207–222.
- Matsumoto N, Minamimoto T, Graybiel AM, Kimura M (2001) Neurons in the thalamic CM-Pf complex supply striatal neurons with information about behaviorally significant sensory events. J Neurophysiol 85:960–976.

- McFarland NR, Haber SN (2000) Convergent inputs from thalamic motor nuclei and frontal cortical areas to the dorsal striatum in the primate. J Neurosci 20:3798–3813.
- McFarland NR, Haber SN (2001) Organization of thalamostriatal terminals from the ventral motor nuclei in the macaque. J Comp Neurol 429:321–336.
- McFarland NR, Haber SN (2002) Thalamic relay nuclei of the basal ganglia form both reciprocal and nonreciprocal cortical connections, linking multiple frontal cortical areas. J Neurosci 22:8117–8132.
- McHaffie JG, Stanford TR, Stein BE, Coizet V, Redgrave P (2005) Subcortical loops through the basal ganglia. Trends Neurosci 28:401–407.
- McKeown MJ, Uthama A, Abugharbieh R, Palmer S, Lewis M, Huang X (2008) Shape (but not volume) changes in the thalami in Parkinson disease. BMC Neurol 8:8.
- Mengual E, de las Heras S, Erro E, Lanciego JL, Gimenez-Amaya JM (1999) Thalamic interaction between the input and the output systems of the basal ganglia. J Chem Neuroanat 16:187–200.
- Meredith GE, Wouterlood FG (1990) Hippocampal and midline thalamic fibers and terminals in relation to the choline acetyltransferaseimmunoreactive neurons in nucleus accumbens of the rat: a light and electron microscopic study. J Comp Neurol 296:204–221.
- Meshul CK, Cogen JP, Cheng HW, Moore C, Krentz L, McNeill TH (2000) Alterations in rat striatal glutamate synapses following a lesion of the cortico- and/or nigrostriatal pathway. Exp Neurol 165:191–206.
- Minamimoto T, Kimura M (2002) Participation of the thalamic CM-Pf complex in attentional orienting. J Neurophysiol 87:3090–3101.
- Minamimoto T, Hori Y, Kimura M (2005) Complementary process to response bias in the centromedian nucleus of the thalamus. Science 308:1798–1801.
- Minamimoto T, Hori Y, Kimura M (2009) Roles of the thalamic CM-PF complex-Basal ganglia circuit in externally driven rebias of action. Brain Res Bull 78:75–79.
- Mink JW, Walkup J, Frey KA, et al. (2006) Patient selection and assessment recommendations for deep brain stimulation in Tourette syndrome. Mov Disord 21:1831–1838.
- Mink JW (2006) Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. Adv Neurol 99:89–98.
- Moss J, Bolam JP (2008) A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. J Neurosci 28:11221–11230.
- Nanda B, Galvan A, Smith Y, Wichmann T (2009) Effects of stimulation of the centromedian nucleus of the thalamus on the activity of striatal cells in awake rhesus monkeys. Eur J Neurosci 29:588–598.
- Neychev VK, Fan X, Mitev VI, Hess EJ, Jinnah HA (2008) The basal ganglia and cerebellum interact in the expression of dystonic movement. Brain 131:2499–2509.
- Ni ZG, Gao DM, Benabid AL, Benazzouz A (2000) Unilateral lesion of the nigrostriatal pathway induces a transient decrease of firing rate with no change in the firing pattern of neurons of the parafascicular nucleus in the rat. Neuroscience 101:993–999.
- Palombo E, Porrino LJ, Bankiewicz KS, Crane AM, Sokoloff L, Kopin IJ (1990) Local cerebral glucose utilization in monkeys with hemiparkinsonism induced by intracarotid infusion of the neurotoxin MPTP. J Neurosci 10:860–869.
- Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 20:91–127.

- Parent M, Parent A (2005) Single-axon tracing and three-dimensional reconstruction of centre median-parafascicular thalamic neurons in primates. J Comp Neurol 481:127–144.
- Peppe A, Gasbarra A, Stefani A, et al. (2008) Deep brain stimulation of CM/PF of thalamus could be the new elective target for tremor in advanced Parkinson's Disease? Parkinsonism Relat Disord 14:501–504.
- Raju DV, and Y. Smith (2005) Differential localization of vesicular glutamate transporters 1 and 2 in the rat striatum. In The Basal Ganglia VIII. Adv Behav Biology: Vol. 56 pp. 601–610. New York: Plenum Press.
- Raju DV, Shah DJ, Wright TM, Hall RA, Smith Y (2006) Differential synaptology of vGluT2-containing thalamostriatal afferents between the patch and matrix compartments in rats. J Comp Neurol 499:231–243.
- Raju DV, Ahern TH, Shah DJ, Wright TM, Standaert DG, Hall RA, Smith Y (2008) Differential synaptic plasticity of the corticostriatal and thalamostriatal systems in an MPTP-treated monkey model of parkinsonism. Eur J Neurosci 27:1647–1658.
- Riley DE, Whitney CM, Maddux BN, Schoenberg MS, Maciunas RJ (2007) Patient selection and assessment recommendations for deep brain stimulation in Tourette syndrome. Mov Disord 22:1366; author reply 1367–1368.
- Sadikot AF, Parent A, Smith Y, Bolam JP (1992a) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a light and electron microscopic study of the thalamostriatal projection in relation to striatal heterogeneity. J Comp Neurol 320:228–242.
- Sadikot AF, Parent A, Francois C (1992b) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a PHA-L study of subcortical projections. J Comp Neurol 315:137–159.
- Sadikot AF, Rymar VV (2009) The primate centromedian-parafascicular complex: anatomical organization with a note on neuromodulation. Brain Res Bull 78:122–130.
- Servello D, Porta M, Sassi M, Brambilla A, Robertson MM (2008) Deep brain stimulation in 18 patients with severe Gilles de la Tourette syndrome refractory to treatment: the surgery and stimulation. J Neurol Neurosurg Psychiatry 79:136–142.
- Shields DC, Cheng ML, Flaherty AW, Gale JT, Eskandar EN (2008) Microelectrode-guided deep brain stimulation for Tourette syndrome: within-subject comparison of different stimulation sites. Stereotact Funct Neurosurg 86:87–91.
- Sidibe M, Smith Y (1996) Differential synaptic innervation of striatofugal neurones projecting to the internal or external segments of the globus pallidus by thalamic afferents in the squirrel monkey. J Comp Neurol 365:445–465.
- Sidibe M, Bevan MD, Bolam JP, Smith Y (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: I. Topography and synaptic organization of the pallidothalamic projection. J Comp Neurol 382:323–347.
- Sidibe M, Smith Y (1999) Thalamic inputs to striatal interneurons in monkeys: synaptic organization and co-localization of calcium binding proteins. Neuroscience 89:1189–1208.
- Sidibe M, Pare JF, Raju D, Smith Y (2002a). Anatomical and functional relationships between intralaminar thalamic nuclei and basal ganglia in monkeys. In The Basal Ganglia VII: Adv Behav Biology, Vol. 52 pp. 409–420, Kluwer Academic/Plenum Publishers. .
- Sidibe M, Pare JF, Smith Y (2002b) Nigral and pallidal inputs to functionally segregated thalamostriatal neurons in the centromedian/

parafascicular intralaminar nuclear complex in monkey. J Comp Neurol 447:286–299.

- Smeal RM, Gaspar RC, Keefe KA, Wilcox KS (2007) A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents. J Neurosci Methods 159:224–235.
- Smeal RM, Keefe KA, Wilcox KS (2008) Differences in excitatory transmission between thalamic and cortical afferents to single spiny efferent neurons of rat dorsal striatum. Eur J Neurosci 28:2041–2052.
- Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13:259–265.
- Smith Y, Parent A (1986) Differential connections of caudate nucleus and putamen in the squirrel monkey (*Saimiri sciureus*). Neuroscience 18:347–371.
- Smith Y, Bennett BD, Bolam JP, Parent A, Sadikot AF (1994) Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. J Comp Neurol 344:1–19.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Smith Y, Raju D, Nanda B, Pare JF, Galvan A, Wichmann T (2009) The thalamostriatal systems: anatomical and functional organization in normal and parkinsonian states. Brain Res Bull 78:60–68.
- Stefani A, Fedele E, Galati S, et al. (2006) Deep brain stimulation in Parkinson's disease patients: biochemical evidence. J Neural Transm Suppl:401–408.
- Stefani A, Peppe A, Pierantozzi M, Galati S, Moschella V, Stanzione P, Mazzone P (2009) Multi-target strategy for Parkinsonian patients: the role of deep brain stimulation in the centromedian-parafascicularis complex. Brain Res Bull 78:113–118.
- Stephens B, Mueller AJ, Shering AF, et al. (2005) Evidence of a breakdown of corticostriatal connections in Parkinson's disease. Neuroscience 132:741–754.
- Taylor RB, Wennberg RA, Lozano AM, Sharpe JA (2000) Central nystagmus induced by deep-brain stimulation for epilepsy. Epilepsia 41:1637–1641.
- Temel Y, van Lankveld JJ, Boon P, Spincemaille GH, van der Linden C, Visser-Vandewalle V (2004) Deep brain stimulation of the thalamus can influence penile erection. Int J Impot Res 16:91–94.
- Temel Y, Visser-Vandewalle V (2004) Surgery in Tourette syndrome. Mov Disord 19:3–14.
- Vandermaelen CP, Kitai ST (1980) Intracellular analysis of synaptic potentials in rat neostriatum following stimulation of the cerebral cortex, thalamus, and substantia nigra. Brain Res Bull 5:725–733.
- Van der Werf YD, Witter MP, Groenewegen HJ (2002) The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. Brain Res Brain Res Rev 39:107–140.
- Vercelli A, Marini G, Tredici G (2003) Anatomical organization of the telencephalic connections of the parafascicular nucleus in adult and developing rats. Eur J Neurosci 18:275–289.
- Villalba RM, Smith Y (2008) Plasticity of vGluT1-containing axospinous synapses in the striatum of MPTP-treated parkinsonian monkey: A 3D ultrastructural analysis. Soc for Neurosci Abstr 670:7.
- Villalba RM, Smith Y (2009) A comparative ultrastructural analysis of cortiocostriatal and thalamostriatal axo-spinous synapses in control and MPTP-treated parkinsonian monkeys. Soc for Neurosci Abstr 845:4.

- Villalba RM, Lee H, Smith Y (2009) Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. Exp Neurol 215:220–227.
- Visser-Vandewalle V, Temel Y, Boon P, et al. (2003) Chronic bilateral thalamic stimulation: a new therapeutic approach in intractable Tourette syndrome. Report of three cases. J Neurosurg 99:1094–1100.
- Visser-Vandewalle V, Temel Y, van der Linden C, Ackermans L, Beuls E (2004) Deep brain stimulation in movement disorders. The applications reconsidered. Acta Neurol Belg 104:33–36.
- Visser-Vandewalle V, Ackermans L, van der Linden C, et al. (2006) Deep brain stimulation in Gilles de la Tourette's syndrome. Neurosurgery 58:E590.
- Visser-Vandewalle V (2007) DBS in Tourette syndrome: rationale, current status and future prospects. Acta Neurochir Suppl 97:215–222.
- Vogt C, Vogt O (1941) Thalamusstudien I-III. I. Zur Einfurung, II. Homogenitat und Grenzgestaldung der Grisea des Thalamus, III. Das Griseum centrale (centrum medianum Luys). J Psychol Neurol (Leipzig) 50:31–154.

- Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, Tkatch T, Lovinger DM, Surmeier DJ (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50:443–452.
- Wilson CJ, Chang HT, Kitai ST (1983) Origins of post synaptic potentials evoked in spiny neostriatal projection neurons by thalamic stimulation in the rat. Exp Brain Res 51:217–226.
- Wilson CJ, Chang HT, Kitai ST (1990) Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J Neurosci 10:508–519.
- Yasukawa T, Kita T, Xue Y, Kita H (2004) Rat intralaminar thalamic nuclei projections to the globus pallidus: a biotinylated dextran amine anterograde tracing study. J Comp Neurol 471:153–167.
- Zackheim J, Abercrombie ED (2005) Thalamic regulation of striatal acetylcholine efflux is both direct and indirect and qualitatively altered in the dopamine-depleted striatum. Neuroscience 131:423–436.

# Subcortical Connections of the Basal Ganglia

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# I. INTRODUCTION

The connections between cortex, basal ganglia and thalamus in the form of cortico-basal ganglia-thalamo-cortical loops are believed to function for the selection of appropriate movements, actions and goals (Mink, 1996; Redgrave et al., 1999; Haber, 2003; Everitt and Robbins, 2005). However, de-corticated rats seem able to make appropriate selections of simple movements, actions and goals. Decorticate rats can move around their environment, effectively orient to sounds, perform coordinated sequences of actions in order to feed or groom, and can discriminate food types (see Humphries et al., 2007). Indeed, the precortical development of the basal ganglia means that fully

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. functional connections will have been established between the basal ganglia and brainstem structures (see Chapter 2). The abilities of de-corticated rats suggest that the evolutionary development of the cerebral cortex has added to the pre-existing machinery rather than remove or render it wholly ineffective. In order to understand fully how the brain selects appropriate movements, actions and goals we must not simply concentrate on cortical processes and connections, but incorporate into our discussions the influence of subcortical inputs to the basal ganglia and the outputs of basal ganglia to the brainstem. In this chapter, we review the functional connections of the basal ganglia with two subcortical sites that have been relatively well studied – the pedunculopontine tegmental nucleus (PPN) and the superior colliculus (SC) – and attempt to develop a framework of basal ganglia function that includes subcortical connections with the midbrain and brainstem.

First, we will provide a brief overview of the anatomical connections of cortico-basal ganglia-thalamo-cortical loops (see also Chapter 1), drawing parallels with subcortical-thalamus-basal ganglia-subcortical looped circuits. Using the PPN and SC as two subcortical examples, we will consider their anatomical connections with basal ganglia and current hypotheses concerning their functions. We will then describe one influential hypothesis on basal ganglia functioning – that it acts as a general-purpose action selector – and discuss ways in which the PPN and SC functionally interact with it. We will conclude that the PPN and SC are critical parts of the interface between brainstem and basal ganglia processing and are therefore key sites contributing to the selection of appropriate movements, actions and goals.

## II. CORTICAL AND SUBCORTICAL LOOPS THROUGH THE BASAL GANGLIA

Multiple cortico-basal ganglia-thalamus-cortico parallel circuit loops have been implicated in processing emotional, cognitive and motor information (Parent and Hazrati, 1995; Joel and Weiner, 2000; Haber, 2003; Voorn et al., 2004). These three functions are associated with three main loop regions that project to the striatum, the major input station to the basal ganglia. These are: (i) motor loops, implicated in selecting particular movements, have projections from caudal premotor, pre-supplementary motor and cingulate motor cortical neurons to dorsolateral striatum; (ii) cognitive loops, which may be responsible for selecting the ideas associated with planning appropriate actions, have projections from dorsolateral prefrontal cortex to central, dorsal striatum; and (iii) limbic loops, which are thought to participate in selecting appropriate motivational goals, include projections from medial and orbital prefrontal cortex to ventromedial striatum.

This tripartite division of "loop regions" based on function is thought to be maintained throughout the internal circuitry of the basal ganglia (Haber, 2003). However, information can cross from one functional loop to another, both within the intrinsic structures of the basal ganglia and between functionally connected regions external to the basal ganglia. For example, there are feed-forward projections between cortical and thalamic neurons and between striatal and midbrain dopamine (DA) neurons (Joel and Weiner, 2000; Haber, 2003; Ikemoto, 2007). Additionally, many components of the looped architecture are reciprocally connected [e.g., subthalamic nucleus (STN)-globus pallidus (GP)-STN and cortico-thalamo-cortical projections] meaning that information does not simply flow one-way around the loops (see also Chapter 24).

This concept of multiple, parallel, largely-closed loops running through the basal ganglia carrying different sets of functional information has been extended to apply to subcortical afferents of the basal ganglia, such as the PPN, SC, inferior colliculus, periaqueductal grey, cuneiform nucleus, and parabrachial nucleus (McHaffie et al., 2005; Hikosaka, 2007). One way that these nuclei can connect into the basal ganglia is via the thalamus. It has been proposed that these subcortical structures form part of a similar looped architecture as cortico-basal gangliathalamus-cortico loops, conceivably even providing a template for the subcortical-thalamus-basal ganglia-subcortical loop structure (McHaffie et al., 2005; Redgrave and Coizet, 2007). The obvious difference between cortical and subcortical loops is that in the former case the thalamic relay is on the output side of the loop, whereas in the latter it is on the input side (see Fig. 23.1). While the full extent to which subcortical loops represent functionally segregated parallel closed-loops remains to be determined, we nevertheless wish to draw attention to two relatively well studied subcortical structures (PPN and SC) that are heavily connected both with the basal ganglia and with the downstream brainstem sites which generate motor output.

# III. FUNCTIONS OF PEDUNCULOPONTINE TEGMENTAL NUCLEUS AND ITS CONNECTIONS WITH BASAL GANGLIA

#### A. Anatomical Connections

The PPN, situated in the mesopontine tegmentum, is a phylogenetically ancient structure with precortically established



**FIGURE 23.1** A simplified schematic showing the difference between cortical and subcortical loops: in the former case the thalamic relay is on the output side of the loop, whereas in the latter it is on the input side.

functional connections with brainstem and basal ganglia. It is comprised of cholinergic neurons of the Ch5 group (Mesulam et al., 1983) and a mixture of unknown cell types, commonly referred to as non-cholinergic neurons. Many of these non-cholinergic neurons contain gamma-aminobutyric acid (GABA) or glutamate (Charara et al., 1996; Parent et al., 1999; Mena-Segovia et al., 2009; Wang and Morales, 2009) but, as with the cholinergic neurons, it is likely that they have the capacity to synthesize other neurotransmitters as well - PPN neurons are known to express a variety of amino acid and neuropeptide transmitters. The PPN is densely interconnected with a variety of brain areas located at different levels of the neuraxis. Its main inputs originate from the motor cortex and from component nuclei of the basal ganglia, notably the internal segment of the globus pallidus (GPi), STN and substantia nigra pars reticulata (SNr). There is some evidence for direct projections to the PPN from the dorsal striatum (the ventrolateral caudate-putamen especially); the ventral striatum appears to have little connection, although the extended amygdala (with which it is closely associated) does (Zahm et al., 2001). In addition to these cortical and basal ganglia connections there are inputs from the locus coeruleus, raphe nuclei, laterodorsal tegmental nucleus and mamillary bodies. It sends outputs to all thalamic nuclei, basal forebrain, lateral hypothalamus, midbrain DA neurons, SC, all main basal ganglia nuclei, pons, medulla and spinal cord (see Winn, 1998).

The PPN has several main routes through which it is connected with the basal ganglia. These are: (i) projections to striatum via different thalamic nuclei; (ii) projections to striatum via midbrain DA neurons in both the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc); (iii) direct projections to basal ganglia nuclei such as the STN; and (iv) afferents from the main basal ganglia output nuclei, the GPi and SNr. Moreover, the PPN is reciprocally connected to every main basal ganglia nucleus (see Fig. 23.2).

Studies examining immediate early gene expression in the thalamus following PPN stimulation have shown activation of centrolateral, ventrolateral and the thalamic reticular nucleus (Ainge et al., 2004). The two principal nuclei, ventrolateral and centrolateral are functionally related to movement and project to the striatum and cortex whereas the thalamic reticular nucleus has more general functions in controlling thalamic state. Importantly, tract tracing studies have revealed that all PPN neurons appear to project to at least one thalamic nucleus (Oakman et al., 1999) and that overlap exists between thalamic areas receiving projections from the PPN with those thalamic areas that project to the striatum (Erro et al., 1999). Moreover, it has recently been reported that in the rat there are direct contacts between terminals of PPN neurons and cells in lateral posterior thalamus that project to the striatum (Kobayashi et al., 2007). Thus, PPN-thalamus connections seem to fit the template of subcortical-thalamus-basal ganglia-subcortical loop structure proposed by McHaffie and his colleagues (McHaffie et al., 2005). Different sets of neurons in the PPN have the potential to influence different corticostriatal loops throughout all regions of the striatum. Although no differential distribution of projections to thalamic nuclei across anterior-posterior sites in the PPN have been described, anterior portions project more densely to the thalamus than posterior portions (Erro et al., 1999).

There are also indirect projections from PPN cholinergic neurons to striatum via its afferents to midbrain DA neurons. In rodents, a functional gradient has been found whereby cholinergic neurons in anterior PPN project to DA neurons in the SNc (which tend to project to more dorsal



**FIGURE 23.2** Schematic diagram of the reciprocal relationships between the PPN and basal ganglia nuclei, and descending connections to brainstem sites of motor control. Note the striking similarities with the connections of the superior colliculus. Abbreviations: GPe, globus pallidus external segment; GPi, globus pallidus internal segment; PPN, pedunculopontine tegmental nucleus; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus.

sites in the striatum) whereas cholinergic neurons in posterior PPN project predominantly to DA neurons in the VTA (which target the ventral striatum) (Oakman et al., 1995; Mena-Segovia et al., 2008). It is important to note that it is collaterals of thalamus-projecting PPN neurons that are the main sources of innervation of midbrain DA neurons (Oakman et al., 1999). Potentially, therefore, one action potential from a single neuron in PPN could provide two different signals to striatum, via thalamus and VTA/SNc, the functional implications of which will be discussed in Section 6. The PPN has ascending projections to the STN and it receives output from the GPi and SNr. In summary, the PPN is connected with the basal ganglia through a variety of both direct and indirect connections with the thalamus and midbrain DA neurons.

The PPN also projects extensively into the medial reticular formation (mRF) of the brainstem (Humphries et al., 2007). Neurons in the mRF are organized into clusters that are not reflective of a sensory or motor topography. Instead, neuronal activity within a cluster is correlated with the recruitment of disparate types of muscles and movements that are idiosyncratic for a particular action. Therefore it has been hypothesized that, because co-activation of different clusters can trigger a coordinated behavioral response, the mRF is a potential action selection system (Humphries et al., 2007).

Aside from differential outputs by anterior and posterior PPN to midbrain DA neurons, the inputs to anterior and posterior PPN also seem to be different and it is becoming clear that the PPN has an internal anatomical dissociation. Posterior PPN, which contains the majority of the cholinergic neurons, receives fast-relayed sensory information. Transmission of visual, auditory and somatosensory information all appear to impact these neurons, evoking neuronal firing with very short latencies of around 8 msec in the case of imperative auditory signals (Dormont et al., 1998; Pan and Hyland, 2005). Indeed, this short transmission speed suggests that the PPN could be in direct contact from neurons positioned at early stages of auditory processing. In contrast, the anterior PPN appears to be targeted by outflow from both basal ganglia and structures of the extended amygdala (Parent and Hazrati, 1995; Zahm et al., 2001) (though whether these target the same neurons, or the same parts of single neurons is not clear). Therefore, it seems possible that the anterior and posterior PPN are differentially incorporated into the corticostriatal architecture: the posterior PPN appears to be in receipt of fast sensory polymodal data that is then directed at VTA DA neurons. In contrast, the anterior PPN receives descending input from the basal ganglia and extended amygdala and has output to the DA neurons of the SNc.

#### **B.** Functions

The functions of the PPN are still not fully established, perhaps because they are so generalized. Traditionally the PPN has been regarded as being important in behavioral state control and locomotion. The large cholinergic neurons in the PPN - the Ch5 group (Mesulam et al., 1983) – do indeed change their activity in characteristic ways across the sleep-wake cycle, while the idea that the PPN was involved with locomotion came primarily from the belief that it was part of the mesencephalic locomotor region. However, contemporary studies suggest that the PPN is an important member of the basal ganglia family of structures (Mena-Segovia et al., 2004), and that it has functions that go beyond the rather automatic processes with which it has traditionally been associated - even to the extent that it appears to have functions related to learning, memory and attention that could be regarded as cognitive (Winn, 1998; Keating and Winn, 2002; Alderson et al., 2004; Taylor et al., 2004; Winn, 2008). As the tripartite functional territories of the basal ganglia have become more clearly delineated (motivational, cognitive and sensorimotor), it is perhaps only to be expected that manipulations of the PPN have been shown modulate motivational, cognitive and sensorimotor operations principally through its effects on learning.

Following excitotoxic lesions of the PPN basic behavioral patterns such as feeding, drinking, locomotion and grooming remain normal yet deficits are found in tasks that involve associative learning for reinforcement. For example, rats with bilateral excitotoxic lesions of the whole PPN could not complete radial maze tasks that are also challenged by interference within corticostriatal systems (Keating and Winn, 2002; Taylor et al., 2004). Additionally, rats with PPN lesions made fewer lever press responses for food reinforcement than control rats when lesions were made prior to any training (learning required post-lesion) but were able to make these responses at normal rates for amphetamine reinforcement when prior to their lesion they had already learnt the same response contingencies for food reinforcement (less learning required post-lesion) (Alderson et al., 2004).

There is evidence that anterior and posterior PPN are functionally different. Behavioral impairments caused by
damage to either anterior or posterior PPN might be best explained by their differential projections to lateral (SNC) or medial (VTA) midbrain DA neurons, respectively. Thus, lesions of the posterior PPN produced no change in baseline levels of locomotion yet altered rats' locomotor response to nicotine and rates of nicotine self-administration (Alderson et al., 2006, 2008). In contrast, lesions of the anterior PPN had no effect on rats' response to nicotine but significantly reduced baseline levels of locomotor activity (Alderson et al., 2006, 2008). These effects are consistent with the idea that the anterior PPN interacts more closely with SNC DA neurons, which project to central and dorsal striatum, and are functionally related to motor events; and that the posterior PPN interacts with VTA DA neurons, which project throughout striatum, and have functions related to novelty, reward and reinforcement. For example, the reinforcing properties of nicotine, clearly affected by posterior PPN lesions, are strongly linked to VTA DA neurons (Corrigall et al., 1994). Intriguingly, interest in the motor functions associated with the PPN has been revived by studies of Parkinsonian patients: low frequency stimulation – most likely in the anterior parts of the PPN, avoiding the densely packed cholinergic neurons of the posterior PPN (pars compactus) - has benefit in the treatment of posture and gait disturbances in Parkinson's disease (Plaha and Gill, 2005; Stefani et al., 2007).

## IV. FUNCTIONS OF SUPERIOR COLLICULUS AND ITS CONNECTIONS WITH BASAL GANGLIA

### A. Background

Like the PPN, the SC is a phylogenetically ancient subcortical structure with precortically established functional connections with brainstem and basal ganglia. Situated in the roof of the midbrain, the SC is responsible for the sensorimotor transformations required to direct eye and head movements towards or away from unexpected, biologically salient events (Stein et al., 1993). Its superficial layers receive only visual input from the retina (and visual cortex in mammals), whereas the deep layers are in receipt of multisensory (visual, auditory and somatosensory [tactile and nociceptive]) and non-sensory modulatory inputs from widespread cortical and subcortical regions. This connectivity has some analogies with PPN, in that it also receives very fast sensory information (primarily to posterior PPN) as well as polymodal sensory input (Dormont et al., 1998; Pan and Hyland, 2005).

Descending outputs from the SC deep layers target regions in the pons and medulla [including the reticular formation and associated pre-cerebellar structures such as the nucleus reticularis tegmentis pontis and the inferior olive (Redgrave et al., 1987b; Chen and May, 2000; Smit et al., 2005)]. It is particularly important that deep layer outputs make direct contact with pre-motor nuclei responsible for directing the animal toward or away from salient sensory cues (Dean et al., 1989; Stein et al., 1993). This parallels the PPN projections to the mRF as these regions also directly correlate with movement activation (Redgrave et al., 1987b; Dean et al., 1989; Chen and May, 2000; Smit et al., 2005). As well as this commonality in their descending outputs, it is noteworthy that there are also connections from the SC to the PPN (Redgrave et al., 1987a) and from PPN to SC (Beninato and Spencer, 1986).

### B. Connections with the Basal Ganglia

As well as these local and descending interactions, the SC is connected with the basal ganglia in a strikingly similar way to the PPN (see Fig. 23.3). Input to basal ganglia from SC is provided via: (i) projections into several thalamic regions that contain neurons that project to striatum (McHaffie et al., [2005]; although it is possible that these terminate on thalamocortical neurons), (ii) connections to midbrain DA neurons that in turn project to striatum (Comoli et al., 2003), and (iii) by direct connections to STN (Redgrave and Coizet, 2007; Coizet et al., 2009). The SC also receives direct inhibitory projections from the basal ganglia output nuclei, SNr and the GPi (Deniau and Chevalier, 1992; Redgrave et al., 1992; Takada et al., 1994; Jiang et al., 2003).

Analogous to cortico-basal ganglia-thalamus-cortico looped architecture, it has been proposed that there are multiple, largely closed but overlapping, SC-thalamusbasal ganglia-SC loops (McHaffie et al., 2005; Redgrave and Coizet, 2007). These loops have been subdivided based on differing SC projections to thalamic nuclei (comparable to the subdivision of cortico-thalamus-basal ganglia-cortico loops based upon differing corticostriatal projections, described in Section II). The three main loop subdivisions are: (i) from superficial layers of SC through visual thalamic nuclei (lateral posterior nucleus and pulvinar) to dorsolateral striatum; (ii) from deep layers of SC through caudal intralaminar thalamic nuclei (centromedian and parafascicular nuclei) to all regions of the striatum; and (iii) from deep layers of SC through rostral intralaminar



**FIGURE 23.3** Schematic showing the relationships between the superior colliculus and basal ganglia nuclei, and descending connections to brainstem sites of motor control. Note the striking similarities with the connections of the pedunculopontine tegmental nucleus. Abbreviations: GPe, globus pallidus external segment; GPi, globus pallidus internal segment; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus.

thalamic nuclei (central lateral, paracentral and central medial nuclei) to all regions of the striatum. Thus, like the PPN, the SC has access to different corticostriatal loops throughout all of the striatum. Since the SC is one of the principal targets of both major output nuclei of the basal ganglia – the SNr and the GPi (Deniau and Chevalier, 1992; Redgrave et al., 1992; Takada et al., 1994; Jiang et al., 2003) – it is presumed that information can flow around the loop from SC-thalamus-striatum-SNr/GPi-SC.

In addition to these loops, just like the PPN, the SC makes direct projections into the basal ganglia via projections to the STN (Redgrave and Coizet, 2007; Coizet et al., 2009). The SC can also gain access to the striatum via projections from deep layers to midbrain DA neurons (Comoli et al., 2003). It is likely that the SC is the main, if not exclusive, source of short-latency visual input to DA neurons in the SNc and VTA (Dommett et al., 2005). The functions of these projections and how they compare with the PPN are discussed further in Section VI.

Overall, both SC and PPN are connected into the basal ganglia similarly with direct connections and indirect projections through thalamus and midbrain DA neurons. In regard to basal ganglia-SC connections, one main way that information related to salient, visual stimuli and oculomotion can reach the basal ganglia is through SC and the principal route through which the basal ganglia can access brainstem motor mechanisms of oculomotor control is through the SC (Haber et al., 2000; McHaffie et al., 2005; Hikosaka, 2007). In regard to basal ganglia-PPN connections, these have perhaps more generalized functions. The PPN has influence on the learning and selection of movements, actions and goals within basal ganglia, whilst the basal ganglia has access to brainstem motor centers such as the "action clusters" in the mRF.

## V. FUNCTION OF BASAL GANGLIA IN RELATION TO CORTICO-BASAL GANGLIA-THALAMO-CORTICAL LOOPS AND THEIR DOPAMINERGIC AFFERENTS

Having considered the functional roles of SC and PPN and their connectivity to basal ganglia, we need to address how they might interact functionally with the basal ganglia. To do so, we must briefly review current thinking on the function of the basal ganglia. This thinking has often been made in relation to its cortically driven loops and/or its afferents from midbrain DA neurons. Many psychological functions are thought to be mediated by these loops, often in interaction with their dopaminergic inputs. These include response selection (Lyon and Robbins, 1975), behavioral switching (Redgrave et al., 1999), behavioral activation (Salamone et al., 2003), incentive salience (Berridge and Robinson, 1998), hedonic attribution (Berridge, 2003), reward learning (Schultz, 1998) and the learning of novel actions (Redgrave and Gurney, 2006). Most researchers agree that cortico-basal ganglia-thalamus-cortico loops and their dopaminergic afferents provide animals with the ability to respond appropriately in instrumental tasks, although the participation of different loops to different components of this process is under debate (Berridge and Robinson, 1998; Schultz, 1998; Prescott et al., 1999; Everitt and Robbins, 2005; Redgrave and Gurney, 2006; Yin and Knowlton, 2006; Atallah et al., 2007; Wickens et al., 2007).

It has been hypothesized that within these loops the basal ganglia provides the essential function of a general-purpose selection mechanism, the solution to "the selection problem" (Redgrave et al., 1999). The selection problem is a fundamental computational issue for the brain that arises when multiple distributed parallel-processing sensory, cognitive and affective systems, each with the potential to influence movement, have to share a limited set of motor resources – the final common motor path (Redgrave et al., 1999). It is not possible to execute two exclusive acts (e.g. approach/ run away) using the same set of muscles (those in the legs) at the same time. Similarly, sensory systems can present simultaneously more than one stimulus that can motivate or guide different movements that have to be exclusive. There are several main arguments in favor of the basal ganglia providing the solution to this.

# A. Internal Circuitry of Basal Ganglia Could Aid Selection

Firstly, the internal circuitry of the basal ganglia lends itself to functioning as a selector of appropriate movements, actions and goals and an inhibitor (de-selector) of the inappropriate ones. Cortical neurons provide glutamatergic (excitatory) inputs to the two main input regions of the basal ganglia (the striatum and the STN). In the direct pathway the output neurons of the striatum, which are inhibitory GABA-containing neurons, project directly to the output stations of the basal ganglia, the GPi and SNr, both of which contain mostly GABAergic (inhibitory) neurons. These neurons project to thalamocortical neurons that are excitatory in nature. In the indirect pathway, striatal output neurons make indirect projections to the main output stations of the basal ganglia, the GPi and SNr, via the external segment of the globus pallidus (GPe; GABAergic, inhibitory neurons) and STN (glutamatergic, excitatory neurons). Finally, in the hyperdirect pathway cortical neurons can bypass the inhibitory links through striatum and project to the basal ganglia output stations via the STN (Mink, 1996; Nambu, 2004). The classic view is that at the final stage of processing in these looped pathways, excitation of thalamocortical neurons determines the selection of appropriate movements, actions or goals, while inhibition of thalamocortical neurons leads to de-selection of inappropriate ones (Mink, 1996; Nambu, 2004). Thus, activation of striatal neurons in the direct pathway causes disinhibition, via double inhibitory links, of thalamocortical neurons (that is, excitation); activation of striatal neurons in the indirect pathway causes inhibition of thalamocortical neurons; and activation of STN via the hyperdirect pathway also causes inhibition. The latter route has been proposed to be a fast route (due to its single excitatory link) and has been linked with inhibiting irrelevant motor programs and/or changing motor plans (Nambu, 2008). It has been proposed in theory that it depends on whether the neurons activated are situated within a motor, cognitive or limbic functional loop (as described in Section II) as to whether selections are made of movements, actions or goals (Redgrave et al., 1999; Haber, 2003). Although this classic view of basal ganglia pathways is important, it is only a first pass to understanding one aspect of how these loops might function given the dynamic reciprocal interactions between different regions, the lack of knowledge on the relative timings of neural responses and the additional functions they may serve (as described at the start of Section V).

# **B.** Basal Ganglia Can Compress Information Aiding Selection

A second main argument in favor of the basal ganglia acting as a general purpose action selection mechanism is that sensory information that is widespread (polysensory, cognitive, affective) becomes compressed (and therefore generalized) as it passes through the basal ganglia (Bar-Gad et al., 2003). There is a massive cortical input to the striatum with approximately  $17 \times 10^6$  corticostriatal neurons present in the rat (Zheng and Wilson, 2002) (with additional striatal inputs from thalamus and subcortical structures; see Section VI). However, the number of striatal projection neurons is a factor of 10 less than this, and the number of output neurons in the basal ganglia output stations a further reduction by a factor of 100-1000 (Bar-Gad et al., 2003). This, coupled with the fact that the basal ganglia outputs to motor regions throughout the brain, promotes the idea that the basal ganglia acts as a solution to the selection problem (Redgrave et al., 1999).

## C. Dopamine Can Modulate Selection Mechanisms within the Basal Ganglia

A third line of evidence consistent with the basal ganglia functioning as a general-purpose selector comes from the neurophysiology of midbrain DA neurons. Dopamine neurons that project into the basal ganglia have been considered to be functionally important in reinforcement learning in a manner which can aid the selection of appropriate movements, actions or goals. Midbrain DA neurons make phasic bursts in activity following the presentation of unexpected reward-related stimuli, conditioned stimuli or novel stimuli and inhibitions to the absence of a predicted reward-related stimulus (Schultz, 1998; Tobler et al., 2005). From these data it has been theorized that DA bursts encode an error in the prediction of the reward value of a stimulus and that this error signal could be used within the striatum as a teaching signal to induce plasticity in corticostriatal synapses (Schultz et al., 1997; Schultz, 1998). Intriguingly, recent neurophysiological data from primate PPN suggests the existence of two populations of neurons, one that fires in a graded manner in response to signals of reward expectation, and another that fires in response to reward delivery, again in proportion to the magnitude of reward (Okada et al., 2009). These could be construed as the necessary component parts of a reward prediction error signal. Alternatively, it has been proposed that the DA responses act in the striatum to "reinforce" salient stimuli or actions that might have caused the sensory-evoked DA pulse (Redgrave and Gurney, 2006). Either way, it seems that DA neurons can influence learning and selection processes throughout cortico-basal gangliathalamus-cortico loops (see Chapter 31 for more discussion on the functions of DA neurons within basal ganglia).

In summary, the basal ganglia can be considered, in the context of its cortically driven loops and its DA afferents, to function as a general-purpose learning and selection machine. We now consider how different subcortical structures such as the SC and PPN might functionally interact with the basal ganglia.

## VI. COMPARISON OF FUNCTIONAL CONNECTIONS OF PEDUNCULOPONTINE TEGMENTAL NUCLEUS AND SUPERIOR COLLICULUS WITH BASAL GANGLIA AND MIDBRAIN DOPAMINE NEURONS

So far we have established that the SC and PPN make similar connections with the basal ganglia and midbrain DA neurons, but have yet to explore fully why they might interact and how they might interact functionally.

# A. Why are there Subcortical Connections with the Basal Ganglia?

Why are the PPN, SC, and other subcortical structures connected with the basal ganglia? To take the SC as an example, it has all the sensorimotor maps required to transform a sensory input at a particular location on the retina to the motor commands which will bring the stimulus onto the fovea or into the mouth. However, lesions that reduce neostriatal DA demonstrate that without a normal-functioning basal ganglia rats are unable to perform behaviors associated with the SC, such as orienting in a normal manner to sensory stimuli (Marshall et al., 1980). It seems that the SC is dependent upon output from the basal ganglia in order for it to function normally. Why might the brain have evolved in this way?

In the early stages of vertebrate evolution, before significant development of the cerebral cortex, the selection problem existed. It is not practical to look at two objects at the same time or to react in two different ways (approach or avoid) to the same stimulus. The basal ganglia provide a mechanism (pre-cortex and post-cortex development) whereby these decisions can be made taking into account an organism's context. Thus, in order for a subcortical structure to gain access to motor output, its "bid" can compete in the basal ganglia with other "bids" from other subcortical structures. These bids can be integrated with contextual and reward information to determine a "winning bid" which is selected and "losing bids" not selected or de-selected. Another potential reason for precortical connections between PPN, SC and basal ganglia is to aid learning new actions to new stimuli in different contexts. As organisms have evolved, behaviors have become more complex and flexible. The addition of the basal ganglia might have facilitated organisms to be able to learn new oculomotor (SC) or specific actions (PPN) in the presence of salient stimuli (see Chapter 31).

# **B.** How might the Input from Subcortical Structures to the Basal Ganglia Function?

How do SC and PPN functionally integrate with the basal ganglia and DA neurons? Single neurons in the SC respond to visual, auditory, somatosensory and nociceptive information and it has been demonstrated that information related to vision (Dommett et al., 2005) is provided directly from SC to DA neurons in VTA and SNc. PPN neurons (primarily in the posterior portion) also respond to visual input (Pan and Hyland, 2005) although there is a bias towards auditory responses (often these have an extremely short latency [mean, 7.7 ms]). Somatosensory responses have also been reported (Grunwerg et al., 1992). PPN activation is essential in driving burst-firing in DA neurons without influencing the overall number of activated DA cells (Lodge and Grace, 2006) and inactivation of PPN was found to reduce the magnitude of responses by DA neurons to conditioned stimuli (Pan and Hyland, 2005). Therefore, it has been suggested that PPN neurons relay sensory information to DA neurons related to the content, rather than the salience, of a given stimulus (Lodge and Grace, 2006; Grace et al., 2007) and might provide the input to DA neurons that aid in the formation of stimulus-reward associations (Pan and

Hyland, 2005). It has been theorized that the PPN might pass on modality-integrated stimulus information, whereas the SC might primarily provide DA neurons with visual input (Dommett et al., 2005; Coizet et al., 2006).

However, the control that the PPN has over DA neurons is complex. There is a mix of neurons in the PPN that use cholinergic, GABAergic, glutamatergic neurotransmission. GABAergic neurons seem to project to the same targets as cholinergic neurons (Mena-Segovia et al., 2008). Following electrical stimulation of PPN cholinergic neurons and activation of different cholinergic receptors muscarinic or nicotinic – on DA neurons in SNc and VTA, a distinctive triphasic pattern of DA release is found in the striatum (Forster and Blaha, 2003; Miller and Blaha, 2005). Initially there is a fast increase in DA efflux (lasting approximately 2 min) that is followed by a decrease (lasting around 9min) and then a subsequent increase of relatively long duration (over around 35 min) and magnitude. The initial DA increase is primarily dependent on nicotinic and ionotropic glutamate receptors in the midbrain, while the subsequent reduction depends on muscarinic M2 receptor activation in the PPN/ laterodorsal tegmental nucleus, and the late increase is considered to be VTA muscarinic receptor-dependent (M5 receptor). Blockade of muscarinic receptors in SNc and VTA results in decreased DA levels in the striatum (Forster and Blaha, 2003; Miller and Blaha, 2005). Functionally, it has been argued that cholinergic PPN neurons might have a dual role in maintaining steadystate, tonic levels of DA and also in driving burst-firing of DA neurons to salient environmental or behavioral events (Mena-Segovia et al., 2008).

If DA neurons are activated to burst fire then the stimulus information provided by the PPN to striatum (via thalamus) can be reinforced (McHaffie et al., 2005). It is interesting that PPN-thalamus neurons send collaterals to DA neurons (Oakman et al., 1999), suggesting that a single PPN neuron could provide information relating to stimulus content (via thalamus) and at the same time 'reinforce' that information in relation to other stimuli or actions via its projections to DA neurons. Given that thalamic oscillatory activity is gated by cholinergic input (McCormick and Prince, 1986; McCormick, 1989) and the PPN projects to all thalamic nuclei including the thalamic reticular nucleus, this provides a mechanism whereby the PPN could influence what is learned in the striatum and when learning can occur. In this way, the information regarding stimulus content that is to be conditioned in the striatum is produced by (primarily posterior) PPN circuitry. Therefore, following lesion of posterior PPN, subcortical information relating to the content of unpredicted, salient stimuli (possibly reward-related) would be unable to induce burst firing in DA neurons. Consequently, the lesioned animal would be unable to learn goal-directed behaviors, which we know to be the case.

# C. How might the Output from the Basal Ganglia to Subcortical Structures Function?

Having considered how the SC and PPN might influence the basal ganglia we should now discuss the reverse situation: that is, how might the basal ganglia influence processing in subcortical structures? The SC and PPN (and other subcortical structures) are kept under inhibitory control by the output neurons of the basal ganglia allowing for focused selections of neurons in subcortical structures (Saitoh et al., 2003; Hikosaka, 2007). What would the basal ganglia be selecting in the cases of SC and PPN? Hikosaka (2007) suggests that each subcortical structure connected to the basal ganglia is associated with particular sets of movements: periaqueductal gray with vocalization, the SC with eye and mouth movements and the PPN and cuneiform nucleus with locomotion and posture. Similarly, it has been proposed that the PPN might output to modify posture and to facilitate locomotion (Takakusaki et al., 2003). This idea is consistent with the findings that low frequency deep brain stimulation in PPN can improve postural instability and gait dysfunction in people with Parkinson's disease (Plaha and Gill, 2005; Stefani et al., 2007).

However, as described above in Section III, PPN lesion studies show clear differentiation between posterior and anterior portions, suggesting a distinct role in learning for the posterior portion and in motor and postural control for the anterior portion. Given this, and the possibility that learned action sequences could be carried out by mRF, it seems worth considering, albeit speculatively, that basal ganglia output via the anterior PPN could instruct particular clusters in mRF to execute particular actions. In this way the anterior PPN could link action representations in the mRF (for example, a lever-press cluster) with instrumental learning procedures throughout basal ganglia.

# D. Subcortical-Basal Ganglia Connections within a Heterarchical Layered Network

It has been posited that these systems might be part of a heterarchical layered network, such that they can work together (as outlined above), independently or compete towards action selection (Prescott et al., 1999; Humphries et al., 2007). Thus, there may be situations where the basal ganglia may subsume the mRF (via PPN connections) or hindbrain pre-motor nuclei (via SC) and gain control. Humphries et al. (2007) suggest this would be most likely to happen when multiple action choices are available of approximately equal salience, as is often the case in new environments where learning is required. Indeed, it is in these situations that DA neurons tend to fire – perhaps this relinquishes control from brainstem circuits to that of the basal ganglia? On the flip side, it is also possible for brainstem circuits not to require the basal ganglia. Thus, the PPN or SC could trigger responding in the brainstem when an action is clearly more salient than its competitors and the environment demands it.

Additionally, the brainstem could act before the basal ganglia, for instance when quick decisions are required, although in these situations the basal ganglia would continue processing in parallel to enable the animal to learn how to respond more effectively in the future. The connections that PPN and SC make with the STN, mRF and hindbrain motor structures provide routes whereby control of behavior can be switched quickly from cortico-basal ganglia loops to subcortical-basal ganglia loops. The most obvious example of this multi-level approach to action selection – low level fast, higher level moderated – is the startle response (Koch et al., 1993; Koch, 1999): the PPN is known to be involved in the production of startle responses to unpredictable stimuli, but its actions can also be brought under learned control by corticostriatal systems.

Finally, in its most general form, occasions where subcortical connections with basal ganglia dominate action control could explain how knowingly irrational actions are sometimes chosen and momentary lapses of control claimed (Berridge, 2002). An example of this might be addiction. Addicts are often able to understand and believe (cortical control of behavior?) that they should change their addictive behavior. However, in particular situations (that are often highly associated with their addictive behaviors) they feel uncontrollable urges and relapse (sub-cortical control of behavior?). Other examples of this might include normal situations involving sex and violence or psychological disorders including most of the phobias, anxiety/panic attacks, post-traumatic stress disorders and psychopathy.

### VII. CONCLUSIONS

Subcortical connections with the basal ganglia form essential components of the mechanism that facilitates the selection of appropriate movements, actions and goals. Moreover, subcortical sites such as the PPN and SC sit at pivotal positions between two action selection centers, basal ganglia and brainstem. Prior to the evolutionary development of cortex, subcortical sites would have required connections with the basal ganglia to aid in selection problems. As cortex has evolved, more selections have become available that are often highly conceptual requiring further integration with basal ganglia, thalamus and subcortical sites. The PPN and SC might allow important switches to occur between slow, complex selections via cortico-basal ganglia loops and faster or more simple selections via subcortical (PPN/SC)–brainstem circuits. These might relate to situations in which we make selections that seem to be ruled by our heads or our heart.

### REFERENCES

- Ainge JA, Jenkins TA, Winn P (2004) Induction of c-fos in specific thalamic nuclei following stimulation of the pedunculopontine tegmental nucleus. Eur J Neurosci 20:1827–1837.
- Alderson HL, Latimer MP, Winn P (2006) Intravenous selfadministration of nicotine is altered by lesions of the posterior, but not anterior, pedunculopontine tegmental nucleus. Eur J Neurosci 23:2169–2175.
- Alderson HL, Latimer MP, Winn P (2008) A functional dissociation of the anterior and posterior pedunculopontine tegmental nucleus: excitotoxic lesions have differential effects on locomotion and the response to nicotine. Brain Struct Funct 213:247–253.
- Alderson HL, Latimer MP, Blaha CD, Phillips AG, Winn P (2004) An examination of D-amphetamine self-administration in pedunculopontine tegmental nucleus-lesioned rats. Neuroscience 125:349–358.
- Atallah HE, Lopez-Paniagua D, Rudy JW, O'Reilly RC (2007) Separate neural substrates for skill learning and performance in the ventral and dorsal striatum. Nat Neurosci 10:126–131.
- Bar-Gad I, Morris G, Bergman H (2003) Information processing, dimensionality reduction and reinforcement learning in the basal ganglia. Prog Neurobiol 71:439–473.
- Beninato M, Spencer RF (1986) A cholinergic projection to the rat superior colliculus demonstrated by retrograde transport of horseradish peroxidase and choline acetyltransferase immunohistochemistry. J Comp Neurol 253:525–538.
- Berridge KC (2002) Irrational pursuit: Hyper-incentive from a visceral brain. In: The Psychology of Economic Decisions (Brocas I, Carrillo JD, eds), pp. 17–40. Oxford: Oxford University Press.
- Berridge KC (2003) Pleasures of the brain. Brain Cogn 52:106-128.
- Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309–369.
- Charara A, Smith Y, Parent A (1996) Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: *Phaseolus vulgaris*-leucoagglutinin anterograde labeling combined with postembedding glutamate and GABA immunohistochemistry. J Comp Neurol 364:254–266.
- Chen B, May PJ (2000) The feedback circuit connecting the superior colliculus and central mesencephalic reticular formation: a direct morphological demonstration. Exp Brain Res 131:10–21.

- Coizet V, Dommett EJ, Redgrave P, Overton PG (2006) Nociceptive responses of midbrain dopaminergic neurones are modulated by the superior colliculus in the rat. Neuroscience 139:1479–1493.
- Coizet V, Graham JH, Moss J, Bolam JP, Savasta M, McHaffie JG, Redgrave P, Overton PG. (2009). Short-Latency visual input to the subthalamic nucleus is provided by the midbrain superior colliculus. J. Neuroscience 29:5701–5709.
- Comoli E, Coizet V, Boyes J, Bolam JP, Canteras NS, Quirk RH, Overton PG, Redgrave P (2003) A direct projection from superior colliculus to substantia nigra for detecting salient visual events. Nat Neurosci 6:974–980.
- Corrigall WA, Coen KM, Adamson KL (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res 653:278–284.
- Dean P, Redgrave P, Westby GW (1989) Event or emergency? Two response systems in the mammalian superior colliculus. Trends Neurosci 12:137–147.
- Deniau JM, Chevalier G (1992) The lamellar organization of the rat substantia nigra pars reticulata: distribution of projection neurons. Neuroscience 46:361–377.
- Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JE, Overton PG, Redgrave P (2005) How visual stimuli activate dopaminergic neurons at short latency. Science 307:1476–1479.
- Dormont JF, Conde H, Farin D (1998) The role of the pedunculopontine tegmental nucleus in relation to conditioned motor performance in the cat. I. Context-dependent and reinforcement-related single unit activity. Exp Brain Res 121:401–410.
- Erro E, Lanciego JL, Gimenez-Amaya JM (1999) Relationships between thalamostriatal neurons and pedunculopontine projections to the thalamus: a neuroanatomical tract-tracing study in the rat. Exp Brain Res 127:162–170.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Forster GL, Blaha CD (2003) Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. Eur J Neurosci 17:751–762.
- Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220–227.
- Grunwerg BS, Krein H, Krauthamer GM (1992) Somatosensory input and thalamic projection of pedunculopontine tegmental neurons. Neuroreport 3:673–675.
- Haber SN (2003) The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Hikosaka O (2007) GABAergic output of the basal ganglia. Prog Brain Res 160:209–226.
- Humphries MD, Gurney K, Prescott TJ (2007) Is there a brainstem substrate for action selection? Philos Trans R Soc Lond B Biol Sci 362:1627–1639.
- Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27–78.
- Jiang H, Stein BE, McHaffie JG (2003) Opposing basal ganglia processes shape midbrain visuomotor activity bilaterally. Nature 423:982–986.

- Joel D, Weiner I (2000) The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. Neuroscience 96:451–474.
- Keating GL, Winn P (2002) Examination of the role of the pedunculopontine tegmental nucleus in radial maze tasks with or without a delay. Neuroscience 112:687–696.
- Kobayashi K, Hoshino K, Homma S, Takagi S, Norita M (2007) A possible monosynaptic pathway links the pedunculopontine tegmental nucleus to thalamostriatal neurons in the hooded rat. Arch Histol Cytol 70:207–214.
- Koch M (1999) The neurobiology of startle. Prog Neurobiol 59:107-128.
- Koch M, Kungel M, Herbert H (1993) Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. Exp Brain Res 97:71–82.
- Lodge DJ, Grace AA (2006) The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. Proc Natl Acad Sci USA 103:5167–5172.
- Lyon M, Robbins TW (1975) The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In: Current Developments in Psychopharmacology (Essman W, Valzelli L, eds). New York: Spectrum.
- Marshall JF, Berrios N, Sawyer S (1980) Neostriatal dopamine and sensory inattention. J Comp Physiol Psychol 94:833–846.
- McCormick DA (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. Trends Neurosci 12:215–221.
- McCormick DA, Prince DA (1986) Acetylcholine induces burst firing in thalamic reticular neurones by activating a potassium conductance. Nature 319:402–405.
- McHaffie JG, Stanford TR, Stein BE, Coizet V, Redgrave P (2005) Subcortical loops through the basal ganglia. Trends Neurosci 28:401–407.
- Mena-Segovia J, Bolam JP, Magill PJ (2004) Pedunculopontine nucleus and basal ganglia: distant relatives or part of the same family? Trends Neurosci 27:585–588.
- Mena-Segovia J, Winn P, Bolam JP (2008) Cholinergic modulation of midbrain dopaminergic systems. Brain Res Rev 58:265–271.
- Mena-Segovia J, Micklem BR, Nair-Roberts RG, Ungless MA, Bolam JP (2009) GABAergic neuron distribution in the pedunculopontine nucleus defines functional subterritories. J Comp Neurol 515: 397-408.
- Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10:1185–1201.
- Miller AD, Blaha CD (2005) Midbrain muscarinic receptor mechanisms underlying regulation of mesoaccumbens and nigrostriatal dopaminergic transmission in the rat. Eur J Neurosci 21:1837–1846.
- Mink JW (1996) The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.
- Nambu A (2004) A new dynamic model of the cortico-basal ganglia loop. Prog Brain Res 143:461–466.
- Nambu A (2008) Seven problems on the basal ganglia. Curr Opin Neurobiol 18:595–604.
- Oakman SA, Faris PL, Cozzari C, Hartman BK (1999) Characterization of the extent of pontomesencephalic cholinergic neurons' projections to the thalamus: comparison with projections to midbrain dopaminergic groups. Neuroscience 94:529–547.
- Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK (1995) Distribution of pontomesencephalic cholinergic neurons projecting to

substantia nigra differs significantly from those projecting to ventral tegmental area. J Neurosci 15:5859–5869.

- Okada K, Toyama K, Inoue Y, Isa T, Kobayashi Y (2009) Different pedunculopontine tegmental neurons signal predicted and actual task rewards. J Neurosci 29:4858-4870.
- Pan WX, Hyland BI (2005) Pedunculopontine tegmental nucleus controls conditioned responses of midbrain dopamine neurons in behaving rats. J Neurosci 25:4725–4732.
- Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 20:91–127.
- Parent A, Parent M, Charara A (1999) Glutamatergic inputs to midbrain dopaminergic neurons in primates. Parkinsonism Relat Disord 5:193–201.
- Plaha P, Gill SS (2005) Bilateral deep brain stimulation of the pedunculopontine nucleus for Parkinson's disease. Neuroreport 16:1883–1887.
- Prescott TJ, Redgrave P, Gurney K (1999) Layered control architectures in robots and vertebrates. Adaptive Behavior 7:99–127.
- Redgrave P, Gurney K (2006) The short-latency dopamine signal: a role in discovering novel actions? Nat Rev Neurosci 7:967–975.
- Redgrave P, Coizet V (2007) Brainstem interactions with the basal ganglia. Parkinsonism Relat Disord 13(Suppl 3):S301–S305.
- Redgrave P, Mitchell IJ, Dean P (1987a) Further evidence for segregated output channels from superior colliculus in rat: ipsilateral tecto-pontine and tecto-cuneiform projections have different cells of origin. Brain Res 413:170–174.
- Redgrave P, Mitchell IJ, Dean P (1987b) Descending projections from the superior colliculus in rat: a study using orthograde transport of wheatgerm-agglutinin conjugated horseradish peroxidase. Exp Brain Res 68:147–167.
- Redgrave P, Marrow L, Dean P (1992) Topographical organization of the nigrotectal projection in rat: evidence for segregated channels. Neuroscience 50:571–595.
- Redgrave P, Prescott TJ, Gurney K (1999) The basal ganglia: a vertebrate solution to the selection problem? Neuroscience 89:1009–1023.
- Saitoh K, Hattori S, Song WJ, Isa T, Takakusaki K (2003) Nigral GABAergic inhibition upon cholinergic neurons in the rat pedunculopontine tegmental nucleus. Eur J Neurosci 18:879–886.
- Salamone JD, Correa M, Mingote S, Weber SM (2003) Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry, and drug abuse. J Pharmacol Exp Ther 305:1–8.
- Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27.
- Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599.
- Smit AE, Zerari-Mailly F, Buisseret P, Buisseret-Delmas C, VanderWerf F (2005) Reticulo-collicular projections: a neuronal tracing study in the rat. Neurosci Lett 380:276–279.

- Stefani A, Lozano AM, Peppe A, et al. (2007) Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. Brain 130:1596–1607.
- Stein BE, Meredith MA, Wallace MT (1993) The visually responsive neuron and beyond: multisensory integration in cat and monkey. Prog Brain Res 95:79–90.
- Takada M, Tokuno H, Ikai Y, Mizuno N (1994) Direct projections from the entopeduncular nucleus to the lower brainstem in the rat. J Comp Neurol 342:409–429.
- Takakusaki K, Habaguchi T, Ohtinata-Sugimoto J, Saitoh K, Sakamoto T (2003) Basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion: a new concept for understanding motor disorders in basal ganglia dysfunction. Neuroscience 119:293–308.
- Taylor CL, Kozak R, Latimer MP, Winn P (2004) Effects of changing reward on performance of the delayed spatial win-shift radial maze task in pedunculopontine tegmental nucleus lesioned rats. Behav Brain Res 153:431–438.
- Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307:1642–1645.
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468–474.
- Wang HL, Morales M (2009) Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur J Neurosci 29: 340–358.
- Wickens JR, Horvitz JC, Costa RM, Killcross S (2007) Dopaminergic mechanisms in actions and habits. J Neurosci 27:8181–8183.
- Winn P (1998) Frontal syndrome as a consequence of lesions in the pedunculopontine tegmental nucleus: a short theoretical review. Brain Res Bull 47:551–563.
- Winn P (2008) Experimental studies of pedunculopontine functions: are they motor, sensory or integrative? Parkinsonism Relat Disord 14(Suppl 2):S194–S198.
- Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci 7:464–476.
- Zahm DS, Williams EA, Latimer MP, Winn P (2001) Ventral mesopontine projections of the caudomedial shell of the nucleus accumbens and extended amygdala in the rat: double dissociation by organization and development. J Comp Neurol 436:111–125.
- Zheng T, Wilson CJ (2002) Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 87: 1007–1017.

# Integrative Networks Across Basal Ganglia Circuits

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## I. INTRODUCTION

The basal ganglia work in concert with the frontal cortex to orchestrate and execute motivated, planned behaviors requiring limbic, cognitive, and motor control systems. The basal ganglia are traditionally considered to process this information in parallel and segregated functional streams consisting of reward (limbic), associative (cognitive), and motor control circuits (Alexander and Crutcher, 1990) (see Chapter 1). Moreover, microcircuits within each region are thought to mediate different aspects of each function (Middleton and Strick, 2002). However, while frontal cortex contains divisions associated with specific functions, expressed behaviors are the result of a combination of complex information processing that involves all of frontal cortex. Indeed, appropriate responses to environmental stimuli require continual updating, and learning to adjust behaviors according to new data. This necessitates coordination between limbic, cognitive, and motor systems, to form smoothly executed, goal-directed behaviors. Parallel processing of functional information through different basal ganglia circuits does not address how information flows

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. between circuits, thereby developing new, or adapting to previously, learned behaviors (or actions). While the anatomical pathways appear to be generally topographic from cortex through BG circuits, and there are some physiological correlates to the functional domains of the striatum, a large growing body of evidence supports a duel processing system. Thus, information is not only processed in parallel streams, but also through integrative mechanisms through which information can be transferred between functional circuits (Percheron and Filion, 1991; Bevan et al., 1997; Bar-Gad et al., 2000; Haber et al., 2000; Kolomiets et al., 2001; McFarland and Haber, 2002; Mena-Segovia et al., 2005; Haber et al., 2006; Belin and Everitt, 2008; Draganski et al., 2008). This chapter will: first, review the basic circuitry that underlies parallel processing; second, outline the anatomical basis for integration across different cortico-basal ganglia circuits; and finally, discuss functional data that support integrative processing through the basal ganglia. The chapter focuses on circuitry in the primate; however, when discussing integrative circuitry, rodent studies are also highlighted.

### II. PARALLEL PROCESSING

### A. Functional Organization of Frontal Cortex

Frontal cortex is organized in a hierarchical manner and can be divided into functional regions (Fuster, 2001): the orbital (OFC) and anterior cingulate (ACC) prefrontal cortex are involved in reward, emotion, and motivation; the dorsal prefrontal cortex (DPFC) are involved in higher cognitive processes or "executive" functions; and the premotor and motor areas are involved in motor planning and the execution of those plans. The ACC is divided into ventral, or subgenual ACC, and dorsal ACC (dACC) areas. The medial orbital areas and the subgenual ACC cortex are collectively referred to as the ventral, medial prefrontal cortex (vmPFC) and are particularly important in the expression of emotion. The vmPFC plays a role in monitoring correct responses based on previous experience and the internal milieu, and is engaged when previously learned responses are no longer appropriate and need to be suppressed (Mayberg et al., 1999; Milad et al., 2005; Milad et al., 2007). These areas play a key role in reward processing, particularly in the anticipation of expected value (Knutson et al., 2005). The more lateral OFC regions play a central role in evaluation of reward value, and in the development of reward and aversive-based learning (Hikosaka and Watanabe, 2000; Schultz et al., 2000; Tremblay and Schultz, 2000; O'Doherty et al., 2001; Wallis and Miller, 2003; Kringelbach and Rolls, 2004; Roesch and Olson, 2004; Padoa-Schioppa and Assad, 2006). This area receives input from multimodal sensory regions and is closely linked to the vmPFC (Barbas, 1992; Price et al., 1996). The dACC is a unique part of frontal cortex, containing diverse frontal lobe functions. However, the overall role of the ACC appears to be involved in monitoring these functions in conflict situations (Paus, 2001; Walton et al., 2003; Vogt et al., 2005). Lesions of the OFC, vmPFC and the dorsal ACC (dACC) areas result in an inability to initiate and carry out goal-directed behaviors, and lead to socially inappropriate and impulsive behaviors (Butter and Snyder, 1972; Fuster, 1989; O'Doherty et al., 2003; Milad and Rauch, 2007). The dorsal prefrontal cortex (DPFC) is involved in working memory, set shifting, and strategic planning, often referred to as 'executive functions' (Goldman-Rakic, 1996; Smith and Jonides, 1997; Fuster, 2000; Passingham and Sakai, 2004; Blumenfeld and Ranganath, 2006). Motor cortices are the most clearly defined of frontal cortex. Caudal motor areas are highly microexcitable, closely timed to the execution of movement, and send a direct descending projection to spinal motor

nuclei. Rostral motor areas are involved in sequence generation, and motor learning. They are less microexcitable than the caudal motor areas, but more so than the prefrontal cortex (Mushiake et al., 1991; Dum and Strick, 1993; He et al., 1993; Tanji and Mushiake, 1996; Strick et al., 1998; Schieber, 1999). Each of these frontal areas projects to specific striatal regions. However, in addition to the welldescribed topographic organization, corticostriatal projections also follow non-topographic rules. We will return to this non-topographic organization later in the chapter.

## B. General Topography of Cortico-Striatal Projections

The striatum is the main input structure of the basal ganglia and receives a massive and topographic input from cerebral cortex and thalamus (see Chapter 1). These afferent projections to the striatum terminate in a general topographic manner, such that the ventromedial striatum receives input from the vmPFC, OFC, and dACC, the central striatum receives input from the DPFC including areas 9 and 46, and the dorsolateral striatum receive input from motor control areas (Fig. 24.1). Together, the frontal regions that mediate reward, motivation, and affect regulation project primarily to the rostral striatum, including the nucleus accumbens, the medial caudate nucleus, and the medial and ventral rostral putamen, collectively referred to as the ventral striatum, which occupies over 20% of the striatum (Haber et al., 2006). In general, this striatal region is also involved in various aspects of reward evaluation and incentive-based learning (Schultz et al., 2000; Knutson et al., 2001; Elliott et al., 2003; Corlett et al., 2004; Tanaka et al., 2004), and are associated with pathological risk-taking and addictive behaviors (Kuhnen and Knutson, 2005; Volkow et al., 2005). Within the ventral striatum, cortical afferent projections are also organized in a somewhat topographic manner (Ferry et al., 2000; Haber et al., 2006). The hippocampal projection is mostly limited to the shell. In contrast, the amygdala projects throughout a wider ventral striatal area (Friedman et al., 2002; Fudge et al., 2002). Projection fields from the vmPFC are the most limited, and are concentrated within and just lateral to the shell. The vmPFC also projects to the medial wall of the caudate nucleus, adjacent to the ventricle. In contrast, the central and lateral parts of the ventral striatum, (including the ventral caudate nucleus and putamen) receive inputs from the OFC. These terminals also extend dorsally, along the medial caudate nucleus, but lateral to those from the



**FIGURE 24.1** Schematic illustrating parallel circuits through corticobasal ganglia pathways. Corresponding shaded striatal and cortical areas demonstrates topographic projections; white, the limbic circuit; light grey, the associative circuit; dark grey, the motor control circuit.

vmPFC. There is some medial to lateral and rostral to caudal topographic organization of the OFC terminal fields. For example, projections from area 11 terminate rostral to those from area 13, and those from area 12 terminate laterally. Projections from the dACC (area 24b) extend from the rostral pole of the striatum to the anterior commissure, and are located in both the central caudate nucleus and putamen. They primarily avoid the shell region. These fibers terminate somewhat lateral to those from the OFC. Thus, the OFC terminal fields are positioned between the vmPFC and dACC.

The DPFC projects to the head of the caudate nucleus and to the putamen, rostral to the anterior commissure. Physiological, imaging, and lesion studies support the idea that these striatal areas are involved in working memory and strategic planning processes, working together with the DPFC in mediating this function (Battig et al., 1960; Levy et al., 1997; Elliott and Dolan, 1999; Pasupathy and Miller, 2005). Caudal to the commissure, this projection is confined to the medial, central portion of the head of the caudate nucleus with few terminals in the central and caudal putamen. There is a complex topography of the focal projections from different regions of areas 9 and 46. At rostral levels, area 9 projects primarily to the caudate nucleus, whereas projections from area 46 also terminate in the putamen. Caudal to the anterior commissure level, the collective DPFC focal projections occupy the same region of the caudate nucleus, and are no longer found in the putamen (Selemon and Goldman-Rakic, 1985; Ferry et al., 2000; Haber et al., 2006; Calzavara et al., 2007). Focal projections from the frontal eye fields are primarily located in the central and lateral caudate nucleus, adjacent to the cell bridges of the internal capsule, with isolated patches in the medial and central putamen, especially at the anterior commissure level. The supplementary eye field projection field terminates primarily lateral to those from the frontal eye fields (Calzavara et al., 2007).

Rostral premotor areas terminate in both the caudate and putamen, bridging the two with a continuous projection at rostral levels. However, at caudal levels, these focal projections primarily occupy the dorsal caudate nucleus. Projections from caudal motor areas terminate almost entirely in the dorsolateral putamen, caudal to the anterior commissure. Few terminals are found rostral to the anterior commissure. Both caudal and rostral motor areas occupy much of the putamen, caudal to the anterior commissure, a region that also receives overlapping projections from somatosensory cortex, resulting in a somatotopically organized sensory-motor area (Kunzle, 1975; Künzle, 1977; Aldridge et al., 1980; Alexander and DeLong, 1985; Kimura, 1986; Flaherty and Graybiel, 1994). In summary, projections from frontal cortex form a functional gradient of inputs from the ventromedial sector through the dorsolateral striatum, with the limbic inputs terminating in the ventromedial part, and the motor cortex terminating in the dorsolateral region. Moreover, there is a fine topography within each system.

## C. General Topography of Thalamo-Striatal Projections

The thalamus provides the second largest input to the striatum (see Chapter 22). As seen with the corticostriatal projection, these projections are also organized in a general topographical manner, such that interconnected and functionally associated thalamic and cortical regions terminate in the same general striatal region (McFarland and Haber, 2000). There are two major thalamo-striatal projections, both topographically organized. First, there is the welldescribed input from the midline and intralaminar nuclei (Dubé et al., 1988; Nakano et al., 1990; Fenelon et al., 1991; Sadikot et al., 1992; Giménez-Amaya et al., 1995). These nuclei are further subdivided and associated with specific functions related to their cortical connections. The midline and medial intralaminar nuclei project to medial prefrontal areas, the amygdala and hippocampus, and, for that reason, are considered the limbic-related thalamic nuclear groups. The intralaminar nuclei central medial (CM) and parafascicularis (Pf) have connections with association areas. The lateral CM nucleus projects to both the primary motor (M1) and sensory cortices and, therefore, considered to be related to motor control (Herkenham, 1986; Groenewegen and Berendse, 1994; Jones, 1998). These midline and intralaminar thalamic nuclei project topographically to the striatum such that the midline and medial Pf nuclei project mainly to ventral (limbic) striatal areas, whereas the more lateral intralaminar nuclei have connections with the dorsolateral (association-sensorimotor) caudate and putamen (Francois et al., 1991; McFarland and Haber, 2000). Thus, the "non-specific" thalamic nuclei project to striatal areas that are consistent with the cortical area they are connected to, thus maintaining the functional distinction of different striatal regions.

Second, in primates there is an equally large input to the dorsal striatum from the BG relay nuclei, the medialis dorsalis nucleus (MD), ventralis anterior (VA) and ventralis lateralis (VL) nuclei (McFarland and Haber, 2001; McFarland and Haber, 2002). These thalamic nuclei are also intimately connected with specific frontal cortical areas (Goldman-Rakic and Porrino, 1985; Wiesendanger and Wiesendanger, 1985; Giguere and Goldman-Rakic, 1988). Different regions of the ventral motor nuclei (VA-VL) have reciprocal projections with specific premotor, motor and cingulate cortices. The MD nucleus is linked to specific prefrontal areas. As with the midline and intralaminar nuclei, interconnected ventral VA/VL MD nuclei and cortical areas project to the same region of the striatum. For example, rostral motor areas, including PMdr, PreSMA, and rostral cingulate motor area (CMAr), receive inputs from the parvicellular part of VA (VApc). Thalamic projections from these areas converge within the putamen and dorsolateral caudate. Thus, there is a tight, anatomical and functional triad of basal ganglia input and output structures, involving the frontal motor cortices, the striatum, and the thalamic relay nuclei (McFarland and Haber, 2000; McFarland and Haber, 2002).

# D. Pathways Through the Basal Ganglia and Back to Cortex

The striatal projection to the pallidal complex and substantia nigra are also generally topographically organized, thus maintaining the functional organization of the striatum in these output nuclei. The ventral striatum terminates in the ventral pallidum and in the dorsal part of the midbrain. Terminals from the central striatum terminate more centrally in both the pallidum and in the SN, while those from the sensorimotor areas of the striatum innervate the ventrolateral part of each pallidal segment and the ventrolateral SN. Finally, the pallidum and SN pars reticulata (SNr) project to the different basal ganglia output nuclei of the thalamus, the mediodorsal, and ventral anterior and ventral lateral cell groups (Szabo, 1979; Haber et al., 1990; Selemon and Goldman-Rakic, 1990; Hedreen and DeLong, 1991; Lynd-Balta and Haber, 1994b; Strick et al., 1995; Middleton and Strick, 2002). The thalamus represents the final basal ganglia link back to cortex, which also maintains a general functional topographic organization. The ventral lateral thalamic complex and the MD nucleus receive the bulk of basal ganglia output (from the pallidum and SNr). Each subdivision of the VA and VL nuclei receives input from different pallidal and nigral regions. The magnocellular part of the VA nucleus (VAmc) receives nigral inputs, whereas the VAmc and VLo nuclei receive pallidal inputs. The MD nucleus receives input from the medial SNr and the ventral pallidum (VP) (Haber et al., 1985; Ilinsky et al., 1985). Regions of VL that receive the main output from motor regions of dorsal pallidum, project to motor and premotor areas of frontal cortex. Likewise, VA is associated with the rostral premotor cortex and DLPFC, and the MD nucleus is linked to the DLPFC, OFC and ACC (Giguere and Goldman-Rakic, 1988; Matelli and Luppino, 1996). Thus, the organization of connections through the cortico-basal ganglia-cortical network preserves a general functional topography within each structure, from the cortex through the striatum, from the striatum to the pallidum/pars reticulata, from these output structures to the thalamus, and finally, back to cortex (Fig. 24.1) (Middleton and Strick, 2000).

This organization of the dense terminal fields from a given cortical area in each basal ganglia nuclei has led to the concept that each functionally identified cortical region drives (and is driven by) a specific basal ganglia loop or circuit. The overall functionally topography has led to the idea of parallel processing of cortical information through segregated basal ganglia circuits (Alexander and Crutcher, 1990). However, this concept does not take into account the detailed analysis of these projection systems, thereby overlooking its complexity. Moreover, fro a functional perspective, the focus has been on the role of the basal ganglia in the selection and implementation of an appropriate motor response, (while inhibiting unwanted ones) (Mink, 1996). This model assumes that the basal ganglia primarily mediates motor output. In other words, it assumes that the behavior has been learned and that the role of the basal ganglia is to carry out a coordinated action. We now know that the cortico-basal ganglia network is critical in mediating the learning process, to adapt and to accommodate past experiences to modify behavioral responses (Aosaki et al., 1994; Wise et al., 1996; Doyon et al., 1997; Hikosaka et al., 1998; Jog et al., 1999; Cools et al., 2004; Pasupathy and Miller, 2005; Muhammad et al., 2006). This process requires links across circuits.

### **III. INTEGRATIVE PATHWAYS**

Growing evidence from both primate and rodent studies have identified possible anatomical substrates through which transfer of information can occur across functional domains (Bevan et al., 1997; Bar-Gad et al., 2000; Haber et al., 2000; Kolomiets et al., 2001; McFarland and Haber, 2002; Mena-Segovia et al., 2005; Haber et al., 2006; Belin and Everitt, 2008; Draganski et al., 2008). This integration between different aspects of reward processing with cognitive and motor control regions occurs at several stations through the system. Moreover, a number of mechanisms serve this integrative function. First, it has been known for some time that axons and dendrites from adjacent cortical regions invade neighboring areas, allowing crosstalk between systems at the edges of functional domains. Therefore, it is not surprising that projections carrying diverse information through the basal ganglia structures would also converge at the edges of different functional domains. Thus, while projections from cortex terminate in a general topography through the BG structures, the dendrites and axons of cells within each functional region often crossfunctional boundaries. For example, the dendritic arbors in the GP extend long distances, well beyond the circumscribed region of its cell body, invading neighboring territories. In this way, striatal axons from one functional region can contact the distal dendrite of an adjacent one. Moreover, convergence is enhanced by the compression of terminals from functionally adjacent fields into progressively smaller BG structures (Percheron and Filion, 1991; Yelnik, 2002; Bar-Gad et al., 2003). Thus, "edges" of functionally identified regions are likely to process mixed signals.

Second, anatomical integration occurs through specific subregions within a functional area that receives input from a different functional region. These are not necessarily located at the edges of functional domains, but rather represent relatively isolated subregions within each domain. Moreover, this phenomenon can occur between terminal fields that are not adjacent. These areas are referred to as nodal points of convergence and have been demonstrated both within specific corticostriatal and cortico-thalamic terminal fields. Third, corticostriatal, and pallidostriatal connections contain a diffuse fiber projection system. This is in addition to the focal dense projections that are used to define the functional territories within each structure. It refers to groups of fibers that travel widely throughout a broad area of the nucleus, giving rise to clusters of terminals, outside of the main, dense, focal terminal field. This scattered, and less densely distributed fiber system is likely to perform a different function than the focal terminal field. Fourth, there are non-reciprocal arrangements between structures that are bidirectionally linked. The non-reciprocal component of these connections provides input from a different functional circuit, and therefore a key element for integration of information. Moreover, in some instances, such as the striato-nigro-striatal and cortico-thalamo-cortical systems, the complex relationships between the reciprocal and non-reciprocal provide a directional flow of information between regions. This flow appears to be generally from limbic through, associative, and then motor control output areas. The following sections will address these integrative mechanisms through each stage of the corticobasal ganglia circuit: (1) The cortico-striatal connections demonstrate integration across functional domains through convergence of terminal fields at the borders of functional domains, at nodes of convergence in within subregions of functional domains, and through a diffuse projection system; (2) The pallidal connections demonstrate integration, through convergence at borders and, (particularly the external segment) a diffuse fiber feedback projection system; (3) The striato-nigro-striatal network and the cortico-thalamocortical network demonstrate integration through a nonreciprocal, directional specific set of connections.

## A. Cortico-Striatal Connections

While there is a general topographic organization to the cortico-striatal terminal fields, this projection system also shows important non-topographic rules: (1) Nodal points of convergent focal projections from different cortical territories; and (2) A diffuse projection system from each cortical region that extends through a wide striatal region. Both of these include convergence at the edges of functional

domains, as well as interfaces deep within a given functional domain (Figs 24.2 and 24.3) (Haber et al., 2006; Calzavara et al., 2007). Focal projection fields are defined as dense clusters of terminals forming the well-known dense corticostriatal patches that can be visualized at relatively low magnification and are the foundation for the concept of parallel and segregated cortico-basal ganglia circuits. The diffuse projections consist of clusters of terminal fibers that are



**FIGURE 24.2** Schematics demonstrating convergence of corticostriatal focal projections from different limbic, associative, and motor areas: (A) and (B) show convergence between projections from different prefrontal regions; (C) shows convergence between prefrontal regions and motor control areas. DPFC, dorsal prefrontal cortex; OFC, orbital prefrontal cortex; vmPFC, ventral medial prefrontal cortex.

widely distributed throughout the striatum, both expanding the borders of the focal terminal fields, but also extending widely throughout other regions of the striatum. These two projection patterns, focal projections and diffuse projections, may represent terminals from two different populations of cortical neurons (Parent and Parent, 2006). Alternatively, they may originate from the collaterals from the same cortical cell. We describe these patterns below in the monkey; however, recent studies show that these same patterns exist in rats (Deniau, in preparation).

# 1. Focal Projections and Nodal Points Convergence

Despite the general topography described above, focal terminal fields from the different frontal areas also show a complex interweaving and convergence, providing an anatomical substrate for modulation between circuits (Haber et al., 2006; Calzavara et al., 2007). As described above, corticostriatal topography forms a general ventromedial to dorsolateral gradient of projections from limbic cognitive and motor control areas respectively. Moreover each of these general areas contains subregions with concentrations of terminals associated with specific cortical areas. As might be expected, there is extensive convergence of fibers from different cortical areas within each functional domain. For example, within the limbic circuits, focal projections from the dACC and OFC regions do not occupy completely separate territories in any part of the striatum, but converge most extensively at rostral levels. Focal projections from the vmPFC converge with those from the dACC and OFC in relatively small, ventral zones.



**FIGURE 24.3** Diffuse and focal corticostriatal projections. (A) Diffuse projections from different prefrontal regions. (B) Diffuse + focal projections from the same prefrontal areas. Red, vmPFC = inputs; dark orange, OFC inputs; light orange, dACC inputs; yellow, DPFC inputs. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

Projections from different parts of areas 9 and 46 terminate in a topographic manner, but also converge at more caudal striatal levels. At the nucleus accumbens level, focal projections from the SEF and FEF converge in the lateral caudate nucleus, adjacent to the internal capsule.

Of particular interest, however, is the convergence of terminal fields from different functional domains (Fig. 24.3). For example, focal projections from dACC and OFC also converge with inputs from the DPFC. At rostral levels, DPFC terminals converge with those from both the dACC and OFC, although each cortical projection also occupies its own territory. Here, projections from all PFC areas occupy a central region, the different cortical projection extending into non-overlapping zones. This convergence does not take place only at the boundaries or edges of different functional domains. Rather, there are dense clusters of invading terminals from, for example, the DPFC embedded deep within the focal projection field of the OFC. Convergence is less prominent caudally, with almost complete separation of the dense terminals from the DPFC and dACC/OFC just rostral to the anterior commissure. At the most rostral striatal levels, projections from the DLPFC occupy a large portion of the dorso-central striatum. In comparison, the area occupied by terminal fields PMdr is relatively small. At this level, projections from PMdr and the DLPFC occupy primarily separate regions. However, at the level of the nucleus accumbens there are nodes of convergence between focal projections from PMdr and DPFC, embedded within each functional domain. This is particularly evident in the dorsal caudate nucleus. At caudal striatal levels, there is little or no convergence between focal projections from areas DPFC and PMdr. In fact, here terminals from these cortical regions are segregated occupying somewhat different, but adjacent locations in the caudate nucleus. As indicated above, convergence between focal projections from different functional regions is not unique to primates, rather extensive convergence between terminals from different functional regions of cortex is also observed in rats (Deniau, in preparation).

Convergence between focal projections from DPFC and limbic regions on the one hand and rostral motor-control areas on the other, takes place in specific and selected striatal regions. At more anterior levels, there is convergence between focal projections from DPFC and those from the limbic areas, OFC, dACC, and vmPFC. At the level of the nucleus accumbens and anterior commissure, convergence is more prominent between the DPFC and FEF and motor control areas. Taken together, projections from the DPFC are in a pivotal position in the striatum, converging at rostral levels with inputs from areas associated with motivation and reward (rostral striatum), and at more posterior levels (nucleus accumbens/anterior commissure levels) with those from cortical areas associated with action planning. These nodes of convergence provide an anatomical substrate for integration between different processing circuits and may represent "hot spots" of plasticity and adaptation. In general, the pivotal position of the DPFC in the striatum is similar to that in cortex, in that it is connected to both premotor and reward-related areas. However, the interaction between cognitive-, motor- and reward-related information may serve a different function in the striatum. The striatum receives focal cortical projections that are funneled into a concentrated region, along with an input from the dopamine cells that signal reward and salience. Thus, the pivotal role of the DLPFC in the striatum may differ from its role in cortex due to the combination of concentrated focal projections in addition to its dopaminergic input, thereby placing it in a position to specifically mediate learning that is associated with habit formation. The complexity of these interactions may be consistent with the concept that integrative aspects of striatal processing are organized around the striatal compartmentation of cortical inputs (Graybiel and Penney, 1999). However, while histochemical compartmentation is associated with cortical connections, this organization is complex and relationships switch depending on the cortical input (Ragsdale and Graybiel, 1990). Therefore, at this point it is not clear whether nodes of convergence are associated with histochemical compartmentation.

#### 2. Diffuse Projections

In addition to the focal projections, each cortical region sends a diffuse fiber projection outside of its focal terminal field, invading striatal regions that receive their focal input from other prefrontal cortex areas (Fig. 24.3) (Haber et al., 2006; Calzavara et al., 2007). These projections are extensive and at some distance from the focal projection field. For example, the diffuse projection from the OFC extends deep into the dorsal, central caudate, and putamen, with extensive convergence with the focal and diffuse projections from both the dACC and the DPFC. Likewise, the diffuse projections from dACC overlap with focal projections from the vmPFC, OFC and DPFC. Moreover, clusters of fibers are found in the dorsal lateral caudate nucleus and in the caudal ventral putamen, areas that do not receive a focal input from other prefrontal regions. Clusters of DPFC fibers terminate throughout the rostral striatum, including the ventral striatum and lateral putamen. Although DPFC focal projections do not reach into the ventro-medial region, its diffuse projection does (Fig. 24.3). Diffuse projections from premotor areas terminate throughout the striatum, with the exception of its ventro-medial part.

Significant and extensive diffuse projections from each frontal cortical region is consistent with the demonstration that a single cortico-striatal axon can innervate 14% of the striatum, demonstrating this pattern exists also in rats (Zheng and Wilson, 2002). However, activation of a medium spiny neuron requires a large coordinated glutamatergic input from many cortical cells (Wilson, 2004). Therefore, the invasions of relatively small fiber clusters from other functional regions are not considered to have much relevance for cortico-striatal information processing and, as a result, anatomical studies have focused on the large, dense focal projections (Selemon and Goldman-Rakic, 1985; Ferry et al., 2000). While under normal conditions in which a routine behavior is executed, these fibers may have little impact; this diffuse projection may serve a separate integrative function. Collectively, these projections represent a large population of axons invading each focal projection field, and, under certain conditions, if collectively activated, they may provide the recruitment strength necessary to modulate the focal signal. This would serve to broadly disseminate cortical activity to a wide striatal region, thus providing an anatomical substrate for crossencoding cortical information to influence the future firing of medium spiny neurons (Kasanetz et al., 2008). Taken together, the combination of focal and diffuse projections from frontal cortex occupies the rostral striatum and continues caudally through the caudate n and putamen. The fronto-striatal network, therefore, constitutes a duel system comprising both topographically organized terminal fields, along with subregions that contain convergent pathways derived from functionally discrete cortical areas (Haber et al., 2006; Draganski et al., 2008).

## **B.** Integration Through Connections of the Pallidum

The globus pallidus, like the striatum, is also organized according to functional domains. However, specific features of the pallidum make this an important way station for potential integration. This arises from the unique morphology of both pallidal segments and from the connections. Integration through the pallidum occurs primarily through convergence at the borders between functional domains. However, in some cases, particularly with connections of the external segment, fibers extend well into other functional domains. Connections through which integration can occur include, external pallido-striatal connections and subthalamo-pallidal connections, and external pallido-nigral connections.

## 1. Unique Morphological Features of the Globus Pallidum

Pallidal cells are large, with long, thick dendrites that are wrapped with striatal axons, forming a dense plexus of synapses that ensheath the axon. This distinct morphology is nicely outlined with immunoreactivity for enkephalin (external pallidal segment-GPe) and substance P (internal pallidal segment-GPi), which shows the extent of these dendrites that are wrapped in striatal fibers. These tubular-like structures, referred to as 'woolly fibers', are particularly useful for determining the boundaries and extent of the pallidum and their expansive span across functional territories (Fox et al., 1974; DiFiglia et al., 1982; Haber and Watson, 1985; Mai et al., 1986). Thus, a given pallidal neuron that lies within a particular dense focal projection, sends its dendrite into a neighboring territory. The proximal part of the dendrite receives a topographic input from a specific cortical area, via the striatum, but distal part of the dendrite is likely to receive input from an other functional region (Percheron and Filion, 1991).

#### 2. Pallido-Striatal Projections

The pallidostriatal pathway, which has received relatively little attention, is extensive and organized in a general dorsal-to-ventral and medial-to-lateral topography (Staines et al., 1981; Beckstead, 1983; Haber et al., 1985; Shu and Peterson, 1988; Kuo and Chang, 1992; Groenewegen et al., 1993; Spooren et al., 1996) (see Chapter 14). These arise from the GPe and parts of the VP in both primates and rats. It is therefore generally considered a simple feedback system. However, pallidostriatal terminal fields from each area of the pallidum contain a dense center that is surrounded by a less dense, albeit wide and extensive innervated area. The dense center region is referred to as the "core", and the collective cores from each pallidostriatal terminal field are topographically organized. However, surrounding each core is a wide-spread terminal region that expands the pallidal influence on the striatum. Thus, although the

pallidostriatal pathway reciprocates the striatopallidal pathway, the terminal arrangement is such that pallidal regions innervate parts of the striatum from which they receive no input, indicating that the pallidostriatal pathway is not a point-to-point feedback for striatopallidal projections. Rather, the pallidum modulates a wider region of the striatum than it receives input from. Striatopallidal projections obey a stricter topographic organization, suggesting that distinct striatopallidal pathways may be more closely maintained in one direction (striatopallidal) but not in its feedback (pallidostriatal). Therefore, GPe and VP regions that form part of distinct cortico-striatopallidal circuits send fibers to the striatum that interact with different cortico-striatopallidal circuits. Of particular interest, however, is that pallidal fibers from limbic regions do not reach motor control areas of the striatum, and those from motor control areas do not reach limbic striatal regions. The nonreciprocal relationship of the pallidum with the striatum subsequently creates both closed and opened loops between the striatum and pallidum. However, these nonreciprocal connections differ from the cortico-striatal pathways in that it is organized in a center (core)-surround configuration. The diffuse fiber projections seen in the cortico-striatal system have a wider distribution and are not organized in a center-surround configuration.

### 3. Subthalamo-Pallidal Projections

A major afferent source of both pallidal segments is the subthalamic nucleus. The GPe has reciprocal connections with the subthalamic nucleus and adjoining lateral hypothalamic area (Kuo and Carpenter, 1973; Kim et al., 1976; DeVito and Anderson, 1982; Harnois and Filion, 1982). Like the striatopallidal projections, the pallido-subthalamic pathway is topographic. However, the subthalamo-pallidal pathway to the GPe is not topographically organized, but rather arranged in bands parallel to the medullary lamina (Smith et al., 1990). Like the pallidostriatal system, distinct regions of the subthalamic nucleus may thus influence wider pallidal regions than it receives input from (Smith et al., 1990; Parent and Hazrati, 1993; Bevan et al., 1994). In other words, while descending projections from the various striatal regions maintain generally separate lines of conduction from the striatum to the pallidum and from the pallidum to the subthalamic nucleus, such segregation is apparently not strictly maintained in the ascending projections from the subthalamic nucleus to the pallidum and from the pallidum to the striatum. Thus,

separation of circuits is maintained in one direction, i.e. the striato-pallido-subthalamic direction, but not in the opposite direction, i.e. the subthalamic-pallido-striatal direction. Hence, a relatively small part of the subthalamic nucleus can influence greater parts of the striatum along these ascending lines of conduction. The general flow of information through the basal ganglia is from cortex to striatum and via the pallidum and thalamus, back to cortex. Information flowing in the opposite direction is seen only in the context of feedback loops. However, this is likely to underestimate the impact of these pathways on the output of the basal ganglia as seen with the extent and organization of both the pallidostriatal and subthalamo-pallidal pathways. As discussed later, this is also the case with the connection of cortex back to the thalamus.

#### 4. Pallido-Nigral Projections

While descending efferent projections from the pallidum terminate topographically in the subthalamic nucleus and thalamus, those projecting from the GPe and VP to the substantia nigra overlap extensively (Haber et al., 1993; Bevan et al., 1996). In particular, fibers from the ventral pallidum converge with those from the dorsal pallidum on single dopaminergic neurons. Therefore, individual substantia nigra cells receive both limbic and nonlimbic input. This convergence has important implications for the role of the dopamine cells in processing diverse information, which in turn is sent back to the striatum (see below). The pars reticulata neurons also receive this diverse information, which is then sent to the thalamus. A unique feature to the ventral pallidum in that it also projects to both the GPi and GPe of the dorsal pallidum. While the GPe does project to the internal segment, neither the internal nor GPe project to the ventral pallidum. This is an example of unilateral interface, in which the limbic system influences the cognitive and/or motor systems, but the reverse does not appear to occur. In summary, there is extensive integrative processing that occurs through the GPe and parts of the VP connections (the indirect pathway). In contrast, the GPi appears to be more involved in parallel processing (the direct pathway).

# C. The Striato-Nigro-Striatal Projection System

The dopamine cells are an integral part of the basal ganglia and are involved mediating all functions, including reward, cognition and motor control. The ventral tegmental area (VTA) is most closely linked to reward and reinforcement. The substantia nigra pars compacta (SNc) is associated with cognition and motor control. While subpopulations of dopamine neurons have been associated with these different functions: the mesolimbic, mesocortical system, and nigrostriatal pathways, respectively, all dopamine cell groups are now thought to be important for the development of reward-based learning and habit formation (Berridge and Robinson, 1998; Matsumoto et al., 1999; Schultz, 2002; Wise, 2004). Before turning to its projections, it is important to understand the organization of the midbrain dopamine cells.

#### 1. The Organization of Dopamine Neurons

Anatomically, the midbrain dopamine neurons are not clearly defined within the mesolimbic, mesocortical, and nigrostriatal categories. The midbrain dopamine neurons are generally divided into the SNc; the VTA; and the retrorubral cell groups (Hokfelt et al., 1984) (see also Chapter 16). In primates, the SNc is further divided into three groups: a dorsal group (the  $\alpha$  group); a main, densocellular region (the  $\beta$  group); and a ventral group (the  $\gamma$  group), or cell columns (Olszewski and Baxter, 1982; Haber et al., 1995). The dorsal group is oriented horizontally and extends dorsolaterally, circumventing the ventral and lateral superior cerebellar peduncle and the red nucleus. These cells merge with the immediately adjacent dopamine cell groups of the VTA to form a continuous mediodorsal band of cells. Calbindin (CaBP), a calcium-binding protein, marks both the VTA and the dorsal SNc. In contrast, the ventral cell groups (the densocellular group and the cell columns) are calbindin negative, and, unlike the dorsal tier, have high expression levels for dopamine transporter and for the D2 receptor mRNAs (Lavoie and Parent, 1991; Haber et al., 1995; McRitchie and Halliday, 1995). Thus, the midbrain cells can be divided in to a dorsal tier that includes the VTA, and the dorsal SNc, and a ventral tier that includes the densocellular group and cell columns (Fig. 24.4).

#### 2. Afferent Projections

Input to the midbrain dopamine neurons is primarily from the striatum, from both the GPe of the globus pallidus and the ventral pallidum, and from the brainstem (for review see Haber and Gdowski, 2004) (see also Chapter 16). Descending projections from the central nucleus of the amygdala also terminate in a wide mediolateral region, but are limited primarily to the dorsal tier cells. In addition, there are



**FIGURE 24.4** Schematic illustrating the organization of the midbrain dopamine neurons into the dorsal and ventral tiers. SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area.

projections to the dorsal tier from the bed nucleus of the stria terminalis and from the sublenticular substantia innominata that travel together with those from the amygdala (Fudge and Haber, 2000; Fudge and Haber, 2001). While the dopamine neurons receive input from these several sources, perhaps the most massive projection is from the striatum. Striatal projections terminate on both the dorsal and ventral tier, in addition to the pars reticulata. This afferent projection is organized with an inverse ventral/dorsal topography. The ventral striatum projects widely to the dorsal tier and much of the dorsal part of the densocellular, pars compacta cells. This ventral striatal terminal field extends laterally to include a large mediolateral region. Descending projections from the extended amygdala also terminate in a wide mediolateral region, but primarily in the dorsal tier. Therefore, the dorsal tier receives a massive limbic input through an indirect projection from the OFC/dACC/vmPFC (via the striatum), and a direct projection from the extended amygdala.

The caudate nucleus, which receives input primarily from the DPFC, projects extensively to the central and ventral parts of the densocellular region, extending into the cell columns and surrounding pars reticulata. Finally, the dorsolateral striatal projection is concentrated in the ventral and lateral part of the substantia nigra. Unlike the widespread terminal fields of the ventral and central striatum, the distribution of efferent fibers from the dorsolateral striatum is more restricted and terminates primarily in the pars reticulata. However, their terminal fields do project to the cell columns of dopamine neurons that penetrate deep into the pars reticulata. Thus, in addition to the inverse dorsoventral topographic organization to the striato-nigral projection, there is an important difference in the extent of



**FIGURE 24.5** Schematic of the substantia nigra showing the combined distribution of striatonigral terminal fields (A and C), nigrostriatal cells (B and C) associated with different functional regions of the striatum. Pink/red = inputs and outputs from the limbic striatum; yellow/orange = inputs and outputs from the associative striatum; blue = inputs and outputs from the motor striatum. CP, cerebral peduncles; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area. To view a color version of this image please visit http://www.elsevierdirect. com/companion/9780123747679

the projections fields from the functional striatal domains. Projections from regions receiving PFC inputs have wide projection fields throughout the midbrain dopamine cells, while those from motor control areas have a relatively limited projection field (Fig. 24.5A).

### 3. Efferent Projections

As with the descending striatonigral pathway, the ascending nigrostriatal projection also exhibits an inverse dorsoventral topographic arrangement. Here, there is also a mediolateral topographic organization. Thus, the dorsal and medial dopamine cells project to the ventral and medial parts of the striatum, while the ventral and lateral cells project to the dorsal and lateral parts of the striatum (Parent et al., 1983; Hedreen and DeLong, 1991; Lynd-Balta and Haber, 1994c, a; Haber et al., 2000). Moreover, as with the striatonigral projection, the proportional distribution of cells that project to different functional domains of the striatum differ (Fig. 24.5B). The shell region of the ventral striatum receives the most limited midbrain input, primarily derived from the VTA. The rest of the ventral striatum receives input primarily from the dorsal tier, including the retrorubral cell group, and from the medial and dorsal region of the densocellular group. The central part of the striatum, which receives input from the DPFC, also receives input from the central part of the dopamine cells. In contrast, the dorsolateral part of the striatum receives input from a wide range of dopamine cells, derived from the ventral tier, including both the densocellular and cell columns groups (Haber et al., 2000) (Fig. 24.5B).

### 4. Relationship Between Reciprocal and Non-Reciprocal Connections

The concept of the ventral striatal influence on the dorsal striatum, via the midbrain dopamine neurons was first proposed based on rodent studies that demonstrated that the ventral striatum projects to SNc cells that, in turn project to the dorsal striatum. This was proposed as one mechanism by which the limbic system directly impacted the motor system (Nauta et al., 1978; Somogyi and Smith, 1979). In primates, the complex relationships between reciprocal and non-reciprocal components create an anatomical substrate for a directional flow of information processing across several circuits (Haber et al., 2000). When considered separately, each limb of the system creates a loose topographic organization. The VTA and medial substantia nigra are associated with the limbic regions, the central substantia nigra with associative regions, and the lateral and ventral substantia nigra are related to the motor control striatal regions (Fig. 24.4C). However, the fact that the descending and ascending limb of each functional striatonigral and nigrostriatal system differs in their proportional projections significantly alters the relationship of different functional striatal areas with the midbrain. The ventral striatum receives a relatively limited midbrain input, but projects to a large region, which includes dorsal and ventral tiers and the dorsal pars reticulata. In contrast, the dorsolateral striatum receives input from a wide range of dopamine cells, but projects to a limited region (Fig. 24.5C).

The proportional differences between inputs and outputs of the dopamine neurons, coupled with their topography results in complex interweaving of functional pathways. In addition, for each striatal region, the afferent and efferent striato-nigro-striatal projection system contains three components in the midbrain. There is a reciprocal connection that is flanked by two non-reciprocal connections. The reciprocal component contains cells that project to a specific striatal area. These cells are embedded within terminals from that same striatal area. Dorsal to this region lies a group of cells that project to the same striatal region, but do not lie within its reciprocal terminal field. In other words, these cells receive a striatal projection from a region that they do not project to. Finally, ventral to the reciprocal component are efferent terminals. However, there are no cells embedded in these terminals that project to that same specific striatal region. The cells located in this terminal field project to a different striatal area. These three components for each striato-nigro-striatal projection system occupy different positions within the midbrain. The ventral striatum system lies dorsomedially, the dorsolateral striatum system lies ventrolaterally, and the central striatal system is positioned between the two. Moreover, as indicated above, each functional region differs in its proportional projections, which significantly alter their relationship to each other. The ventral striatum receives a limited midbrain input but projects to a large region. In



FIGURE 24.6 Schematic illustrating the striato-nigro-striatal projections. The colored gradient in cortex and striatum illustrates the organization of functional corticostriatal inputs. Midbrain projections from the shell target both the VTA and ventromedial SNc (red arrows). Midbrain projections from the VTA to the shell form a "closed," reciprocal SNS loop (red arrow). Projections from the medial SN feed-forward to the central ventral striatum forming the first part of a spiral (orange arrow). The spiral continues through the striato-nigro-striatal projections (yellow, green, and blue arrows) with pathways originating in the ventral striatum and projecting more dorsally. In this way ventral striatal regions influence more dorsal striatal regions via these spiraling projections. IC, internal capsule; vmPFC/OFC, ventral medial prefrontal cortex/orbital prefrontal cortex; DPFC, dorsal prefrontal cortex. (see Color Plate Section to view the color version of this figure)

contrast, the dorsolateral striatum receives a wide input but projects to a limited region. In other words, the ventral striatum influences a wide range of dopamine neurons, but is itself influenced by a relatively limited group of dopamine cells. On the other hand, the dorsolateral striatum influences a limited midbrain region, but is affected by a relatively large midbrain region.

Thus, the combination of proportional differences coupled with the three components, creates an arrangement in which information from the limbic system flows through a series of connections to reach the motor system. The ventral striatum receives input from limbic regions and projects to the dorsal tier. The dorsal tier projects back to the ventral striatum. However, the ventral striatum efferent projection to the midbrain extends beyond the tight ventral striatal/dorsal tier/ventral striatal circuit, terminating lateral and ventral to the dorsal tier. This area of terminal projection does not project back to the ventral striatum. Rather, cells in this region project more dorsally, into the striatal area that receives input from the DPFC. Through this connection, the same cortical information that influences the dorsal tier through the ventral striatum also modulates the densocellular region that projects to the central striatum. This central striatal region is reciprocally connected to the densocellular region. But it also projects to the ventral densocellular area and into the cell columns. Thus, projections from the DPFC, via the striatum, are in a position to influence cells that project to motor control areas of the striatum. The dorsolateral striatum is reciprocally connected to the ventral densocellular region and cell columns. The confined distribution of efferent dorsolateral striatal fibers limits the influence of the motor striatum to a relatively small region involving the cell columns and the pars reticulata. Taken together, the interface between different striatal regions via the midbrain dopamine cells is organized in an ascending spiral interconnecting different functional regions of the striatum (Fig. 24.6). This creates a feed forward organization. Through this spiral of inputs and outputs between the striatum and midbrain dopamine neurons, information can be channeled from the shell and ventral striatum, through the central striatum, and to the dorsolateral striatum. In this way, information can flow from limbic to cognitive to motor circuits, allowing a mechanism by which motivation and cognition can influence motor decision-making processes, and appropriate responses to environmental cues.

# D. The Place of the Thalamus in Basal Ganglia Circuitry

The thalamic-cortical pathway is the last link in the circuit and is often treated as a simple "one-way relay" back to cortex. However this pathway does not transfer information passively but rather plays a key role in regulating cortical ensembles of neurons through its non-reciprocal connections with cortex. This occurs in two ways. First, the thalamus projects to different cortical layers. Therefore, while the thalamus receives input from the deep cortical layers, the thalamic projection to cortex, from the BG relay nuclei, terminates in superficial, middle, and deep layers (layers I/II, III/IV, and V, respectively) (McFarland and Haber, 2002; Erickson and Lewis, 2004). Projections that terminate in layer V form both direct thalamo-corticothalamic and thalamo-corticostriatal loops, thus sustaining information processing from the thalamus through each specific cortico-BG circuit. However, projections to the superficial layers play a key role in corticocortical processing. These are particularly interesting in that they have a more global recruiting action response effecting wide networks of cortical activity. In contrast to the topographicallyspecific thalamo-cortical projections to middle layers, the more widespread, diffuse terminals to layer I are in a position to modulate neuronal activity from all cortical layers with apical dendrites ascending into layer I. Moreover, this projection can provide an important mechanism for cross-communication between basal ganglia circuits. Projections to superficial layers also interface with corticocortical connections. These cortical regions, in turn, send axons to the striatum, thereby potentially modulating a different loop.

Second, while corticothalamic projections to specific relay nuclei are thought to follow a general rule of reciprocity, corticothalamic projections to VA/VL and central MD sites, as seen in other thalamocortical systems, are more extensive than thalamocortical projections (Catsman-Berrevoets and Kuypers, 1978; Hoogland et al., 1987; Sherman and Guillery, 1996; Deschenes et al., 1998; Jones, 1998; Darian-Smith et al., 1999; McFarland and Haber, 2002). Furthermore, they are derived from areas not innervated by the same thalamic region, indicating non-reciprocal corticothalamic projections to specific basal ganglia relay nuclei (McFarland and Haber, 2002). Although each thalamic nucleus completes the cortico-BG segregated circuit, the non-reciprocal component is derived from a functionally distinct frontal cortical area. For example, the central MD has reciprocal connections with the lateral and orbital prefrontal areas and also a non-reciprocal input from medial prefrontal areas; VA has reciprocal connections with dorsal premotor areas, and caudal DLPFC and also a non-reciprocal connection from medial prefrontal areas; and VLo has reciprocal connections with caudal motor areas along with a non-reciprocal connection from rostral motor regions. The potential for relaying information between circuits through thalamic connections, therefore, is accomplished both through the organization of projections to different layers and through the non-reciprocal corticothalamic pathways. Thus, similar to the striato-nigro-striatal project system, the thalamic relay nuclei from the BG, also appear to mediate information flow from higher cortical "association" areas of the prefrontal cortex to rostral motor areas involved in "cognitive" or integrative aspects of motor control to primary motor areas that direct movement execution.

### **IV. FUNCTIONAL CONSIDERATIONS**

A key component for developing appropriate goal directed behaviors is the ability to first correctly evaluate different aspects of reward, including value versus risk and predictability, and inhibit maladaptive choices, based on previous experience. These calculations rely on integration between different aspects of reward processing and cognition to develop and execute appropriate action plans. While parallel networks that mediate different functions are critical to maintaining coordinated behaviors, cross talk between functional circuits during learning and adaptation is critical. Indeed, reward, associative, and motor control functions are not clearly and completely separated within the striatum. For example, consistent with human imaging studies, reward-responsive neurons are not restricted to the ventral striatum, but rather they are found throughout the striatum. Moreover, cells responding in working memory tasks are often found also in the ventral striatum (Apicella et al., 1991; Levy et al., 1997; Hassani et al., 2001; Takikawa et al., 2002; Cromwell and Schultz, 2003; Watanabe et al., 2003; Tanaka et al., 2004; Delgado et al., 2005).

As described above, embedded within limbic, associative, and motor control striatal territories, are subregions containing convergent terminals between different reward processing cortical areas, between these projections and those from the DPFC, and between the DPFC and rostral motor control areas. These nodes of converging terminals may represent "hot spots" that may be particularly sensitive to synchronizing information across functional areas to impact on long-term strategic planning, and habit formation (Kasanetz et al., 2008). Indeed, cells in the dorsal striatum are progressively recruited during different types of learning, from simple motor tasks to drug self administration (Porrino et al., 2004; Lehericy et al., 2005; Pasupathy and Miller, 2005; Volkow et al., 2006). The existence of convergent fibers from cortex within the ventral striatum, taken together with hippocampal and amygdalo-striatal projections, places the ventral striatum in a key entry port to processing emotional and motivational information that, in turn, drives basal ganglia action output. The ventral, reward-based striatal region, and the associative, central striatal region can impact on motor output circuits, not only through convergent terminal fields within the striatum, but also through the striato-nigro-striatal pathways. One can hypothesize that initially the nodal points of interface between the reward and associative circuits, for example, send a coordinated signal to dopamine cells. This pathway is in a pivotal position for temporal "training" dopamine cells. In turn, these nodal points may be further reinforced through the burst firing activity of the nigro-striatal pathway, thus transferring that impact back to the striatum. Thus, through the striato-nigro-striatal system, information is linked transferred to other functional regions, during learning and habit formation (Everitt and Robbins, 2005; Volkow et al., 2006; Porrino et al., 2007; Belin et al., 2008). As demonstrated in rodent, monkey and human studies, when the striato-nigro-striatal circuit is interrupted, information transfer from Pavlovian to instrumental learning does not take place (Belin and Everitt, 2008).

In addition, the direct cortico-thalamic and corticosubthalamic signals arrive in those structures prior to signal generated through the striatum. This likely sets the stage for information passed through the striato-pallidal loop. Through feed back loops of the pallido-striatal, subthalamo-pallidal, and cortico-thalamic systems information is continually updated. In this respect, it is of interest that we consider the loop running from cortex through the striatum, back to cortex via the thalamus and not the reverse and rises the interesting question, of where is the central processing station? In any event, the signal then enters the parallel system and, via the pallidum and thalamus, carries an integrated signal back to cortex (Fig. 24.7).

Parallel circuits and integrative circuits must work together, allowing the coordinated behaviors to be maintained, and focused (via parallel networks), but also to be modified and changed according to external and internal stimuli (via integrative networks) (Fig. 24.7). Both the ability to maintain focus in the execution of specific behaviors, as well as the ability to adapt appropriately to external and internal cues, are key deficits in basal ganglia diseases which affect these aspects of motor control, cognition and motivation. As outlined in this chapter, within each interconnected cortico-basal ganglia loop, there are subregions that cross functional domains.



FIGURE 24.7 Schematic illustrating both dual parallel and integrative processing through cortico-basal ganglia pathways. Corresponding shaded striatal and cortical areas demonstrates topographic of projections. multiple shaded areas indicate substriatal regions where convergence between terminals from different cortical areas occurs. Arrows connecting the striatum and substantia nigra illustrate how ventral striatum can influence the dorsal striatum through the midbrain dopamine cells. The connections between integrated areas also enter the parallel processing system, back to cortex, as indicated by arrows connecting the striatum via the pallidum and thalamus. DPFC, dorsolateral prefrontal cortex; GP/SNr, globus pallidus/substantia nigra pars reticulata; OFC/ACC, orbital prefrontal/anterior cingulate cortex; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area.

### ABBREVIATIONS

- dACC dorsal anterior cingulate cortex
- DPFC dorsal prefrontal cortex
- GPe globus pallidus, external segment
- GPi globus pallidus, internal segment
- OFC orbital prefrontal cortex
- SNc substantia nigra, pars compacta
- SNr substantia nigra, pars reticulata
- vmPFC ventromedial prefrontal cortex
- VP ventral pallidum

### REFERENCES

- Aldridge JW, Anderson RJ, Murphy JT (1980) Sensory-motor processing in the caudate nucleus and globus pallidus: a single-unit study in behaving primates. Can J Physiol Pharmacol 58:1192–1201.
- Alexander GE, DeLong MR (1985) Microstimulation of the primate neostriatum. II. Somatotopic organization of striatal microexcitable zones

and their relation to neuronal response properties. J Neurophysiol 53:1417–1430.

- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Aosaki T, Graybiel AM, Kimura M (1994) Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. Science 265:410–412.
- Apicella P, Ljungberg T, Scarnati E, Schultz W (1991) Responses to reward in monkey dorsal and ventral striatum. Exp Brain Res 85:491–500.
- Bar-Gad I, Morris G, Bergman H (2003) Information processing, dimensionality reduction and reinforcement learning in the basal ganglia. Prog Neurobiol 71:439–473.
- Bar-Gad I, Havazelet-Heimer G, Goldberg JA, Ruppin E, Bergman H (2000) Reinforcement-driven dimensionality reduction – a model for information processing in the basal ganglia. J Basic Clin Physiol Pharmacol 11:305–320.
- Barbas H (1992) Architecture and cortical connections of the prefrontal cortex in the rhesus monkey. In: Advances in Neurology (Chauvel P, Delgado-Escueta AV, eds), pp. 91–115. New York: Raven Press.
- Battig K, Rosvold HE, Mishkin M (1960) Comparison of the effect of frontal and caudate lesions on delayed response and alternation in monkeys. J Comp Physiol Psychol 53:400–404.

- Beckstead RM (1983) A pallidostriatal projection in the cat and monkey. Brain Res Bull 11:629–632.
- Belin D, Everitt BJ (2008) Cocaine Seeking Habits Depend upon Dopamine-Dependent Serial Connectivity Linking the Ventral with the Dorsal Striatum. Neuron 57:432–441.
- Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320:1352–1355.
- Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309–369.
- Bevan MD, Crossman AR, Bolam JP (1994) Neurons projecting from the entopeduncular nucleus to the thalamus receive convergent synaptic inputs from the subthalamic nucleus and the neostriatum in the rat. Brain Res 659:99–109.
- Bevan MD, Smith AD, Bolam JP (1996) The substantia nigra as a site of synaptic integration of functionally diverse information arising from the ventral pallidum and the globus pallidus in the rat. Neuroscience 75:5–12.
- Bevan MD, Clarke NP, Bolam JP (1997) Synaptic integration of functionally diverse pallidal information in the entopeduncular nucleus and subthalamic nucleus in the rat. J Neurosci 17:308–324.
- Blumenfeld RS, Ranganath C (2006) Dorsolateral prefrontal cortex promotes long-term memory formation through its role in working memory organization. J Neurosci 26:916–925.
- Butter CM, Snyder DR (1972) Alterations in aversive and aggressive behaviors following orbital frontal lesions in rhesus monkeys. Acta Neurobiol Exp 32:525–565.
- Calzavara R, Mailly P, Haber SN (2007) Relationship between the corticostriatal terminals from areas 9 and 46, and those from area 8A, dorsal and rostral premotor cortex and area 24c: an anatomical substrate for cognition to action. Eur J Neurosci 26:2005–2024.
- Catsman-Berrevoets CE, Kuypers HG (1978) Differential laminar distribution of corticothalamic neurons projecting to the VL and the center median. An HRP study in the cynomolgus monkey. Brain Res 154:359–365.
- Cools R, Clark L, Robbins TW (2004) Differential responses in human striatum and prefrontal cortex to changes in object and rule relevance. J Neurosci 24:1129–1135.
- Corlett PR, Aitken MR, Dickinson A, Shanks DR, Honey GD, Honey RA, Robbins TW, Bullmore ET, Fletcher PC (2004) Prediction error during retrospective revaluation of causal associations in humans: fMRI evidence in favor of an associative model of learning. Neuron 44:877–888.
- Cromwell HC, Schultz W (2003) Effects of expectations for different reward magnitudes on neuronal activity in primate striatum. J Neurophysiol 89:2823–2838.
- Darian-Smith C, Tan A, Edwards S (1999) Comparing thalamocortical and corticothalamic microstructure and spatial reciprocity in the macaque ventral posterolateral nucleus (VPLc) and medial pulvinar. J Comp Neurol 410:211–234.
- Delgado MR, Miller MM, Inati S, Phelps EA (2005) An fMRI study of reward-related probability learning. Neuroimage 24:862–873.
- Deschenes M, Veinante P, Zhang ZW (1998) The organization of corticothalamic projections: reciprocity versus parity. Brain Res Rev 28:286–308.
- DeVito JL, Anderson ME (1982) An autoradiographic study of efferent connections of the globus pallidus in *Macaca mulatta*. Exp Brain Res 46:107–117.

- DiFiglia M, Aronin N, Martin JB (1982) Light and electron microscopic localization of immunoreactive leu-enkephalin in the monkey basal ganglia. J Neurosci 2(3):303–320.
- Doyon J, Gaudreau D, Laforce R Jr, Castonguay M, Bedard PJ, Bedard F, Bouchard JP (1997) Role of the striatum, cerebellum, and frontal lobes in the learning of a visuomotor sequence. Brain Cogn 34:218–245.
- Draganski B, Kherif F, Kloppel S, Cook PA, Alexander DC, Parker GJ, Deichmann R, Ashburner J, Frackowiak RS (2008) Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. J Neurosci 28:7143–7152.
- Dubé L, Smith AD, Bolam JP (1988) Identification of synaptic terminals of thalamic or cortical origin in contact with distinct medium-size spiny neurons in the rat neurostriatum. J Comp Neurol 267:455–471.
- Dum RP, Strick PL (1993) Cingulate motor areas. In: Neurobiology of Cingulate Cortex and Limbic Thalamus: A Comprehensive Treatise (Vogt BA, Gabriel M, eds), pp. 415–441. Boston: Birkhauser.
- Elliott R, Dolan RJ (1999) Differential neural responses during performance of matching and nonmatching to sample tasks at two delay intervals. J Neurosci 19:5066–5073.
- Elliott R, Newman JL, Longe OA, Deakin JF (2003) Differential response patterns in the striatum and orbitofrontal cortex to financial reward in humans: a parametric functional magnetic resonance imaging study. J Neurosci 23:303–307.
- Erickson SL, Lewis DA (2004) Cortical connections of the lateral mediodorsal thalamus in cynomolgus monkeys. J Comp Neurol 473:107–127.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Fenelon G, Francois C, Percheron G, Yelnik J (1991) Topographic distribution of the neurons of the central complex (Centre median-parafascicular complex) and of other thalamic neurons projecting to the striatum in macaques. Neuroscience 45:495–510.
- Ferry AT, Ongur D, An X, Price JL (2000) Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks. J Comp Neurol 425:447–470.
- Flaherty AW, Graybiel AM (1994) Input-output organization of the sensorimotor striatum in the squirrel monkey. J Neurosci 14:599–610.
- Fox CH, Andrade HN, Du Qui IJ, Rafols JA (1974) The primate globus pallidus. A Golgi and electron microscope study. J R Hirnforschung 15:75–93.
- Francois C, Percheron G, Parent A, Sadikot AF, Fenelon G, Yelnik J (1991) Topography of the projection from the central complex of the thalamus to the sensorimotor striatal territory in monkeys. J Comp Neurol 305:17–34.
- Friedman DP, Aggleton JP, Saunders RC (2002) Comparison of hippocampal, amygdala, and perirhinal projections to the nucleus accumbens: combined anterograde and retrograde tracing study in the Macaque brain. J Comp Neurol 450:345–365.
- Fudge JL, Haber SN (2000) The central nucleus of the amygdala projection to dopamine subpopulations in primates. Neuroscience 97:479–494.
- Fudge JL, Haber SN (2001) Bed nucleus of the stria terminalis and extended amygdala inputs to dopamine subpopulations in primates. Neuroscience 104:807–827.
- Fudge JL, Kunishio K, Walsh C, Richard D, Haber SN (2002) Amygdaloid projections to ventromedial striatal subterritories in the primate. Neuroscience 110:257–275.

- Fuster JM (1989). Lesion Studies. In: The Prefrontal Cortex Anatomy, Physiology, and Neuropsychology of the Frontal Lobe, 2nd edn, pp. 51–82: Raven Press. New York.
- Fuster JM (2000) Prefrontal neurons in networks of executive memory. Brain Res Bull 52:331–336.
- Fuster JM (2001) The prefrontal cortex--an update: time is of the essence. Neuron 30:319–333.
- Giguere M, Goldman-Rakic PS (1988) Mediodorsal nucleus: area 1 laminar and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkeys. J Comp Neurol 277:195–213.
- Giménez-Amaya JM, McFarland NR, de las Heras S, Haber SN (1995) Organization of thalamic projections to the ventral striatum in the primate. J Comp Neurol 354:127–149.
- Goldman-Rakic PS (1996) The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Phil Trans Roy Soc Lond – Ser B: Biol Sci 351:1445–1453.
- Goldman-Rakic PS, Porrino LJ (1985) The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. J Comp Neurol 242:535–560.
- Graybiel AM, Penney JB (1999) Chemical architecture of the basal ganglia. In: Handbook of Chemical Neuroanatomy (Bloom FE, Bjorklund A, Hokfelt T, eds), pp. 227–284. New York: Elsevier Science.
- Groenewegen HJ, Berendse HW (1994) The specificity of the 'nonspecific' midline and intralaminar thalamic nuclei. Trends Neurosci 17:50.
- Groenewegen HJ, Berendse HW, Haber SN (1993) Organization of the output of the ventral striatopallidal system in the rat: Ventral pallidal efferents. Neuroscience 57:113–142.
- Haber SN, Watson SJ (1985) The comparative distribution of enkephalin, dynorphin and substance P in the human globus pallidus and basal forebrain. Neuroscience 14:1011–1024.
- Haber SN, Gdowski MJ (2004) The Basal Ganglia. In: The Human Nervous System, 2nd edn (Paxinos G, Mai JK, eds), pp. 677–738: Elsevier Press.
- Haber SN, Lynd-Balta E, Mitchell SJ (1993) The organization of the descending ventral pallidal projections in the monkey. J Comp Neurol 329(1):111–129.
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Haber SN, Groenewegen HJ, Grove EA, Nauta WJH (1985) Efferent connections of the ventral pallidum. Evidence of a dual striatopallidofugal pathway. J Comp Neurol 235:322–335.
- Haber SN, Lynd E, Klein C, Groenewegen HJ (1990) Topographic organization of the ventral striatal efferent projections in the rhesus monkey: An anterograde tracing study. J Comp Neurol 293:282–298.
- Haber SN, Ryoo H, Cox C, Lu W (1995) Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: Comparison with the mRNA for the D2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. J Comp Neurol 362:400–410.
- Haber SN, Kim KS, Mailly P, Calzavara R (2006) Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical inputs, providing a substrate for incentive-based learning. J Neurosci 26:8368–8376.
- Harnois C, Filion M (1982) Pallidofugal projections to thalamus and midbrain: A quantitative antidromic activation study in monkeys and cats. Exp Brain Res 47:277–285.

- Hassani OK, Cromwell HC, Schultz W (2001) Influence of expectation of different rewards on behavior-related neuronal activity in the striatum. J Neurophysiol 85:2477–2489.
- He SQ, Dum RP, Strick PL (1993) Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. J Neurosci 13:952–980.
- Hedreen JC, DeLong MR (1991) Organization of striatopallidal, striatonigral, and nigrostriatal projections in the Macaque. J Comp Neurol 304:569–595.
- Herkenham M (1986) New perspectives on the organization and evolution of nonspecific thalamocortical projections. In: Cerebral Cortex: Sensory-Motor Areas and Aspects of Cortical Connectivity (Jones EG, Peters A, eds), pp. 403–445. New York: Plenum Press.
- Hikosaka K, Watanabe M (2000) Delay activity of orbital and lateral prefrontal neurons of the monkey varying with different rewards. Cerebral Cortex 10:263–271.
- Hikosaka O, Miyashita K, Miyachi S, Sakai K, Lu X (1998) Differential roles of the frontal cortex, basal ganglia, and cerebellum in visuomotor sequence learning. Neurobiol Learn Mem 70:137–149.
- Hokfelt T, Martensson R, Bjorklund A, Kleinau S, Goldstein M (1984). Distributional maps of tyrosine-hydroxylase immunoreactive neurons in the rat brain. In: Handbook of Chemical Neuroanatomy, Vol. II: Classical Neurotransmitters in the CNS, I (Bjorklund A, Hokfelt T, eds), pp. 277–379: Elsevier. Amsterdam.
- Hoogland PV, Welker E, Van der Loos H (1987) Organization of the projections from barrel cortex to thalamus in mice studied with *Phaseolus vulgaris*-leucoagglutinin and HRP. Exp Brain Res 68:73–87.
- Ilinsky IA, Jouandet ML, Goldman-Rakic PS (1985) Organization of the nigrothalamocortical system in the rhesus monkey. J Comp Neurol 236:315–330.
- Jog MS, Kubota Y, Connolly CI, Hillegaart V, Graybiel AM (1999) Building neural representations of habits. Science 286:1745–1749.
- Jones EG (1998) The thalamus of primates. In: The Primate Nervous System, Part II (Bloom FE, Björklund A, Hökfelt T, eds), pp. 1–298. Amsterdam: Elsevier Science.
- Kasanetz F, Riquelme LA, Della-Maggiore V, O'Donnell P, Murer MG (2008) Functional integration across a gradient of corticostriatal channels controls UP state transitions in the dorsal striatum. Proc Natl Acad Sci USA 105:8124–8129.
- Kim R, Nakano K, Jayaraman A, Carpenter MB (1976) Projections of the globus pallidus and adjacent structures: an autoradiographic study in the monkey. J Comp Neurol 169:263–290.
- Kimura M (1986) The role of primate putamen neurons in the association of sensory stimulus with movement. Neurosci Res 3:436–443.
- Knutson B, Adams CM, Fong GW, Hommer D (2001) Anticipation of increasing monetary reward selectively recruits nucleus accumbens. J Neurosci 21:RC159.
- Knutson B, Taylor J, Kaufman M, Peterson R, Glover G (2005) Distributed neural representation of expected value. J Neurosci 25:4806–4812.
- Kolomiets BP, Deniau JM, Mailly P, Menetrey A, Glowinski J, Thierry AM (2001) Segregation and convergence of information flow through the cortico-subthalamic pathways. J Neurosci 21:5764–5772.
- Kringelbach ML, Rolls ET (2004) The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol 72:341–372.
- Kuhnen CM, Knutson B (2005) The neural basis of financial risk taking. Neuron 47:763–770.

- Kunzle H (1975) Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in *Macaca fascicularis*. Brain Res 88:195–209.
- Künzle H (1977) Projections from the primary somatosensory cortex to basal ganglia and thalamus in the monkey. Exp Brain Res 30:481–492.
- Kuo H, Chang HT (1992) Ventral pallido-striatal pathway in the rat brain: A light and electron microscopic study. J Comp Neurol 321:626–636.
- Kuo J, Carpenter MB (1973) Organization of pallidothalamic projections in the rhesus monkey. J Comp Neurol 151:201–236.
- Lavoie B, Parent A (1991) Dopaminergic neurons expressing calbindin in normal and parkinsonian monkeys. Neuroreport 2:601–604.
- Lehericy S, Benali H, Van de Moortele PF, Pelegrini-Issac M, Waechter T, Ugurbil K, Doyon J (2005) Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. Proc Natl Acad Sci USA 102:12566–12571.
- Levy R, Friedman HR, Davachi L, Goldman-Rakic PS (1997) Differential activation of the caudate nucleus in primates performing spatial and nonspatial working memory tasks. J Neurosci 17:3870–3882.
- Lynd-Balta E, Haber SN (1994a) The organization of midbrain projections to the striatum in the primate: Sensorimotor-related striatum versus ventral striatum. Neuroscience 59:625–640.
- Lynd-Balta E, Haber SN (1994b) Primate striatonigral projections: A comparison of the sensorimotor-related striatum and the ventral striatum. J Comp Neurol 345:562–578.
- Lynd-Balta E, Haber SN (1994c) The organization of midbrain projections to the ventral striatum in the primate. Neuroscience 59:609–623.
- Mai JK, Stephens PH, Hopf A, Cuello AC (1986) Substance P in the human brain. Neuroscience 17:709–739.
- Matelli M, Luppino G (1996) Thalamic input to mesial and superior area 6 in the Macaque monkey. J Comp Neurol 372:59–87.
- Matsumoto N, Hanakawa T, Maki S, Graybiel AM, Kimura M (1999) Nigrostriatal dopamine system in learning to perform sequential motor tasks in a predictive manner. J Neurophysiol 82:978–998.
- Mayberg HS, Liotti M, Brannan SK, et al. (1999) Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. Am J Psychiat 156:675–682.
- McFarland NR, Haber SN (2000) Convergent inputs from thalamic motor nuclei and frontal cortical areas to the dorsal striatum in the primate. J Neurosci 20:3798–3813.
- McFarland NR, Haber SN (2001) Organization of thalamostriatal terminals from the ventral motor nuclei in the macaque. J Comp Neurol 429:321–336.
- McFarland NR, Haber SN (2002) Thalamic relay nuclei of the basal ganglia form both reciprocal and nonreciprocal cortical connections, linking multiple frontal cortical areas. J Neurosci 22:8117–8132.
- McRitchie DA, Halliday GM (1995) Calbindin D28K-containing neurons are restricted to the medial substantia nigra in humans. Neuroscience 65:87–91.
- Mena-Segovia J, Ross HM, Magill PJ, Bolam JP (2005) The pedunculopontine nucleus: towards a functional integration with the basal ganglia. New York: Springer Science and Business Media.
- Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Rev 31:236–250.
- Middleton FA, Strick PL (2002) Basal-ganglia 'projections' to the prefrontal cortex of the primate. Cereb Cortex 12:926–935.
- Milad MR, Rauch SL (2007) The role of the orbitofrontal cortex in anxiety disorders. Ann NY Acad Sci 1121:546–561.
- Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL (2005) Thickness of ventromedial prefrontal cortex in humans

is correlated with extinction memory. Proc Natl Acad Sci USA 102:10706–10711.

- Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL (2007) Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiatry 62:446–454.
- Mink JW (1996) The basal ganglia: focused selection and inhibition of competing motor programs. Progress in Neurobiology 50:381–425.
- Muhammad R, Wallis JD, Miller EK (2006) A comparison of abstract rules in the prefrontal cortex, premotor cortex, inferior temporal cortex, and striatum. J Cogn Neurosci 18:974–989.
- Mushiake H, Inase M, Tanji J (1991) Neuronal activity in the primate premotor, supplementary, and precentral motor cortex during visually guided and internally determined sequential movements. J Neurophysiol 66:705–718.
- Nakano K, Hasegawa Y, Tokushige A, Nakagawa S, Kayahara T, Mizuno N (1990) Topographical projections from the thalamus, subthalamic nucleus and pedunculopontine tegmental nucleus to the striatum in the Japanese monkey, *Macaca fuscata*. Brain Res 537:54–68.
- Nauta WJH, Smith GP, Faull RLM, Domesick VB (1978) Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience 3:385–401.
- O'Doherty J, Critchley H, Deichmann R, Dolan RJ (2003) Dissociating valence of outcome from behavioral control in human orbital and ventral prefrontal cortices. J Neurosci 23:7931–7939.
- O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C (2001) Abstract reward and punishment representations in the human orbitofrontal cortex. Nat Neurosci 4:95–102.
- Olszewski J, Baxter D (1982) Cytoarchitecture of the human brain stem, 2nd edn. Basel: S. Karger.
- Padoa-Schioppa C, Assad JA (2006) Neurons in the orbitofrontal cortex encode economic value. Nature 441:223–226.
- Parent A, Hazrati LN (1993) Anatomical aspects of information processing in primate basal ganglia [see comments]. Trends Neurosci 16:111–116.
- Parent A, Mackey A, De Bellefeuille L (1983) The subcortical afferents to caudate nucleus and putamen in primate: a fluorescence retrograde double labeling study. Neuroscience 10:1137–1150.
- Parent M, Parent A (2006) Single-axon tracing study of corticostriatal projections arising from primary motor cortex in primates. J Comp Neurol 496:202–213.
- Passingham D, Sakai K (2004) The prefrontal cortex and working memory: physiology and brain imaging. Curr Opin Neurobiol 14:163–168.
- Pasupathy A, Miller EK (2005) Different time courses of learning-related activity in the prefrontal cortex and striatum. Nature 433:873–876.
- Paus T (2001) Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci 2:417–424.
- Percheron G, Filion M (1991) Parallel processing in the basal ganglia: Up to a point. Trends Neurosci 14:55–59.
- Porrino LJ, Smith HR, Nader MA, Beveridge TJ (2007) The effects of cocaine: a shifting target over the course of addiction. Prog Neuropsychopharmacol Biol Psychiatry 31:1593–1600.
- Porrino LJ, Lyons D, Smith HR, Daunais JB, Nader MA (2004) Cocaine self-administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci 24:3554–3562.
- Price JL, Carmichael ST, Drevets WC (1996) Networks related to the orbital and medial prefrontal cortex; a substrate for emotional behavior? Prog Brain Res 107:523–536.

- Ragsdale CW Jr, Graybiel AM (1990) A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. Proc Natl Acad Sci USA 87:6196–6199.
- Roesch MR, Olson CR (2004) Neuronal activity related to reward value and motivation in primate frontal cortex. Science 304:307–310.
- Sadikot AF, Parent A, Francois C (1992) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: A PHA-L study of subcortical projections. J Comp Neurol 315:137–159.
- Schieber MH (1999). Voluntary Descending Control. In Fundamental Neuroscience, pp. 931–949: Academic Press. New York.
- Schultz W (2002) Getting formal with dopamine and reward. Neuron 36:241–263.
- Schultz W, Tremblay L, Hollerman JR (2000) Reward processing in primate orbitofrontal cortex and basal ganglia. Cerebral Cortex 10:272–284.
- Selemon LD, Goldman-Rakic PS (1985) Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. J Neurosci 5:776–794.
- Selemon LD, Goldman-Rakic PS (1990) Topographic intermingling of striatonigral and striatopallidal neurons in the rhesus monkey. J Comp Neurol 297:359–376.
- Sherman SM, Guillery RW (1996) Functional organization of thalamocortical relays. J Neurophysiol 76:1367–1395.
- Shu SY, Peterson GM (1988) Anterograde and retrograde axonal transport of phaseolus vulgaris leucoagglutinin (PHA-L) from the globus pallidus to the striatum of the rat. J Neurosci Methods 25:175–180.
- Smith EE, Jonides J (1997) Working memory: a view from neuroimaging. Cognit Psychol 33:5–42.
- Smith Y, Hazrati L-N, Parent A (1990) Efferent projections of the subthalamic nucleus in the squirrel monkey as studied by the PHA-L anterograde tracing method. J Comp Neurol 294:306–323.
- Somogyi P, Smith AD (1979) Projection of neostriatal spiny neurons to the substantia nigra. Application of a combined Golgi-staining and horseradish peroxidase transport procedure at both light and electron microscopic levels. Brain Res 178:3–15.
- Spooren WPJM, Lynd-Balta E, Mitchell S, Haber SN (1996) Ventral pallidostriatal pathway in the monkey: Evidence for modulation of basal ganglia circuits. J Comp Neurol 370:295–312.
- Staines WA, Atmadja S, Fibiger HC (1981) Demonstration of a pallidostriatal pathway by retrograde transport of HRP-labeled lectin. Brain Res 206:446–450.
- Strick PL, Dum RP, Mushiake H (1995) Basal Ganglia "Loops" with the Cerebral Cortex. In: Functions of the Cortico-Basal Ganglia Loop (Kimura M, Graybiel AM, eds), pp. 106–124. New York: Springer-Verlag.
- Strick PL, Dum RP, Picard N (1998) Motor areas on the medial wall of the hemisphere. Novartis Foundation Symposium 218:64–75 discussion 75-80.

- Szabo J (1979) Strionigral and nigrostriatal connections. Anatomical studies. Appl Neurophysiol 42:9–12.
- Takikawa Y, Kawagoe R, Hikosaka O (2002) Reward-dependent spatial selectivity of anticipatory activity in monkey caudate neurons. J Neurophysiol 87:508–515.
- Tanaka SC, Doya K, Okada G, Ueda K, Okamoto Y, Yamawaki S (2004) Prediction of immediate and future rewards differentially recruits cortico-basal ganglia loops. Nat Neurosci 7:887–893.
- Tanji J, Mushiake H (1996) Comparison of neuronal activity in the supplementary motor area and primary motor cortex. Cogn Brain Res 3:143–150.
- Tremblay L, Schultz W (2000) Reward-related neuronal activity during go-nogo task performance in primate orbitofrontal cortex. J Neurophysiol 83:1864–1876.
- Vogt BA, Vogt L, Farber NB, Bush G (2005) Architecture and neurocytology of monkey cingulate gyrus. J Comp Neurol 485:218–239.
- Volkow ND, Wang GJ, Ma Y, Fowler JS, Wong C, Ding YS, Hitzemann R, Swanson JM, Kalivas P (2005) Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: relevance to addiction. J Neurosci 25:3932–3939.
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26:6583–6588.
- Wallis JD, Miller EK (2003) Neuronal activity in primate dorsolateral and orbital prefrontal cortex during performance of a reward preference task. Eur J Neurosci 18:2069–2081.
- Walton ME, Bannerman DM, Alterescu K, Rushworth MF (2003) Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. J Neurosci 23:6475–6479.
- Watanabe K, Lauwereyns J, Hikosaka O (2003) Neural correlates of rewarded and unrewarded eye movements in the primate caudate nucleus. J Neurosci 23:10052–10057.
- Wiesendanger R, Wiesendanger M (1985) The thalamic connections with medial area 6 (supplementary motor cortex) in the monkey (*Macaca fascicularis*). Exp Brain Res 59:91–104.
- Wilson CJ (2004) The basal ganglia. In: Synaptic Organization of the Brain, 5th edn (Shepherd GM ed), pp. 361–413. New York, NY: Oxford University Press.
- Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483–494.
- Wise SP, Murray EA, Gerfen CR (1996) The frontal cortex-basal ganglia system in primates. Crit Rev Neurobiol 10:317–356.
- Yelnik J (2002) Functional anatomy of the basal ganglia. Mov Disord 17(Suppl 3):S15–S21.
- Zheng T, Wilson CJ (2002) Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 87: 1007–1017.

# Synchronous Activity in Basal Ganglia Circuits

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I. INTRODUCTION

Early interest in synchronous activity in basal ganglia circuits focused on how changes in tonic firing rates in the striatum might affect tonic activity throughout the basal ganglia-thalamocortical network. In the late 1980s – early 1990s, an operating model of the basal ganglia was proposed that incorporated data from in situ hybridization techniques, receptor binding studies and dopamine receptor cloning (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990). This "dual circuit", "rate", or "Albin DeLong" model (Fig. 25.1) was widely used for generating hypotheses about the impact of increases and decreases in striatal dopamine receptor stimulation on activity in the basal ganglia output nuclei, the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr) (see Chapter 1).

A therapeutically relevant prediction of the rate model was that the dysfunctional motor effects of dopamine agonist administration and dopamine loss correlate with decreases and increases, respectively, in firing rates of neurons in basal ganglia output nuclei. Increased stimulation of the D2 dopamine receptor subtype, preferentially expressed by striatopallidal neurons of the "indirect" pathway, was predicted to reduce activity in the striatopallidal pathway, thereby enhancing activity in the external segment of the globus pallidus (GPe) and inducing a net decrease in basal ganglia output. Similarly, increased stimulation of the D1 receptor subtype, preferentially expressed by striatal projections of the "direct" pathway to the SNr and GPi, was also predicted to induce a net decrease in basal ganglia output (Albin et al., 1989; Gerfen et al., 1990; Gerfen, 1992). The hyperactivity associated with dopamine receptor stimulating agents was thereby attributed to decreases in inhibitory output from the basal ganglia and disinhibition of thalamocortical activity. Loss of dopamine, conversely, was predicted to induce increases in inhibitory output from the basal ganglia and inhibition of thalamocortical activity, leading to akinesia, as occurs in Parkinson's disease.

## II. TESTING PREDICTIONS OF THE RATE-BASED MODEL: EFFECTS OF INCREASED DOPAMINE RECEPTOR STIMULATION

The availability of drugs selective for D1 and D2 receptor subtypes made it feasible to examine the predictions of the



**FIGURE 25.1** Schematic diagram of the basal ganglia circuitry. Abbreviations: GPe, globus pallidus external; STN, subthalamic nucleus; DA, dopamine neurons of the substantia nigra pars compacta; SNr, substantia nigra pars reticulata; GPi, globus pallidus internal; ENK, enkephalin; SP, substance P; " + " and open square, excitatory transmission; "–" and black square, inhibitory transmission.

rate-based dual circuit model. Neurophysiological recording studies conducted in an immobilized, locally anesthetized rat preparation in the 1980s and 1990s showed that systemically administered dopamine agonists or dopamine uptake blockers triggered robust increases in mean firing rate in the GPe, as the rate model predicted. However, this increase in GPe firing rate only occurred with non-selective D1/D2 agonists or when both D1 and D2 selective agonists were coadministered (Bergstrom and Walters, 1981; Bergstrom et al., 1984; Carlson et al., 1986, 1987, 1988, 1990; Walters et al., 1987, 1998). In contrast to predictions, D2 agonists alone exerted only modest rate increasing effects on GPe firing rates, and these modest effects appeared dependent on intact endogenous dopamine tone at D1 receptors (Carlson et al., 1988). Similarly, D1 agonists, given alone, also induced only modest rate increasing effects on GPe activity. However, D1 agonists were unexpectedly effective at enhancing activity of subthalamic nucleus (STN) neurons while D2 agonists had little effect on STN activity (Kreiss et al., 1996, 1997). Other results from these studies were also at odds with predictions of the rate model. For example, given alone, D1 agonists induced only mild increases

in activity in the SNr while drugs stimulating both D1 and D2 receptors exerted a mix of increases, decreases and no change in activity (Waszczak et al., 1984a, 1984b; Weick and Walters, 1987a; Walters et al., 1998).

While many of these early neurophysiological results were inconsistent with the rate model's predictions, the synergistic effects of D1 and D2 agonists on mean firing rates in the GPe and SNr were notably consistent with observations emerging from behavioral studies showing that concurrent stimulation of D1 and D2 receptor subtypes is required to induce the behavioral hyperactivity associated with dopamine agonist treatment (Gershanik et al., 1983; Molloy and Waddington, 1985; Pugh et al., 1985; Walters et al., 1987). Taken together, the behavioral and neurophysiological studies highlighted the importance of synergistic interactions between D1 and D2 receptor subtypes in mediating functionally significant effects of dopamine, but the specific mechanisms underlying these effects still remain to be clarified.

## III. TESTING PREDICTIONS OF THE RATE-BASED MODEL: EFFECTS OF DOPAMINE LOSS

The effects of dopamine cell lesion on mean firing rates of basal ganglia nuclei were also explored in animal models of Parkinson's disease. Here, results have been more consistent with the rate model predictions. Notably, mean firing rates in the STN were significantly increased following dopamine cell lesion in primate models of Parkinson's disease (Miller and DeLong, 1987; Bergman et al., 1990, 1994; Bézard et al., 1999; Wichmann et al., 2002; Soares et al., 2004; Wichmann and Soares, 2006) and in rats with unilateral dopamine cell lesions (Hassani et al., 1996; Kreiss et al., 1997; Allers et al., 2000, 2005; Perier et al., 2000; Vila et al., 2000; Breit et al., 2001; Magill et al., 2001; Parr-Brownlie et al., 2007, 2009; Walters et al., 2007, 2009; Mallet et al., 2008b). In addition, in dopamine cell lesioned rats, administration of a combination of D1 and D2 agonists caused significant decreases in SNpr firing rates, in contrast to the variable rate changes observed in intact rats ((Weick and Walters, 1987a, 1987b; Walters et al., 1998), supporting the idea that loss of dopamine induces an enhanced response to dopamine receptor stimulation in the denervated striatum resulting in increased activity in the striatonigral inhibitory pathway. Some observations were less consistent with rate model predictions, however. For example, dopamine cell lesion-induced increases in

STN firing rates were accompanied by modest or insignificant changes in firing rates in the SNr and GPi (Filion, 1979; Sanderson et al., 1986; Miller and DeLong, 1987; Weick and Walters, 1987a; MacLeod et al., 1990; Filion and Tremblay, 1991; Bergman et al., 1994; Burbaud et al., 1995; Murer et al., 1997; Rohlfs et al., 1997; Boraud et al., 1998; Bezard et al., 1999; Wichmann et al., 1999; Raz et al., 2000; Tseng et al., 2000; Ruskin et al., 2002). In addition, systemic administration of D1 agonists alone still induced increases in STN firing rates after dopamine cell lesion, while stimulation of both D1 and D2 receptors appeared required to reduce firing rates in the overactive STN (Kreiss et al., 1997). Thus, although predictions of the rate-based model were more consistent with observations in dopamine cell lesioned rats than intact rats (Waszczak et al., 1984a, 1984b; Weick and Walters, 1987a, 1987b; Huang and Walters, 1994; Walters et al., 1998; Ruskin et al., 1999b), overall, results from these studies, and, as discussed below, many subsequent observations in parkinsonian patients and in animal models of Parkinson's disease, did not support the idea that differential rate changes in the direct and indirect pathways and simple increases or decreases in basal ganglia output could account for the hyperactivity and hypoactivity induced by tonic changes in dopamine receptor activation.

In spite of limitations of the rate model, the prediction that lesioning the STN or GPi (Bergman et al., 1990) would be an effective treatment for advanced Parkinson's disease was remarkably consistent with clinical outcome. Lesion of the GPi proved to be highly therapeutic for many advanced Parkinson's disease patients (Bergman et al., 1990; Baron et al., 1996; Lang et al., 1997; Lozano and Lang, 1998; Krack et al., 2000). The success of this strategy for reducing activity in basal ganglia output led to examination of the efficacy of lesioning the STN, directly upstream from the GPi. Subsequently, deep brain stimulation (DBS) was applied to these nuclei as a reversible means for altering basal ganglia output (Gill and Heywood, 1997; Limousin et al., 1998; Krack et al., 2000; Lozano, 2001; Obeso et al., 2001; Benabid, 2003) (see also Chapter 39). However, while GPi and STN lesion and DBS treatment have proven effective in reducing motor symptoms in advanced Parkinson's disease, in the years since the introduction of these therapies it has become clear that the underlying mechanisms involve more than a simple reversal of increased firing rate in basal ganglia output. At the circuit level, the rate-based model is limited in explaining how loss of dopamine promotes both bradykinesia and tremor, how D1 and D2 receptor mediated effects interact to induce stereotypy, how intermittent dopamine receptor stimulation promotes dyskinesias and how lesions of the GPi alleviate not only the hypokinetic aspects of Parkinson's disease but also the hyperkinetic symptoms. In addition, data from a variety of sources have further highlighted the complexity of basal ganglia circuitry. Research has called attention to the STN as an input nucleus to the basal ganglia (Mink, 1996; Gurney et al., 2001; Nambu, 2004, 2005), the colocalization as well as segregation of D1/D2 receptor subtypes in the striatum (Gerfen et al., 1990; Gerfen, 1992; Surmeier et al., 1996; Aizman et al., 2000) (see also Chapters 6 and 28), multiple dopamine receptor subtypes (Sealfon and Olanow, 2000) and distributed connections between basal ganglia nuclei (Bolam et al., 2000; Wu et al., 2000). Meanwhile, software that allows on-line evaluation of neuronal activity in both time and frequency domains has made it easier to assess synchronization of neuronal firing patterns, as well as firing rate, throughout the basal ganglia network. These advances, together with evidence that dopamine loss affects expression of oscillatory activity in the basal ganglia, as discussed below, have focused new attention on the role of synchronized changes in firing pattern, in addition to rate, in basal ganglia circuits.

## IV. SYNCHRONOUS FIRING PATTERNS IN BASAL GANGLIA CIRCUITS

The last decade has seen growing interest in the potential for basal ganglia circuits to engage in both functionally and dysfunctionally synchronized rhythmic activity. In vitro studies have shown that many basal ganglia cell types are autonomous pacemakers, capable of firing regularly without input (Bevan and Wilson, 1999; Surmeier et al., 2005), and recordings in slices and organotypic cell cultures have called attention to the potential for rhythmic activity to emerge in the basal ganglia, especially in the reciprocally connected STN and GPe nuclei (Plenz and Kital, 1999; Bevan et al., 2002b). However, evidence for a role for synchronized and oscillatory activity within basal ganglia circuits has lagged behind indications of significance of this phenomenon in the cortex, even though the basal ganglia is a major target of cortical output. In part, this could relate to the fact that neurons in basal ganglia nuclei are relatively unaligned, in contrast to neurons in the cortex or hippocampus. Synchronized oscillations in neuronal activity are more likely to generate larger fluctuations in net voltage when neurons are aligned in columns or layers, and the lack of such an alignment in basal ganglia nuclei has led to questions about whether local field potentials (LFP) recorded in these structures can be appropriately attributed to local activity. LFPs are thought to reflect net potential change in tissue immediately surrounding the electrode, but they can be contaminated by electrical field fluctuations from nearby structures if these structures generate sufficient power (Berke, 2005). Assessment of synchronized activity in in vivo studies has extended beyond LFPs, however, to include multielectrode recordings of spiking activity within and between basal ganglia nuclei and functional imaging studies, techniques assessing synchronization of neuronal activity on short and long time scales, respectively. Notably, studies utilizing paired or multiunit recordings to assess tightly coupled spiking activity have commented upon the striking absence of functionally correlated activity in several neuronal populations the basal ganglia of normal awake behaving animals (Bar-Gad et al., 2003; Nevet et al., 2007; Berke, 2008). However, interest in a pathological role for such activity in basal ganglia structures has been promoted by advances in Parkinson's disease research showing that dopamine cell death is associated with increased synchronization and oscillatory activity in the basal ganglia in a range of frequencies, suggesting that increased synchronization of basal ganglia output may be responsible for some aspects of motor dysfunction in Parkinson's disease. The sections below describe investigation of oscillatory activity in the basal ganglia in four frequency ranges: multisecond or ultraslow oscillations (<0.1 Hz), slow oscillations  $(\sim 1 \text{ Hz})$  and faster frequency ranges including theta, alpha, beta (4-30 Hz) and gamma (>30 Hz) range oscillations.

### A. Multisecond Oscillations

Evidence for synchronized ultraslow oscillations in neuronal activity (<0.1 Hz) came initially from EEG recordings from awake animals in the 1950s (Aladjalova, 1957) and subsequently from a number of direct and indirect measures of neuronal activity, including spiking activity in the hippocampus, thalamus and the developing retina, heart rate variation, cerebral blood flow and functional magnetic resonance imaging (fMRI) of the brain blood oxygenation level dependent (BOLD) signal (Norton and Jewett, 1965; Ehlers and Foote, 1984; Biswal et al., 1995; Mayhew et al., 1996; Penttonen et al., 1999; Obrig et al., 2000; Montano et al., 2001; Firth et al., 2005; Lorincz et al., 2009). While it is not clear what processes generate oscillatory neuronal activity in this ultraslow frequency range, the literature suggests these slow fluctuations in firing rate play a role in activity-based synaptic connectivity and homeostasis, as well as central coordination of autonomic function (Feller, 1999; Turrigiano, 1999; Pagani and Malliani, 2000; Roerig and Feller, 2000). The observation that changes in brain activity in the ultraslow frequency band could be visualized using fMRI has lead, over the past decade, to the widespread use of ultraslow fluctuations in BOLD signal fMRI as a tool to identify brain areas engaged in synchronous and correlated activity (Raichle and Mintun, 2006; Fox and Raichle, 2007; Fair et al., 2008; Uddin et al., 2008).

Ultraslow oscillations (<0.1 Hz) have been observed in basal ganglia activity in two types of studies. One approach has involved the use of the BOLD signal to assess functional relationships within the basal ganglia and between the basal ganglia and other brain regions. Data from the basal ganglia fMRI BOLD studies show correlated activity between basal ganglia nuclei and other brain regions in humans consistent with anatomical evidence for motor, cognitive and affective subdivisions in the striatum, and parallel and integrative basal ganglia-thalamocortical loop models described in animals (Di Martino et al., 2008).

Fluctuations in the ultraslow frequency range have also been demonstrated in the basal ganglia in spike trains recorded in awake rats and monkeys (Ruskin et al., 1999a; Allers et al., 2000; Walters et al., 2000; Wichmann et al., 2002; Walters and Bergstrom, 2009). In awake immobilized rats, 30-70% of spike trains recorded in the SNr, GPe, GPi and STN and 20% in the substantia nigra pars compacta show significant oscillatory activity with ultraslow periodicity, in the range of 0.017-0.5 Hz) (Ruskin et al., 1999a, 1999c, 2001, 2003; Allers et al., 2000, 2002; Walters et al., 2000). Paired recordings from the STN or GPe nuclei in opposite hemispheres, and from GPe and STN or GPe and SNr in the same hemisphere demonstrate that firing rate oscillations of individual neurons in these rats are frequently ( $\sim$ 30%) correlated in the 10–60 second time scale and provided evidence that neurons participating in these oscillations are widespread throughout the basal ganglia (Allers et al., 2002; Tierney et al., 2002; Ruskin et al., 2003; Walters and Bergstrom, 2009) (Fig. 25.2). The observation that increased dopamine receptor stimulation significantly increases the incidence of correlated ultraslow activity in basal ganglia circuits suggests that increased synchronization in these ultraslow frequency ranges could



**FIGURE 25.2** Correlated multisecond oscillations in GPe and SNr firing rates, and in GPe firing rate and hippocampal (HP) theta local field potential (LFP) power after dopamine agonist administration in awake immobilized rats. Following D1/D2 dopamine agonist (apomorphine) administration, multisecond oscillations were correlated and antiphasic in simultaneously recorded GPe and SNr spike trains (A). As shown in bar graph, the % of pairs showing correlated oscillations was significantly increased after agonist administration, and this effect was reversed by dopamine antagonist (haloperido) administration. Multisecond oscillations were correlated in simultaneously recorded GPe spike trains and HP LFPs filtered for theta range activity (4–7 Hz). After dopamine agonist administration (B), in phase multisecond oscillations were correlated in 73% of simultaneously recorded GPe spike trains and HP LFPs theta activity. Bar graph shows that HP LFP power in the theta range was significantly greater after agonist administration, relative to baseline, and this effect was reversed by dopamine antagonist treatment.

contribute to dopamine's effects on motor activity and attention. Single unit recording studies have also utilized correlated activity in the ultraslow frequency range to explore the impact of dopamine receptor stimulation on the dynamics of the basal ganglia. These studies show that dopamine agonist administration increases the incidence of correlations between the GPe and SNr in the ultraslow frequency range, and affects their phase relationships network (Ruskin et al., 2003). Results suggest a model of oscillation flow through the basal ganglia circuit in which, after agonist treatment, oscillations in the GPe are consistently shaped by STN input, and have the potential of entraining oscillatory activity in the SNr, especially if the oscillations from the GPe arrive in phase with oscillations via the direct pathway from the striatum. In these studies, coherence was also observed between ultraslow oscillations in basal ganglia spike trains and hippocampal theta range activity (Allers et al., 2002; Tierney et al., 2002), supporting a role for synchronized activity in the ultraslow time scale in interactions between basal ganglia and other brain regions.

#### **B. 1 Hz Oscillations**

In the basal ganglia, 1 Hz "slow wave" oscillatory activity has been most frequently studied in systemically anesthetized rodents. In this preparation, cortical activity is highly synchronized in the 0.3–2.5 Hz range, with the peak frequency dependent on the depth and type of anesthetic. This activity is reflected in large amplitude EEG oscillations and in recordings of cortical LFP (Steriade et al., 1993; Contreras and Steriade, 1995, 1997; Amzica and Steriade, 1998; Steriade, 2001; Mahon et al., 2001). As this synchronized state is relatively stable over time, these oscillations can be viewed as a probe or input signal for assessing the ability of cortical activity to synchronize basal ganglia networks. Thus, the 1 Hz oscillations prominent in the cortex of the anesthetized rats have become useful, like the multisecond oscillations in awake subjects, for examining the functional dynamics of activity in basal ganglia networks. Magill and coworkers have used this approach to investigate functional connectivity between the cortex and basal ganglia in intact rats anesthetized with a combination of urethane and ketamine, and have shown that GPe and STN spike trains exhibit frequent periods of  $\sim 1 \text{ Hz}$  rate fluctuations that are coherent with cortical EEG 1 Hz activity (Magill et al., 2000, 2004; Sharott et al., 2005a). Interestingly, the expression of 1 Hz oscillatory activity in the basal ganglia of anesthetized rats is markedly enhanced after lesion of the dopamine neurons. A number of investigations have collectively demonstrated that firing patterns throughout the basal ganglia, in the striatum, GPe, STN, EPN and SNr, become more bursty and/or oscillatory in the 1 Hz range in anesthetized rats after unilateral 6hydroxydopamine-induced dopamine cell lesion (Fig. 25.3), and further shown that this bursty activity correlates with



**FIGURE 25.3** Simultaneous recordings of SNr spike trains and SNr LFPs ipsilateral (A) and contralateral (B) to a unilateral dopamine cell lesion in urethane anesthetized rats. SNr spike trains recorded in the lesioned hemisphere exhibited marked burstiness; spectral analysis showed that these firing patterns were significantly more oscillatory than those in the non-lesioned hemisphere (D). SNr LFP peak frequencies (C) in the slow  $\sim 1$  Hz range were recorded in both non-lesioned and lesioned hemispheres with LFP power significantly greater in the lesioned hemisphere. SNr spike-triggered waveform averages (E) illustrate that SNr spikes occurred most frequently at or near the trough of the SNr LFP in the lesioned hemisphere (from recording in A, shown in E inset) and significantly greater correlations were between SNr spike train spiking and LFP oscillations in the lesioned hemisphere than those in the non-lesioned hemisphere. Correlations between SNr spike from the lesioned hemisphere and SNr LFP oscillations from the non-lesioned hemisphere than spiking/LFP correlations from the non-lesioned hemisphere. Data in part from Walters et al. (2007). \*Significantly different from non-lesioned.

slow oscillations in cortical EEG/LFP (Sanderson et al., 1986; MacLeod et al., 1990; Hollerman and Grace, 1992; Burbaud et al., 1995; Hassani et al., 1996; Murer et al., 1997, 2002; Rohlfs et al., 1997; Perier et al., 2000; Tseng et al., 2000, 2001a, 2001b; Vila et al., 2000; Breit et al., 2001, 2005; Magill et al., 2001; Ni et al., 2001; Belluscio et al., 2003, 2007; Rodriguez Díaz et al., 2003; Tai et al., 2003; Parr-Brownlie et al., 2007, 2009; Walters et al., 2007; Zold et al., 2007). Important insight into this phenomenon was provided by Murer, Tseng and coworkers who demonstrated that striatal neurons are more depolarized after dopamine cell lesion and fire more frequently in conjunction with the slow oscillations in cortical EEG in anesthetized rats (Tseng et al., 2001b; Murer et al., 2002). Together with additional contributions of Mallet and coworkers (Mallet et al., 2006), these findings argue that loss of dopamine induces alterations in striatal processing of oscillatory cortical input that promote entrainment of 1 Hz synchronized, oscillatory activity throughout the basal ganglia network of anesthetized rats. Further perspective on how loss of dopamine affects passage of oscillatory activity through the excitatory and inhibitory pathways in the basal ganglia network has emerged with analyses of phase relationships between slow oscillations in striatal, GPe, STN and SNr spike trains in the anesthetized rat model of Parkinson's disease (Walters et al., 2007). As shown in Fig. 25.4, phase relationship studies in this model argue that increased phasic oscillatory activity in the GPe entrained by striatal output, in conjunction with convergent oscillatory input from cortex, contributes to oscillatory activity in the STN. In addition, increased oscillatory activity in SNr spike trains is consistent with convergent inhibitory and excitatory oscillatory input from the GPe and STN, respectively.

These studies make a strong case for the idea that loss of dopamine disrupts striatal "filtering" of oscillatory components of cortical activity (Murer et al., 2002; Mallet et al., 2006). In the anesthetized animal, reduced transmission of cortical activity through the direct pathway and increased transmission through the indirect pathway appears to facilitate convergence of 1 Hz phasic input from GPe and STN to promote oscillations in basal ganglia output from the SNr and GPi (Walters et al., 2007) (Fig. 25.4). However, whether this mechanism contributes to the emergence of



FIGURE 25.4 Phase model illustrating a hypothesized scheme for passage of oscillatory signals in the slow 0.3–2.5 Hz range through the cortico-basal ganglia pathway after dopamine loss in anesthetized rats. Left diagram: Examples of striatal, GPe, STN, and SNr spiking activity recorded simultaneously with SNr LFP are shown with SNr LFP as a common temporal reference (indicated in black). Thick black lines with black arrows indicate excitatory connections between nuclei and thin lines with open arrows or bars indicate inhibitory connections. Cortical oscillatory inputs to the striatum and STN are indicated. Right diagram: Results from data on left are consistent with a model showing loss of dopamine enhancing transmission of oscillatory activity primarily through the indirect pathway, resulting in robust oscillatory activity in the SNr. Implied in this model are: (1) D2 receptor-bearing striatopallidal neurons transmitting patterned activity from the cortex to the GPe; (2) Striatally-mediated pauses in inhibitory GPe activity contributing to the timing of bursts in STN neuronal activity; and (3) Pauses in inhibitory GPe output coinciding with bursts in excitatory STN output supporting enhanced oscillatory activity in SNr/GPi spike trains. Data from Walters et al. (2007).

faster frequency activity in Parkinson's disease patients, as discussed below, remains to be determined.

### C. 4–30 Hz Oscillations

Oscillatory and synchronized activity in the 4–30 Hz range in the cortex has been generally associated with motor activity (theta rhythm, 4–8 Hz) and "idling" or motor planning activity (alpha, beta 8–30 Hz). Recently, investigators have begun exploring the role of oscillatory activity in this range in the striatum in chronic recordings from rodents and monkeys during learning and motor tasks (Courtemanche et al., 2003; Berke, 2005; DeCoteau et al., 2007a, 2007b; Tort et al., 2008; Berke et al., 2009). However, interest in the role of oscillatory activity in the basal ganglia in the 4–30 Hz range has been most influenced by indications that dopamine depletion is associated with dysfunctional synchronization of basal ganglia activity in this frequency range in Parkinson's disease.

In 1979, Filion (Filion, 1979) described changes in GPe and GPi firing patterns in monkeys after electrolytic lesion of the nigrostriatal dopamine pathway. A few years later, the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively destroys dopamine neurons in primates, provided a new research tool for further investigation of the effects of dopamine loss on basal ganglia activity (Burns et al., 1983; Jenner et al., 1984; Langston et al., 1984; Bankiewicz et al., 1986). Studies in MPTPtreated monkeys showed, in addition to faster STN firing rates, increases in oscillatory firing patterns in the 4-18Hz range in the GPi, GPe and STN (Miller and DeLong, 1987; Filion and Tremblay, 1991; Bergman et al., 1994, 1998; Nini et al., 1995; Boraud et al., 1998; Wichmann et al., 1999; Raz et al., 2000, 2001; Heimer et al., 2002, 2006; Goldberg et al., 2004) (see also Chapter 38).

Data collected from the GPi and STN in parkinsonian patients during implantation of DBS electrodes have also shown apparent increases in expression of LFP beta range activity. Oscillations in the beta frequency range are of special interest in the context of akinesia, as power in this range in STN and GPi LFPs in parkinsonian patients during rest is reduced by L-DOPA treatment and movement (Brown et al., 2001; Levy et al., 2001, 2002; Cassidy et al., 2002; Priori et al., 2002; Williams et al., 2002; Brown, 2003, 2007; Kuhn et al., 2004, 2006; Alegre et al., 2005; Doyle et al., 2005; Foffani et al., 2006; Fogelson et al., 2005; Alonso-Frech et al., 2006; Chen et al., 2006; Weinberger et al., 2006).

Similar changes in firing pattern have also been observed in the rodent models of Parkinson's disease, in awake rats and in anesthetized rats during periods of cortical desynchronization (Ruskin et al., 2002; Sharott et al., 2005b; Costa et al., 2006; Mallet et al., 2008a, 2008b; Degos et al., 2009; Walters et al., 2009). In these studies, loss of dopamine has been associated with increased incidence of beta range oscillations in LFP and spiking activity in recordings from striatum, GPe, GPi and the STN (Fig. 25.5). While data from the anesthetized rat, as discussed above, argue that altered processing of cortical input in the striatum and convergent activity from cortex and GPe in the STN and from GPe and STN in the SNr play a significant role in the emergence of slow wave oscillatory activity in the basal ganglia after dopamine cell lesion, it remains unclear whether similar processes account for the increase in beta range activity in the basal ganglia in the awake parkinsonian animal or patient. Studies in brain slices suggest that loss of dopaminergic innervation in the STN may contribute to changes promoting STN/GPe resonance in the beta frequency range (Bevan et al., 2002a; Baufreton et al., 2005).

### D. Gamma Frequency Oscillations

In addition to finding evidence for synchronization of neuronal activity in the beta range in the STN of Parkinson's disease patients, researchers have also reported evidence for gamma range (>30Hz) synchronization in recordings from DBS electrodes, most commonly focused around 70Hz (Cassidy et al., 2002; Williams et al., 2002; Brown and Williams, 2005; Fogelson et al., 2005; Pogosyan et al., 2006; Trottenberg et al., 2006). Notably, however, power in the gamma range is not increased during rest in these recordings; rather, it is more evident during movement and after L-DOPA treatment. The presence of gamma range activity has also been demonstrated in the STN and ventral striatum in awake behaving rats (Brown et al., 2002; Berke et al., 2004; Masimore et al., 2005; van der Meer and Redish, 2009) and in STN in monkeys (Gatev and Wichmann, 2009). These reports are interesting with respect to a potential role for gamma range activity in attention, plasticity and learning, and the possibility for further insight into mechanisms underlying these processes in basal ganglia circuits in future studies.

### V. CONCLUSIONS

Modeling of the basal ganglia network in the late 1980s and early 1990s played an important role in generating and



**FIGURE 25.5** GPe and STN spike-triggered waveform averages (STWAs) of beta frequency local field potential (LFP) activity in intact and dopamine cell lesioned rats. LFPs were filtered for beta frequency activity (15–30 Hz). GPe spike-triggered STN beta range LFP waveform average amplitudes (A) were significantly increased following dopamine cell lesion. Graph on left shows a GPe spike-triggered STN LFP waveform from a single GPe spike/ STN LFP paired recording; timing of the GPe spikes was consistently aligned with the peak of the STN beta LFP oscillations. Averages of waveform amplitudes presented in the bar graph (B) show that synchronization between GPe spikes and STN beta LFPs was significantly decreased by dopamine agonist administration in both intact and lesioned rats. This desynchronizing effect was antagonized by haloperidol effectively in intact rats and partially in lesioned rats. STN spike-triggered GPe beta LFP waveform average amplitudes of beta frequency LFP activity (C) were significantly increased following dopamine cell lesion. Graph at left shows a STN spike-triggered GPe LFP waveform from a single STN spike-GPe LFP paired recording; STN neurons tended to spike shortly after the peaks of the GPe LFPs. Averages of waveform amplitudes are presented in the bar graph. Cross-correlogram (D) shows that GPe and STN spiking activity was significantly correlated and with an antiphase relationship (~180°). Lomb periodogram power spectrum shows the dominant frequency in the beta frequency range. Bar graphs represent means of results from 7–9 spike/LFP paired recordings with one recording per rat in awake immobilized rats. Data in part from Walters et al. (2009). \*Significantly different from intact; #significantly different from baseline.

consolidating ideas about basal ganglia function. These models led to predictions about relationships between changes in striatal activity and alterations tonic firing rate in basal ganglia circuits, and triggered research that has evolved to show that firing pattern as well as rate contribute to the functional dynamics of normal and pathological activity in basal ganglia circuits. It has become clear that under some conditions synchronized oscillatory neuronal activity in ultraslow, slow and faster frequency ranges are observed in basal ganglia circuits. As synchronized activity has enhanced impact on postsynaptic structures, these oscillations are useful tools for probing basal ganglia function as well as assessing functional connectivity within and between the basal ganglia nuclei and downstream sites. They may also provide insight into mechanisms underlying disease-related processes in the basal ganglia. The observation that removal of dopaminergic input or administration of dopamine agonists alters not only firing rates in the basal ganglia network, but also the extent of synchronization of oscillatory firing patterns may constitute important insight into processes underlying the symptomology of Parkinson's disease. These observations also argue that an appropriate level of synchronization of neuronal activity in the basal ganglia may be necessary for optimal execution of behaviors know to be modulated by dopamine. However, the processes regulating synchronized and oscillatory activity in the basal ganglia, the role of these patterns in normal basal ganglia function and the mechanisms underlying the ability of neuromodulators like dopamine to regulate the transmission of these patterns through basal ganglia circuits are not yet well understood and provide opportunities for research endeavors in the coming decade.
## REFERENCES

- Aizman O, Brismar H, Uhlen P, Zettergren E, Levey AI, Forssberg H, Greengard P, Aperia A (2000) Anatomical and physiological evidence for D-1 and D-2 dopamine receptor colocalization in neostriatal neurons. Nature Neurosci 3:226–230.
- Aladjalova NA (1957) Infra-slow rhythmic oscillations of the steady potential of the cerebral cortex. Nature 179:957–959.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–374.
- Alegre M, Alonso-Frech F, Rodriguez-Oroz M, Guridi J, Zamarbide I, Valencia M, Manrique M, Obeso J, Artieda J (2005) Movementrelated changes in oscillatory activity in the human nucleus: ipsilateral vs. contralateral movements. Eur J Neurosci 22:2315–2324.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Allers KA, Bergstrom DA, Ghazi LJ, Kreiss DS, Walters JR (2005) MK801 and amantadine exert different effects on subthalamic neuronal activity in a rodent model of Parkinson's disease. Exp Neurol 191:104–118.
- Allers KA, Kreiss DS, Walters JR (2000) Multisecond oscillations in the subthalamic nucleus: effects of apomorphine and dopamine cell lesion. Synapse 38:38–50.
- Allers KA, Ruskin DN, Bergstrom DA, Freeman LE, Ghazi LJ, Tierney PL, Walters JR (2002) Multisecond periodicities in basal ganglia firing rates correlate with theta bursts in transcortical and hippocampal EEG. J Neurophysiol 87:1118–1122.
- Alonso-Frech F, Zamarbide I, Alegre M, et al. (2006) Slow oscillatory activity and levodopa-induced dyskinesias in Parkinson's disease. Brain 129:1748–1757.
- Amzica F, Steriade M (1998) Cellular substrates and laminar profile of sleep K-complex. Neuroscience 82:671–686.
- Bankiewicz KS, Oldfield EH, Chiueh CC, Doppman JL, Jacobowitz DM, Kopin IJ (1986) Hemiparkinsonism in monkeys after unilateral internal carotid-artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Life Sci 39:7–16.
- Bar-Gad I, Heimer G, Ritov Y, Bergman H (2003) Functional correlations between neighboring neurons in the primate globus pallidus are weak or nonexistent. J Neurosci 23:4012–4016.
- Baron MS, Vitek JL, Bakay RAE, et al. (1996) Treatment of advanced Parkinson's disease by posterior GPi pallidotomy: 1-year results of a pilot study. Ann Neurol 40:355–366.
- Baufreton J, Atherton JF, Surmeier DJ, Bevan MD (2005) Enhancement of excitatory synaptic integration by GABAergic inhibition in the subthalamic nucleus. J Neurosci 25:8505–8517.
- Belluscio MA, Kasanetz F, Riquelme LA, Murer MG (2003) Spreading of slow cortical rhythms to the basal ganglia output nuclei in rats with nigrostriatal lesions. Eur J Neurosci 17:1046–1052.
- Belluscio MA, Riquelme LA, Murer MG (2007) Striatal dysfunction increases basal ganglia output during motor cortex activation in parkinsonian rats. Eur J Neurosci 25:2791–2804.
- Benabid AL (2003) Deep brain stimulation for Parkinson's disease. Curr Opin Neurobiol 13:696–706.
- Bergman H, Feingold A, Nini A, Raz A, Slovin H, Abeles M, Vaadia E (1998) Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. Trends Neurosci 21:32–38.

- Bergman H, Wichmann T, DeLong MR (1990) Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 249:1436–1438.
- Bergman H, Wichmann T, Karmon B, DeLong MR (1994) The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol 72:507–520.
- Bergstrom DA, Bromley SD, Walters JR (1984) Dopamine agonists increase pallidal unit activity: attenuation by agonist pretreatment and anesthesia. Eur J Pharmacol 100:3–12.
- Bergstrom DA, Walters JR (1981) Neuronal responses of the globus pallidus to systemic administration of d-amphetamine: investigation of the involvement of dopamine, norepinephrine, and serotonin. J Neurosci 1:292–299.
- Berke JD (2005) Participation of striatal neurons in large-scale oscillatory networks. In: The Basal Ganglia VIII (Bolam JP, Ingham CA, Magill PJ, eds), pp. 25–35. New York: Springer Science + Business Media, Inc.
- Berke JD (2008) Uncoordinated firing rate changes of striatal fastspiking interneurons during behavioral task performance. J Neurosci 28:10075–10080.
- Berke JD, Breck JT, Eichenbaum H (2009) Striatal versus hippocampal representations during win-stay maze performance. J Neurophysiol 101:1575–1587.
- Bevan MD, Magill PJ, Hallworth NE, Bolam JP, Wilson CJ (2002a) Regulation of the timing and pattern of action potential generation in rat subthalamic neurons in vitro by GABA-A IPSPs. J Neurophysiol 87:1348–1362.
- Bevan MD, Magill PJ, Terman D, Bolam JP, Wilson CJ (2002b) Move to the rhythm: oscillations in the subthalamic nucleus-external globus pallidus network. Trends Neurosci 25:525–531.
- Bevan MD, Wilson CJ (1999) Mechanisms underlying spontaneous oscillation and rhythmic firing in rat subthalamic neurons. J Neurosci 19:7617–7628.
- Bezard E, Boraud T, Bioulac B, Gross CE (1999) Involvement of the subthalamic nucleus in glutamatergic compensatory mechanisms. Eur J Neurosci 11:2167–2170.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 34:537–541.
- Bolam JP, Hanley JJ, Booth PAC, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196:527–542.
- Boraud T, Bezard E, Guehl D, Bioulac B, Gross C (1998) Effects of L-DOPA on neuronal activity of the globus pallidus externalis (GPe) and globus pallidus internalis (GPi) in the MPTP-treated monkey. Brain Res 787:157–160.
- Breit S, Bouali-Benazzouz R, Benabid AL, Benazzouz A (2001) Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal activity of the pedunculopontine nucleus, which is reversed by the lesion of the subthalamic nucleus in the rat. Eur J Neurosci 14:1833–1842.
- Breit S, Lessmann L, Benazzouz A, Schulz JB (2005) Unilateral lesion of the pedunculopontine nucleus induces hyperactivity in the subthalamic nucleus and substantia nigra in the rat. Eur J Neurosci 22:2283–2294.
- Brown P (2007) Abnormal oscillatory synchronisation in the motor system leads to impaired movement. Curr Opin Neurobiol 17:656–664.
- Brown P (2003) Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson's disease. Mov Disord 18:357–363.

- Brown P, Kupsch A, Magill PJ, Sharott A, Harnack D, Meissner W (2002) Oscillatory local field potentials recorded from the subthalamic nucleus of the alert rat. Exp Neurol 177:581–585.
- Brown P, Oliviero A, Mazzone P, Insola A, Tonali P, Di Lazzaro V (2001) Dopamine dependency of oscillations between subthalamic nucleus and pallidum in Parkinson's disease. J Neurosci 21:1033–1038.
- Brown P, Williams D (2005) Basal ganglia local field potential activity: character and functional significance in the human. Clin Neurophysiol 116:2510–2519.
- Burbaud P, Gross C, Benazzouz A, Coussemacq M, Bioulac B (1995) Reduction of apomorphine-induced rotational behaviour by subthalamic lesion in 6-OHDA lesioned rats is associated with a normalization of firing rate and discharge pattern of pars reticulata neurons. Exp Brain Res 105:48–58.
- Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ (1983) A primate model of parkinsonism – selective destruction of dopaminergic-neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci USA 80:4546–4550.
- Carlson JH, Bergstrom DA, Demo SD, Walters JR (1990) Nigrostriatal lesion alters neurophysiological responses to selective and nonselective D-1 and D-2 dopamine agonists in rat globus pallidus. Synapse 5:83–93.
- Carlson JH, Bergstrom DA, Demo SD, Walters JR (1988) Acute reduction of dopamine levels alters responses of basal ganglia neurons to selective D-1 and D-2 dopamine receptor stimulation. Eur J Pharmacol 152:289–300.
- Carlson JH, Bergstrom DA, Walters JR (1986) Neurophysiological evidence that D-1 dopamine receptor blockade attenuates postsynaptic but not autoreceptor-mediated effects of dopamine agonists. Eur J Pharmacol 123:237–251.
- Carlson JH, Bergstrom DA, Walters JR (1987) Stimulation of both D1 and D2 dopamine receptors appears necessary for full expression of postsynaptic effects of dopamine agonists: a neurophysiological study. Brain Res 400:205–218.
- Cassidy M, Mazzone P, Oliviero A, Insola A, Tonali P, Di Lazzaro V, Brown P (2002) Movement-related changes in synchronization in the human basal ganglia. Brain 125:1235–1246.
- Chen C, Kuhn A, Hoffman KT, et al. (2006) Oscillatory pallidal local field potential activity correlates with involuntary EMG in dystonia. Neurology 66:418–420.
- Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15:604–622.
- Contreras D, Steriade M (1997) Synchronization of low-frequency rhythms in corticothalamic networks. Neuroscience 76:11–24.
- Costa RM, Lin SC, Sotnikova TD, Cyr M, Gainetdinov RR, Caron MG, Nicolelis MAL (2006) Rapid alterations in corticostriatal ensemble coordination during acute dopamine-dependent motor dysfunction. Neuron 52:359–369.
- Courtemanche R, Fujii N, Graybiel AM (2003) Synchronous, focally modulated beta-band oscillations characterize local field potential activity in the striatum of awake behaving monkeys. J Neurosci 23:11741–11752.
- DeCoteau WE, Thorn C, Gibson DJ, Courtemanche R, Mitra P, Kubota Y, Graybiel AM (2007a) Learning-related coordination of striatal and hippocampal theta rhythms during acquisition of a procedural maze task. Proc Natl Acad Sci USA 104:5644–5649.

- DeCoteau WE, Thorn C, Gibson DJ, Courtemanche R, Mitra P, Kubota Y, Graybiel AM (2007b) Oscillations of local field potentials in the rat dorsal striatum during spontaneous and instructed behaviors. J Neurophysiol 97:3800–3805.
- Degos B, Deniau J-M, Chavez M, Maurice N (2009) Chronic but not acute dopaminergic transmission interruption promotes a progressive increase in cortical beta frequency synchronization: relationships to vigilance state and akinesia. Cereb Cortex 19:1616–1630.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Di Martino A, Scheres A, Margulies DS, et al. (2008) Functional connectivity of human striatum: a resting state fMRI study. Cereb Cortex 18:2735–2747.
- Doyle LMF, Kuhn AA, Hariz M, Kupsch A, Schneider GH, Brown P (2005) Levodopa-induced modulation of subthalamic beta oscillations during self-paced movements in patients with Parkinson's disease. Eur J Neurosci 21:1403–1412.
- Ehlers CL, Foote SL (1984) Ultradian periodicities in EEG and behavior in the squirrel monkey (*Saimiri sciureus*). Am J Primatol 7:381–389.
- Fair DA, Cohen AL, Dosenbach NUF, et al. (2008) The maturing architecture of the brain's default network. Proc Natl Acad Sci USA 105:4028–4032.
- Feller MB (1999) Spontaneous correlated activity in developing neural circuits. Neuron 22:653–656.
- Filion M (1979) Effects of interruption of the nigrostriatal and of dopaminergic agents on the spontaneous activity of globus pallidus neurons in the awake monkey. Brain Res 179:425–441.
- Filion M, Tremblay L (1991) Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res 547:142–151.
- Firth SI, Wang CT, Feller MB (2005) Retinal waves: mechanisms and function in visual system development. Cell Calcium 37:425–432.
- Foffani G, Bianchi A, Baselli G, Priori A (2005) Movement-related frequency modulation of beta oscillatory activity in the human subthalamic nucleus. J Physiol 568:699–711.
- Fogelson N, Pogosyan A, Kuhn AA, et al. (2005) Reciprocal interactions between oscillatory activities of different frequencies in the subthalamic region of patients with Parkinson's disease. Eur J Neurosci 22:257–266.
- Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 8:700–711.
- Gatev P, Wichmann T (2009) Interactions between cortical rhythms and spiking activity of single basal ganglia neurons in the normal and parkinsonian state. Cereb Cortex 19:1330–1344.
- Gerfen CR (1992) The neostriatal mosaic multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285–320.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Sibley DR (1990) D1 and D2 dopamine receptor regulated geneexpression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gershanik O, Heikkila RE, Duvoisin RC (1983) Behavioral correlations of dopamine receptor activation. Neurology 33:1489–1492.
- Gill SS, Heywood P (1997) Bilateral dorsolateral subthalamotomy for advanced Parkinson's disease. Lancet 350:1224.
- Goldberg JA, Rokni U, Boraud T, Vaadia E, Bergman H (2004) Spike synchronization in the cortex-basal ganglia networks of

parkinsonian primates reflects global dynamics of the local field potentials. J Neurosci 24:6003–6010.

- Gurney K, Prescott TJ, Redgrave P (2001) A computational model of action selection in the basal ganglia. I. A new functional anatomy. Biol Cybern 84:401–410.
- Hassani OK, Mouroux M, Féger J (1996) Increased subthalamic neuronal activity after nigral dopaminergic lesion independent of disinhibition via the globus pallidus. Neuroscience 72:105–115.
- Heimer G, Bar-Gad I, Goldberg JA, Bergman H (2002) Dopamine replacement therapy reverses abnormal synchronization of pallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of parkinsonism. J Neurosci 22:7850–7855.
- Heimer G, Rivlin-Etzion M, Bar-Gad I, Goldberg JA, Haber SN, Bergman H (2006) Dopamine replacement therapy does not restore the full spectrum of normal pallidal activity in the 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine primate model of parkinsonism. J Neurosci 26:8101–8114.
- Hollerman JR, Grace AA (1992) Subthalamic nucleus cell firing in the 6-OHDA-treated rat: basal activity and response to haloperidol. Brain Res 590:291–299.
- Huang K-X, Walters JR (1994) Electrophysiological effects of SKF-38393 in rats with reserpine treatment and 6-hydroxydopamineinduced nigrostriatal lesions reveal two types of plasticity in D1 dopamine-receptor modulation of basal ganglia output. J Pharmacol Exp Ther 271:1434–1443.
- Jenner P, Rupniak NMJ, Rose S, Kelly E, Kilpatrick G, Lees A, Marsden CD (1984) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. Neurosci Lett 50:85–90.
- Krack P, Poepping M, Weinert D, Schrader B, Deuschl C (2000) Thalamic, pallidal, or subthalamic surgery for Parkinson's disease? J Neurol 247:122–134.
- Kreiss DS, Anderson LA, Walters JR (1996) Apomorphine and dopamine D<sub>1</sub> receptor agonists increase the firing rates of subthalamic nucleus neurons. Neuroscience 72:863–876.
- Kreiss DS, Mastropietro CW, Rawji SS, Walters JR (1997) The response of subthalamic nucleus neurons to dopamine receptor stimulation in a rodent model of Parkinson's disease. J Neurosci 17:6807–6819.
- Kuhn AA, Kupsch A, Schneider GH, Brown P (2006) Reduction in subthalamic 8-35 Hz oscillatory activity correlates with clinical improvement in Parkinson's disease. Eur J Neurosci 23: 1956–1960.
- Kuhn AA, Williams D, Kupsch A, Limousin P, Hariz M, Schneider GH, Yarrow. K. Brown,P (2004) Event-related beta desynchronization in human subthalamic nucleus correlates with motor performance. Brain 127:735–746.
- Lang AE, Lozano AM, Montgomery E, Duff J, Tasker R, Hutchinson W (1997) Posteroventral medial pallidotomy in advanced Parkinson's disease. NEJM 337:1036–1042.
- Langston JW, Forno LS, Rebert CS, Irwin I (1984) Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6tetrahydropyrine (MPTP) in the squirrel-monkey. Brain Res 292:390–394.
- Levy R, Ashby P, Hutchison WD, Lang AE, Lozano AM, Dostrovsky JO (2002) Dependence of subthalamic nucleus oscillations on movement and dopamine in Parkinson's disease. Brain 125:1196–1209.

- Levy R, Dostrovsky JO, Lang AE, Sime E, Hutchison WD, Lozano AM (2001) Effects of apomorphine on subthalamic nucleus and globus pallidus internus neurons in patients with Parkinson's disease. J Neurophysiol 86:249–260.
- Limousin P, Krack P, Pollak P, Benazzouz A, Ardouin C, Hoffmann D, Benabid AL (1998) Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. NEJM 339:1105–1111.
- Lorincz ML, Geall F, Bao Y, Crunelli V, Hughes SW (2009) ATP-dependent infra-slow (<0.1 Hz) oscillations in thalamic networks. PLoS ONE 4:e4447.
- Lozano AM (2001) Deep brain stimulation for Parkinson's disease. Parkinsonism Relat Disord 7:199–203.
- Lozano AM, Lang AE (1998) Pallidotomy for Parkinson's disease. Neurosurg Clin N Am 9:325–336.
- MacLeod NK, Ryman A, Arbuthnott GW (1990) Electrophysiological properties of nigrothalamic neurons after 6-hydroxydopamine lesions in the rat. Neuroscience 38:447–456.
- Magill PJ, Bolam JP, Bevan MD (2000) Relationship of activity in the subthalamic nucleus- globus pallidus network to cortical electroencephalogram. J Neurosci 20:820–833.
- Magill PJ, Bolam JP, Bevan MD (2001) Dopamine regulates the impact of the cerebral cortex on the subthalamic nucleus-globus pallidus network. Neuroscience 106:313–330.
- Magill PJ, Sharott A, Bolam JP, Brown P (2004) Brain state-dependency of coherent oscillatory activity in the cerebral cortex and basal ganglia of the rat. J Neurophysiol 92:2122–2136.
- Mahon S, Deniau J-M, Charpier S (2001) Relationship between EEG potentials and intracellular activity of striatal and cortico-striatal neurons: an *in vivo* study under different anesthetics. Cereb Cortex 11:360–373.
- Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 26:3875–3884.
- Mallet N, Pogosyan A, Marton LF, Bolam JP, Brown P, Magill PJ (2008a) Parkinsonian beta oscillations in the external globus pallidus and their relationship with subthalamic nucleus activity. J Neurosci 28:14245–14258.
- Mallet N, Pogosyan A, Sharott A, Csicsvari J, Bolam JP, Brown P, Magill PJ (2008b) Disrupted dopamine transmission and the emergence of exaggerated beta oscillations in subthalamic nucleus and cerebral cortex. J Neurosci 28:4795–4806.
- Masimore B, Schmitzer-Torbert NC, Kakalios J, Redish AD (2005) Transient striatal gamma local field potentials signal movement initiation in rats. NeuroReport 16:2021–2024.
- Mayhew JEW, Askew S, Zheng Y, Porrill J, Westby GWM, Redgrave P, Rector DM, Harper RM (1996) Cerebral vasomotion: a 0.1-Hz oscillation in reflected light imaging of neural activity. NeuroImage 4:183–193.
- Miller WC, DeLong MR (1987) Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism. In: The Basal Ganglia II (Carpenter MB, Jayaraman A, eds), pp. 415–427. New York: Plenum Press.
- Mink JW (1996) The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.
- Molloy AG, Waddington JL (1985) The enantiomers of SK&F 83566, a new selective D-1 dopamine antagonist, stereospecifically block

stereotyped behaviour induced by apomorphine and by the selective D-2 agonist RU 24213. Eur J Pharmacol 116:183–186.

- Montano N, Porta A, Malliani A (2001) Evidence for central organization of cardiovascular rhythms. Ann N Y Acad Sci 940:299–306.
- Murer MG, Riquelme LA, Tseng KY, Pazo JH (1997) Substantia nigra pars reticulata single unit activity in normal and 6OHDA-lesioned rats: effects of intrastriatal apomorphine and subthalamic lesions. Synapse 27:278–293.
- Murer MG, Tseng KY, Kasanetz F, Belluscio M, Riquelme LA (2002) Brain oscillations, medium spiny neurons, and dopamine. Cell Mol Neurobiol 22:611–632.
- Nambu A (2004) A new dynamic model of the cortico-basal ganglia loop. Prog Brain Res 143:461–466.
- Nambu A (2005) A new approach to understand the pathophysiology of Parkinson's disease. J Neurol 252:1–4.
- Nevet A, Morris G, Saban G, Arkadir D, Bergman H (2007) Lack of spike-count and spike-time correlations in the substantia nigra reticulata despite overlap of neural responses. J Neurophysiol 98:2232–2243.
- Ni ZG, Bouali-Benazzouz R, Gao DM, Benabid AL, Benazzouz A (2001) Time-course of changes in firing rates and firing patterns of subthalamic nucleus neuronal activity after 6-OHDA-induced dopamine depletion in rats. Brain Res 899:142–147.
- Nini A, Feingold A, Slovin H, Bergman H (1995) Neurons in the globus pallidus do not show correlated activity in the normal monkey, but phase-locked oscillations appear in the MPTP model of parkinsonism. J Neurophysiol 74:1800–1805.
- Norton S, Jewett RE (1965) Frequencies of slow potential oscillations in the cortex of cats. Electroencephalogr Clin Neurophysiol 19:377–386.
- Obeso JA, et al. (2001) Deep-brain stimulation of the subthalamic nucleus or the pars interna of the globus pallidus in Parkinson's disease. NEJM 345:956–963.
- Obrig H, Neufang M, Wenzel R, Kohl M, Steinbrink J, Einhaupl K, Villringer A (2000) Spontaneous low frequency oscillations of cerebral hemodynamics and metabolism in human adults. NeuroImage 12:623–639.
- Pagani M, Malliani A (2000) Interpreting oscillations of muscle sympathetic nerve activity and heart rate variability. J Hypertens 18:1709–1719.
- Parr-Brownlie LC, Poloskey SL, Bergstrom DA, Walters JR (2009) Parafascicular thalamic nucleus activity in a rat model of Parkinson's disease. Exp Neurol 217:269–281.
- Parr-Brownlie LC, Poloskey SL, Flanagan KK, Eisenhofer G, Bergstrom DA, Walters JR (2007) Dopamine lesion-induced changes in subthalamic nucleus activity are not associated with alterations in firing rate or pattern in layer V neurons of the anterior cingulate cortex in anesthetized rats. Eur J Neurosci 26:1925–1939.
- Penttonen M, Nurminen N, Miettinen R, Sirvio J, Henze DA, Csicsvari J, Buzsaki G (1999) Ultra-slow oscillation (0.025 Hz) triggers hippocampal afterdischarges in Wistar rats. Neuroscience 94:735–743.
- Perier C, Agid Y, Hirsch EC, Feger J (2000) Ipsilateral and contralateral subthalamic activity after unilateral dopaminergic lesion. NeuroReport 11:3275–3278.
- Plenz D, Kital ST (1999) A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. Nature 400:677–682.

- Pogosyan A, Kuhn AA, Trottenberg T, Schneider GH, Kupsch A, Brown P (2006) Elevations in local gamma activity are accompanied by changes in the firing rate and information coding capacity of neurons in the region of the subthalamic nucleus in Parkinson's disease. Exp Neurol 202:271–279.
- Priori A, Foffani G, Pesenti A, et al. (2002) Movement-related modulation of neural activity in human basal ganglia and its L-DOPA dependency: recordings from deep brain stimulation electrodes in patients with Parkinson's disease. Neurol Sci 23:S101–S102.
- Pugh MT, O'Boyle KM, Molloy AG, Waddington JL (1985) Effects of the putative D-1 antagonist SCH 23390 on stereotyped behaviour induced by the D-2 agonist RU24213. Psychopharmacology (Berl) 87:308–312.
- Raichle ME, Mintun MA (2006) Brain work and brain imaging. Annu Rev Neurosci 29:449–476.
- Raz A, Frechter-Mazar V, Feingold A, Abeles M, Vaadia E, Bergman H (2001) Activity of pallidal and striatal tonically active neurons is correlated in MPTP-treated monkeys but not in normal monkeys. J Neurosci 21:1–5 RC128.
- Raz A, Vaadia E, Bergman H (2000) Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine vervet model of parkinsonism. J Neurosci 20:8559–8571.
- Rodriguez Díaz M, Barroso-Chinea P, Acevedo A, Gonzalez-Hernandez T (2003) Effects of dopaminergic cell degeneration on electrophysiological characteristics and GAD65/GAD67 expression in the substantia nigra: different action on GABA cell subpopulations. Mov Disord 18:254–266.
- Roerig B, Feller MB (2000) Neurotransmitters and gap junctions in developing neural circuits. Brain Res Rev 32:86–114.
- Rohlfs A, Nikkhah G, Rosenthal C, Rundfeldt C, Brandis A, Samii M, Löscher W (1997) Hemispheric asymmetries in spontaneous firing characteristics of substantia nigra pars reticulata neurons following a unilateral 6-hydroxydopamine lesion of the rat nigrostriatal pathway. Brain Res 761:352–356.
- Ruskin DN, Bergstrom DA, Baek D, Freeman LE, Walters JR (2001) Cocaine or selective block of dopamine transporters influences multisecond oscillations in firing rate in the globus pallidus. Neuropsychopharmacology 25:28–40.
- Ruskin DN, Bergstrom DA, Kaneoke Y, Patel BN, Twery MJ, Walters JR (1999a) Multisecond oscillations in firing rate in the basal ganglia: robust modulation by dopamine receptor and anesthesia. J Neurophysiol 81:2046–2055.
- Ruskin DN, Bergstrom DA, Mastropietro CW, Twery MJ, Walters JR (1999b) Dopamine receptor-mediated rotation in rats with unilateral nigrostriatal lesions is not dependent on net inhibitions of rate in basal ganglia output nuclei. Neuroscience 91:935–946.
- Ruskin DN, Bergstrom DA, Tierney PL, Walters JR (2003) Correlated multisecond oscillations in firing rate in the basal ganglia: modulation by dopamine and the subthalamic nucleus. Neuroscience 117:427–438.
- Ruskin DN, Bergstrom DA, Walters JR (1999c) Multisecond oscillations in firing rate in the globus pallidus: synergistic modulation by D1 nd D2 dopamine receptors. J Pharmacol Exp Ther 290:1493–1501.
- Ruskin DN, Bergstrom DA, Walters JR (2002) Nigrostriatal lesion and dopamine agonists affect firing patterns of rodent entopeduncular nucleus neurons. J Neurophysiol 88:487–496.
- Sanderson P, Mavoungou R, Albe-Fessard D (1986) Changes in substantia nigra pars reticulata activity following lesions of the substantia nigra pars compacta. Neurosci Lett 67:25–30.

- Sealfon SC, Olanow CW (2000) Dopamine receptors: from structure to behavior. Trends Neurosci 23:S34–S40.
- Sharott A, Magill PJ, Bolam JP, Brown P (2005a) Directional analysis of coherent oscillatory field potentials in the cerebral cortex and basal ganglia of the rat. J Physiol Lond 562:951–963.
- Sharott A, Magill PJ, Harnack D, Kupsch A, Meissner W, Brown P (2005b) Dopamine depletion increases the power and coherence of beta-oscillations in the cerebral cortex and subthalamic nucleus of the awake rat. Eur J Neurosci 21:1413–1422.
- Soares J, Kliem MA, Betarbet R, Greenamyre JT, Yamamoto B, Wichmann T (2004) Role of external pallidal segment in primate parkinsonism: comparison of the effects of 1-methyl-4-phenyl-1,2,3,6tetrahydropyridine-induced parkinsonism and lesions of the external pallidal segment. J Neurosci 24:6417–6426.
- Steriade M (2001) Impact of network activities on neuronal properties in corticothalamic systems. J Neurophysiol 86:1–39.
- Steriade M, Contreras D, Curro DR, Nunez A (1993) The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J Neurosci 13:3284–3299.
- Surmeier DJ, Mercer JN, Chan CS (2005) Autonomous pacemakers in the basal ganglia: who needs excitatory synapses anyway? Curr Opin Neurobiol 15:312–318.
- Surmeier DJ, Song WJ, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
- Tai CH, Boraud T, Bezard E, Bioulac B, Gross C, Benazzouz A (2003) Electrophysiological and metabolic evidence that high-frequency stimulation of the subthalamic nucleus bridles neuronal activity in the subthalamic nucleus and the substantia nigra reticulata. FASEB J 17:1820–1830.
- Tierney PL, Bergstrom DA, Ruskin DN, Walters JR (2002) Correlations between multisecond oscillations in firing rate in basal ganglia neurons: modulation by dopamine receptor stimulation and association with hippocampal theta rhythm. Soc Neurosci Abstr OnlineProgram No. 765.3.
- Tort ABL, Kramer MA, Thorn C, Gibson DJ, Kubota Y, Graybiel AM, Kopell NJ (2008) Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. Proc Natl Acad Sci USA 105:20517–20522.
- Trottenberg T, Fogelson N, Kuhn AA, Kivi A, Kupsch A, Schneider GH, Brown P (2006) Subthalamic gamma activity in patients with Parkinson's disease. Exp Neurol 200:56–65.
- Tseng KY, Kasanetz F, Kargieman L, Pazo JH, Murer MG, Riquelme LA (2001a) Subthalamic nucleus lesions reduce low frequency oscillatory firing of substantia nigra pars reticulata neurons in a rat model of Parkinson's disease. Brain Res 904:93–103.
- Tseng KY, Kasanetz F, Kargieman L, Riquelme LA, Murer MG (2001b) Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions. J Neurosci 21:6430–6439.
- Tseng KY, Riquelme LA, Belforte JE, Pazo JH, Murer MG (2000) Substantia nigra pars reticulata units in 6-hydroxydopamine-lesioned rats: responses to striatal D2 dopamine receptor stimulation and subthalamic lesions. Eur J Neurosci 12:247–256.
- Turrigiano GG (1999) Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. Trends Neurosci 22:221–227.

- Uddin LQ, Kelly AMC, Biswal BB, et al. (2008) Network homogeneity reveals decreased integrity of default-mode network in ADHD. J Neurosci Methods 169:249–254.
- van der Meer MAA, Redish AD (2009) Low and high gamma oscillations in rat ventral striatum have distinct relationships to behavior, reward, and spiking activity on a learned spatial decision task. Front Integr Neurosci 3:9 doi:10.3389/neuro.07.009.2009.
- Vila M, Périer C, Féger J, Yelnik J, Faucheux B, Ruberg M, Raisman-Vozari R, Agid Y, Hirsch EC (2000) Evolution of changes in neuronal activity in the subthalamic nucleus of rats with unilateral lesion of the substantia nigra assessed by metabolic and electrophysiological measurements. Eur J Neurosci 12:337–344.
- Walters JR, Bergstrom DA (2009) Basal ganglia network synchronization in animal models of Parkinson's disease. In: Cortico-Subcortical Dynamics in Parkinson's Disease (Tseng K-Y ed), pp. 117–142. New York: Humana Press.
- Walters JR, Bergstrom DA, Carlson JH, Chase TN, Braun AR (1987) D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. Science 236:719–722.
- Walters JR, Bergstrom DA, Carlson JH, Weick BG, Pan HS (1998) Electrophysiological investigation of D-1/D-2 receptor interactions in the substantia nigra and basal ganglia. In: Pharmacology and Functional Regulation of Dopamine Systems (Beart PM, Woodruff GN, Jackson DM, eds), pp. 96–102. London: Macmillan Press.
- Walters JR, Hu D, Itoga CA, Parr-Brownlie LC, Bergstrom DA (2007) Phase relationships support a role for coordinated activity in the indirect pathway in organizing slow oscillations in basal ganglia output after loss of dopamine. Neuroscience 144:762–776.
- Walters JR, Ruskin DN, Allers KA, Bergstrom DA (2000) Pre- and postsynaptic aspects of dopamine-mediated transmission. Trends Neurosci 23:S41–S47.
- Walters JR, Tierney PL, Bergstrom DA (2009) Oscillatory activity and synchronization in the basal ganglia network in rodent models of Parkinson's disease. In: The Basal Ganglia IX (Groenewegen HJ, Voorn P, Berendse HW, Mulder AB, Cools AR, eds), pp. 443–459 New York: Springer.
- Waszczak BL, Lee EK, Ferraro T, Hare TA, Walters JR (1984a) Single unit responses of substantia nigra pars reticulata neurons to apomorphine: effects of striatal lesions and anesthesia. Brain Res 306:307–318.
- Waszczak BL, Lee EK, Tamminga CA, Walters JR (1984b) Effect of dopamine system activation on substantia nigra pars reticulata output neurons: variable single-unit responses in normal rats and inhibition in 6-hydroxydopamine-lesioned rats. J Neurosci 4: 2369–2375.
- Weick BG, Walters JR (1987a) D-1 dopamine receptor stimulation potentiates neurophysiological effects of bromocriptine in rats with lesions of the nigrostriatal dopamine pathway. Neuropharmacology 26:641–644.
- Weick BG, Walters JR (1987b) Effects of D1 and D2 dopamine receptor stimulation on the activity of substantia nigra pars reticulata neurons in 6-hydroxydopamine lesioned rats: D1/D2 coactivation induces potentiated responses. Brain Res 405:234–246.
- Weinberger M, Mahant N, Hutchison W, Lozano A, Moro E, Hodaie M, Lang A, Dostrovsky J (2006) Beta oscillatory activity in the subthalamic nucleus and its relation to dopaminergic response in Parkinson's disease. J Neurophysiol 96:3248–3256.

- Wichmann T, Bergman H, Starr PA, Subramanian T, Watts RL, DeLong MR (1999) Comparison of MPTP-induced changes in spontaneous neuronal discharge in the internal pallidal segment and in the substantia nigra pars reticulata in primates. Exp Brain Res 125:397–409.
- Wichmann T, Kliem MA, Soares J (2002) Slow oscillatory discharge in the primate basal ganglia. J Neurophysiol 87:1145–1148.
- Wichmann T, Soares J (2006) Neuronal firing before and after burst discharges in the monkey basal ganglia is predictably patterned in the normal state and altered in parkinsonism. J Neurophysiol 95:2120–2133.
- Williams D, Tijssen M, van Bruggen G, et al. (2002) Dopaminedependent changes in the functional connectivity between basal ganglia and cerebral cortex in humans. Brain 125:1558–1569.
- Wu Y, Richard S, Parent A (2000) The organization of the striatal output system: a single-cell juxtacellular labeling study in the rat. Neurosci Res 38:49–62.
- Zold CL, Ballion B, Riquelme LA, Gonon F, Murer MG (2007) Nigrostriatal lesion induces D2-modulated phase-locked activity in the basal ganglia of rats. Eur J Neurosci 25:2131–2144.

## Chapter 26

## **Second-Messenger Cascades**

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I. Introduction

- II. Second-Messenger Pathways A. G-Protein-Coupled Receptors
- B. Ionotropic Receptors
  C. Ca<sup>2+</sup> Signaling
  D. Ras/MAP Kinase Signaling

III. Conclusions and Outlook References

## I. INTRODUCTION

Second messengers are vital elements of intracellular signaling pathways that can be activated by receptor-ligand interaction at postsynaptic as well as presynaptic membranes. The nuclei of the basal ganglia participate in the modulation of a diverse range of behaviors, emotions, and motor functions in humans. Second messenger pathways in the basal ganglia have been studied extensively and many components of these pathways are targets of pharmaceutical therapies for neurological illnesses. This chapter will introduce the main second messenger pathways that participate in neurotransmission in the basal ganglia and will identify the key molecules that are needed for normal basal ganglia function.

Despite a limited number of molecules that function as second messengers, second messenger pathways show a high degree of specificity in linking particular receptors to cellular responses. For example,  $Ca^{2+}$  is a ubiquitous signaling molecule used by various G-protein coupled receptors, ionotropic receptors and ion channels, yet there is a high degree of specificity contingent on the source, location and timing of  $Ca^{2+}$  influx into the cytoplasm. Although we

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. will focus here on common themes of signaling pathways, it is important to keep in mind that neurons have the ability to navigate these pathways to very selective targets. These targets are usually proteins that, through covalent modifications such as phosphorylation, alter their enzymatic activity, affect membrane permeability or mediate vesicle fusion. The initial, rapid effects of signal transduction pathways are not dependent on protein synthesis, but can be followed by long-lasting responses that involve the activation of transcription factors, induction of gene expression and protein synthesis, and ultimately rearrangements of synapses.

In this chapter we will highlight the major second messenger pathways that are involved in basal ganglia neurotransmission (see Chapter 1 for an overview of basal ganglia circuits). As discussed in other chapters in this book, different receptor types co-exist in any given neuron in any particular brain region (see Chapter 4). These receptors can activate multiple second messenger pathways simultaneously in opposing or synergistic fashion. Thus, cells can integrate information from multiple brain areas. The dynamic integration of various signals is of interest for therapeutic approaches that target disorders of basal ganglia nuclei.

## II. SECOND-MESSENGER PATHWAYS

The receptors in the basal ganglia can be broadly categorized into three groups: G-protein-coupled receptors (GPCRs) that activate second messenger cascades; ionotropic receptors that gate ion channels; and tyrosine kinase receptors that have intracellular kinase properties. Downstream of these immediate actions, both types of receptors are able to reciprocally activate ion channels or signal transduction pathways.

## A. G-Protein-Coupled Receptors

GPCRs are a diverse family of integral membrane proteins that mediate signals from neurotransmitters, hormones, neuropeptides and cytokines. All GPCRs possess seven transmembrane domains and derive their name from the interaction with intracellular heterotrimeric G-proteins,  $G_{\alpha\beta\gamma}$ . Under basal conditions, G-proteins are bound to GDP. Upon activation by GPCRs, GDP is exchanged for GTP, causing dissociation of the  $G_{\alpha}$ -subunit from the  $G_{\beta\gamma}$ subunit (Fig. 26.1). A plethora of  $G_{\alpha}$ ,  $G_{\beta}$ , and  $G_{\gamma}$  subunits leads to a diversity of downstream effects, yet G-proteins are most commonly categorized by their  $G_{\alpha}$  subunit into  $G_{\alpha s/olf},~G_{\alpha i},~G_{\alpha q/11}$  and  $G_{\alpha 12/13}$  (Strathmann and Simon, 1991; Fields and Casey, 1997; Bridges and Lindsley, 2008). Dissociation of the  $G_{\alpha}$ -subunit from the  $G_{\beta\gamma}$ -subunit activates effector molecules. Effectors are enzymes that, upon G-protein binding, produce second messengers. The effector for both  $G_{\alpha s/olf}$  and  $G_{\alpha i}$  is adenylyl cyclase (AC; see Table 26.1 for all abbreviations). In response to stimulation by  $G_{\alpha s/olf}$ , AC generates the second messenger cyclic adenosine mono-phosphate (AMP), whereas activation of  $G_{\alpha i}$  halts cyclic AMP production by inhibiting AC. The primary effector for  $G_{\alpha q/11}$  is phospholipase C- $\beta$  (PLC- $\beta$ ), which converts phosphatidylinositol-4,5-bisphosphate into the second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3).  $G_{\alpha 12/13}$  plays a role in cellular proliferation and cancer biology and is linked to the mitogen-activated protein kinase (MAPK) cascade as well as to small monomeric G-proteins such as Ras, Rac and Rho (Dhanasekaran and Dermott, 1996; Spiegelberg and Hamm, 2007; Worzfeld et al., 2008).

After activation of the effector, the G-protein uses its inherent GTPase activity to hydrolyze GTP back to GDP, which leads to the re-association of the  $G_{\beta\gamma}$ -subunit with the inactive heterotrimer. The extracellular ligand, such as a neurotransmitter or a pharmacological agent, facilitates or prevents the coupling of the receptor to the G-protein. GPCR agonists increase the affinity of the receptor for the G-protein, inverse agonists decrease the affinity, and antagonists prevent other ligands from binding. Binding of different ligands to GPCRs can furthermore lead to desensitization and internalization (Bridges and Lindsley, 2008). In addition to the G-protein heterotrimers, a variety of other factors can confer specificity toward particular second messengers. These include various accessory proteins such as regulators of G-protein signaling (RGS-proteins), receptor-independent activators of G-protein-mediated signaling (AGS-proteins), or beta-arrestins (Bouvier, 2001; McDonald and Lefkowitz, 2001; Offermanns, 2003; Sato et al., 2006).

The effector activated by the G-protein generates the second messenger. Second messengers trigger biochemical cascades that can directly or indirectly affect ion channels permeable to  $Ca^{2+}$ , potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) (see, for example, Chapter 6). Second messengers also activate kinases that phosphorylate substrate proteins and activate transcription factors (see Chapters 27 and 30). The production of second messengers can result in signal amplification long after the ligand-receptor and G-protein-effector complexes have been dissociated.

A central player in the basal ganglia is the modulatory neurotransmitter dopamine, which is produced and released by neurons originating in the substantia nigra (SN). Dopamine interacts with G-protein coupled dopamine receptors on medium spiny neurons in the striatum (see Chapter 6). Movement disorders such as Parkinson's disease (see Chapter 34), Huntington's disease or hemiballism (Albin et al., 1989) are attributed to pathological changes in the basal ganglia motor circuits that involve dopamine receptors, as well as glutamatergic and GABAergic receptors. Of equal importance, the basal ganglia are involved in circuits of emotional function, reward, and memory processing, and have raised attention in psychiatric disorders such as schizophrenia, mood disorders, attention deficit disorder and drug addiction (Busatto and Kerwin, 1997; Lafer et al., 1997; Ring and Serra-Mestres, 2002; Casey et al., 2007; Haber, 2008). A great variety of signaling molecules play a role in the modulation and coordination of intracellular responses to neurotransmission in the basal ganglia, and the precise interaction of these molecules is necessary to ensure proper motor and behavioral function.

GPCRs are linked to a variety of second messengers. We will first highlight the signaling pathways of the traditional second messengers cyclic AMP, DAG and IP3.



**FIGURE 26.1** Selected routes of GPCR signaling. A, After binding of the ligand to the GPCR (1), the  $G_{\beta\gamma}$  subunits dissociate from the  $G_{\alpha}$  figure is cut off at the bottom subunit (2). Depending on the properties of  $G_{\alpha}$ , specific second messenger pathways are activated. Moreover, the dissociated  $G_{\beta\gamma}$  subunit can activate signal transduction pathways in its own right. Accumulation of cyclic AMP by AC can furthermore stimulate HCNs and cation influx from the extracellular space (3). B, Activation of inhibitor-1 (I1) or DARPP-32 blocks PP1 and prevents PP1 from counteracting PKA effects. C, Elements of the  $G_{\alpha\alpha/11}$ -PLC signal transduction pathway.

The majority of GPCRs modulate levels of one or more of these molecules. Second, we will discuss the ubiquitous role of  $Ca^{2+}$ , which serves as a second messenger after IP3 activation or via entry through ionotropic receptors and ion channels. Finally, we address the receptor tyrosine kinases (RTK), which stimulate the PLC- $\gamma$  pathway and play an

important role in growth and development. In addition, we will describe signaling properties of the  $_{\beta\gamma}$  components of G-proteins as well as a non-canonical signaling pathway described for dopamine receptors. The combination of these signaling pathways provides specificity as well as diversity in the basal ganglia.

TABLE 26.1 Com	mon Abbreviations and Terminology
АКАР	<u>A K</u> inase <u>A</u> nchoring- <u>P</u> rotein
Ca <sup>2+</sup>	calcium
CaM	calmodulin
CaMK	Ca <sup>2+</sup> /CaM-dependent kinase
cAMP	cyclic adenosine mono-phosphate
CaN	calcineurin or protein phosphatase 2B (PP2B)
DAG	diacylglycerol
DGK	diacylglycerol kinase
ERK	extracellular signal-regulated kinase
GPCR	G-protein coupled receptor
HCN	hyperpolarization-activated cyclic nucleotide-gated channel
IP3	inositol-1,4,5-trisphosphate
K <sup>+</sup>	potassium
марк	mitogen-activated protein kinase
mGluR	metabotropic glutamate receptor
Na <sup>+</sup>	sodium
PDE	phosphodiesterase
PIP2	phosphatidylinositol-4,5-bisphosphate
РКА	protein kinase A
РКС	protein kinase C
PLC	phospholipase C
РР	protein phosphatase
RasGAP	GTPase-activating protein
RasGEF	guanine nucleotide exchange factor
RasGRP/RasGRF	ras-guanine nucleotide-releasing protein/factor
RTK	receptor tyrosine kinase
TRPC	transient receptor potential channels
VGCC	voltage-gated calcium channel

## 1. Adenylyl Cyclase Modulation and the Cyclic AMP Pathway

The  $D_1$  class of dopamine receptors  $(D_1, D_5)$  is expressed extensively in the striatum (see Chapter 1) and is the primary activator of the cyclic AMP pathway in the basal ganglia. D<sub>1</sub> receptors couple to the stimulatory G-protein  $G_{\alpha s/olf}$ , named for its ability to stimulate the activation of AC (Fig. 26.1A). In addition to the  $D_1$  class of receptors, adenosine A2A receptors in the basal ganglia are G<sub>os</sub>-coupled receptors (Zezula and Freissmuth, 2008). Adenosine A2A receptors are concentrated in the GABAergic striatopallidal neurons, where they can form heteromeric complexes with dopamine D<sub>2</sub> receptors (Morelli et al., 2007) (see Chapter 11). A2A receptors antagonize the action of D<sub>2</sub> receptors via the formation of heteromers, but also via the opposite action of their second messenger pathways. Thus, the adenosine A2A receptor has emerged as a potential target in the treatment of Parkinson's disease (Schwarzschild et al., 2006).

Another AC-linked G-protein,  $G_{\alpha i}$ , is widespread in the basal ganglia and coupled to a variety of GPCRs such as D<sub>2</sub> like dopamine receptors (D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>), metabotropic glutamate receptors, opioid receptors, cannabinoid receptors, and muscarinic acetylcholine receptors (Levey et al., 1991; Jiang and North, 1992; Conn et al., 2005; Matyas et al., 2006).  $G_{\alpha i}$  is an inhibitory G-protein, named for its ability to inhibit AC activity and to downregulate cyclic AMP production (Fig. 26.1A).  $G_{\alpha i}$  -coupled receptors oppose directly Gas/olf activation of AC and promote hyperpolarization of the cell membrane by inhibiting Na<sup>+</sup> and Ca<sup>2+</sup> ion channels while opening K<sup>+</sup> channels (Surmeier et al., 2007).  $G_{\alpha i}$  receptors such as  $D_2$  and cannabinoid receptor 1 (CB1) are present on postsynaptic and presynaptic terminals. As presynaptic autoreceptors they inhibit neurotransmitter release (Wallmichrath and Szabo, 2002; Fisone et al., 2007) (see Chapter 17). D<sub>2</sub> autoreceptors are expressed at nigro-striatal synapses and are activated after dopamine is released from SN neurons. Endocannabinoids, the endogenous ligands for CB1, retrogradely diffuse from postsynaptic cells in the striatum to CB1 receptors on presynaptic terminals (see Chapter 9). CB1 receptors mediate long term depression (LTD), a decrease in presynaptic glutamate release of cortico-striatal neurons (Wickens, 2009), and a decrease in GABA neurotransmission (Wallmichrath and Szabo, 2002). Both CB1 and  $D_2$  hyperpolarize the presynaptic terminals to inhibit subsequent neurotransmitter release. In addition, the muscarinic M4 receptor dampens  $D_1$  receptor activation of cyclic AMP in the striatum (Sanchez-Lemus and Arias-Montano, 2006). M4 couples to  $G_{\alpha i}$  and is expressed in cholinergic neurons as well as  $D_1$  receptor-expressing neurons (Bernard et al., 1992).

The presence of multiple GPCRs in a given cell (see Chapter 4) allows for specificity in intracellular responses. Integration of signaling properties from different G-proteins plays an important role in determining how the cell responds to multiple neurotransmitters that are active in basal ganglia nuclei. The interaction of A2A and  $D_2$ receptors in striatopallidal neurons, mentioned above, is one such example. Heteromers of A2A and  $D_2$  receptors within the membrane couple to  $G_{\alpha q/11}$ , while homomers of A2A and  $D_2$  receptors couple to  $G_{\alpha s}$  and  $G_{\alpha i}$  respectively (Ferre et al., 2008). Furthermore, although it is well established that  $D_1$  and  $D_2$  receptors are segregated in the striatum (Gerfen et al., 1990; Le Moine et al., 1991; Gerfen et al., 1998), in areas where they are co-expressed they can form hetero-oligomers which couple to  $G_{\alpha\alpha/11}$  (Rashid et al., 2007). The following is a list of intracellular molecules that are intricate parts of cyclic AMP pathways in the basal ganglia (Fig. 26.1A).

- 1. Adenylyl cyclase (AC): After binding GTP,  $G_{\alpha s/olf}$ activates AC, a 12-transmembrane domain protein that converts ATP to cyclic AMP (Patel et al., 2001).  $G_{\alpha s}$  in the GTP-bound form displays a tenfold greater affinity for activating AC compared to the GDP-bound form (Sunahara et al., 1997). The AC enzyme serves as the effector and cyclic AMP as the second messenger signal. The complex of AC and  $G_{\alpha s/olf}$  functions as an active enzyme with AC comprising the catalytic unit and  $G_{\alpha s/olf}$  the regulatory unit (Kandel, 2000). AC production of cyclic AMP is terminated when  $G_{\alpha s/olf}$  dissociates from the complex. Currently, ten distinct human isoforms of AC are listed in the Entrez Gene database. Of these, AC5 is the main isoform in the striatum (Sadana and Dessauer, 2009). In medium spiny neurons, AC5 activity is regulated by the activation of dopamine receptors. D1 receptors stimulate its activity while D<sub>2</sub> receptors inhibit AC5. Animal models of AC5-/- knockout mice show impairment in dopamine receptor-activated signaling, emphasizing the importance of this isoform in normal basal ganglia neurotransmission.
- 2. Cyclic AMP-dependent protein kinase (PKA): A major role of cyclic AMP is to activate PKA (Montminy, 1997). The PKA holoenzyme is a tetrameric

complex containing two catalytic subunits bound to two regulatory subunits. On binding cyclic AMP, the catalytic subunits are released and activated. PKA transfers a phosphate from ATP onto the amino acids serine (Ser) or threonine (Thr) within consensus amino acid sequences in substrate proteins (Ubersax and Ferrell, 2007). PKA can increase neuronal excitability by increasing ion flux through Na<sup>+</sup> and Ca<sup>2+</sup> channels, and decreasing ion flux through K<sup>+</sup> channels. PKA can also facilitate NMDA receptor function by phosphorylating the NR1 subunit of the NMDA receptor (Dudman et al., 2003). The activation of  $D_1$  receptors and the cyclic AMP pathway acts to synergistically propagate glutamate neurotransmission (Cepeda and Levine, 1998; Konradi, 1998) (Fig. 26.3) (see Chapter 35). In addition to local effects on membrane depolarization, PKA can phosphorylate transcription factors to stimulate gene expression (see Chapter 27). For example, the transcription factor cyclic AMP response element binding protein (CREB) is activated by PKA, as are kinases involved in the MAP kinase pathway (Montminy, 1997). PKA is inhibited by protein kinase inhibitor peptides, a group of three small proteins that inhibit the function of the catalytic subunit.

- **3.** Hyperpolarization-activated cyclic nucleotide-gated channels (HCN): Cyclic AMP can bind directly to HCNs to modulate ion permeability (Fig. 26.1A), (Craven and Zagotta, 2006). HCNs have a unique reverse voltage dependence that leads to activation upon hyperpolarization (Wahl-Schott and Biel, 2009). When activated, HCN channels conduct inward current and depolarize the cell toward the threshold of voltage-gated Ca<sup>2+</sup> channels. HCN channels mediate pacemaker activity in the heart as well as the nervous system. In the globus pallidus, HCNs regulate tonic release of GABA (Boyes et al., 2007).
- 4. Phosphodiesterases (PDEs): PDEs break down intracellular cyclic AMP to 5'-AMP and are thus the counterparts to ACs (Fig. 26.1A). These enzymes consist of a diverse group of proteins, the products of at least 21 genes with multiple splice variants, divided into 11 families (Conti and Beavo, 2007). The PDE families hydrolyze cyclic AMP, cyclic GMP or both cyclic nucleotides with varying efficiencies. Phosphorylation of PDE enzymes by PKA increases their catalytic activity. The striatum expresses seven families of PDEs (Menniti et al., 2006). Each PDE isotype displays a high degree of specificity, allowing for multiple

dimensions of feedback control at basal ganglia synapses. The predominantly expressed subtypes in the striatum are PDE1B, PDE7B, and PDE10A. PDE1B and PDE10A are both potential targets for treating basal ganglia disorders (Menniti et al., 2006). PDE1B inhibition has been shown to potentiate dopamineactivated pathways in medium spiny neurons, while PDE10A inhibition has the opposite effect. Patients with movement disorders such as Parkinson's disease and Huntington's disease might benefit from these treatments as PDE-targeting drugs can affect the excitability of neurons in the basal ganglia motor circuitry.

- 5. <u>A Kinase Anchoring-Proteins</u> (AKAPs): The scaffolding/anchoring AKAPs compartmentalize PKA and affiliated substrates. As such, they regulate the spatial and temporal organization of the cyclic AMP-PKA pathway (Wong and Scott, 2004). AKAPs exist as a large and diverse group of anchoring proteins (over 50 members) that are differentially expressed within various cell and tissue types. All AKAPs have a PKA-anchoring domain, unique localization signals, and the ability to form complexes with other signaling molecules. AKAPs play an essential role in the specificity of signal transduction pathways by creating microenvironments that bring together the factors of selective pathways within a cell.
- 6. Protein phosphatases: Two phosphatases, protein phosphatase 1 and 2A (PP1 and PP2A) are the major Ser/Thr phosphatases in the basal ganglia that counteract PKA (Fig. 26.1A). Protein phosphatases form heteromers by associating one or more catalytic subunit(s) with scaffolding proteins and regulatory subunits (Mansuy and Shenolikar, 2006). Protein phosphatases are metalloenzymes with two divalent metal ions at the center of the catalytic site (Barford, 1996). PPI regulates PKA signaling by dephosphorylating PKA substrate proteins. However, PKA can promote amplification of its own kinase activity by phosphorylating inhibitor-1/DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein). After phosphorylation, inhibitor-1 associates with and blocks PP1 (Svenningsson et al., 2004), (Fig. 26.1B). This inhibition is counteracted by the protein phosphatase 2B/calcineurin (see below). Moreover, metabotropic glutamate receptor 5 (mGluR5), co-expressed in medium spiny neurons with D<sub>1</sub> receptors, can activate cyclin dependent kinase 5 (cdk5) to phosphorylate DARPP-32 (Conn et al., 2005). When phosphorylated by cdk5,

DARPP-32 no longer associates with inhibitor-1 and directly inhibits PKA activity.

## 2. DAG and IP3 Second Messengers

 $G_{\alpha q/11}$  activates PLC, which converts phosphatidylinositol-4, 5-bisphosphate into DAG and IP3 (Fig. 26.1A). To date, 13 mammalian PLC isozymes have been identified, and they are divided into six groups: PLC- $\beta$ , - $\gamma$ , - $\delta$ , - $\varepsilon$ , - $\zeta$  and - $\eta$ (Suh et al., 2008). Of these, PLC- $\beta$  and - $\varepsilon$  are known to be activated by  $G_{\alpha q/11}$ . Other PLC isozymes are stimulated by  $G_{\beta \gamma}$  dimers, tyrosine kinase pathways with the small G-proteins Rap, Rho, and Ras as effectors, and intracellular Ca<sup>2+</sup> (Smrcka and Sternweis, 1993; Suh et al., 2008) (Fig. 26.1C).

- 1. Diacylglycerol (DAG): DAG is a glycerol derivative, which is found at low levels in biological membranes during resting potentials. Upon stimulation, the PLC isozymes cleave phosphatidylinositol-4,5-bisphosphate (PIP2) into DAG and IP3. Phospholipase D (PLD) can also metabolize phosphatidylcholine to form DAG (Brose et al., 2004). The most prominent target of DAG is the protein kinase C (PKC) family of Ser/Thr kinases. However, a number of alternative targets with PKC homology domains exist as well. These include protein kinase D (PKD), diacylglycerol kinase (DGK), Ras guanyl nucleotide-releasing protein (RasGRP), chimaerin, and mammalian uncoordinated 13 (Munc13) (Brose et al., 2004). Each target comes in a variety of homologs and is discussed below (Fig. 26.1C).
  - The PKC (protein kinase C) family is group of enzymes that are involved in all aspects of neuronal function and malfunction. From neurotransmitter release and uptake, to receptor and ion channel function, to gene expression regulation, the PKC family modulates neuronal development, neuronal excitability, neuronal death and ultimately learning and memory (Mellor and Parker, 1998). Most neuronal processes, if not all, are affected by PKCs in a variety of ways. PKCs contain a catalytic domain which is linked to a regulatory domain that maintains the enzyme in an inactive conformation (Steinberg, 2008). At least 12 isoenzymes of PKC are described which are subdivided into three subfamilies based on structural differences in their NH2-terminal regulatory domain. The conventional PKCs – PKC $\alpha$ , - $\beta$ I, - $\beta$ II, and  $-\gamma$  – require DAG, phosphatidylserine and Ca<sup>2+</sup>

for activation. The novel PKCs-,  $\delta$ ,  $\varepsilon$ ,  $\eta$  and  $\theta$  isotypes are Ca<sup>2+</sup> insensitive, but are dependent on DAG.

- *PKD* (protein kinase D), a family of three Ser/Thr protein kinases, is a direct target of DAG but also lies downstream of PKC (Rozengurt et al., 2005). Initially termed "atypical PKCµ", PKD is implicated in the regulation of a variety of biological processes, including membrane trafficking and apoptosis, with many of these functions interdependent with other signaling pathways. PKC seems to be an essential factor in the activation of PKD (Wang, 2006).
- DGK (diacylglycerol kinase), a family of at least 11 paralogs in human, catalyzes phosphorylation of DAG to phosphatidic acid, and thus has a major role in deactivating DAG and its affiliated substrates, such as RasGRP and Munc-13 (Merida et al., 2008). Phosphatidic acid itself serves as a lipid second messenger, and has been reported to regulate cell growth, membrane trafficking, differentiation and migration (Goto and Kondo, 2004). In the basal ganglia, the DGK isoform, DGKβ is enriched in medium spiny neurons of the striatonigral and striatopallidal pathways (Hozumi et al., 2008).
- RasGEFs (Ras guanine nucleotide exchange factors) promote GDP/GTP exchange and activation of Ras GTPases, which leads to the modulation of the ERK/MAP kinase signal transduction cascade (Fig. 26.2). Among RasGEFs are RasGRF, RasGRP, Sos, and CalDAG-GEFs. RasGRP1 and RasGRP2 are expressed in striatal striosome and matrix compartments respectively, and they are inversely regulated in L-DOPA-induced dyskinesia in a rat model of Parkinson's Disease (Crittenden et al., 2009). In the dopamine-depleted striatum, L-DOPA treatment produces up-regulation of RasGRP1 (activator of MAP kinase cascade; Yang and Kazanietz, 2003) and down-regulation of RasGRP2 (inhibitor of the ERK/MAP kinase cascade; Kawasaki et al., 1998). These findings are in concordance with increased ERK phosphorylation observed in a similar model of L-DOPA-induced dyskinesia (Westin et al., 2007). Ras-GRF1 is an important factor in dopamine  $D_1$ and glutamate-mediated phosphorylation/activation of ERK1/2, as activation of the ERK1/2 pathway in Ras-GRF1-deficient striatal cells is impaired (Fasano et al., 2009).
- *Chimaerins* derive their names from the observation that they resemble a "chimaera" between the C1



**FIGURE 26.2** Selected routes of TRK receptor/MAP kinase signaling. A, Ligand binding leads to dimerization of TRK receptors and the phosphorylation of their intracellular domains. Receptor phosphorylation activates various effectors such as PLC $\gamma$  or GRB2, leads to recruitment of the MAP kinase pathways and to the release of Ca<sup>2+</sup> from the endoplasmic reticulum (see also Fig. 26.3). B, Small monomeric G-proteins (GTPases) interact with GTP in their active conformation and catalyze the hydrolysis of the terminal phosphate group of GTP to GDP to assume an inactive conformation. Guanine nucleotide exchange factors (GEFs; i.e. RasGRF, RasGRP and SoS) increase the rate of GDP to GTP exchange, while GTPase-activating proteins (RasGAP) facilitate the internal GTPase activity of the G-proteins. A third group of modulatory proteins, guanine nucleotide dissociation inhibitors, prevent nucleotide exchange (not shown).

domain of PKC isozymes and the GAP domain of the breakpoint cluster region protein (BCR), which is involved in chronic myelogenous leukemia (Yang and Kazanietz, 2003). They are Rho GTPase-activating proteins with at least four known paralogs that inactivate the small GTPase Rac by accelerating the intrinsic GTPase activity of Rac (Yang and Kazanietz, 2007). Chimaerins are strongly expressed in the brain and are implicated in diverse cellular processes such as neurocytoskeleton organization and growth cone guidance.

- Munc13 (Mammalian uncoordinated) proteins are the mammalian homologs of *Caenorhabditis elegans* Unc13. Mammals express four Munc13 isoforms that are localized to presynaptic active zones in the central nervous system. Munc13s are vesicle-priming proteins that promote short-term synaptic plasticity. Munc13-1 has a major role in neurotransmitter release, particularly of glutamatergic neurons. It has been shown to be essential for the membrane fusion of vesicles of the readily-releasable vesicle pool (Brose et al., 2000).
- Transient receptor potential (TRP) channels (Soboloff et al., 2007) are a family of conserved Ca<sup>2+</sup> -permeable cation channels that mediate depolarization in GABA projection neurons of the substantia nigra pars reticulata (Zhou et al., 2008). TRPCs are involved in mGluR1/5-mediated excitation of cholinergic interneurons in the striatum (Berg et al., 2007), and it seems reasonable to assume that future studies will demonstrate a major role for these channels in the basal ganglia.
- 2. IP3, the second metabolite of phosphatidylinositol-4, 5-bisphosphate, mobilizes intracellular  $Ca^{2+}$  release through its interaction with IP3 receptors (IP3R). IP3Rs are intracellular  $Ca^{2+}$  channels that are located primarily in the endoplasmic reticulum and are regulated by IP3 and  $Ca^{2+}$ . Three isoforms of the IP3R have been identified to date (Dawson, 1997). Activation of IP3Rs results in a variety of oscillatory changes in free  $Ca^{2+}$ . The far-reaching consequences of  $Ca^{2+}$  signaling are addressed below.

## 3. $G_{\beta\gamma}$ signaling

 $G_{\beta\gamma}$  plays an important role in the interaction with GPCRs (Smrcka, 2008). It is also required for  $G_{\alpha}$ -mediated nucleotide exchange and for the inactivation of  $G_{\alpha}$  subunits. In addition,  $G_{\beta\gamma}$  it is capable of independently activating signal transduction pathways. Among the effectors that bind directly to  $G_{\beta\gamma}$ , the following were shown to have an important physiological role: the inwardly-rectifying K<sup>+</sup> channel, the beta-adrenergic receptor kinase (BARK), N-type and P/Q type Ca<sup>2+</sup> channels, phosphoinositide 3-kinases, PLC $\beta$ 1, and ACs (Logothetis et al., 1987; Tang and Gilman, 1991; Camps et al., 1992; Pitcher et al., 1992; Stephens et al., 1994; Herlitze et al., 1996; Ikeda, 1996), (Fig. 26.1).

On the presynaptic side, the  $G_{\beta\gamma}$ -subunit can affect  $Ca^{2+}$  channel mediated neurotransmitter release. It can directly bind to presynaptic  $Ca^{2+}$  channels and reduce the sensitivity to membrane depolarization (Dolphin, 2003). Moreover,

it has a direct inhibitory effect on the transmitter release machinery by binding to proteins of the SNARE complex (Blackmer et al., 2005; Gerachshenko et al., 2005). Finally, there is some indication that  $G_{\beta\gamma}$  might be translocated to the cell nucleus where it interacts with transcription factors and histone modifying enzymes (Spiegelberg and Hamm, 2007).

## 4. Novel GPCR-Mediated Second Messenger Pathways

After binding to an agonist, GPCRs are phosphorylated by one of a number of GPCR Ser/Thr kinases (GRKs). Phosphorylation of the receptor promotes the high-affinity binding of an arrestin protein, which prevents further coupling to G-proteins (Kohout and Lefkowitz, 2003). An internalization complex is formed comprising the GPCR,  $\beta$ -arrestin, adaptor protein 2 (AP2), and clathrin, leading to receptor internalization through clathrin-mediated endocytosis (Beaulieu et al., 2007). The classical role of  $\beta$ -arrestins was thus thought to be GPCR desensitization. However, recently it has been shown that  $\beta$ -arrestins can act as receptorregulated scaffolds and mediate a variety of receptor signaling and regulatory processes (Lefkowitz and Shenoy, 2005).

An example of this "non-canonical" signaling pathway, i.e., G-protein and cyclic AMP-independent signaling of GPCRs, has been observed in neurons expressing the dopamine D<sub>2</sub> receptor. Recent studies have shown that D<sub>2</sub>-GPCRs exert some of their effects in vivo through cyclic AMP-independent mechanisms (Beaulieu et al., 2007). This new mode of dopamine receptor signaling involves β-arrestin, protein kinase B (Akt) and protein phosphatase 2A (PP2A), proteins that have been classically implicated in GPCR desensitization. The formation of this complex results in the dephosphorylation/inactivation of Akt by PP2A and the subsequent stimulation of GSK-3-mediated signaling (Beaulieu et al., 2005). GSK3 is a regulator of many cellular functions, including cell architecture, motility, and survival (Jope and Johnson, 2004). Interestingly, GSK3 is inhibited by the mood stabilizing salt, lithium (Gould and Manji, 2005), and would presumably also be inhibited by conventional antipsychotic drugs that are antagonists of the D<sub>2</sub> receptor (Seeman, 1987).

## **B.** Ionotropic Receptors

Ligand-gated ion channels are transmembrane protein complexes that conduct ion flow through a channel pore in response to the binding of a neurotransmitter. They are different from voltage-gated ion channels, which are sensitive to membrane potentials, and GPCRs, which use second messengers. Although ionotropic receptors are commonly regarded as postsynaptic elements, they can also been found on presynaptic membranes near release sites, where they contribute to vesicle fusion (Engelman and MacDermott, 2004).

Ionotropic receptors include glutamate receptors (NMDA and AMPA/kainate receptors), serotonin-5-HT3 receptors, cholinergic nicotinic receptors, GABA-A receptors and glycine receptors (see Chapter 4). These receptors are either cation or anion selective, leading to their involvement in either excitatory (NMDA, AMPA/kainate, 5-HT3, nicotinic receptors), or inhibitory (GABA-A, glycine) neurotransmission.

The major ions permeating these receptors are  $Ca^{2+}$ ,  $Na^+$ ,  $K^+$  and  $Cl^-$ . Receptors that are permeable to  $Ca^{2+}$  will generally conduct monovalent cations as well, while receptors permeable to monovalent cations such as  $Na^+$ , conduct  $Ca^{2+}$  only under special circumstances associated with subtype composition and mRNA editing (discussed in other chapters).

NMDA receptors, 5-HT3, and nicotinic receptors are permeable to  $Ca^{2+}$ , whereas AMPA/kainate receptors are predominantly permeable to Na<sup>+</sup>. GABA and glycine receptors are permeable to Cl<sup>-</sup>. Signal transduction and gene expression are driven mostly by Ca<sup>2+</sup>, while anion channels might down-modulate these pathways. While Ca<sup>2+</sup>-permeable receptors can drive signal transduction autonomously, influx of Na<sup>+</sup> and other cations can depolarize the plasma membrane and trigger opening of voltagegated Ca<sup>2+</sup> channels (VGCCs). The activation of L-type VGCCs leads to increases in intracellular Ca<sup>2+</sup> and activation of signal transduction pathways (see below).

## C. Ca<sup>2+</sup> Signaling

Calcium accumulation in the postsynaptic area initiates a cascade of events that can translocate to the nucleus and result in the activation of gene expression. This alteration of gene and protein expression patterns can promote dendritic growth, improve synaptic strength, and affect neuronal plasticity (Greer and Greenberg, 2008) (Fig. 26.3). At the postsynaptic membrane,  $Ca^{2+}$  activates kinases and phosphatases that affect the function and trafficking of various receptors and channels. At the presynaptic site, localized  $Ca^{2+}$  signals drive vesicle fusion at and near active zones, and thus regulate neurotransmitter release (Schneggenburger and Neher, 2005). These important actions of  $Ca^{2+}$ , together with the fact that high levels

of  $Ca^{2+}$  are neurotoxic (Choi, 1988), require neurons to expend considerable energy to maintain low basal levels of intracellular  $Ca^{2+}$  under homeostatic conditions. Calcium is pumped into the extracellular space or into internal  $Ca^{2+}$ storage sites such as the endoplasmic reticulum or mitochondria (Verkhratsky and Petersen, 1998).

Cytoplasmic Ca<sup>2+</sup> levels are rapidly increased by Ca<sup>2+</sup> influx from *extracellular* sources or from *internal* Ca<sup>2+</sup> stores. Calcium release from intracellular stores can be mediated by a cascade of  $G_{\alpha q/11} \rightarrow PLC \rightarrow IP3 \rightarrow IP3Rs$  (Fig. 26.3). In addition to IP3Rs, ryanodine receptors can release Ca<sup>2+</sup> from internal stores. Both receptor types are distributed throughout the endoplasmic reticulum and are also sensitive to Ca<sup>2+</sup> levels. Increased Ca<sup>2+</sup> levels induce Ca<sup>2+</sup> release which leads to propagated Ca<sup>2+</sup> waves (Berridge, 1998). Calcium can therefore be a messenger that induces its own release.

Extracellular Ca<sup>2+</sup> can enter the cytoplasm through voltage-gated Ca<sup>2+</sup> channels (VGCCs) or through ligandgated ion channels. VGCCs have a role in second messenger pathways as well as in presynaptic transmitter release. The  $Ca_{V21}$  to  $Ca_{V23}$  groups of VGCCs, which includes P/Q-type, N-type and R-type Ca<sup>2+</sup> channels, has a major role in presynaptic plasticity (Pietrobon, 2005; Catterall and Few, 2008). Dihydropyridine-sensitive L-type  $Ca^{2+}$  channels, which belong to the Ca<sub>V1.1</sub> to Ca<sub>V1.4</sub> group, couple membrane depolarization to signal transduction and gene expression (Lipscombe et al., 2004). These channels have a high conductance for  $Ca^{2+}$  and are localized at the cell soma and on the dendrites. Thus, they are uniquely suited to propagate Ca<sup>2+</sup> signals to the nucleus (Westenbroek et al., 1990; Catterall, 2000), since it has been shown that an elevation of Ca<sup>2+</sup> concentration within the nucleus is required in some cases for the induction of gene expression (Greer and Greenberg, 2008) (see also Chapter 30). A number of signaling pathways involving protein kinases and phosphatases, GPCRs, scaffolding proteins, and Ca<sup>2+</sup>-binding proteins lead to a dynamic regulation of VGCCs (Calin-Jageman and Lee, 2008).

Principal Ca<sup>2+</sup>-conducting ligand-gated ion channels are the glutamatergic N-methyl-D-aspartate (NMDA) receptor and, in some cases, the  $\alpha$ -amino-3-hydroxy-5methyl-4-isoxazolepropionate type (AMPA) glutamate receptor. Other ligand-gated ion channels such as the 5-HT3 receptor (Brown et al., 1998) and the  $\alpha$ 7 nicotinic receptor (Bertrand et al., 1993), can be permeable to Ca<sup>2+</sup> as well, although overall their participation to Ca<sup>2+</sup> influx might be of lesser magnitude.



**FIGURE 26.3** Neuronal Ca<sup>2+</sup> pathways. Ca<sup>2+</sup> enters neurons from the extracellular space via voltage-gated Ca<sup>2+</sup> channels (VGCC) or via ionotropic receptors (NMDA receptor shown as an example). Ca<sup>2+</sup> can also enter the cytoplasm from intracellular Ca<sup>2+</sup> stores via the G $\alpha_{q11}$ /PLC/IP3 receptor pathway. G $\alpha_{s/olf}$  signal transduction can modulate ion channel function. Ca<sup>2+</sup> activates calmodulin which in turn affects other signal transduction molecules.

Although each of the routes of  $Ca^{2+}$  entry increases intracellular  $Ca^{2+}$  concentrations, the mode of entry determines responses in terms of gene induction (Bading et al., 1993; Ginty, 1997). Channel conductance properties, channel open times, localization of channels, the association of a channel with key signaling molecules, and the ability of a channel to propagate the signal to the nucleus, all contribute to the specificity of the response (Greer and Greenberg, 2008). Subcellular location of  $Ca^{2+}$  in microdomains, which are formed at sites where  $Ca^{2+}$  enters the cytoplasm, contribute to signaling specificity (Berridge, 2006).

The cytosolic rise in  $Ca^{2+}$ , due to influx from external sources or from internal storage organelles, can activate a number of signal transduction pathways such as the cyclic AMP/PKA pathway, the  $Ca^{2+}$ /calmodulin (CaM)/CaM kinase pathway, and MAP kinase pathway (Figs 26.2, 26.3).

 Calmodulin (CaM) is a 17kDa Ca<sup>2+</sup>-binding protein that binds four Ca<sup>2+</sup> ions. Upon binding to Ca<sup>2+</sup>, CaM undergoes a conformational switch enabling it to bind to and activate a number of effector proteins in the nucleus and the cytoplasm, including  $Ca^{2+}/CaM$ dependent kinases, ACs, and the phosphatase calcineurin. Interestingly, CaM itself is a relatively poor  $Ca^{2+}$ sensor but increases its  $Ca^{2+}$  affinity in the presence of its target molecules (Swulius and Waxham, 2008).

- 2.  $Ca^{2+}$ -activated AC: Of the ten members of the AC family, AC1 and AC8 are activated by  $Ca^{2+}/CaM$  (Wang and Storm, 2003). Like all other ACs, AC1 and AC8 generate intracellular cyclic AMP, albeit in specific response to rises in intracellular  $Ca^{2+}$ . Both AC isoforms play critical roles in long-term potentiation and memory formation (Ferguson and Storm, 2004). Interestingly, AC1 is a coincidence detector as its response to an increase in intracellular  $Ca^{2+}$  levels is amplified by  $G_{cos}$ -coupled receptor activation.
- **3.** Ca<sup>2+</sup>/calmodulin kinases (CaMK) are activated by Ca<sup>2+</sup>/CaM and they have an important function in memory and plasticity (Mayford, 2007). Members of

the CaM-kinase family are Ser/Thr kinases. CaM kinases are separated into two groups: multifunctional CaM-kinases (CaM kinase kinase, CaMKI, CaMKII and CaMKIV) which have multiple downstream targets, and substrate-specific CaM-kinases (CaMKIII, phosphorylase kinase, and myosin light chain kinases) with only one known downstream target (Swulius and Waxham, 2008). Kinase activation is dependent on the binding of Ca<sup>2+</sup>/CaM. At basal Ca<sup>2+</sup> levels, CaMKs are inhibited by an autoinhibitory domain, which prevents substrate binding to the catalytic domain. A rise in intracellular Ca<sup>2+</sup> concentration and subsequent saturation of CaM with four Ca<sup>2+</sup> molecules leads to the binding of CaM to CaMK and disruption of autoinhibition. Similar to the MAP kinase cascade, CaMK can be activated/phosphorylated by a CaM kinase kinase (CaMKK) in a CamK cascade (Soderling, 1999). CaMKII is among the most prominent proteins in the post-synaptic density, where it plays a major role in the modulation of activity of ion channels (Kennedy et al., 1983). It is the only CaMK with known homomultimerization (dodecamers) and it is thought to be the primary initiating signal in NMDA-receptor-mediated long-term potentiation and gene expression (Mayford, 2007; Swulius and Waxham, 2008). CaMKIV is activated by Ca<sup>2+</sup>/CaM and CaMKK. It is involved in the phosphorylation of a number of activity-dependent transcription factors such as CREB (Soderling, 1999). CaMKIV is highly expressed in the striatum and can be found in both the cytosol and the nucleus.

- 4. Calcineurin (CaN; protein phosphatase 2B) is a phosphatase that is enriched in the post-synaptic density and the cell soma. CaN is assembled with a catalytic and a regulatory subunit. CaN is activated by binding of  $Ca^{2+}/CaM$  to the catalytic subunit together with concurrent binding of  $Ca^{2+}$  to the regulatory subunit. CaN has a higher affinity for  $Ca^{2+}/CaM$  than CaMK, thus elevations in intracellular  $Ca^{2+}$  will affect CaN activity before affecting CaMK activity. Only after prolonged  $Ca^{2+}$  influx will the balance be shifted to CaMKs. CaN has many functions, including disinhibition of protein phosphatase 1 (PP1), and the modulation of neurotransmitter synthesis, release and receptor activity (Groth et al., 2003).
  - Disinhibition/activation of the Ser/Thr phosphatase PP1 via DARPP-32: The activity of PP1 is regulated by inhibitor-1 or DARPP-32. Phosphorylation of inhibitor-1/DARPP-32 by PKA activates inhibitor-1

and inhibits PP1 (Fig. 26.1B). CaN dephosphorylates inhibitor-1/ DARPP-32, thus increasing PP1 activity.

 Neurotransmitter synthesis, release and receptor activation: CaN modulates the activity of enzymes involved in neurotransmitter synthesis such as glutamate decarboxylase (GAD) and neuronal nitric oxide synthase (nNOS). Both GAD and nNOS are activated by dephosphorylation. CaN dephosphorylation of synapsin I prevents vesicles from joining the readily releasable pool at the synapse and thus downmodulates neurotransmitter release. CaN is also involved in synaptic vesicle endocytosis. Finally, many neurotransmitter receptors are modulated by phosphorylation status and CaN plays an important role in the regulation of receptor activity and receptor internalization.

#### D. Ras/MAP Kinase Signaling

## 1. Calcium-Activated MAP Kinase Signaling

Ras GTPases (H-Ras, N-Ras, K-Ras4A and K-Ras4B) are responsive to rises in cytosolic Ca<sup>2+</sup> levels, and they are activated by the exchange of GDP for GTP (Cullen and Lockyer, 2002), (Fig. 26.2). Calcium can activate kinases, which phosphorylate Ras guanine nucleotide exchange factors (RasGEFs). RasGEFs induce the dissociation of GDP and association with GTP (Avraham et al., 2000; Cullen and Lockyer, 2002), (Fig. 26.2B) and thus activate the MAP kinase pathway (Fig. 26.3).

## 2. Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) are a family of cell surface receptors with an intracellular domain that has tyrosine kinase activity (Fig. 26.2). They mediate a host of cellular programs including cell proliferation, cell differentiation, cell death and inflammation. Ligands for RTKs include cytokines, growth factors, and hormones. Ligand binding to RTKs causes the inactive monomers to form dimers and to induce the autophosphorylation of tyrosine residues in the intracellular domain. Phosphorylation leads to recruitment of Grb2/Sos complexes and activation of Ras. Ras triggers the sequential phosphorylation of hierarchically organized mitogen-activated protein kinase (MAPK) cascades, i.e. MAP kinase kinase kinase (MAPKKK), MAP kinase kinase and MAP kinase (MAPK), (McKay and Morrison, 2007) (Fig. 26.2). Phosphorylation of RTKs can also lead to the activation of PLC $\gamma$ , IP3, DAG and Ca<sup>2+</sup>, and thus activate MAPK pathways (Suh et al., 2008). Known MAPK pathways include extracellular signal-regulated kinase (ERK) 1 and 2, c-Jun N-terminal kinase (JNK) 1, 2 and 3, p38 MAP kinases  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ , and ERK5 (Turjanski et al., 2007).

MAP kinases such as ERK1/2 can translocate to the nucleus where they phosphorylate/activate downstream kinases and transcription factors such as CREB, SRF, c-Jun, c-Fos and others (Vanhoutte et al., 1999; Turjanski et al., 2007) (see also Chapter 30).

## **III. CONCLUSIONS AND OUTLOOK**

Although many signal transduction pathways seem to involve cyclic AMP and Ca<sup>2+</sup> as second messengers, a surprising level of specificity is achieved for individual pathways. Many factors contribute to specificity including (i) scaffolding and anchoring proteins; (ii) modulators of enzymatic activity; (iii) protein families with similar properties but different downstream effects; and (iv) splice variants of these proteins. These factors make for attractive targets in drug discovery pursuits with the goal to treat disorders ranging from neuro-psychiatric disorders to cancer. In the context of the basal ganglia, neurologic disorders such as Parkinson's disease or Huntington's disease, and psychiatric disorders such as mood disorders or schizophrenia, may soon be treated with drugs that specifically target defined signaling pathways in disease-causing subsets of neurons.

## REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Avraham H, Park SY, Schinkmann K, Avraham S (2000) RAFTK/Pyk2mediated cellular signalling. Cell Signal 12:123–133.
- Bading H, Ginty DD, Greenberg ME (1993) Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 260:181–186.
- Barford D (1996) Molecular mechanisms of the protein serine/threonine phosphatases. Trends Biochem Sci 21:407–412.
- Beaulieu JM, Gainetdinov RR, Caron MG (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 28:166–172.
- Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG (2005) An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 122:261–273.
- Berg AP, Sen N, Bayliss DA (2007) TrpC3/C7 and Slo2.1 are molecular targets for metabotropic glutamate receptor signaling in rat striatal cholinergic interneurons. J Neurosci 27:8845–8856.

Bernard V, Normand E, Bloch B (1992) Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. J Neurosci 12:3591–3600.

Berridge MJ (1998) Neuronal calcium signaling. Neuron 21:13-26.

- Berridge MJ (2006) Calcium microdomains: organization and function. Cell Calcium 40:405–412.
- Bertrand D, Galzi JL, Devillers-Thiery A, Bertrand S, Changeux JP (1993) Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal alpha 7 nicotinic receptor. Proc Natl Acad Sci USA 90:6971–6975.
- Blackmer T, Larsen EC, Bartleson C, et al. (2005) G protein betagamma directly regulates SNARE protein fusion machinery for secretory granule exocytosis. Nat Neurosci 8:421–425.
- Bouvier M (2001) Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2:274–286.
- Boyes J, Bolam JP, Shigemoto R, Stanford IM (2007) Functional presynaptic HCN channels in the rat globus pallidus. Eur J Neurosci 25:2081–2092.
- Bridges TM, Lindsley CW (2008) G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. ACS Chem Biol 3:530–541.
- Brose N, Rosenmund C, Rettig J (2000) Regulation of transmitter release by Unc-13 and its homologues. Curr Opin Neurobiol 10: 303–311.
- Brose N, Betz A, Wegmeyer H (2004) Divergent and convergent signaling by the diacylglycerol second messenger pathway in mammals. Curr Opin Neurobiol 14:328–340.
- Brown AM, Hope AG, Lambert JJ, Peters JA (1998) Ion permeation and conduction in a human recombinant 5-HT3 receptor subunit (h5-HT3A). J Physiol 507(Pt 3):653–665.
- Busatto GF, Kerwin RW (1997) Schizophrenia, psychosis, and the basal ganglia. Psychiatr Clin North Am 20:897–910.
- Calin-Jageman I, Lee A (2008) Ca(v)1 L-type Ca<sup>2+</sup> channel signaling complexes in neurons. J Neurochem 105:573–583.
- Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P (1992) Isozyme-selective stimulation of phospholipase C-beta 2 by G protein beta gamma-subunits. Nature 360:684–686.
- Casey BJ, Nigg JT, Durston S (2007) New potential leads in the biology and treatment of attention deficit-hyperactivity disorder. Curr Opin Neurol 20:119–124.
- Catterall WA (2000) Structure and regulation of voltage-gated Ca<sup>2+</sup> channels. Annu Rev Cell Dev Biol 16:521–555.
- Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. Neuron 59:882–901.
- Cepeda C, Levine MS (1998) Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. Dev Neurosci 20:1–18.
- Choi DW (1988) Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci 11:465–469.
- Conn PJ, Battaglia G, Marino MJ, Nicoletti F (2005) Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat Rev Neurosci 6:787–798.
- Conti M, Beavo J (2007) Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. Annu Rev Biochem 76:481–511.
- Craven KB, Zagotta WN (2006) CNG and HCN channels: two peas, one pod. Annu Rev Physiol 68:375–401.
- Crittenden JR, Cantuti-Castelvetri I, Saka E, et al. (2009) Dysregulation of CalDAG-GEFI and CalDAG-GEFII predicts the severity of motor

side-effects induced by anti-parkinsonian therapy. Proc Natl Acad Sci USA 106:2892–2896.

- Cullen PJ, Lockyer PJ (2002) Integration of calcium and Ras signalling. Nat Rev Mol Cell Biol 3:339–348.
- Dawson AP (1997) Calcium signalling: how do IP3 receptors work? Curr Biol 7:R544–R547.
- Dhanasekaran N, Dermott JM (1996) Signaling by the G12 class of G proteins. Cell Signal 8:235–245.
- Dolphin AC (2003) G protein modulation of voltage-gated calcium channels. Pharmacol Rev 55:607–627.
- Dudman JT, Eaton ME, Rajadhyaksha A, et al. (2003) Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. J Neurochem 87:922–934.
- Engelman HS, MacDermott AB (2004) Presynaptic ionotropic receptors and control of transmitter release. Nat Rev Neurosci 5:135–145.
- Fasano S, D'Antoni A, Orban PC, et al. (2009) Ras-Guanine Nucleotide-Releasing Factor 1 (Ras-GRF1) Controls Activation of Extracellular Signal-Regulated Kinase (ERK) Signaling in the Striatum and Long-Term Behavioral Responses to Cocaine. Biol Psychiatry 66:758–768.
- Ferguson GD, Storm DR (2004) Why calcium-stimulated adenylyl cyclases? Physiology (Bethesda) 19:271–276.
- Ferre S, Quiroz C, Woods AS, et al. (2008) An update on adenosine A2A-dopamine D2 receptor interactions: implications for the function of G protein-coupled receptors. Curr Pharm Des 14:1468–1474.
- Fields TA, Casey PJ (1997) Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. Biochem J 321(Pt 3): 561–571.
- Fisone G, Hakansson K, Borgkvist A, Santini E (2007) Signaling in the basal ganglia: postsynaptic and presynaptic mechanisms. Physiol Behav 92:8–14.
- Gerachshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S (2005) Gbetagamma acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. Nat Neurosci 8:597–605.
- Gerfen CR, Keefe KA, Steiner H (1998) Dopamine-mediated gene regulation in the striatum. Adv Pharmacol 42:670–673.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Ginty DD (1997) Calcium regulation of gene expression: isn't that spatial? Neuron 18:183–186.
- Goto K, Kondo H (2004) Functional implications of the diacylglycerol kinase family. Adv Enzyme Regul 44:187–199.
- Gould TD, Manji HK (2005) Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. Neuropsychopharmacology 30:1223–1237.
- Greer PL, Greenberg ME (2008) From synapse to nucleus: calciumdependent gene transcription in the control of synapse development and function. Neuron 59:846–860.
- Groth RD, Dunbar RL, Mermelstein PG (2003) Calcineurin regulation of neuronal plasticity. Biochem Biophys Res Commun 311:1159–1171.
- Haber S (2008) Parallel and integrative processing through the Basal Ganglia reward circuit: lessons from addiction. Biol Psychiatry 64:173–174.
- Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA (1996) Modulation of Ca<sup>2+</sup> channels by G-protein beta gamma subunits. Nature 380:258–262.
- Hozumi Y, Fukaya M, Adachi N, Saito N, Otani K, Kondo H, Watanabe M, Goto K (2008) Diacylglycerol kinase beta accumulates on the perisynaptic site of medium spiny neurons in the striatum. Eur J Neurosci 28:2409–2422.

- Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. Nature 380:255–258.
- Jiang ZG, North RA (1992) Pre- and postsynaptic inhibition by opioids in rat striatum. J Neurosci 12:356–361.
- Jope RS, Johnson GV (2004) The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29:95–102.
- Kandel ER, Schwartz JH, Jessel TM (2000) Principles of Neural Science: McGraw-Hill Companies, Inc.
- Kawasaki H, Springett GM, Toki S, et al. (1998) A Rap guanine nucleotide exchange factor enriched highly in the basal ganglia. Proc Natl Acad Sci USA 95:13278–13283.
- Kennedy MB, Bennett MK, Erondu NE (1983) Biochemical and immunochemical evidence that the "major postsynaptic density protein" is a subunit of a calmodulin-dependent protein kinase. Proc Natl Acad Sci USA 80:7357–7361.
- Kohout TA, Lefkowitz RJ (2003) Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. Mol Pharmacol 63:9–18.
- Konradi C (1998) The molecular basis of dopamine and glutamate interactions in the striatum. Adv Pharmacol 42:729–733.
- Lafer B, Renshaw PF, Sachs GS (1997) Major depression and the basal ganglia. Psychiatr Clin North Am 20:885–896.
- Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.
- Lefkowitz RJ, Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. Science 308:512–517.
- Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218–3226.
- Lipscombe D, Helton TD, Xu W (2004) L-type calcium channels: the low down. J Neurophysiol 92:2633–2641.
- Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE (1987) The beta gamma subunits of GTP-binding proteins activate the muscarinic K<sup>+</sup> channel in heart. Nature 325:321–326.
- Mansuy IM, Shenolikar S (2006) Protein serine/threonine phosphatases in neuronal plasticity and disorders of learning and memory. Trends Neurosci 29:679–686.
- Matyas F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF (2006) Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. Neuroscience 137:337–361.
- Mayford M (2007) Protein kinase signaling in synaptic plasticity and memory. Curr Opin Neurobiol 17:313–317.
- McDonald PH, Lefkowitz RJ (2001) Beta-Arrestins: new roles in regulating heptahelical receptors' functions. Cell Signal 13:683–689.
- McKay MM, Morrison DK (2007) Integrating signals from RTKs to ERK/MAPK. Oncogene 26:3113–3121.
- Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332(Pt 2):281–292.
- Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. Nat Rev Drug Discov 5:660–670.
- Merida I, Avila-Flores A, Merino E (2008) Diacylglycerol kinases: at the hub of cell signalling. Biochem J 409:1–18.
- Montminy M (1997) Transcriptional regulation by cyclic AMP. Annu Rev Biochem 66:807–822.
- Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA (2007) Role of adenosine A2A receptors in parkinsonian motor impairment and 1-DOPA-induced motor complications. Prog Neurobiol 83:293–309.

- Offermanns S (2003) G-proteins as transducers in transmembrane signalling. Prog Biophys Mol Biol 83:101–130.
- Patel TB, Du Z, Pierre S, Cartin L, Scholich K (2001) Molecular biological approaches to unravel adenylyl cyclase signaling and function. Gene 269:13–25.
- Pietrobon D (2005) Function and dysfunction of synaptic calcium channels: insights from mouse models. Curr Opin Neurobiol 15:257–265.
- Pitcher JA, Inglese J, Higgins JB, et al. (1992) Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. Science 257:1264–1267.
- Rashid AJ, O'Dowd BF, Verma V, George SR (2007) Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. Trends Pharmacol Sci 28:551–555.
- Ring HA, Serra-Mestres J (2002) Neuropsychiatry of the basal ganglia. J Neurol Neurosurg Psychiatry 72:12–21.
- Rozengurt E, Rey O, Waldron RT (2005) Protein kinase D signaling. J Biol Chem 280:13205–13208.
- Sadana R, Dessauer CW (2009) Physiological roles for G proteinregulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. Neurosignals 17:5–22.
- Sanchez-Lemus E, Arias-Montano JA (2006) M1 muscarinic receptors contribute to, whereas M4 receptors inhibit, dopamine D1 receptor-induced [3H]-cyclic AMP accumulation in rat striatal slices. Neurochem Res 31:555–561.
- Sato M, Blumer JB, Simon V, Lanier SM (2006) Accessory proteins for G proteins: partners in signaling. Annu Rev Pharmacol Toxicol 46:151–187.
- Schneggenburger R, Neher E (2005) Presynaptic calcium and control of vesicle fusion. Curr Opin Neurobiol 15:266–274.
- Schwarzschild MA, Agnati L, Fuxe K, Chen JF, Morelli M (2006) Targeting adenosine A2A receptors in Parkinson's disease. Trends Neurosci 29:647–654.
- Seeman P (1987) Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1:133–152.
- Smrcka AV (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. Cell Mol Life Sci 65:2191–2214.
- Smrcka AV, Sternweis PC (1993) Regulation of purified subtypes of phosphatidylinositol-specific phospholipase C beta by G protein alpha and beta gamma subunits. J Biol Chem 268:9667–9674.
- Soboloff J, Spassova M, Hewavitharana T, et al. (2007) TRPC channels: integrators of multiple cellular signals. Handb Exp Pharmacol:575–591.
- Soderling TR (1999) The Ca-calmodulin-dependent protein kinase cascade. Trends Biochem Sci 24:232–236.
- Spiegelberg BD, Hamm HE (2007) Roles of G-protein-coupled receptor signaling in cancer biology and gene transcription. Curr Opin Genet Dev 17:40–44.
- Steinberg SF (2008) Structural basis of protein kinase C isoform function. Physiol Rev 88:1341–1378.
- Stephens L, Smrcka A, Cooke FT, Jackson TR, Sternweis PC, Hawkins PT (1994) A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein beta gamma subunits. Cell 77:83–93.
- Strathmann MP, Simon MI (1991) G alpha 12 and G alpha 13 subunits define a fourth class of G protein alpha subunits. Proc Natl Acad Sci USA 88:5582–5586.
- Suh PG, Park JI, Manzoli L, Cocco L, Peak JC, Katan M, Fukami K, Kataoka T, Yun S, Ryu SH (2008) Multiple roles of phosphoinositidespecific phospholipase C isozymes. BMB Rep 41:415–434.

- Sunahara RK, Dessauer CW, Whisnant RE, Kleuss C, Gilman AG (1997) Interaction of Gsalpha with the cytosolic domains of mammalian adenylyl cyclase. J Biol Chem 272:22265–22271.
- Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopaminereceptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30:228–235.
- Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol 44:269–296.
- Swulius MT, Waxham MN (2008) Ca(2+)/calmodulin-dependent protein kinases. Cell Mol Life Sci 65:2637–2657.
- Tang WJ, Gilman AG (1991) Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits.. Science 254:1500–1503.
- Turjanski AG, Vaque JP, Gutkind JS (2007) MAP kinases and the control of nuclear events. Oncogene 26:3240–3253.
- Ubersax JA, Ferrell JE Jr. (2007) Mechanisms of specificity in protein phosphorylation. Nat Rev Mol Cell Biol 8:530–541.
- Vanhoutte P, Barnier JV, Guibert B, Pages C, Besson MJ, Hipskind RA, Caboche J (1999) Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. Mol Cell Biol 19:136–146.
- Verkhratsky AJ, Petersen OH (1998) Neuronal calcium stores. Cell Calcium 24:333–343.
- Wahl-Schott C, Biel M (2009) HCN channels: structure, cellular regulation and physiological function. Cell Mol Life Sci 66:470–494.
- Wallmichrath I, Szabo B (2002) Cannabinoids inhibit striatonigral GABAergic neurotransmission in the mouse. Neuroscience 113:671–682.
- Wang H, Storm DR (2003) Calmodulin-regulated adenylyl cyclases: cross-talk and plasticity in the central nervous system. Mol Pharmacol 63:463–468.
- Wang QJ (2006) PKD at the crossroads of DAG and PKC signaling. Trends Pharmacol Sci 27:317–323.
- Westenbroek RE, Ahlijanian MK, Catterall WA (1990) Clustering of L-type Ca<sup>2+</sup> channels at the base of major dendrites in hippocampal pyramidal neurons. Nature 347:281–284.
- Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA (2007) Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol Psychiatry 62:800–810.
- Wickens JR (2009) Synaptic plasticity in the basal ganglia. Behav Brain Res 199:119–128.
- Wong W, Scott JD (2004) AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5:959–970.
- Worzfeld T, Wettschureck N, Offermanns S (2008) G(12)/G(13)-mediated signalling in mammalian physiology and disease. Trends Pharmacol Sci 29:582–589.
- Yang C, Kazanietz MG (2003) Divergence and complexities in DAG signaling: looking beyond PKC. Trends Pharmacol Sci 24:602–608.
- Yang C, Kazanietz MG (2007) Chimaerins: GAPs that bridge diacylglycerol signalling and the small G-protein Rac. Biochem J 403:1–12.
- Zezula J, Freissmuth M (2008) The A(2A)-adenosine receptor: a GPCR with unique features? Br J Pharmacol 153(Suppl 1):S184–S190.
- Zhou FW, Matta SG, Zhou FM (2008) Constitutively active TRPC3 channels regulate basal ganglia output neurons. J Neurosci 28:473–482.

# Neurotransmitter Regulation of Basal Ganglia Gene Expression

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## I. Introduction

- II. Regulation by Glutamate

   A. α-Amino-3-hydroxy-5-methyl-4-isoxazole-propionate
  - (AMPA)/Kainate Receptors B. *N*-Methyl-D-aspartate (NMDA) Receptors
  - C. Metabotropic Glutamate Receptors
- III. Regulation by Dopamine
  - A. D1 Receptors
  - B. D2 Receptors

- C. "D1–D2 Receptor Synergy" D. Gene Expression in the
- Globus Pallidus
- IV. Regulation by Adenosine
  - A. A1 Receptors
  - B. A2a Receptors
  - C. A1-A2a Receptor Interactions
  - D. Gene Expression in the
  - Globus Pallidus
- V. Regulation by Acetylcholine
  - A. Muscarinic Receptors
  - B. Nicotinic Receptors

- VI. Regulation by Serotonin
  - A.  $5-HT_1$  Receptors
  - B.  $5-HT_2$  Receptors
  - C. 5-HT<sub>3</sub> Receptors
  - D. 5-HT<sub>4</sub> Receptors
  - E. 5-HT<sub>6</sub> Receptors
  - F. 5-HT<sub>7</sub> Receptors
- VII. Regulation by Neuropeptides
  - A. Opioids
  - B. Tachykinins
  - C. Neurotensin **References**

## I. INTRODUCTION

Over the past 15 years, considerable research has examined the regulation of basal ganglia gene expression by neurotransmitters. A significant portion of this work has focused on unraveling interactions between neurochemical systems that regulate basal ganglia circuitry (see Chapter 1), and the results strongly support the concept of broad regulation of basal ganglia gene expression by interactions between multiple neurotransmitter systems, especially those signaling through G-protein-coupled receptors. The goal of this chapter is to review the data implicating numerous neurotransmitter systems in the regulation of gene expression in the basal ganglia, with a major focus on the regulation of gene expression in the striatum. While regulation of gene expression has been examined in other basal ganglia nuclei and is reviewed to some extent in this chapter [e.g., regulation of gene expression in the globus pallidus (GP) and substantia nigra/ventral tegmental area (VTA)], the review is largely focused on the dorsal striatum as this is the area on which the vast majority of studies have been conducted to date and in which the neurotransmitter systems and their interactions have been most extensively examined. The review is not focused on any particular gene or class of genes (e.g., immediate early genes, IEGs), but rather attempts to provide a relatively comprehensive detailing of the available literature on effects of neurotransmitter receptor activation or blockade on gene expression overall. Throughout, we have tried to identify genes by their commonly used abbreviations in the field; however, Table 27.1 delineates the genes covered, their commonly used abbreviations, and their "official symbol" from NCBI Entrez Gene.

The functional significance of the changes in gene expression detailed in this chapter often remains elusive. Increases in IEG or neuropeptide expression may be

Gene name	Common abbreviation	Official symbo
activity regulated, cytoskeleton-associated	arc	Arc
adenosine A2a receptor	A2ar	Adora2a
c-fos/FBJ osteosarcoma oncogene	c-fos	Fos
cholecystokinin	cck	Cck
clathrin heavy chain	chc	Cltc
cyclin L1	ania-6	Ccnl1
dopamine receptor D1A	D1r	Drd1a
dopamine receptor D2	D2r	Drd2
dopamine receptor D3	D3r	Drd3
early growth response 1	zif268; ngfi-a	Egr1
early growth response 2	krox20	egr2
early growth response 4	ngfi-c	egr4
fosB/FBJ osteosarcoma oncogene B	fosB	Fosb
glial fibrillary acidic protein	gfap	Gfap
glutamate decarboxylase 65	gad65	gad2
glutamate decarboxylase 67	gad67	gad1
glutamate receptor, ionotropic, AMPA 1	glur-A	Gria1
homer homolog 1	homer /ania-3	Homer1
glutamate transporter 1/solute carrier family 1	eaac1/eaat3	Slc1a1
growth associated protein 43	Gap43	Gap43
jun oncogene	c-jun	Jun
junB proto-oncogene	junB	Junb
JunD proto-oncogene	junD	jund
kappa opioid receptor/opioid receptor, kappa 1	kor	oprk1
melanocortin 4 receptor	Mc4r	Mc4r
microtubule-associated protein tau	tau	Mapt
mu opioid receptor/opioid receptor, mu 1	mora	oprm1
neuroglycan C/chondroitin sulfate proteoglycan	Ngc	Cspg5

TABLE 27.1 (Continued)				
Gene wname	Common abbreviation	Official symbol		
neurotensin/neuromedin n	nt/n	Nts		
neurotensin receptor 1	Ntsr	Ntsr1		
nuclear receptor subfamily 4, group A, member 1	ngfi-b	Nr4a1		
nuclear receptor subfamily 4, group A, member 3	nor1	Nr4a3		
preprodynorphin/prodynorphin	ppd	Pdyn		
preproenkephalin/proenkephalin 1	ppe	Penk1		
preprotachykinin/tachykinin 1	ppt	Tac1		
prepronociceptin/proorphaninFQ/N	OFQ/N	Pnoc		
serine racemase	Srr	Srr		
serotonin receptor 6	5-HT6	Htr6		
stathmin 1	Stmn1	Stmn1		
synapsin IIa	Syn2	Syn2		
synuclein, alpha	Snca	Snca		
tachykinin receptor 1	Tac1r	Tacr1		
tubulin, alpha 1A	Tuba1a	Tuba1a		
tyrosine hydroxylase	th	Th		

suggestive of changes in the functional activity of particular neurons/nuclei in the basal ganglia or the role of basal ganglia nuclei in particular behaviors or pathologies. However, there clearly are examples of instances in which there is a mismatch between the gene expression changes in and the electrophysiological activity/functional output of the neurons or nuclei in question (e.g., Keefe and Gerfen, 1999; LaHoste et al., 2000; Hanson et al., 2002) or lack of correlation between gene expression in a brain region and a particular behavior being examined, despite there being a general increase in gene expression in that part of the brain (Guzowski et al., 2001). Therefore, while we have commented in places and in reference to ideas put forth in the literature about the possible functional significance of particular changes, such discussion is truly speculative and we therefore have limited its amount in this chapter. Conclusively establishing the functional significance of the

changes in gene expression that have been observed to date in the basal ganglia undoubtedly will be the challenge for the coming years in this field.

## **II. REGULATION BY GLUTAMATE**

Glutamate plays a pivotal role in acute and long-term regulation of central nervous system function. In the basal ganglia, then, glutamate-induced changes in gene expression presumably may be interpreted as reflecting underlying changes in the function of basal ganglia circuitries and/or long-term plastic changes underlying normal basal ganglia-mediated learning and memory functions, as well as pathological changes associated with basal ganglia-related disorders. As the main input nucleus of the basal ganglia, the striatum receives prominent glutamate afferents from the cerebral cortex and thalamus (Kemp and Powell, 1970; Beckstead, 1984; McGeorge and Faull, 1989; Berendse and Groenewegen, 1990; McFarland and Haber, 2000; Smith et al., 2004) and these afferents are known to play a significant role in the regulation of striatal neuron function (see Chapter 1). Activation of corticostriatal afferents through direct electrical stimulation (Fu and Beckstead, 1992; Liste et al., 1995; Sgambato et al., 1997; Sgambato et al., 1999; Miyachi et al., 2005) or chemical disinhibition (Berretta et al., 1997) produces increases in gene expression in striatal neurons. Therefore, it seems likely that gene expression in striatal neurons is regulated, at least under conditions of ongoing cortical or thalamic input, by glutamate.

## A. $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/Kainate Receptors

The contribution of AMPA/kainate receptor activation to basal and drug-induced gene expression has been examined through a number of approaches. Administration of AMPA can induce IEG or neuropeptide gene expression in striatal neurons in vivo or in vitro (Vaccarino et al., 1992; Page and Everitt, 1993; Beckstead, 1995; Keefe and Gerfen, 1999; Rajadhyaksha et al., 1999). However, results from a number of studies suggest that basal gene expression in striatum is not heavily regulated by ongoing excitatory input through AMPA/kainate receptors. That is, intrastriatal or systemic administration of AMPA/kainate receptor antagonists does not alter basal zif268 (egr1) or c-fos mRNA expression (Wang et al., 1994b; Keefe and Gerfen, 1999), although in the work by Wang and colleagues there is some variability and thus some suggestion that basal levels of zif 268 expression may be attenuated by systemic administration of the AMPA/kainate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Wang et al., 1994b; Wang and McGinty, 1996a), perhaps under experimental conditions in which there is greater cortical or thalamic input. Likewise, AMPA/kainate receptor blockade does not decrease basal preprodynorphin (ppd; Pdyn) (Wang et al., 1994b; Wang and McGinty, 1996a), preproenkephalin (ppe; penkl) (Wang and McGinty, 1996a; Périer et al., 2002; Mao and Wang, 2003a) or cholecystokinin (cck; Cck) (Ding and Mocchetti, 1992) mRNA expression in the striatum. Additionally, administration of AMPAkines - positive modulators of AMPA receptor function (Lynch, 2006) – does not enhance, and in fact may possibly decrease, basal c-fos mRNA expression in dorsal striatum (Palmer et al., 1997; Ferguson and Robinson, 2004). Given that AMPA/kainate receptors are major

determinants of striatal neuron electrophysiological activity, these data suggest that the basal expression of multiple genes in the striatum does not likely reflect ongoing electrophysiological activity of striatal neurons or require ongoing glutamate input through AMPA/kainate receptors.

Although basal gene expression in the striatum appears to be relatively insensitive to manipulations of AMPA/kainate receptors, evoked changes in the expression of some genes do appear to be dependent on AMPA receptor activation. For example, systemic administration of DNOX blocks amphetamine (AMPH)- or methamphetamine (METH)-induced increases in ppd mRNA expression in dorsal striatum (Wang et al., 1994b; Wang and McGinty, 1996a), as well as AMPH-induced increases in zif268 and ppe mRNA expression (Wang et al., 1994b; Mao and Wang, 2003a). Likewise, the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) administered locally in striatum blocked c-fos mRNA expression induced by electrical stimulation of the cortex (Sgambato et al., 1999). Interestingly, however, DNQX did not block METH-induced increases in striatal zif268 or ppe mRNA expression (Wang and McGinty, 1996a), and striatal IEG expression induced by chemical disinhibition of the cortex also was not blocked, and in some cases was enhanced, by systemic AMPA/kainate receptor blockade with GYKI 52466 (Berretta et al., 1997). Furthermore, coadministration of an AMPAkine augmented AMPH-induced increases in the numbers of c-fos+/ppe+ (i.e. striatopallidal) neurons (Ferguson and Robinson, 2004), but not the AMPHinduced increase in the numbers of c-fos + /preprotachykininpositive (ppt+; i.e. striatonigral) neurons, in the dorsal striatum (Ferguson and Robinson, 2004) or METH-induced increases in striatal c-fos expression (Hess et al., 2003). These data suggest that induction of some genes in striatum under some circumstances (i.e. certain drugs or electrical activation of cortical afferents) may require ongoing AMPA receptor activation or be secondary to increased glutamate release and activation of AMPA/kainate receptors arising from the manipulation. However, it also is clear that evoked gene expression in striatum is not universally dependent on such activation. In particular the data suggest potential intriguing differences in AMPA receptor mediation of gene expression induced by AMPH vs. METH, the expression of different genes (e.g., peptides vs. IEGs), and expression induced in striatal efferent neuron subpopulations.

An additional unknown at the present time is the extent to which observed effects of AMPA receptor manipulations on striatal gene expression reflect the role of AMPA receptors in the striatum per se, versus system-level changes

arising due to changes in AMPA receptor function outside of the striatum. On the one hand, the data from Sgambato and colleagues (Sgambato et al., 1999) suggest that blockade of striatal AMPA receptors inhibits striatal gene expression induced by activation of corticostriatal afferents. On the other hand, the work from Berretta and colleagues (Berretta et al., 1997) suggest that systemic blockade of AMPA/kainate receptors does not block, and may actually enhance striatal gene expression evoked by activation of corticostriatal afferents. It is likely that delineation of the contribution of striatal vs. extrastriatal AMPA receptors to any evoked changes in striatal gene expression will have to be empirically determined for each condition being examined. In addition, if striatal AMPA receptors do play a role in evoked gene expression, that role may largely be a permissive one. For example, the ability of AMPAkines to enhance amphetamine-induced c-fos mRNA expression in striatopallidal neurons is thought to arise as a consequence of AMPAkine-mediated enhancement of cortical activity based on the topographic relation of the striatal gene expression to cortical areas with increased activity (Ferguson and Robinson, 2004). Robinson and colleagues have furthered argued that the enhanced cortical input then drives striatal gene expression via activation of NMDA receptors. Thus, striatal AMPA/kainate receptor activation may just provide the necessary depolarization to allow for current flow through NMDA receptors and subsequent activation of signaling cascades leading to gene expression changes (Rajadhyaksha et al., 1999; Sgambato et al., 1999; Ferguson and Robinson, 2004) (Fig. 27.1).

#### **B.** *N*-Methyl-D-aspartate (NMDA) Receptors

In addition to AMPA/kainate receptors, glutamate regulates neuronal function through activation of N-methyl-Daspartate (NMDA) receptors. Because of the voltagedependence of activation of these receptors, the more prolonged currents associated with them, and their permeability to calcium, these receptors play a critical role in mediating neuroplastic changes in the brain. Given these properties, it seems reasonable to speculate that NMDA receptors will play a critical role in the regulation of gene expression in the basal ganglia, and several laboratories have hypothesized that glutamate regulation of striatal gene expression is predominately mediated by NMDA receptor activation (Rajadhyaksha et al., 1999; Sgambato et al., 1999; Ferguson and Robinson, 2004). Multiple lines of evidence in fact support a role for NMDA receptors in striatal gene expression. Activation of NMDA receptors with glutamate, NMDA or quinolinic acid increases c-fos, ppe, and cck mRNA expression and Fos protein levels in striatal neuron cultures and in vivo (Berretta et al., 1992; Ding and



**FIGURE 27.1** Schematic depiction of the contribution of AMPA and NMDA subtypes of glutamate receptors to gene expression in striatum induced by D1 and D2 receptor manipulations. (A) D1 receptor-mediated changes in gene expression are dependent on activation of synaptic diheteromeric (NR1/2A) or triheteromeric (NR1/2A/2B) NMDA receptors (solid black lines/arrows). AMPA receptors regulate the gene expression by providing the necessary depolarization for relief of the Mg<sup>2+</sup> blockade of the NMDA receptor channel (wider gray arrows). (B) D2 receptor-mediated changes in gene expression involve extrasynaptic diheteromeric (NR1/2B) NMDA receptors. The extent to which AMPA receptor-mediated depolarization is necessary for activation of the extrasynaptic NMDA receptors (dashed gray line/arrow) and thus for gene expression induced by D2 receptor blockade is currently unknown.

Mocchetti, 1992; Vaccarino et al., 1992; Beckstead, 1995; Das et al., 1997; Hollen et al., 1997; Rajadhyaksha et al., 1999). Likewise, increases in IEG expression (c-*fos*, *fosB*, *junB*, *zif268*, *arc*) induced by electrical or chemical activation of corticostriatal afferents are markedly attenuated by pretreatment of animals with NMDA receptor antagonists (Liste et al., 1995; Berretta et al., 1997; Miyachi et al., 2005). Taken together, these data suggest that activation of NMDA receptors by excitatory afferents to striatum is capable of driving striatal gene expression.

The extent to which NMDA receptor activation is involved in regulating basal gene expression in striatum, however, varies within, as well as across, laboratories. For example, the basal expression of zif268 mRNA expression in the intact striatum was reported to be significantly suppressed by systemic administration of NMDA receptor antagonists such as MK-801 and 3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) (Wang et al., 1994a; Wang and McGinty, 1996a). Likewise, systemic or intrastriatal administration of MK-801 was reported to decrease basal glutamate decarboxylase 65 (gad65; gad2) (Laprade and Soghomonian, 1995), serotonin receptor subtype 6 (Healy and Meador-Woodruff, 1999), Cck (Ding and Mocchetti, 1992), and ppt and ppe (Jolkkonen et al., 1994; Noailles et al., 1996) mRNA expression in the dorsal striatum. However, in other reports the basal expression of zif268, c-fos, and ppe mRNA was not blocked by these or other (CGS 19755, dextrorphan, ifenprodil) NMDA receptor antagonists (Wang and McGinty, 1996a; Keefe and Adams, 1998; Keefe and Ganguly, 1998; Chartoff et al., 1999; Ganguly and Keefe, 2000; Hussain et al., 2001; Ferguson et al., 2003; Mao and Wang, 2003a). Likewise, systemic administration of MK-801 and CPP did not decrease basal ppd (Wang et al., 1994a; Wang and McGinty, 1996a) or glutamate decarboxylase 67 (gad67; gad1) mRNA expression in the striatum (Laprade and Soghomonian, 1995). Further complicating the picture are reports that acute or repeated administration of MK-801 increases ppe, ppt, and ppd mRNA expression in various regions of the striatum (Angulo et al., 1993; Angulo et al., 1995). These differences cannot be simply explained by the NMDA receptor antagonist used nor the dose and duration of NMDA receptor antagonism. Rather, it seems likely that uncontrolled variables, such as differences in the degree of activation of excitatory afferents to the striatum at the time of the experiment or sensitivity of the assays for the gene expression being examined may contribute to the different reports regarding the role of NMDA receptors in the regulation of basal gene expression in the striatum.

Assessment of actual changes in transcriptional activity of the genes of interest in the setting of intrastriatal NMDA receptor blockade or conditional site- or cell-specific knockdown of NMDA receptors will be necessary to more clearly delineate the role of ongoing NMDA receptor activation in basal gene expression in striatum.

While the role of NMDA receptors in the regulation of basal striatal gene expression is unclear at present, the involvement of NMDA receptors in the induction of gene expression in the striatum by a number of drugs/interactions with other neurotransmitter systems is quite evident. For example, pre-treatment of rats with NMDA receptor antagonists generally suppresses (although the degree of attenuation varies somewhat across studies) the induction of IEGs in the striatum by psychostimulants including 3,4methylenedioxy-N-methamphetamine (MDMA) (Dragunow et al., 1991), cocaine (Torres and Rivier, 1993), AMPH (Wang et al., 1994a; Konradi et al., 1996; Ferguson et al., 2003), and METH (Ohno et al., 1994; Wang and McGinty, 1996a). Likewise, NMDA receptor antagonists block the induction of IEGs in the intact striatum by D1 dopamine receptor agonists (Keefe and Ganguly, 1998; Nakazato et al., 1998; Radulovic et al., 2000). In addition, the induction of IEGs in rodent striatum by nicotine (Kiba and Jayaraman, 1994), morphine (Liu et al., 1994; Sharp et al., 1995), amantadine (Tomitaka et al., 1995), caffeine (Svenningsson et al., 1996), and fenfluramine (Guerra et al., 1998) is blocked by systemic pretreatment with NMDA receptor antagonists. Interestingly, the induction of striatal IEGs by all of these agents also is blocked by D1 receptor antagonists, supporting a general model in which release of dopamine by any of a number of agents/conditions and subsequent activation of D1 receptors leads to induction of IEGs in striatal neurons through a mechanism that requires ongoing NMDA receptor activation (Konradi et al., 1996; Guerra et al., 1998; Konradi, 1998) (Fig. 27.1A).

In addition to the necessity for ongoing NMDA receptor activation for D1 receptor-mediated changes in striatal gene expression, NMDA receptor activation also appears to be necessary for changes in gene expression in the dorsal striatum induced by D2 receptor blockade. Thus haloperidol-induced IEG expression is blocked by a number of NMDA receptor antagonists including MK-801 (Dragunow et al., 1990; Ziólkowska and Höllt, 1993; Boegman and Vincent, 1996; de Souza and Meredith, 1999; Hussain et al., 2001; Lee and Rajakumar, 2003; Yanahashi et al., 2004) (but see Boegman and Vincent, 1996), CPP-101,606 and ifenprodil (Yanahashi et al., 2004), and RO 25-6981 (Lee and Rajakumar, 2003). Likewise, increases in striatal IEG expression induced by eticlopride are blocked by MK-801, CGS 19755, and ifenprodil (Keefe and Adams, 1998; Adams and Keefe, 2000). These data in general suggest a requirement for ongoing NMDA receptor activation for the induction of IEG expression in striatal efferent neurons by D2 receptor blockade (Fig. 27.1B), as well as D1 receptor activation. However, two interesting issues arise when considering the studies examining these interactions between dopamine- and NMDA-receptor-mediated effects on striatal gene expression.

The first issue is that although IEG expression induced by dopamine receptor manipulations is often dependent on ongoing NMDA receptor activation, it is not always so. For example, whereas the increase in *zif 268*, c-fos, and ppt mRNA expression induced by direct-acting D1 receptor agonists in the intact striatum is completely blocked by NMDA receptor antagonists (Keefe and Ganguly, 1998; Nakazato et al., 1998; Campbell et al., 2006), intrastriatal or intraventricular infusion of NMDA receptor antagonists fails to block induction of *zif268* or *ppt* mRNA in the dopamine-depleted striatum by the D1 agonist SKF 38393 or L-DOPA and only partially (20-40%) decreases c-fos expression (Keefe and Gerfen, 1996; Adams et al., 2000). Likewise, systemic administration of MK-801 also fails to block *zif268* and *ppt* mRNA and is less efficacious in blocking c-fos expression in the dopamine-depleted striatum (Ganguly and Keefe, 2000; Campbell et al., 2006). Therefore, ongoing NMDA receptor activation is necessary for D1 agonist-mediated increases in gene expression in the intact, but not the dopamine-depleted, striatum. The basis for this shift in the involvement of NMDA receptors is not specifically known, although two potential mechanisms have been suggested. Recruitment of the extracellular regulated 1/2 kinase (ERK)/mitogen-activated protein kinase (MAPK) cascade by D1 receptor activation in the denervated, but not the intact, striatum may lead to gene expression through pathways not dependent on NMDA receptor activation, as suggested by work from Gerfen and colleagues (Gerfen et al., 2002). Alternatively, greater ("supersensitive") activation of adenylyl cyclase/protein kinase A (PKA) by D1 receptor activation in the dopamine-depleted striatum may lead to loss of NMDA receptor involvement in the gene expression, as prior work from Konradi and colleagues has suggested that the degree of PKA activation may influence sensitivity to NMDA receptor blockade (Rajadhyaksha et al., 1998).

Prior work from our laboratory provides additional support for the idea that a greater degree of PKA activation may render striatal gene expression independent of NMDA receptor activation. Specifically, the degree to which an NMDA receptor antagonist blocks eticlopride-induced IEG expression varies regionally in striatum in relation to the magnitude of the induction. The lower level of eticloprideinduced IEG expression in the medial and central aspects of the striatum is more sensitive to NMDA receptor blockade than is the greater degree of IEG induction in the lateral aspects of striatum (Keefe and Adams, 1998). A similar result for haloperidol-induced c-fos expression in the dorsolateral striatum also has been reported (Chartoff et al., 1999) (but see Hussain et al., 2001; Lee and Rajakumar, 2003). We subsequently determined that the sensitivity of eticlopride-induced gene expression in lateral striatum to NMDA receptor blockade was greater when the degree of induction was less, for example, as in response to a lower dose of eticlopride (Adams and Keefe, 2000). Conversely, the sensitivity of eticlopride-induced IEG expression throughout the medial-lateral extent of the striatum to NMDA receptor blockade was reduced by combined treatment of rats with eticlopride and a phosphodiesterase inhibitor (Adams and Keefe, 2000). It is clear from these data that NMDA receptors can be, but are not always, involved in the regulation of striatal gene expression by dopamine receptor manipulations. Thus, as was the case for AMPA receptor involvement, delineation of the involvement of glutamate input through NMDA receptors in the regulation of striatal gene expression will need to be empirically determined for the specific situations being examined. Furthermore, the extent to which the changes in gene expression mediate different functional effects depending on whether they are dependent on glutamate receptor activation or not remains to be determined.

The second significant issue is the potential differential contribution of distinct subtypes of NMDA receptors comprised of different subunits in the gene expression induced by D1 and D2 receptor manipulations and, therefore, the potential differential contribution of synaptic vs. extrasynaptic NMDA receptors in the evoked expression. NMDA receptors consist of two obligatory NR1 subunits and two NR2 subunits, of which there are four different ones (NR2A-D). Most data suggest that in the adult rodent forebrain, including the dorsal striatum, synaptic NMDA receptors are triheteromeric, containing both NR2A and NR2B subunits (Dunah and Standaert, 2003), whereas extrasynaptic NMDA receptors are predominately diheteromeric, containing NR1/2B subunits (Tovar and Westbrook, 1999). Consistent with the biochemical studies of Dunah and Standaert (Dunah and Standaert, 2003), electrophysiological studies in our lab (Smeal et al., 2008) indicate that excitatory corticostriatal and thalamostriatal synaptic transmission through NMDA receptors in the mature striatum is mediated by triheteromeric (i.e. NR1/2A/2B) or diheteromeric NR1/2A/2A NMDA receptors that are insensitive to blockade by ifenprodil, which is selective for diheteromeric NR1/2B subunitcontaining NMDA receptors (Hatton and Paoletti, 2005). Gene expression studies show that systemic administration NR2B-subunit selective antagonists such as ifenprodil do not attenuate gene expression induced by D1 receptor activation (Keefe and Ganguly, 1998; Adams et al., 2000). Interestingly, however, other studies show that the NR2B-selective NMDA receptor antagonists ifenprodil and RO 25-6981 do attenuate IEG expression induced in striatum by D2 receptor blockade (Keefe and Adams, 1998; Lee and Rajakumar, 2003; Yanahashi et al., 2004). These findings suggest an interaction between D1 receptors and glutamate input through synaptic (i.e. NR2A subunit-containing) NMDA receptors and D2 receptors and glutamate signaling through extrasynaptic (i.e. NR2B subunit-containing) NMDA receptors in the regulation of evoked striatal gene expression (Fig. 27.1). Given that synaptic and extrasynaptic NMDA receptors differentially regulate intracellular signaling cascades leading to cell death, cell survival, and plasticity (Papadia and Hardingham, 2007), it is likely that the functional consequences of the changes in striatal gene expression observed under these different conditions will vary, even though the changes themselves (e.g. increases in IEG expression) may seem similar on the surface.

## C. Metabotropic Glutamate Receptors

In addition to activating the ionotropic glutamate receptors reviewed above, glutamate activates metabotropic glutamate receptors (mGluRs) to influence striatal gene expression. Of the three groups of mGluRs – Group 1 (mGluR1, mGluR5), Group 2 (mGluR2, mGluR3), and Group 3 (mGluR4, mGluR6, mGluR7, mGluR8) – the role of Group 1 mGluRs in regulating striatal gene expression has been most extensively examined by the laboratories of Wang and Ossowska. Furthermore, each of these laboratories has published recent reviews of their findings (Ossowska et al., 2007; Mao et al., 2008). Therefore, the role of mGluRs in regulating striatal gene expression will be simply summarized in this paragraph. Briefly, basal IEG or neuropeptide gene expression does not appear to be regulated by Group 1 mGluR receptors, as antagonists of Group I mGluRs do not alter such basal gene expression (Wang and McGinty, 1996c; Mao and Wang, 2001; Ossowska et al., 2002; Ossowska et al., 2003; Parelkar and Wang, 2003; Wardas et al., 2003; Parelkar and Wang, 2004; Konieczny et al., 2007), and basal levels of ppt, ppd, and ppe mRNA expression are not different between mGluR1 knockout mice and wildtype controls (Mao et al., 2001, 2002). However, Group I mGluRs can regulate striatal gene expression, as agonists for Group 1 MGluRs increase striatal IEG and neuropeptide gene expression in vivo and in vitro (Wang, 1998; Wang and McGinty, 1998; Mao and Wang, 2001, 2003c, 2003b; Parelkar and Wang, 2003; Yang et al., 2004; Mao et al., 2005) (although see Kaatz and Albin, 1995; Kearney et al., 1997). Also, antagonists of Group I mGluRs attenuate AMPH-evoked increases in c-fos, ppd, ppt, and ppe (but, interestingly, not zif 268) mRNA expression (Wang and McGinty, 1996c; Mao and Wang, 2002; Parelkar and Wang, 2004) and D2 receptor antagonistinduced increases in ppe mRNA expression (Ossowska et al., 2002; Ossowska et al., 2003; Parelkar and Wang, 2003; Wardas et al., 2003; Konieczny et al., 2007). Mice with deletion of the mGluR1 gene also show decreased AMPH- and D1 receptor agonist-induced ppd (but not ppt or ppe) mRNA expression in striatum (Mao et al., 2001, 2002). Thus, group I mGluRs play a role in evoked, but not basal, gene expression in striatum.

While a contribution of Group I mGluRs to evoked gene expression changes in the striatum is clear, the role of Group II and Group III mGluRs in the regulation of striatal gene expression has been less extensively examined. Group II mGluRs, which are thought to function as inhibitory autoreceptors, may indirectly play a role in the regulation of striatal gene expression through glutamate input, as an antagonist of Group II mGluRs increased c-fos expression in the striatum (Linden et al., 2005). However, agonists of Group II and Group III mGluRs do not consistently affect basal or haloperidol-induced ppe mRNA expression (Wardas et al., 2003; Ossowska et al., 2004; Ossowska et al., 2007). Given that the group II and group III mGluRs function both pre- and post-synaptically in the striatum, it is not surprising that their role in the regulation of striatal gene expression is presently unclear. Full elaboration of the function of these receptors in the regulation of striatal gene expression likely will require conditional, cell-specific knock-out of the different receptors and subsequent examination of basal and evoked gene expression.

## **III. REGULATION BY DOPAMINE**

By the early 1990s, it was already clear that activation of D1 dopamine receptors or blockade of D2 receptors induced gene expression in the striatum (see also Chapter 28), and the results of those studies have been elegantly reviewed by Robertson, Graybiel and others whose laboratories made those seminal contributions (Robertson et al., 1991; Berretta et al., 1993; Graybiel, 1993; Fibiger, 1994). This review will therefore emphasize developments regarding those points since that time.

## A. D1 Receptors

Activation of D1 receptors stimulates gene expression in the striatum. This stimulatory effect is evident both in the effects of acute administration of D1 receptor antagonists, as well as in the effects of dopamine-depleting brain lesions. For example, blockade of D1 receptors with SCH23390 decreases basal zif268 and c-fos (Wang and McGinty, 1996e), as well as *ppt* and *ppd* mRNA expression in the striatum (Wang and McGinty, 1996b, 1997a). Interestingly, SCH23390 also has been reported to decrease ppe mRNA expression in striatum (Wang and McGinty, 1996b, 1997a), perhaps by inducing an increase in dopamine release (Saklayen et al., 2004). Depletion of nigrostriatal dopamine also selectively decreases the basal expression of *ppt* and ppd mRNA (c.f. Gerfen et al., 1990; Nisenbaum et al., 1994; Nisenbaum et al., 1996), as well as the basal expression of zif268 in striatonigral efferent neurons (Gerfen et al., 1995). Therefore, gene expression in striatonigral neurons is stimulated by ongoing dopamine-mediated signaling.

It is interesting that whereas large dopamine depleting brain lesions are needed to see the effects of dopamine depletions on gene expression in striatopallidal neurons, the decrease in ppt mRNA expression in striatonigral neurons is evident across a wider range of dopamine depletions induced by 6-hydroxydopamine (Nisenbaum et al., 1996). Partial (50-60%) dopamine loss induced by a neurotoxic regimen of METH also is associated with decreased basal ppt mRNA expression (Chapman et al., 2001; Johnson-Davis et al., 2002), as well as decreased learning-induced arc mRNA expression in striatonigral neurons (Daberkow et al., 2008). The sensitivity of striatonigral neuron gene expression to partial dopamine loss may reflect greater sensitivity of D1 receptors on those neurons to phasic dopamine transmission. Data suggest that such partial dopamine depletions may selectively impair phasic, rather than tonic, dopamine release (Bergstrom and Garris, 2003). Furthermore, several lines of evidence suggest that gene expression in striatonigral efferent neurons is sensitive to phasic dopamine transmission. First, stimulation of the medial forebrain bundle in a manner that mimics phasic firing of dopamine neurons induces zif268 mRNA expression selectively in striatonigral efferent neurons (Chergui et al., 1997). Second, the decrease in ppt mRNA expression associated with complete loss of striatal dopamine is reversed by grafts of ventral mesencephalon tissue only in striatal regions that are most densely innervated by the graft (Cenci et al., 1993) - presumably where the graft is capable of generating higher dopamine levels consistent with phasic signaling. Finally, intermittent, but not continuous administration of L-DOPA or a D1 receptor agonist reverses the depletion-induced changes in ppt and ppd mRNA expression, but not ppe (Gerfen et al., 1990; Engber et al., 1991), whereas the opposite holds true for the restoration of ppe mRNA levels by a D2 receptor agonist (Gerfen et al., 1990). These data suggest that although basal gene expression in both striatonigral and striatopallidal efferent neurons is under the control of dopamine transmission, gene expression in the two populations of neurons may be differentially regulated by phasic vs. tonic modes of dopamine signaling through D1 and D2 receptors, respectively (Fig. 27.2). It is likely, therefore, that the gene expression regulated in these two populations of neurons by these two modes of dopamine neurotransmission will mediate different aspects of neural plasticity underlying basal ganglia-mediated behaviors.

The effects of psychostimulants and direct-acting D1 receptor agonists also support a stimulatory role of D1 receptors on striatal gene expression. Because a review of the effects of psychostimulants on striatal gene expression (see Chapter 29) is beyond the scope of this chapter and two excellent recent reviews related to this subject are available (Yano and Steiner, 2007; McGinty et al., 2008), we will focus on the effects of direct-acting D1 receptor agonists here in relation to this point. In rats with unilateral depletions of striatal dopamine, administration of direct-acting D1 receptor agonists induces the expression of numerous genes including c-fos, zif268, Nts, ppt, and ania-6 (Ccnl1) (Morelli et al., 1993; Morelli et al., 1994; Keefe and Gerfen, 1996; Morelli et al., 1996; Sandstrom et al., 1996; Steiner and Gerfen, 1996; Berke et al., 1998; van de Witte et al., 1998; Keefe and Gerfen, 1999; Ganguly and Keefe, 2000; Berke et al., 2001; Van De Witte et al., 2002). Likewise, in the intact striatum full-efficacy D1 receptor agonists, such



**FIGURE 27.2** Schematic depiction of the regulation of gene expression in striatopaindal and striatopaindal neurons by tonic and phasic dopamine neurotransmission and D2 and D1 receptors, respectively (top). Dopamine generally inhibits gene expression in striatopallidal neurons via tonic activation of D2 receptors whereas it stimulates gene expression in striatonigral neurons through phasic activation of D1 receptors. Partial dopamine loss ( $\sim$ 50–85%) alters phasic, but not tonic, dopamine neurotransmission and, thus, selectively affects (decreases) gene expression in striatonigral neurons (bottom, left). Near-complete loss of dopamine (>90%), which compromises both tonic and phasic dopamine transmission, leads to disinhibition of gene expression in striatopallidal neurons and decreased gene expression in striatonigral neurons (bottom, right).

as SKF82958 and SKF81297 or the mixed agonist apomorphine, also increase the expression of a number of these genes (Cenci et al., 1992; Wang and McGinty, 1996e; Le Moine et al., 1997; Wang and McGinty, 1997a; Nakazato et al., 1998; Hanson and Keefe, 1999; Mao et al., 2002; Diaz Heijtz and Castellanos, 2006).

Interestingly, although D1 receptor activation in the dopamine-depleted striatum predominately increases gene expression in striatonigral efferent neurons (c.f. Robertson et al., 1992; Gerfen et al., 1995) (see also Chapter 28), the extent to which there is a selective effect of these agonists on gene expression in striatonigral neurons of the intact striatum is less clear. On the one hand, administration of the partial efficacy agonist SKF38393 to intact animals in combination with a D2 receptor agonist induces *zif268* 

mRNA expression in striatonigral neurons (Alonso et al., 1999), similar to its effects in the dopamine-depleted striatum (Gerfen et al., 1995). Also, administration of the mixed D1–D2 receptor agonist apomorphine induces gene expression specifically in striatonigral neurons (Cenci et al., 1992). On the other hand, administration of the full efficacy D1 receptor agonist SKF82958 increases c-*fos* mRNA expression in both striatonigral and striatopallidal efferent neurons (Le Moine et al., 1997), and this same drug also increases *ppe* mRNA expression in the intact striatum (Wang and McGinty, 1997a; Mao et al., 2002). It has been speculated that the ability of SKF82958 to induce gene expression in striatopallidal neurons reflects a D1 receptor-mediated inhibition of dopamine neuron firing and consequent decrease in dopamine release. However, it is also possible that this induction in striatopallidal neurons by SKF82958 reflects the fact that this particular compound, in addition to its efficacy at D1 receptors, also functions as a partialefficacy D2 receptor agonist (Mannoury la Cour et al., 2007) that can suppress dopamine neuron firing via activation of D2 autoreceptors (Ruskin et al., 1998). Inhibition of dopamine release via this mechanism may therefore lead to the induction of gene expression in striatopallidal neurons. Clarification of the extent to which activation of D1 receptors selectively induces gene expression in striatonigral efferent neurons will depend upon studies examining the phenotype of neurons in the intact striatum in which gene expression is induced by full-efficacy D1 receptor agonists, such as SKF81297 and A-77636, that lack agonist activity at D2 receptors. This caveat aside, the data available in the field to date indicate that dopamine, possibly that released phasically, regulates both basal and evoked gene expression in the dorsal striatum via activation of D1 receptors.

## **B. D2 Receptors**

In contrast to the stimulatory effects of D1 receptors on striatal gene expression, three lines of evidence suggest that dopamine, via activation of D2 receptors, plays a major inhibitory role in regulating the basal expression of numerous genes in the striatum. First, both the initial (Dragunow et al., 1990; Miller, 1990) and numerous subsequent studies have established that D2 receptor antagonists, by removing this inhibitory influence, increase gene expression in the dorsal striatum. Such D2 receptor antagonist-mediated increases in gene expression are evident for c-fos (Merchant and Dorsa, 1993; Ziólkowska and Höllt, 1993; Sirinathsinghji et al., 1994; Decker et al., 1995; MacGibbon et al., 1995; Boegman and Vincent, 1996; Keefe and Adams, 1998; de Souza and Meredith, 1999; Steiner and Gerfen, 1999; Adams and Keefe, 2000; Hussain et al., 2002; Pillot et al., 2002; Lee and Rajakumar, 2003; Binder et al., 2004; Ethier et al., 2004; Yanahashi et al., 2004; Robbins et al., 2008), zif268 (MacGibbon et al., 1995; Keefe and Adams, 1998; Steiner and Gerfen, 1999; Adams and Keefe, 2000), neurotensin/neuromedin N (Nts) (Merchant and Dorsa, 1993; Senger et al., 1993; Sirinathsinghji et al., 1994; Decker et al., 1995; Augood et al., 1997; Pillot et al., 2003), fosB (MacGibbon et al., 1995), junB (Sirinathsinghji et al., 1994; MacGibbon et al., 1995), junD (MacGibbon et al., 1995), krox20 (egr2) (MacGibbon et al., 1995; Robbins et al., 2008), ngfi-B (Nr4a1) (Beaudry et al., 2000), homer1/ania-3 (de Bartolomeis et al., 2002; Polese et al., 2002; Tomasetti et al., 2007), ppe (Pillot et al., 2003), and arc (Nakahara et al., 2000; Robbins et al., 2008; Fumagalli et al., 2009). Second, D2 receptor agonists can suppress gene expression (e.g., zif268, c-fos, ngfi-B, junB) in striatum (Gerfen et al., 1995; Svenningsson et al., 1995; Le Moine et al., 1997), suggesting that tonic dopamine signaling does not maximally inhibit basal expression. Both the D2 receptor antagonist- and agonist-induced changes in gene expression occur predominately in striatopallidal efferent neurons (Robertson et al., 1992; Gerfen et al., 1995; Le Moine et al., 1997; Alonso et al., 1999; Bertran-Gonzalez et al., 2008), consistent with the preferential segregation of D2 receptors to striatopallidal neurons (Gerfen et al., 1990; Le Moine et al., 1990; Hersch et al., 1995; Le Moine and Bloch, 1995; Aubert et al., 2000). Finally, depletions of striatal dopamine due to neurotoxin-induced insult or reserpine treatment are associated with increases in the expression of a number of genes in striatopallidal neurons, including c-fos, ppe, and Nts mRNA expression (Angulo et al., 1986; Sivam et al., 1986; Normand et al., 1988; Gerfen et al., 1990; Jian et al., 1993; Cole and Di Figlia, 1994; Pollack and Fink, 1995; Nisenbaum et al., 1996; Hanson and Keefe, 1999; Pollack et al., 1999; Svenningsson et al., 1999). Interestingly, these latter changes appear to require large (i.e.  $\geq \sim 90\%$ ) depletions of striatal dopamine (Nisenbaum et al., 1996; Hanson and Keefe, 1999), similar to those required to produce decreases in tonic, extracellular concentrations of striatal dopamine (Abercrombie et al., 1990; Castañeda et al., 1990; Robinson et al., 1990). Taken together, these data suggest that tonic signaling through the D2 receptor by basal levels of dopamine in the extracellular fluid suppresses, albeit incompletely, the basal expression of numerous genes in striatopallidal efferent neurons. Such expression can be further suppressed by acute administration of D2 receptor agonists, suggesting that the tonic activation of D2 receptors and inhibition of gene expression by basal extracellular levels of dopamine is not maximal. Given the breadth of genes affected by D2 receptor manipulations, the functional consequences of these dopamine-related changes in gene expression are likely to be extensive.

## C. "D1–D2 Receptor Synergy"

In addition to the ability of either D1 or D2 receptors to independently regulate gene expression in the striatum, combined activation of D1 and D2 receptors evokes quantitatively greater and qualitatively different patterns of gene expression in the striatum - a manifestation of so-called "D1–D2 synergy". Paul and colleagues (Paul et al., 1992) provided the first evidence that combined administration of a D1 and a D2 receptor agonist resulted in synergistic striatal Fos protein expression. This quantitative increase in gene expression reflects greater expression in striatonigral efferent neurons (Gerfen et al., 1995; Le Moine et al., 1997; Alonso et al., 1999). Qualitatively, combined administration of D1 and D2 receptor agonists also changes the pattern of gene expression from a more homogeneous to a "patchy" distribution with greater expression in the patch/striosome compartment relative to the matrix, particularly in the rostral striatum (LaHoste et al., 1993; Wirtshafter and Asin, 1994; Le Moine et al., 1997; Alonso et al., 1999; Wirtshafter and Asin, 2001; Capper-Loup et al., 2002). The basis for D1–D2 synergy with respect to the quantitative and qualitative effects on gene expression remains to be determined. One study has suggested a critical role for neurotensin in this process, as the neurotensin antagonist SR48692 blocks the enhanced gene expression in striatonigral neurons (Alonso et al., 1999). Work by LaHoste and colleagues (2000) has further demonstrated that D1–D2 synergy is insensitive to local administration of the voltage-sensitive sodium channel blocker tetrodotoxin (TTX), suggesting that the basis for the synergy is independent of action potential activity. However, the actual mechanisms - whether intracellular or intercellular - by which the synergistic quantitative and qualitative effects of combined D1/D2 agonist administration are realized are currently unknown.

## D. Gene Expression in the Globus Pallidus

In addition to regulating gene expression in striatum, dopamine receptor agonists and antagonists also alter gene expression in the GP. Contrary to what was initially predicted by models of basal ganglia function, both D2 receptor antagonists and agonists increase c-*fos* expression or Fos-like immunoreactivity in the GP (Paul et al., 1992; Robertson et al., 1992; Marshall et al., 1993; Ruskin and Marshall, 1995; Fenu et al., 1997; Le Moine et al., 1997; Ruskin and Marshall, 1997; Svenningsson and Fredholm, 1997; Alonso et al., 1999; Miwa et al., 2001; Hoover and Marshall, 2002; Billings and Marshall, 2003). Activation of D1 receptors alone in dopamine-depleted animals or in combination with D2 receptor activation in intact animals ("D1–D2 synergy") also increases c-*fos* expression in the GP (Marshall et al., 1993; Ruskin and Marshall, 1995; Le

Moine et al., 1997; Ruskin and Marshall, 1997; Hoover and Marshall, 2002). Extensive work by Marshall and colleagues has shown that the populations of pallidal neurons in which c-fos expression is induced varies as a consequence of the dopamine receptor manipulation. Stimulation of D2 receptors alone or blockade of D2 receptors in intact animals increases c-fos exclusively in parvalbumin-negative (PV-neg) neurons in the GP (Ruskin and Marshall, 1997; Billings and Marshall, 2003). Combined D1-D2 agonistinduced c-fos expression in the GP of intact rats also occurs preferentially (although no longer exclusively) in PV-neg neurons (Billings and Marshall, 2003). However, in the case of D2 antagonists, the c-fos induction occurs specifically in pallidostriatal neurons (Ruskin and Marshall, 1997; Miwa et al., 2001) that were subsequently shown to express ppe mRNA (Hoover and Marshall, 2002) (see also Chapter 14). Conversely, the induction in response to combined D1-D2 agonist administration occurs not only in pallidostriatal neurons, but also in pallidosubthalamic, pallidoentopeduncular, and pallidonigral neurons (Ruskin and Marshall, 1997). Further work suggests that the effect of D2 receptor blockade is secondary to blockade of D2 receptors (or D2 family receptors) in the GP per se (Marshall et al., 2001; Billings and Marshall, 2003). Interestingly, intrapallidal infusion of quinpirole, a D2 receptor agonist, also increases Fos protein expression in the GP, and this expression occurs to a greater extent in PV-positive (PV-pos) neurons (Billings and Marshall, 2003).

In addition to Fos expression, dopamine also regulates the expression of gad67 (gad1) mRNA in the GP, which may reflect alterations in the electrophysiological activity of GP neurons. Dopamine loss induced by 6-hydroxydopamine is associated with increased gad67 mRNA expression in the GP (Soghomonian and Chesselet, 1992; Soghomonian et al., 1994; Delfs et al., 1995; Billings and Marshall, 2004). A similar increase in gad67 mRNA also is observed with repeated daily injections of a D2 receptor antagonist (Billings and Marshall, 2004). Under these slightly more chronic conditions, the increase in gad67 mRNA occurs in both PV-neg and PV-pos neurons, although the increase is greater in PV-neg neurons (Billings and Marshall, 2004). The increase in gad67 mRNA expression in both populations of GP neurons is blocked by excitotoxic lesion of the subthalamic nucleus (Delfs et al., 1995; Billings and Marshall, 2004). Lesion of the subthalamic nucleus also blocks haloperidol-induced increase in Fos protein expression in the GP (Miwa et al.,

2001). These data therefore suggest that dopamine can act at multiple sites and via systems-level changes in basal ganglia function to regulate gene expression in different neuronal populations of the GP.

## **IV. REGULATION BY ADENOSINE**

## A. A1 Receptors

A1 adenosine receptors are expressed to a limited degree in a minority of striatal neurons, particularly striatonigral neurons. They also are present presynaptically on glutamate, dopamine, and acetylcholine terminals in the striatum and inhibit neurotransmitter release from those terminals (Mahan et al., 1991; Rivkees et al., 1995; Ferre et al., 1996). Administration of an A1 receptor agonist alone has been reported to increase (Karcz-Kubicha et al., 2003), decrease (Gotoh et al., 2002), or have no effect (Ferré et al., 1999) on Fos-like immunoreactivity in the accumbens or dorsal striatum. Administration of an A1 agonist also was reported to inhibit IEG expression induced by D1 receptor activation (Ferré et al., 1999). Conversely, blockade of A1 receptors increases *zif268* and *ngfi-B* mRNA expression in the lateral aspects of the dorsal striatum (Svenningsson et al., 1997), and also increases Fos immunoreactivity in both striatonigral and striatopallidal efferent neurons of the dorsal striatum (Dassesse et al., 1999). High doses of caffeine, a non-selective antagonist of A1 and A2 receptors, also increase the striatal expression of c-fos, zif268, and arc (Svenningsson et al., 1995; Svenningsson et al., 1996; Bennett and Semba, 1998; Dassesse et al., 1999). These A1 antagonist-induced increases in striatal IEG expression are thought to reflect increased dopamine, glutamate, and acetylcholine release secondary to blockade of inhibitory, presynaptic A1 receptors in striatum (Dassesse et al., 1999). Given the effects of A1 receptor activation on the release of the major neurotransmitters in the striatum, adenosine signaling through these receptors is likely to have complex effects on the regulation of gene expression in the basal ganglia.

## **B.** A2a Receptors

In addition to A1 receptors, adenosine regulates gene expression in striatum via effects on the A2a subtype of adenosine receptor, which is selectively expressed by striatopallidal efferent neurons (Fink et al., 1992; Schiffmann and Vanderhaeghen, 1993). Several recent reviews (Schiffmann et al., 2003; Schiffmann et al., 2007; Ferré et al., 2008) have discussed the effects of A2a receptors on basal ganglia function and interactions of these receptors with A1 and other neurotransmitter receptors (e.g., D2 and mGluR5 glutamate receptors) (see also Chapter 11). Therefore, the role of A2a receptors in the regulation of basal ganglia gene expression will just be summarized briefly here, and the reader is referred to the references above for detailed discussion of receptor interactions. Stimulation of A2a receptors has been reported to have no effect (Morelli et al., 1995; Ferré et al., 2002; Karcz-Kubicha et al., 2003) or to increase (Pinna et al., 1997) Fos expression in the intact striatum. Similarly, in the dopamine-depleted striatum, an A2a agonist has been reported to have no effect on (Pinna et al., 1997) or to increase (Morelli et al., 1995) Fos expression. These discrepancies may reflect differences in the extent to which basal adenosine tone is activating the A2a receptors in different studies or the extent to which endogenous dopamine activation of D2 receptors antagonizes any effect of adenosine.

Blockade of A2a receptors with selective antagonists or low doses of caffeine decreases basal c-fos and zif268 mRNA expression in the striatum (Svenningsson et al., 1995; Le Moine et al., 1997; Svenningsson et al., 1997), and attenuates the increase in c-fos expression induced by reserpine (Pollack and Fink, 1995), haloperidol (Boegman and Vincent, 1996; Chartoff et al., 1999; Pinna et al., 1999; Ward and Dorsa, 1999; Hussain et al., 2002), raclopride (Svenningsson et al., 1995), clozapine (Pinna et al., 1999), or acute administration of 6-hydroxydopamine into the medial forebrain bundle (Svenningsson et al., 1999). Increases in Nts and ppe mRNA expression in striatopallidal neurons induced by D2 receptor antagonists (Chartoff et al., 1999; Ward and Dorsa, 1999), 6-hydroxydopamine-induced dopamine depletion (Richardson et al., 1997), or D2 receptor knock-out (Aoyama et al., 2000) also are attenuated by A2a receptor blockade. Mice lacking the A2a receptor also show decreased basal (Ledent et al., 1997; Dassesse et al., 2001a; Dassesse et al., 2001b) and haloperidol-induced (Chen et al., 2001) ppe mRNA expression. Interestingly, A2a-/- mice also show increased Gad67 and D1 receptor (Drd1a) mRNA (Dassesse et al., 2001a; Dassesse et al., 2001b) and decreased ppt mRNA expression in the striatum (Ledent et al., 1997; Dassesse et al., 2001a; Dassesse et al., 2001b). Taken together, these data suggest that adenosine acting through A2a receptors tonically and positively regulates gene expression in striatopallidal neurons, and also exerts modulatory influences on the regulation of genes in other striatal neurons. Clearly future studies will be needed to clarify the mechanisms by which A2a receptor manipulations alter gene expression in, for example, striatonigral efferent neurons.

## C. A1–A2a Receptor Interactions

As the data cited immediately above suggest, there are complex effects of adenosine on gene expression in the basal ganglia. Not surprisingly, then, there are interactions between A1 and A2a receptors in the regulation of such gene expression. In particular, work from Morales and colleagues (Karcz-Kubicha et al., 2003; Karcz-Kubicha et al., 2006) has shown that co-administration of A1 and A2 agonists, at doses that alone produce little change in c-fos expression, results in synergistic increases in c-fos and ppe mRNA expression in the dorsal and ventral striatum, but no change in ppd mRNA expression. The c-fos expression occurs selectively in striatopallidal neurons (Karcz-Kubicha et al., 2006). These findings suggest that decreased dopamine release due to A1 receptor activation and consequent loss of D2 receptor activation unmasks the ability of A2a receptor activation to drive gene expression in striatopallidal efferent neurons.

## D. Gene Expression in the Globus Pallidus

Adenosine also can regulate gene expression in the GP, most likely through its effects on the A2a receptor localized to striatopallidal neurons and consequent changes in striatopallidal neuron input to the GP. A low dose of caffeine increases c-fos expression in the GP (Fenu et al., 1997; Svenningsson and Fredholm, 1997; Bennett and Semba, 1998). This increase presumably is due to blockade of A2a receptors and thus decreased striatopallidal neuron function, because this induction is mimicked by and is additive or synergistic with that induced by D2 receptor activation (Fenu et al., 1997; Svenningsson and Fredholm, 1997). A2a receptor blockade also potentiates c-fos mRNA or protein expression induced in the GP by L-DOPA or a D1 receptor agonist (Fenu et al., 1997; Le Moine et al., 1997). An A2a agonist also at least partially reverses c-fos expression in the GP induced by the D2 receptor agonist quinpirole (Morelli et al., 1995). These data suggest that manipulations of adenosine alter gene expression in the GP by affecting striatopallidal neuron function and interactions between striatopallidal and striatonigral neurons.

## **V. REGULATION BY ACETYLCHOLINE**

## A. Muscarinic Receptors

A number of studies have examined the role of cholinergic signaling via muscarinic receptors in the regulation of striatal gene expression. In particular, work by McGinty and colleagues has led to a model in which the acetylcholine release from cholinergic interneurons serves to enhance gene expression in striatopallidal neurons and inhibit gene expression in striatonigral neurons (Wang and McGinty, 1997a). This model is based on the observations that stimulation of muscarinic receptors increases Fos protein (Bernard et al., 1993) as well as ppe mRNA (Wang and McGinty, 1996d, 1997b) expression in striatopallidal neurons. Furthermore, although basal levels of ppe expression are not altered by acute administration of a muscarinic antagonist (Wang and McGinty, 1996d, 1997b), increases in c-fos or ppe expression induced in striatum by D2 receptor blockade or reserpine pre-treatment are attenuated by the muscarinic antagonist scopolamine (Guo et al., 1992; Pollack and Wooten, 1992; Wang and McGinty, 1997a; Hussain et al., 2002; Pollack and Angerer, 2005). Conversely, stimulation of muscarinic receptors suppresses D1 receptor-induced c-fos, ppd, and ppt mRNA expression in striatum (Chou et al., 1992; Wang and McGinty, 1996d, 1997b), whereas blockade of muscarinic receptors enhances basal ppd and ppt mRNA expression (Wang and McGinty, 1996d, 1997a, 1997b), as well as D1 receptormediated increases in c-fos, zif268, ppd, and ppt mRNA expression (Chou et al., 1992; Bernard et al., 1993; Morelli et al., 1993; Morelli et al., 1994; Sandstrom et al., 1996; Wang and McGinty, 1996d, 1996e, 1997a, 1997b; Wirtshafter and Asin, 2001).

This model by McGinty and colleagues (Wang and McGinty, 1997a) also puts forth the idea that the cholinergic interneurons may mediate some aspects of D1–D2 synergy in the regulation of gene expression, particularly the ability of D1 receptor activation to increase *ppe* mRNA expression and the apparent involvement of D2 receptor activation in SKF 82958-induced *ppd* and *ppt* mRNA expression (Wang and McGinty, 1997a). However, the combination of a muscarinic receptor antagonist with a D1 receptor agonist does not induce a "patchy" pattern of enhanced gene expression, whereas combined administration of D1 and D2 receptor agonists does (Wirtshafter and Asin, 2001). Also, the work by LaHoste and colleagues cited above suggests that D1–D2 synergy is insensitive to blockade of action potential activity by TTX, which would render interneuronal interactions through the cholinergic interneuron inoperative. Therefore, further elaboration of the mechanisms underlying potentiation of D1 receptor-mediated effects and suppression of gene expression in the matrix compartment by D2 agonists in the context of D1–D2 synergy clearly is necessary. This caveat notwithstanding, the results of studies from multiple laboratories reviewed above suggest a critical role for cholinergic signaling through muscarinic receptors in both basal and evoked gene expression in the striatum.

## **B.** Nicotinic Receptors

In addition to acting through metabotropic muscarinic receptors, acetylcholine can activate ionotropic nicotinic receptors. A number of studies examining the effects of acute administration of nicotine or nicotine agonists suggest that acetylcholine can regulate basal ganglia gene expression via nicotinic receptors. For example, acute administration of nicotine increases ppe mRNA expression in the striatum, and this effect is blocked by the non-selective nicotine receptor antagonist mecamylamine (Dhatt et al., 1995; Houdi et al., 1998). Additionally, acute administration of nicotine increases ppd mRNA and dynorphin protein expression (Isola et al., 2009) and Fos expression in the striatum (Kiba and Jayaraman, 1994). Either a D1 or a D2 receptor antagonist or an NMDA receptor antagonist blocks the increases in dynorphin protein levels induced by acute administration of nicotine (Isola et al., 2009), whereas only a D1 or NMDA receptor antagonist blocks the increase in Fos (Kiba and Jayaraman, 1994). It should be noted, however, that other studies have failed to find an effect of acute nicotine on striatal ppd or ppe mRNA expression (Höllt and Horn, 1992; Le Foll et al., 2003) or have reported a decrease in *ppd* and *ppt* expression in the nucleus accumbens shell after an acute administration of nicotine (Le Foll et al., 2003). Finally, nicotine also increases c-fos mRNA in the nucleus accumbens shell, and this increase in c-fos mRNA in the accumbens is blocked by the alpha-7 subunit-selective antagonist methyllycaconitine, but not the alpha4/beta2\* selective antagonist dihydro-beta-erythroidine (DHBE) (Schilström et al., 2003). Selective agonists of alpha-7-containing nicotinic receptors mimic these effects of nicotine, increasing c-fos and arc mRNA expression in the nucleus accumbens shell (Hansen et al., 2007; Thomsen et al., 2008), as well as the accumbens core and the dorsomedial striatum, although these latter effects are more apparent in juvenile, as opposed to adult, rats (Thomsen et al., 2008). Overall, these data suggest that cholinergic input through nicotinic receptors can alter gene expression in the striatum, possibly through increased dopamine release and in concert with NMDA receptor activation. Clearly, additional work is needed to fully elaborate the impact of nicotine on dorsal vs. ventral striatal gene expression and on other genes (e.g., *Nts*) in the basal ganglia, and the role of different nicotinic receptor subtypes in the regulation of basal ganglia gene expression.

Nicotine also alters gene expression in the ventral tegmental area (VTA). For example, acute administration of nicotine increases Fos protein in the VTA (Pang et al., 1993), consistent with the ability of nicotine to increase dopamine neuron firing, [e.g. (Pidoplichko et al., 1997)]. Also, infusion of nicotine into the VTA increases the expression of tyrosine hydroxylase (*th*) and the GluR1 subunit of the AMPA subtype of glutamate receptor (*glur-A; Gria1*) in the VTA, although these changes occur 24 hrs after the administration of nicotine (Ferrari et al., 2002). It is therefore clear that acetylcholine activation of nicotinic receptors in multiple locations likely plays a role in the regulation of basal ganglia gene expression.

## **VI. REGULATION BY SEROTONIN**

The serotonin (5-HT) system sends extensive projections to many regions of the brain, including the basal ganglia. In particular, the striatum receives dense serotonergic innervation from the raphe nucleus, and several 5-HT receptor subtypes are found in the striatum, including 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>4</sub> and 5-HT<sub>6</sub> subtypes (Sijbesma et al., 1991; Jackson and Westlind-Danielsson, 1994; Pompeiano et al., 1994; Villaró et al., 2005). The majority of studies involving the effects of 5-HT on gene expression in the basal ganglia have focused on the striatum and we will focus on this region of the basal ganglia in this section. Other 5-HT receptor subtypes that are not found in the striatum can also influence striatal gene expression. For example, 5-HT<sub>1A</sub> receptors are expressed on the serotonergic neurons of the raphe nucleus, where they serve as autoreceptors, 5-HT<sub>3</sub> receptors are found in areas that contain dense dopaminergic inputs, and 5-HT7 receptors are found in the thalamus (Sijbesma et al., 1991; Jackson and Westlind-Danielsson, 1994; Bourson et al., 1998; Kinsey et al., 2001). Thus, activation of 5-HT receptors can alter gene expression in the neurons of the striatum via direct activation of striatal 5-HT receptors or by modifying 5-HT,

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dopamine or glutamate input to the striatum. Serotonergic regulation of gene expression in the striatum is thought to contribute to a number of conditions, including obsessivecompulsive disorder, behavioral effects of psychostimulants, therapeutic effects of antidepressants and psychostimulant withdrawal.

## A. 5-HT<sub>1</sub> Receptors

The majority of studies examining the role of 5-HT<sub>1</sub> receptors in the regulation of basal ganglia gene expression have focused on 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors. Treatment with the 5-HT<sub>1A</sub> agonist 8-OH-DPAT [8-hydroxy-2-(di-npropylamino)tetralin] decreases the expression of the transcription factor ngfi-B in the striatum, but has no effect on the striatal expression of c-fos, ngfi-c (Egr4) or ngfi-b (Tilakaratne and Friedman, 1996; Gervais et al., 1999). These data indicate that 5-HT<sub>1A</sub> receptors may preferentially modulate the expression of nerve growth factor in striatum; however, the functional significance of this finding is unclear. On the other hand, repeated stimulation of 5-HT<sub>1A</sub> receptors with 8-OH-DPAT decreases ppe and *ppt* mRNA expression, indicating that maintenance of serotonergic tone is critical for maintaining neuropeptide expression in the striatum (Walker et al., 1996). These receptors may also play a role antidepressant-induced gene expression, as pretreatment with the 5-HT<sub>1A</sub> antagonist WAY 100635 potentiates arc and c-fos expression induced by a selective serotonin reuptake inhibitor in striatum (Castro et al., 2003). Thus, 5- $HT_{1A}$  receptor activation may be an important adjunct in the therapeutic effects of antidepressants.

5-HT<sub>1B</sub> receptors also regulate gene expression in the basal ganglia, as treatment with the 5-HT<sub>1B</sub> agonist RU24969 increases c-*fos* mRNA expression in the striatum (Lucas et al., 1997). These receptors also modulate psychostimulant-induced gene expression in the striatum, since mice lacking 5-HT<sub>1B</sub> receptors display reduced cocaineevoked c-*fos* mRNA expression (Lucas et al., 1997). These data suggest that 5-HT<sub>1B</sub> receptors may play a role in psychostimulant-induced IEG expression, and could contribute to changes in striatal plasticity that underlie addiction.

Alternatively,  $5\text{-HT}_{1D}$  receptors appear to play little, if any, role in cocaine-induced gene expression in the basal ganglia (Lucas et al., 1997), and it is not known whether  $5\text{-HT}_{1D}$  agonists, such as sumatriptan (which is a popular anti-migraine medication), alter basal ganglia gene expression.

## **B.** 5-HT<sub>2</sub> Receptors

Studies examining 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor activation and basal ganglia gene expression have been complicated by the fact that most of the available 5-HT<sub>2</sub> agonists are non-selective. Nevertheless, a number of studies have investigated the effects of 5-HT<sub>2A/2C</sub> receptor activation on IEG and neuropeptide gene expression in the basal ganglia. In the striatum, activation of 5-HT<sub>2A/2C</sub> receptors differentially regulates IEG expression. For example, treatment with the 5-TH<sub>2A/2C</sub> agonist 1-(2,5-dimethoxy-4iodophenyl)-propan-2-amine (DOI) increases ngfi-b mRNA expression in the medial striatum, weakly increases striatal arc mRNA expression, and has no effect on striatal c-fos or ngfi-c expression (Tilakaratne and Friedman, 1996; Gervais et al., 1999; Pei et al., 2000). The 5-HT<sub>2A/2C</sub> receptor also regulates striatal neuropeptide gene expression in a regionand dose-specific manner. Acute treatment with DOI increases ppe and ppt mRNA expression only in the dorsocaudal and dorsolateral anterior striatum (Walker et al., 1996). Alternatively, repeated DOI treatment decreases ppt mRNA expression in the dorsocaudal striatum (Gresch and Walker, 1999b). These studies indicate that  $5-HT_{2A/2C}$ receptor-mediated regulation of ppe and ppt expression is mainly restricted to caudal regions of striatum, which is in line with the observation that the caudal striatum contains a higher density of 5-HT<sub>2</sub> receptors (Pompeiano et al., 1994). Interestingly, however, chronic DOI treatment decreases ppd mRNA expression uniformly in the striatum (Mijnster et al., 1998a). Furthermore ppd, but not ppt, mRNA expression is tonically regulated by 5-HT<sub>2</sub> receptor-mediated inhibition, as treatment with the 5-HT<sub>2A/2C</sub> antagonist ritanserin increases ppd, but not ppt, mRNA expression in the striatum (Mijnster et al., 1998b; Gresch and Walker, 1999a). Thus, the regulation of different genes in striatum by 5-HT<sub>2</sub> receptors may vary as a function of tonic vs. phasic serotonin signaling and regional differences in receptor expression.

## C. 5-HT<sub>3</sub> Receptors

The 5-HT<sub>3</sub> receptor is located in brain regions that contain a dense concentration of DA, and these receptors are thought to be involved in interactions between the 5-HT and dopamine systems. Indeed, studies involving drugs of abuse (which elicit an increase in dopamine transmission) point to a role for 5-HT<sub>3</sub> receptors in cocaine-induced gene expression in the striatum, although it is not clear if 5-HT<sub>3</sub>-induced alterations in dopamine transmission are responsible for the
observed effects. Blockade of 5-HT<sub>3</sub> receptors with tropisetron attenuates cocaine-induced c-*fos* and *zif/268* expression in the striatum (Humblot et al., 1998). Activation of 5-HT<sub>3</sub> receptors induces significant c-*fos* and *zif/268* expression in striatum, but to a lesser degree than what was observed with cocaine treatment (Humblot et al., 1998). It is not clear, however, whether 5-HT<sub>3</sub>-induced increases in IEG expression in the striatum are due to alterations in dopamine transmission or are a direct result of 5-HT<sub>3</sub> receptor activation on postsynaptic neurons. Nevertheless, these data indicate that the 5-HT system can influence striatal IEG expression induced by drugs of abuse, via activation of 5-HT<sub>3</sub> receptors. Specifically, 5-HT<sub>3</sub> receptors may be involved in the long-term changes in neuronal function that are induced by drugs of abuse.

#### D. 5-HT<sub>4</sub> Receptors

While 5-HT<sub>4</sub> receptors are expressed in the basal ganglia (Villaró et al., 2005), their involvement in the regulation of basal ganglia gene expression has not heretofore been reported.

### E. 5-HT<sub>6</sub> Receptors

Despite the fact that 5-HT<sub>6</sub> receptor mRNA and protein is found in the striatum, little is known about the regulation of striatal gene expression by these receptors. However, recent data indicate that treatment with the 5-HT<sub>6</sub> agonist EMDT (2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine) induces significant c-*fos* expression in the striatum (Svenningson et al., 2007). Interestingly, activation of 5-HT<sub>6</sub> receptors also appears to be necessary for the behavioral and biochemical effects of selective serotonin reuptake inhibitors, and 5-HT<sub>6</sub> agonists themselves exhibit antidepressant effects (Svenningson et al., 2007). These data suggest that 5-HT<sub>6</sub> receptors play a role in emotional processing and may serve as a target for new antidepressant therapies.

#### F. 5-HT<sub>7</sub> Receptors

5-HT<sub>7</sub> receptors are found in the thalamus (Kinsey et al., 2001), but it is not known whether their activation alters basal ganglia gene expression.

## **VII. REGULATION BY NEUROPEPTIDES**

Neuropeptides act as gain control devices in the basal ganglia, adjusting the activity of classical neurotransmitter systems, particularly the dopamine system (Alonso et al., 1999). Thus, neuropeptides can alter gene expression in the basal ganglia via their association with classical neurotransmitter systems, and can also exert direct effects on gene expression. Within the basal ganglia and particularly in the striatum, neuropeptide-mediated alterations in gene expression may underlie the pathophysiology of certain neurological or psychiatric disorders, may contribute to or oppose the addictive properties of drugs of abuse and could underlie the therapeutic properties of medications used to treat psychiatric conditions, such as schizophrenia. This section will focus on the regulation of gene expression by neuropeptides in the striatum, as the majority of studies involving the effects of neuropeptides on basal ganglia function have focused on the striatum, due to the relatively high levels of neuropeptides and neuropeptide receptors found in this region.

### A. Opioids

#### 1. Mu Receptors

A great deal is known about the regulation of gene expression in the striatum by mu opioid receptors since these receptors are densely expressed in the striatum and due to the large number of studies that have involved the mu receptor agonist, morphine. However, it is important to note that unlike most other mu receptor agonists, morphine does not induce internalization of mu receptors and may not induce mu receptor down-regulation in vivo (Arden et al., 1995; Burford et al., 1998). Morphine has also been described as a partial agonist at mu receptors (Kovoor et al., 1998). Thus, it is possible that the ability of morphine to alter gene expression in the striatum is related to its unique effects on mu receptor regulation and/or its partial agonist activity, and may not fully reflect or represent the effects of mu receptor activation on striatal gene expression per se. Nevertheless, these studies are important, as morphine is a widely used analgesic and its effects on striatal gene expression could be related to its addictive properties.

Several studies have examined the effects of acute and repeated morphine treatment on IEG expression in the striatum, often with conflicting results. Acute morphine treatment increases *arc* (Ammon et al., 2003; Ziółkowska et al., 2005) and *junB* (Liu et al., 1994) expression in the striatum, whereas *fosB*, *c-jun* (*jun*) and *junD* show no change (Liu et al., 1994). On the other hand, c-*fos* has been shown to increase (Liu et al., 1994; Yukhananov and Handa, 1997) or show little to no change after acute morphine treatment (Curran et al., 1996; Erdtmann-Vourliotis et al., 1998).

Repeated treatment (i.e., daily injections) with morphine has been shown to either increase (Curran et al., 1996; Erdtmann-Vourliotis et al., 1998; Marie-Claire et al., 2004) or not alter (Marie-Claire et al., 2004; Ziółkowska et al., 2005) c-fos and arc expression in the striatum. Continuous stimulation of mu receptors via implantation of a subcutaneous morphine pellet results in only a weak increase in c-fos mRNA expression in the striatum that is most likely related to the constant and low rate of morphine released by subcutaneous morphine implantation, suggesting that tolerance to the effects of morphine on IEG expression may develop with prolonged and continuous treatment (Georges et al., 2000). It is possible that the differences observed with these studies are the result of differences in the dosing regimen or species differences. Nevertheless, despite the disparities observed with these studies, it is clear that morphine can induce changes in IEG expression at potentially any stage of treatment. Furthermore, the changes in IEG expression induced by morphine may contribute to plastic alterations in striatal neurons that contribute to opioid addiction. It is also important to note that while morphineinduced IEG expression in the striatum is dependent upon D1 and NMDA receptor activation, mu receptor activation can induce IEG expression, suggesting that direct stimulation of these receptors may also contribute to the striatal plasticity and addiction.

Similar to what is has been observed for IEG expression, the effects of morphine on neuropeptide expression have proven to also be disparate. Acute stimulation of mu receptors with morphine does not alter ppd or ppe expression in striatum, but does increase expression of proorphaninFQ/N (Pnoc) (Przewłocka et al., 1996; Tjon et al., 1997; Turchan et al., 1997; Yukhananov and Handa, 1997; Romualdi et al., 2002). Chronic treatment with morphine decreases *Pnoc* expression, but increases expression of *ppe* and *ppd*, although decreases in *ppd* and *ppe* expression also have been reported with chronic morphine treatment (Przewłocka et al., 1996; Tjon et al., 1997; Turchan et al., 1997; Yukhananov and Handa, 1997; Romualdi et al., 2002). Implantation of a subcutaneous morphine pellet has been shown to decrease ppd, ppe and ppt expression in the striatum, although others report seeing a lack of effect on ppe (Gudehithlu and Bhargava, 1995; Georges et al., 1999). These morphine-induced alterations in striatal peptide expression may be related to morphine's unique regulation of mu receptors, but may also represent a complex feedback system that serves to regulate the activity of striatal endogenous opioid peptides in response to exogenous

opioid treatment. It is unclear, however, why differences in opioid peptide gene expression exist in chronic treatment vs. subcutaneous pellet implantation. It is possible that chronic administration leads to increased activity of other neurotransmitter systems (e.g., the dopamine system) and increases in opioid peptide expression may serve to dampen excessive activation of these systems. On the other hand, the constant and low rate of morphine released by subcutaneous pellets could result in a continuous level of mu receptor activation, which may signal the system to shut down endogenous production of opioid peptides. On the other hand, chronic mu receptor blockade has been shown to increase ppd and ppe expression in the striatum (Romualdi et al., 1995; Mavridis and Besson, 1999) - an effect that has also been observed in response to chronic morphine treatment - suggesting that decreased activation of mu receptors may serve as a signal to the endogenous opioid peptide system to increase synthesis.

Acute and chronic treatment with morphine does not alter the expression of mu receptors in the striatum (Castelli et al., 1997; Zhou et al., 2006), although downregulation of both kappa (oprk1) and mu (oprm1) receptors in the striatum has been reported (Teodorov et al., 2006). D1 receptor (Drd1a) expression does not appear to be regulated by mu receptors, whereas chronic morphine treatment increases D3 receptor (Drd3) expression (Georges et al., 1999; Spangler et al., 2003). The role of mu receptor activation in the regulation of D2 receptor (Drd2) expression is equivocal, as decreases in D2 expression have been observed with chronic morphine treatment, whereas others have reported a lack of effect of morphine treatment on D2 receptor expression (Turchan et al., 1997; Georges et al., 1999; Spangler et al., 2003). Chronic morphine treatment also down-regulates melanocortin-4 receptor (Mc4r) expression in the striatum (Alvaro et al., 1996). The presence of endogenous opioid modulation compounds (e.g., Tyr-W-MIF) might interfere with the ability of morphine to alter the expression of mu receptors (Harrison et al., 1998). However, it is not clear what mechanisms are responsible for the ability of morphine to alter the expression of D2, D3, kappa opioid and melanocortin receptors. It is possible that these effects are due to overall alterations in neurotransmitter release induced by morphine treatment. Clearly additional studies are needed to resolve this issue.

Mu receptor activation also regulates the expression of a number of other genes in the striatum. Chronic morphine treatment increases the expression of *ngfi-b* (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997;

Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008), nor1 (Nr4a3) (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997; Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008),  $G_{\alpha s}$  (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997; Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008), serine racemase (srr) (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997; Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008), neuroglycan C (Cspg5) (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997; Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008), glial fibrillary acidic protein (Gfap) (Mackler and Eberwine, 1994; Ozawa et al., 2001; Marie-Claire et al., 2004; Ziolkowska et al., 2005), and synapsin IIa (Syn2) (Basheer and Tempel, 1993; Matus-Leibovitch et al., 1997; Werme et al., 2000; Ishikawa et al., 2006; Yoshikawa et al., 2008), but decreases the expression of glutamate transporter-1 (Slc1a2), several cytoskeletal-associated proteins including growth associated protein 43 (Gap43), clathrin heavy chain (Cltc), alpha-tubulin (Tuba1a), Tau (Mapt), stathmin (*Stmn1*), alpha-synuclein (*Snca*), and the  $K_v1$  and  $K_v2$ voltage-gated K<sup>+</sup> channels (Mackler and Eberwine, 1994; Ozawa et al., 2001; Marie-Claire et al., 2004; Ziolkowska et al., 2005). These diverse findings indicate that morphine can alter the expression of a wide range of genes in the striatum that could contribute to the deleterious and addictive properties of this drug.

Mu receptors also modulate psychostimulant-induced gene expression, as pretreatment with a mu receptor antagonist attenuates AMPH and METH-induced neuropeptide and IEG expression in the striatum (Gonzalez-Nicolini et al., 2003; Horner and Keefe, 2006). It has been suggested that striatal mu receptor blockade might reduce psychostimulant-induced dopamine release into the striatum (but see Schad et al., 1996), which may be responsible for the ability of mu receptor blockade to attenuate psychostimulant-induced gene expression (Gonzalez-Nicolini et al., 2003; Horner and Keefe, 2006). Alternatively, blockade of striatal mu receptors may actually result in a decrease in the transcriptional activity of striatal neurons, as mu receptor activation is linked to increased activation of MAP kinase and cyclase-response element (CRE)-mediated transcription (Shoda et al., 2001; Bilecki et al., 2004). Several immediate early genes and peptides have CRE sites present on their promoters and their expression may also be induced by activation of MAP kinase (Konradi et al., 1994; Cole et al., 1995; Curran et al., 1996). Thus, it is possible that mu receptor blockade decreases MAP kinase activity and CRE-mediated transcription, thereby reducing the ability of psychostimulants to induce gene expression in the striatum.

#### 2. Delta Receptors

The majority of studies examining delta opioid receptor regulation of basal ganglia gene expression have focused on the ability of delta receptors to modulate gene expression induced by D2 receptor manipulations. For example, enkephalin and delta receptors regulate the response of striatopallidal neurons to D2 receptor blockade, as stimulation of striatal delta receptors with enkephalin suppresses D2 receptor antagonist-induced IEG expression (Steiner and Gerfen, 1999). These data suggest that when dopamine transmission is compromised (e.g., Parkinson's disease) or when there is chronic D2 receptor blockade (e.g., antipsychotic treatment), the enkephalin system may act in a compensatory manner to counteract the effects of disinhibition of the indirect pathway and normalize basal ganglia function (Steiner and Gerfen, 1998). That is, the alterations in motor function that are observed with inadequate D2 receptor activation may recover to a certain degree when there is up-regulation of endogenous enkephalin and activation delta receptors (Steiner and Gerfen, 1998).

Delta receptor blockade also oppositely alters psychostimulant-induced gene expression in the striatum. In this case, blockade of delta receptors with DADLE ([D-Ala2-D-Leu-5]Enkephalin) attenuated METH-induced c-fos expression in the striatum, whereas pretreatment with the delta antagonist TIPP $\Psi$  blocked AMPH-induced *ppt*, *ppd* and ppe mRNA expression in the striatum (Hayashi et al., 1999; Gonzalez-Nicolini et al., 2003) It is thought that delta receptor blockade inhibits AMPH-induced dopamine release in the striatum by preventing endogenous delta recptor ligands from hyperpolarizing GABAergic interneurons in the substantia nigra pars compacta, leaving the inhibitory influence of these interneurons on the dopaminergic neurons in this region intact (Schad et al., 1996). Delta receptor blockade also attenuates AMPH-induced glutamate release in the striatum, which may involve increased acetylcholine release from striatal interneurons (Mulder et al., 1984; Rawls and McGinty, 2000). Thus, the ability of delta receptor antagonists to reduce psychostimulant-induced gene expression may lie in their ability to attenuate amphetamine-induced increases in extracellular dopamine and glutamate in the striatum. These data point to the delta receptor as a potential target for the treatment of psychostimulant abuse. The expression of A2a receptors (*adora2a*), which are found on neurons of the striatopallidal pathway, is also regulated by delta receptors, as treatment with the delta agonist SNC80 produces an increase in striatal *adora2a* mRNA expression (Halimi et al., 2000), although the mechanism underlying this effect, as well as its functional significance are not clear.

#### 3. Kappa Receptors

The dynorphin system regulates dopamine transmission in the basal ganglia and regulates the responsiveness of direct pathway neurons in the striatum (Steiner and Gerfen, 1998; see Chapter 29). In addition, up-regulation of the dynorphin system in the basal ganglia during psychostimulant withdrawal may contribute to relapse into drug-taking behavior via induction of a depressive-like state (Steiner and Gerfen, 1998). As such, several studies have examined the effects of kappa receptor activation on gene expression in the basal ganglia, as well as its role in dopamine-regulated gene expression in the striatum. Dynorphin regulates its own expression, as repeated stimulation of kappa receptors with the kappa receptor-specific agonist U-69593 decreases ppd mRNA expression in striatum (Collins et al., 2002). Interestingly, the levels of *ppd* mRNA in the striatum are increased 22 days after termination of repeated treatment with U-69593, suggesting that kappa receptor regulation of ppd expression is a dynamic process (Collins et al., 2002). Dynorphin signaling through kappa receptors also regulates the response of striatonigral neurons to D1 receptor stimulation. Treatment with a kappa receptor agonist attenuates D1 receptor-induced IEG expression in striatum (Steiner and Gerfen, 1995, 1996), as well as cocaine-induced IEG expression (Steiner and Gerfen, 1995) and AMPH-induced ppd, ppt and ppe expression in striatum (Tzaferis and McGinty, 2001). Dynorphin/kappa receptor activation is thought to suppress striatonigral neuron responsiveness by activating inhibitory presynaptic kappa receptors on nigrostriatal dopamine nerve terminals, because kappa agonist treatment no longer attenuates D1 receptor-mediated IEG expression in striatum when nigrostriatal dopamine neurons are ablated (Steiner and Gerfen, 1996). These data suggest that the dynorphin system may act as a homeostatic mechanism to counteract the effects of over-stimulation of the dopamine system, like that observed during psychostimulant treatment, making the kappa receptor an attractive therapeutic target for treatment of psychostimulant addiction.

## **B.** Tachykinins

Of the tachykinins, substance P has garnered the most attention due to its high concentration in the neurons of the direct pathway, its ability to modulate dopamine transmission, and the ability of dopamine to regulate *ppt* expression. It has been suggested that the neurokinin-1 (NK-1) receptor may be a therapeutic target in situations in which dopamine transmission in the basal ganglia is altered. As such, the effects of NK-1 receptor manipulation on gene expression in the basal ganglia, and in particular the striatum has been investigated by a number of groups, and to this point has involved the regulation of the substance P system by NK-1 receptors. Chronic blockade of NK-1 receptors with CP-122,721 increases ppt and NK-1 receptor (Tacr1) mRNA expression in the striatum, indicating that NK-1 receptors regulate their own expression, as well as the expression of ppt mRNA, and may serve to re-set target cell sensitivity to substance P (McCarson et al., 1998). Administration of the NK-1 receptor agonist  $[Sar^9Met(O_2)^{11}]SP$  or the NK-3 receptor agonist [MePhe7]NKB directly into the substantia nigra alters dopamine metabolism, but does not decrease ppt mRNA expression in the striatum, indicating that a threshold may exist, in terms of the degree of alteration in the dopamine system that is needed to induce a change in the tachykinin system (Humpel and Saria, 1993). The role of NK-1 activation in dopamine-mediated events (e.g., psychostimulant-induced gene expression in the striatum) has also been investigated. Acute pretreatment with the NK-1-specific antagonist LY306740 attenuates AMPH-induced ppd, ppt, and ppe mRNA expression in the striatum (Gonzalez-Nicolini and McGinty, 2002). Thus, AMPH-induced release of substance P may be an important modulator of psychostimulant-induced gene expression in the striatum and could possibly contribute to psychostimulant-induced changes in gene expression that may underlie addiction.

## C. Neurotensin

Neurotensin is thought to be an endogenous neuroleptic and is intimately involved in dopamine transmission in the basal ganglia. Alterations in the neurotensin system may underlie the pathophysiology of a number of neurological and neuropsychiatric conditions, including Parkinson's disease, Huntington's disease, schizophrenia and psychostimulant addiction (Fadel et al., 2001). Neurotensin agonists, through their ability to modulate the dopamine system, may be effective in treating these psychiatric disorders, and

several studies have investigated the effects of neurotensin receptor activation on gene expression in the basal ganglia, as well as the role of neurotensin receptor activation on neuroleptic- and psychostimulant-induced gene expression in this region. Activation of neurotensin receptors alone does not induce IEG expression in the basal ganglia, nor does activation of neurotensin receptors influence neurotensin gene (nts) expression in striatum (Yamada et al., 1995; Alonso et al., 1999; Fadel et al., 2001; Binder et al., 2004; Fadel et al., 2006). However, neurotensin exerts inhibitory control on neurotensin receptor (ntsrl) mRNA expression in the basal ganglia, which may be important in regions of the brain where neurotensin is tonically released (Azzi et al., 1996). Repeated treatment with the neurotensin receptor 1 (Ntsr1) agonist NT69L decreases ntsr1 mRNA expression in nigrostriatal neurons, whereas chronic treatment with the Ntsr1 antagonist SR 48692 increases ntsr1 receptor mRNA expression in the substantia nigra pars compacta (Yamada et al., 1995; Azzi et al., 1996; Wang et al., 2005). This effect is most likely specific for neurotensin receptors, as repeated Ntsr1 receptor activation had no effect on D1 or D2 receptor mRNA expression in striatum (Wang et al., 2005). Ntsr1 receptor activation also appears to facilitate the effects of both direct- and indirect-acting dopamine receptor agonists and D2 receptor antagonists on IEG expression in striatum. That is, AMPHinduced IEG expression is attenuated in neurotensin-null mice, and pre-treatment with an Ntsr1 antagonist attenuates IEG expression induced by AMPH, combined D1/D2 receptor activation, or haloperidol (Alonso et al., 1999; Fadel et al., 2001; Binder et al., 2004; Fadel et al., 2006). Interestingly, blockade of neurotensin receptors suppresses haloperidol-induced IEG expression in the patch compartment of striatum, suggesting that neurotensin may play a role in the cognitive and affective functions of haloperidol treatment (Fadel et al., 2001). Activation of Ntsr1 also decreases DOPA decarboxylase (Ddc) mRNA expression in striatum and tyrosine hydroxylase mRNA expression in the substantia nigra and increases expression of Nurr-1 (Nr4a2) mRNA in striatum (Wang et al., 2005). These data further support the notion that neurotensin acts as an endogenous neuroleptic by modulating dopamine neurotransmission.

#### REFERENCES

Abercrombie ED, Bonatz AE, Zigmond MJ (1990) Effects of 1-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopaminetreated rats. Brain Res 525:36–44.

- Adams AC, Keefe KA (2000) Degree of immediate early gene induction in striatum by eticlopride determines sensitivity to *N*-methyl-D-aspartate receptor blockade. Brain Res 885:201–207.
- Adams AC, Layer RT, McCabe RT, Keefe KA (2000) Effects of conantokins on L-3,4-dihydroxyphenylalanine-induced behavior and immediate early gene expression. Eur J Pharmacol 404:303–313.
- Alonso R, Gnanadicom H, Fréchin N, Fournier M, Le Fur G, Soubrié P (1999) Blockade of neurotensin receptors suppresses the dopamine D1/D2 synergism on immediate early gene expression in the rat brain. Eur J Neurosci 11:967–974.
- Alvaro JD, Tatro JB, Quillan JM, Fogliano M, Eisenhard M, Lerner MR, Nestler EJ, Duman RS (1996) Morphine down-regulates melanocortin-4 receptor expression in brain regions that mediate opiate addiction. Mol Pharmacol 50:583–591.
- Ammon S, Mayer P, Riechert U, Tischmeyer H, Hollt V (2003) Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone-precipitated withdrawal. Brain Res Mol Brain Res 112:113–125.
- Angulo JA, Davis LG, Burkhart BA, Christoph GR (1986) Reduction of striatal dopaminergic neurotransmission elevates striatal proenkephalin mRNA. Eur J Pharmacol 130:341–343.
- Angulo JA, Watanabe Y, Cadet J, Ledoux M, McEwen BS (1993) Upregulation of forebrain proenkephalin mRNA subsequent to NMDA receptor blockade. Eur J Pharmacol 244:317–318.
- Angulo JA, Williams A, Ledoux M, Watanabe Y, McEwen BS (1995) Elevation of striatal and accumbal preproenkephalin, preprotachykinin and preprodynorphin mRNA abundance subsequent to *N*-methyl-Daspartate receptor blockade with MK-801. Brain Res Mol Brain Res 29:15–22.
- Aoyama S, Kase H, Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. J Neurosci 20:5848–5852.
- Arden J, Segredo W, Wang Z, Lameh J, Sadee W (1995) Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK293 cells. J Neurochem 65:1636–1645.
- Aubert I, Ghorayeb I, Normand E, Bloch B (2000) Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 418:22–32.
- Augood SJ, Westmore K, Emson PC (1997) Phenotypic characterization of neurotensin messenger RNA-expressing cells in the neuroleptictreated rat striatum: a detailed cellular co-expression study. Neuroscience 76:763–774.
- Azzi M, Boudin H, Mahmudi N, Pelaprat D, Rostene W, Berod A (1996) In vivo regulation of neurotensin receptors following long-term pharmacological blockade with a specific receptor antagonist. Brain Res Mol Brain Res 42:221–231.
- Basheer R, Tempel A (1993) Morphine-induced reciprocal alterations in G alpha s and opioid peptide mRNA levels in discrete brain regions. J Neurosci Res 36:551–557.
- Beaudry G, Langlois M-C, Weppe I, Rouillard C, Levesque D (2000) Contrasting patterns and cellular specificity of transcriptional regulation of the nuclear receptor nerve growth factor-inducible B by haloperidol and clozapine in the rat forebrain. J Neurochem 75:1694–1702.
- Beckstead RM (1984) The thalamostriatal projection in the cat. J Comp Neurol 223:313–346.
- Beckstead RM (1995) *N*-methyl-D-aspartate acutely increases proenkephalin mRNA in the rat striatum. Synapse 21:342–347.

- Bennett HJ, Semba K (1998) Immunohistochemical localization of caffeine-induced c-Fos protein expression in the rat brain. J Comp Neurol 401:89–108.
- Berendse HW, Groenewegen HJ (1990) Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. J Comp Neurol 299:187–228.
- Bergstrom BP, Garris PA (2003) "Passive stabilization" of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. J Neurochem 87:1224–1236.
- Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR (1998) A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci 18:5301–5310.
- Berke JD, Sgambato V, Zhu PP, Lavoie B, Vincent M, Krause M, Hyman SE (2001) Dopamine and glutamate induce distinct striatal splice forms of Ania-6, an RNA polymerase II-associated cyclin. Neuron 32:277–287.
- Bernard V, Dumartin B, Lamy E, Bloch B (1993) Fos immunoreactivity after stimulation or inhibition of muscarinic receptors indicates anatomical specificity for cholinergic control of striatal efferent neurons and cortical neurons in the rat. Eur J Neurosci 5:1218–1225.
- Berretta S, Parthasarathy HB, Graybiel AM (1997) Local release of GABAergic inhibition in the motor cortex induces immediate-early gene expression in indirect pathway neurons of the striatum. J Neurosci 17:4752–4763.
- Berretta S, Robertson HA, Graybiel AM (1992) Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. J Neurophysiol 68:767–777.
- Berretta S, Robertson HA, Graybiel AM (1993) Neurochemically specialized projection neurons of the striatum respond differentially to psychomotor stimulants. Prog Brain Res 99:201–205.
- Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Hervé D, Valjent E, Girault JA (2008) Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 28:5671–5685.
- Bilecki W, Wawrzczak-Bargiela A, Przewlocki R (2004) Activation of AP-1 and CRE-dependent gene expression via mu-opioid receptor. J Neurochem 90:874–882.
- Billings LM, Marshall JF (2003) D2 antagonist-induced c-fos in an identified subpopulation of globus pallidus neurons by a direct intrapallidal action. Brain Res 964:237–243.
- Billings LM, Marshall JF (2004) Glutamic acid decarboxylase 67 mRNA regulation in two globus pallidus neuron populations by dopamine and the subthalamic nucleus. J Neurosci 24:3094–3103.
- Binder EB, Kinkead B, Owens MJ, Nemerof CB (2004) Neurotensin receptor antagonist SR 142948A alters Fos expression and extrapyramidal side effect profile of typical and atypical antipsychotic drugs. Neuropsychopharmacology 29:2200–2207.
- Boegman RJ, Vincent SR (1996) Involvement of adenosine and glutamate receptors in the induction of c-fos in the striatum by haloperidol. Synapse 22:70–77.
- Bourson A, Boess FG, Bos N, Sleight AJ (1998) Involvement of 5-HT6 receptors in nigro-striatal function in rodents. Br J Pharmacol 125:1562–1566.
- Burford N, Tobler L, Sadee W (1998) Specific G protein activation and μ-opioid receptor internalization caused by morphine, DAMGO and endomorphin-1. Eur J Pharmcol 342:123–126.
- Campbell BM, Kreipke CW, Walker PD (2006) Failure of MK-801 to suppress D1 receptor-mediated induction of locomotor activity and striatal preprotachykinin mRNA expression in the dopamine-depleted rat. Neuroscience 137:505–517.

- Capper-Loup C, Canales JJ, Kadaba N, Graybiel AM (2002) Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. J Neurosci 22:6218–6227.
- Castañeda E, Whishaw IQ, Robinson TE (1990) Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: variation as a function of lesion size. J Neurosci 10:1847–1854.
- Castelli MP, Melis M, Mameli M, Fadda P, Diaz G, Gessa GL (1997) Chronic morphine and naltrexone fail to modify mu-opioid receptor mRNA levles in the rat brain. Brain Res Mol Brain Res 45:149–153.
- Castro E, Tordera RM, Hughes ZA, Pei Q, Sharp T (2003) Use of Arc expression as a molecular marker of increased postsynaptic 5-HT function after SSRI/5-HT1A receptor antagonist co-administration. J Neurochem 85:1480–1487.
- Cenci MA, Campbell K, Bjorklund A (1993) Neuropeptide messenger RNA expression in the 6-hydroxydopamine-lesioned rat striatum reinnervated by fetal dopaminergic transplants: differential effects of the grafts on preproenkephalin, preprotachykinin and prodynorphin messenger RNA levels. Neuroscience 57:275–296.
- Cenci MA, Campbell K, Wictorin K, Björklund A (1992) Striatal c-fos induction by cocaine or apomorphine occurs preferentially in output neurons projecting to the substantia nigra in the rat. Eur J Neurosci 4:376–380.
- Chapman DE, Hanson GR, Kesner RP, Keefe KA (2001) Long-term changes in basal ganglia function after a neurotoxic regimen of methamphetamine. J Pharmacol Exp Ther 296:520–527.
- Chartoff EH, Ward RP, Dorsa DM (1999) Role of adenosine and N-methyl-D-aspartate receptors in mediating haloperidol-induced gene expression and catalepsy. J Pharmacol Exp Ther 291:531–537.
- Chen JF, Moratalla R, Impagnatiello F, et al. (2001) The role of the D(2) dopamine receptor [D(2)R] in A(2A) adenosine receptor [A(2A)R]-mediated behavioral and cellular responses as revealed by A(2A) and D(2) receptor knockout mice. Proc Natl Acad Sci USA 98:1970–1975.
- Chergui K, Svenningsson P, Nomikos GG, Gonon F, Fredholm BB, Svennson TH (1997) Increased expression of NGFI-A mRNA in the rat striatum following burst stimulation of the medial forebrain bundle. Eur J Neurosci 9:2370–2382.
- Chou H, Ogawa N, Asanuma M, Hirata H, Mori A (1992) Muscarinic cholinergic receptor-mediated modulation on striatal c-fos mRNA expression induced by levodopa in rat brain. J Neural Transm Gen Sect 90:171–181.
- Cole DG, Di Figlia M (1994) Reserpine increases Fos activity in the rat basal ganglia via a quinpirole-sensitive mechanism. Neuroscience 60:115–123.
- Cole RL, Konradi C, Douglass J, Hyman SE (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 14:813–823.
- Collins S, D'Addario C, Romualdi P, Candeletti S, Izenwasser S (2002) Regulation of dynorphin gene expression by kappa-opioid agonist treatment. Neuroreport 13:107–109.
- Curran EJ, Akil H, Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. Neurochem Res 21:1425–1435.
- Daberkow DP, Riedy MD, Kesner RP, Keefe KA (2008) Effect of methamphetamine neurotoxicity on learning-induced Arc mRNA expression in identified striatal efferent neurons. Neurotox Res 14:307–315.

- Das S, Grunert M, Williams L, Vincent SR (1997) NMDA and D1 receptors regulate the phosphorylation of CREB and the induction of c-fos in striatal neurons in primary culture. Synapse 25:227–233.
- Dassesse D, Ledent C, Parmentier M, Schiffmann SN (2001a) Acute and chronic caffeine administration differentially alters striatal gene expression in wild-type and adenosine A(2A) receptor-deficient mice. Synapse 42:63–76.
- Dassesse D, Massie A, Ferrari R, Ledent C, Parmentier M, Arckens L, Zoli M, Schiffmann SN (2001b) Functional striatal hypodopaminergic activity in mice lacking adenosine A(2A) receptors. J Neurochem 78:183–198.
- Dassesse D, Vanderwinden JM, Goldberg I, Vanderhaeghen JJ, Schiffmann SN (1999) Caffeine-mediated induction of c-fos, zif-268 and arc expression through A1 receptors in the striatum: different interactions with the dopaminergic system. Eur J Neurosci 11:3101–3114.
- de Bartolomeis A, Aloj L, Ambesi-Impiombato A, Bravi D, Caracò C, Muscettola G, Barone P (2002) Acute administration of antipsychotics modulates Homer striatal gene expression differentially. Brain Res Mol Brain Res 98:124–129.
- de Souza IE, Meredith GE (1999) NMDA receptor blockade attenuates the haloperidol induction of Fos protein in the dorsal but not the ventral striatum. Synapse 32:243–253.
- Decker KP, Roy-Byrne PP, Merchant KM (1995) Effect of muscimol on haloperidol-induced alteration of neurotensin gene expression in the striatum and nucleus accumbens in the rat. Brain Res 691:9–17.
- Delfs JM, Ciaramitaro VM, Parry TJ, Chesselet MF (1995) Subthalamic nucleus lesions: widespread effects on changes in gene expression induced by nigrostriatal dopamine depletion in rats. J Neurosci 15:6562–6575.
- Dhatt RK, Gudehithlu KP, Wemlinger TA, Tejwani GA, Neff NH, Hadjiconstantinou M (1995) Preproenkephalin mRNA and methionine-enkephalin content are increased in mouse striatum after treatment with nicotine. J Neurochem 64:1878–1883.
- Diaz Heijtz R, Castellanos FX (2006) Differential effects of a selective dopamine D1-like receptor agonist on motor activity and c-fos expression in the frontal-striatal circuitry of SHR and Wistar-Kyoto rats. Behav Brain Func 2:18.
- Ding XZ, Mocchetti I (1992) Regulation of cholecystokinin mRNA content in rat striatum: a glutamatergic hypothesis. J Pharmacol Exp Ther 263:368–373.
- Dragunow M, Logan B, Laverty R (1991) 3,4-Methylenedioxymethamp hetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801. Eur J Pharmacol 206:255–258.
- Dragunow M, Robertson GS, Faull RL, Robertson HA, Jansen K (1990) D2 dopamine receptor antagonists induce Fos and related proteins in rat striatal neurons. Neuroscience 37:287–294.
- Dunah AW, Standaert DG (2003) Subcellular segregation of distinct heteromeric NMDA glutamate receptors in the striatum. J Neurochem 85:935–943.
- Engber TM, Susel Z, Kuo S, Gerfen CR, Chase TN (1991) Levodopa replacement therapy alters enzyme activities in striatum and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats. Brain Res 552:113–118.
- Erdtmann-Vourliotis M, Mayer P, Riechert U, Crecksch G, Hollt V (1998) Identification of brain regions that are markedly activated by morphine in tolerant but not naive rats. Brain Res Mol Brain Res 61:51–61.
- Ethier I, Beaudry G, St-Hilaire M, Milbrandt J, Rouillard C, Lévesque D (2004) The transcription factor NGFI-B (Nur77) and retinoids play a critical role in acute neuroleptic-induced extrapyramidal effect and

striatal neuropeptide gene expression. Neuropsychopharmacology 29:335–346.

- Fadel J, Dobner PR, Deutch AY (2001) The neurotensin antagonist SR 49892 attenuates haloperidol-induced striatal Fos expression in the rat. Neurosci Lett 303:17–20.
- Fadel J, Dobner PR, Deutch AY (2006) Amphetamine-elicited striatal Fos expression is attenuated in neurotensin null mutant mice. Neurosci Lett 402:97–101.
- Fenu S, Pinna A, Ongini E, Morelli M (1997) Adenosine A2A receptor antagonism potentiates L-DOPA-induced turning behaviour and c-fos expression in 6-hydroxydopamine-lesioned rats. Eur J Pharmacol 321:143–147.
- Ferguson SM, Norton CS, Watson SJ, Akil H, Robinson TE (2003) Amphetamine-evoked c-fos mRNA expression in the caudate-putamen: the effects of DA and NMDA receptor antagonists vary as a function of neuronal phenotype and environmental context. J Neurochem 86:33–44.
- Ferguson SM, Robinson TE (2004) Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade. J Neurochem 91:337–348.
- Ferrari R, Le Novère N, Picciotto MR, Changeux JP, Zoli M (2002) Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. Eur J Neurosci 15:1810–1818.
- Ferre S, O'Connor WT, Svenningsson P, et al. (1996) Dopamine D1 receptor-mediated facilitation of GABAergic neurotransmission in the rat strioentopenduncular pathway and its modulation by adenosine A1 receptor-mediated mechanisms. Eur J Neurosci 8:1545–1553.
- Ferré S, Karcz-Kubicha M, Hope BT, et al. (2002) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: Implications for striatal neuronal function. Proc Natl Acad Sci USA 99:11940–11945.
- Ferré S, Quiroz C, Woods AS, Cunha R, Popoli P, Ciruela F, Lluis C, Franco R, Azdad K, Schiffmann SN (2008) An update on adenosine A2A-dopamine D2 receptor interactions: implications for the function of G protein-coupled receptors. Curr Pharm Des 14:1468–1474.
- Ferré S, Rimondini R, Popoli P, Reggio R, Pèzzola A, Hansson AC, Andersson A, Fuxe K (1999) Stimulation of adenosine A1 receptors attenuates dopamine D1 receptor-mediated increase of NGFI-A, c-fos and jun-B mRNA levels in the dopamine-denervated striatum and dopamine D1 receptor-mediated turning behaviour. Eur J Neurosci 11:3884–3892.
- Fibiger HC (1994) Neuroanatomical targets of neuroleptic drugs as revealed by Fos immunochemistry. J Clin Psychiatry 55(Suppl B):33–36.
- Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM, Reppert SM (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. Brain Res Mol Brain Res 14:186–195.
- Fu L, Beckstead RM (1992) Cortical stimulation induces fos expression in striatal neurons. Neuroscience 46:329–334.
- Fumagalli F, Frasca A, Racagni G, Riva MA (2009) Antipsychotic drugs modulate Arc expression in the rat brain. Eur Neuropsychopharmacol 19:109–115.
- Ganguly A, Keefe KA (2000) Effects of MK-801 on D1 dopamine receptormediated immediate early gene expression in the dopamine-depleted striatum. Brain Res 871:156–159.
- Georges F, Stinus L, Bloch B, Le Moine C (1999) Chronic morphine exposure and spontaneous withdrawal are associated with modifications of dopamine receptor and neuropeptide gene expression in the rat striatum. Eur J Neurosci 11:481–490.

- Georges F, Stinus L, Le Moine C (2000) Mapping of c-fos gene expresion in the brain during morphine dependence and precipitated withdrawal, and phenotypic identification of the striatal neurons involved. Eur J Neurosci 12:4475–4486.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gerfen CR, Keefe KA, Gauda EB (1995) D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. J Neurosci 15:8167–8176.
- Gerfen CR, Miyachi S, Paletzki R, Brown P (2002) D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 22:5042–5054.
- Gervais J, Soghomonian JJ, Richard D, Rouillard C (1999) Dopamine and serotonin interactions in the modulation of the expression of the immediate-early transcription factor, nerve growth factor-inducible B, in the striatum. Neuroscience 91:1045–1054.
- Gonzalez-Nicolini MV, Berglind W, Cole KS, Keogh CL, McGinty JF (2003) Local mu and delta opioid receptors regulate amphetamineinduced behavior and neuropeptide mRNA in the striatum. Neuroscience 121:387–398.
- Gonzalez-Nicolini V, McGinty JF (2002) NK-1 receptor blockade decreases amphetamine-induced behavior and neuropeptide mRNA expression in the striatum. Brain Res 931:41–49.
- Gotoh L, Kawanami N, Nakahara T, et al. (2002) Effects of the adenosine A(1) receptor agonist N(6)-cyclopentyladenosine on phencyclidineinduced behavior and expression of the immediate-early genes in the discrete brain regions of rats. Brain Res Mol Brain Res 100:1–12.
- Graybiel AM (1993) Acute effects of psychomotor stimulant drugs on gene expression in the striatum. NIDA Res Monogr 125:72–81.
- Gresch PJ, Walker PD (1999a) Acute *p*-chloroamphetamine increases striatal preprotachykinin mRNA: role of the serotonin 2A/2C receptor. Brain Res Mol Brain Res 67:190–193.
- Gresch PJ, Walker PD (1999b) Serotonin-2 receptor stimulation normalizes striatal preprotachykinin messenger RNA in an animal model of Parkinson's disease. Neuroscience 93:831–841.
- Gudehithlu KP, Bhargava HN (1995) Modulation of preproenkphalin mRNA levels in brain regions and spinal cord of rats treated chronically with morphine. Peptides 16:415–419.
- Guerra MJ, Liste I, Labandeira-Garcia JL (1998) Interaction between the serotonergic, dopaminergic, and glutamatergic systems in fenfluramineinduced Fos expression in striatal neurons. Synapse 28:71–82.
- Guo N, Robertson GS, Fibiger HC (1992) Scopolamine attenuates haloperidol-induced c-fos expression in the striatum. Brain Res 588:164–167.
- Guzowski J, Setlow B, Wagner E, McGaugh J (2001) Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. J Neurosci 21:5089–5098.
- Halimi G, Devaux C, Clot-Faybesse O, Sampol J, Legof L, Rochat H, Guieu R (2000) Modulation of adenosine concentration by opioid receptor agonist in rat striatum. Eur J Pharmacol 398:217–224.
- Hansen HH, Timmermann DB, Peters D, Walters C, Damaj MI, Mikkelsen JD (2007) Alpha-7 nicotinic acetylcholine receptor agonists selectively activate limbic regions of the rat forebrain: an effect similar to antipsychotics. J Neurosci Res 85:1810–1818.

- Hanson G, Bush L, Keefe K, Alburges M (2002) Distinct responses of basal ganglia substance P systems to low and high doses of methamphetamine. J Neurochem 82:1171–1178.
- Hanson GR, Keefe KA (1999) Dopamine D-1 regulation of caudate neurotensin mRNA in the presence or absence of the nigrostriatal dopamine pathway. Brain Res Mol Brain Res 66:111–121.
- Harrison LM, Kastin AJ, Zadina JE (1998) Tyr-W-MIF-1 attenuates down-regulation of opiate receptors in SH-SY5Y human neuroblastoma cells. J Pharmacol Exp Ther 284:611–617.
- Hatton CJ, Paoletti P (2005) Modulation of triheteromeric NMDA receptors by N-terminal domain ligands. Neuron 46:261–274.
- Hayashi T, Tsao L, Cadet JL, Su TP (1999) [D-Ala2, D-Leu5]enkephalin blocks the methamphetamine-induced c-fos mRNA increase in mouse striatum. Eur J Pharmacol 366:R7–R8.
- Healy DJ, Meador-Woodruff JH (1999) Ionotropic glutamate receptor modulation of 5-HT6 and 5-HT7 mRNA expression in rat brain. Neuropsychopharmacology 21:341–351.
- Hersch SM, Ciliax BJ, Gutekunst CA, et al. (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Hess US, Whalen SP, Sandoval LM, Lynch G, Gall CM (2003) Ampakines reduce methamphetamine-driven rotation and activate neocortex in a regionally selective fashion. Neuroscience 121:509–521.
- Hollen KM, Nakabeppu Y, Davies SW (1997) Changes in expression of delta FosB and the Fos family proteins following NMDA receptor activation in the rat striatum. Brain Res Mol Brain Res 47:31–43.
- Hoover BR, Marshall JF (2002) Further characterization of preproenkephalin mRNA-containing cells in the rodent globus pallidus. Neuroscience 111:111–125.
- Horner KA, Keefe KA (2006) Regulation of psychostimulant-induced preprodynorphin, c-fos and zif/268 messenger RNA expression in the rat dorsal striatum by mu opioid receptor blockade. Eur J Pharmacol 532:61–73.
- Houdi AA, Dasgupta R, Kindy MS (1998) Effect of nicotine use and withdrawal on brain preproenkephalin A mRNA. Brain Res 799:257–263.
- Humblot N, Thiriet N, Gobaille S, Aunis D, Zwiller J (1998) The serotonergic system modulates the cocaine-induced expression of the immediate early genes egr-1 and c-fos in rat brain. Ann NY Acad Sci 844:7–20.
- Humpel C, Saria A (1993) Intranigral injection of selective neurokinin-1 and neurokinin-3 but not neurokinin-2 receptor agonists biphasically modulate striatal dopamine metabolism but not striatal preprotachykinin-A mRNA in the rat. Neurosci Lett 157:223–226.
- Hussain N, Flumerfelt BA, Rajakumar N (2001) Glutamatergic regulation of haloperidol-induced c-fos expression in the rat striatum and nucleus accumbens. Neuroscience 102:391–399.
- Hussain N, Flumerfelt BA, Rajakumar N (2002) Muscarinic, adenosine A(2) and histamine H(3) receptor modulation of haloperidolinduced c-fos expression in the striatum and nucleus accumbens. Neuroscience 112:427–438.
- Höllt V, Horn G (1992) Effect of nicotine on mRNA levels encoding opioid peptides, vasopressin and alpha 3 nicotinic receptor subunit in the rat. The Clinical investigator 70:224–231.
- Ishikawa K, Nitta A, Mizoguchi H, Mohri A, Murai R, Miyamoto Y, Noda Y, Kitaichi K, Yamada K, Nabeshima T (2006) Effects of single and repeated administration of methamphetamine or morphine on neuroglycan C gene expression in the rat brain. Int J Neuropsychopharmacology 9:407–415.

- Isola R, Zhang H, Tejwani GA, Neff NH, Hadjiconstantinou M (2009) Acute nicotine changes dynorphin and prodynorphin mRNA in the striatum. Psychopharmacology (Berl) 201:507–516.
- Jackson DM, Westlind-Danielsson A (1994) Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 64:291–370.
- Jian M, Staines WA, Iadarola MJ, Robertson GS (1993) Destruction of the nigrostriatal pathway increases Fos-like immunoreactivity predominantly in striatopallidal neurons. Brain Res Mol Brain Res 19:156–160.
- Johnson-Davis KL, Hanson GR, Keefe KA (2002) Long-term post-synaptic consequences of methamphetamine on preprotachykinin mRNA expression. J Neurochem 82:1472–1479.
- Jolkkonen J, Jenner P, Marsden CD (1994) GABAergic modulation of striatal peptide expression in rats and the alterations induced by dopamine antagonist treatment. Neurosci Lett 180:273–276.
- Kaatz KW, Albin RL (1995) Intrastriatal and intrasubthalamic stimulation of metabotropic glutamate receptors: a behavioral and Fos immunohistochemical study. Neuroscience 66:55–65.
- Karcz-Kubicha M, Ferré S, Díaz-Ruiz O, Quiroz-Molina C, Goldberg SR, Hope BT, Morales M (2006) Stimulation of adenosine receptors selectively activates gene expression in striatal enkephalinergic neurons. Neuropsychopharmacology 31:2173–2179.
- Karcz-Kubicha M, Quarta D, Hope BT, et al. (2003) Enabling role of adenosine A1 receptors in adenosine A2A receptor-mediated striatal expression of c-fos. Eur J Neurosci 18:296–302.
- Kearney JA, Frey KA, Albin RL (1997) Metabotropic glutamate agonist-induced rotation: a pharmacological, Fos immunohistochemical, and [14C]-2-deoxyglucose autoradiographic study. J Neurosci 17:4415–4425.
- Keefe KA, Adams AC (1998) Differential effects of *N*-methyl-D-aspartate receptor blockade on eticlopride-induced immediate early gene expression in the medial and lateral striatum. J Pharmacol Exp Ther 287:1076–1083.
- Keefe KA, Ganguly A (1998) Effects of NMDA receptor antagonists on D1 dopamine receptor-mediated changes in striatal immediate early gene expression: Evidence for involvement of pharmacologically distinct NMDA receptors? Dev Neurosci 20:216–228.
- Keefe KA, Gerfen CR (1996) D1 dopamine receptor-mediated induction of zif268 and c-fos in the dopamine-depleted striatum: differential regulation and independence from NMDA receptors. J Comp Neurol 367:165–176.
- Keefe KA, Gerfen CR (1999) Local infusion of the (+/-)-alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione does not block D1 dopamine receptor-mediated increases in immediate early gene expression in the dopamine-depleted striatum. Neuroscience 89:491–504.
- Kemp JM, Powell TP (1970) The cortico-striate projection in the monkey. Brain 93:525–546.
- Kiba H, Jayaraman A (1994) Nicotine induced c-fos expression in the striatum is mediated mostly by dopamine D1 receptor and is dependent on NMDA stimulation. Brain Res Mol Brain Res 23:1–13.
- Kinsey AM, Wainwright A, Heavens R, Sirinathsinghji DJ, Oliver KR (2001) Distribution of 5-ht(5A), 5-ht(5B), 5-ht(6) and 5-HT(7) receptor mRNAs in the rat brain. Brain Res Mol Brain Res 88:194–198.
- Konieczny J, Wardas J, Kuter K, Pilc A, Ossowska K (2007) The influence of group III metabotropic glutamate receptor stimulation by (1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid on the

parkinsonian-like akinesia and striatal proenkephalin and prodynorphin mRNA expression in rats. Neuroscience 145:611–620.

- Konradi C (1998) The molecular basis of dopamine and glutamate interactions in the striatum. In: Advances in Pharmacology; Catecholamines: Bridging Basic Science with Clinical Medicine (Goldstein DS, Eisenhofer G, McCarty R, eds), pp. 729–733. San Diego: Academic Press.
- Konradi C, Cole RL, Heckers S, Hyman SE (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 14:5623–5634.
- Konradi C, Leveque JC, Hyman SE (1996) Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J Neurosci 16:4231–4239.
- Kovoor A, Celver J, Wu A, Chavin C (1998) Agonist-induced homologous desensitization of mu opioid receptors mediated by G protein-coupled kinases is dependent on agonist efficacy. Mol Pharm 54:704–711.
- LaHoste GJ, Henry BL, Marshall JF (2000) Dopamine D1 receptors synergize with D2, but not D3 or D4, receptors in the striatum without the involvement of action potentials. J Neurosci 20:6666–6671.
- LaHoste GJ, Yu J, Marshall JF (1993) Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci USA 90:7451–7455.
- Laprade N, Soghomonian JJ (1995) MK-801 decreases striatal and cortical GAD65 mRNA levels. Neuroreport 6:1885–1889.
- Le Foll B, Diaz J, Sokoloff P (2003) Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. Synapse 47:176–183.
- Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: Sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc Natl Acad Sci USA 87:230–234.
- Le Moine C, Svenningsson P, Fredholm BB, Bloch B (1997) Dopamineadenosine interactions in the striatum and the globus pallidus: Inhibition of striatopallidal neurons through either D2 or A2A receptors enhances D1 receptor-mediated effects on c-fos expression. J Neurosci 17:8038–8048.
- Ledent C, Vaugeois JM, Schiffmann SN, et al. (1997) Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature 388:674–678.
- Lee J, Rajakumar N (2003) Role of NR2B-containing *N*-methyl-D-aspartate receptors in haloperidol-induced c-Fos expression in the striatum and nucleus accumbens. Neuroscience 122:739–745.
- Linden AM, Bergeron M, Schoepp DD (2005) Comparison of c-Fos induction in the brain by the mGlu2/3 receptor antagonist LY341495 and agonist LY354740: evidence for widespread endogenous tone at brain mGlu2/3 receptors in vivo. Neuropharmacology 49(Suppl 1):120–134.
- Liste I, Rozas G, Guerra MJ, Labandeira-Garcia JL (1995) Cortical stimulation induces Fos expression in striatal neurons via NMDA glutamate and dopamine receptors. Brain Res 700:1–12.
- Liu J, Nickolenko J, Sharp FR (1994) Morphine induces c-fos and junB in striatum and nucleus accumbens via D1 and *N*-methyl-D-aspartate receptors. Proc Natl Acad Sci USA 91:8537–8541.
- Lucas JJ, Segu L, Hen R (1997) 5-Hydroxytryptamine1B receptors modulate the effect of cocaine on c-fos expression: converging evidence using 5-hydroxytryptamine1B knockout mice and the

5-hydroxytryptamine1B/1D antagonist GR127935. Mol Pharmacol 51:755–763.

- Lynch G (2006) Glutamate-based therapeutic approaches: ampakines. Curr Opin Pharmacol 6:82–88.
- MacGibbon GA, Lawlor PA, Hughes P, Young D, Dragunow M (1995) Differential expression of inducible transcription factors in basal ganglia neurons. Brain Res Mol Brain Res 34:294–302.
- Mackler SA, Eberwine JH (1994) Cellular adaptation to opiates alters ion-channel mRNA levels. Proc Natl Acad Sci USA 91:385–389.
- Mahan LC, McVittie LD, Smyk-Randall EM, Nakata H, Monsma FJ, Gerfen CR, Sibley DR (1991) Cloning and expression of an A1 adenosine receptor from rat brain. Mol Pharmacol 40:1–7.
- Mannoury la Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ (2007) Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: a scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. Neuropharmacology 52:1003–1014.
- Mao L, Conquet F, Wang JQ (2001) Augmented motor activity and reduced striatal preprodynorphin mRNA induction in response to acute amphetamine administration in metabotropic glutamate receptor 1 knockout mice. Neuroscience 106:303–312.
- Mao L, Conquet F, Wang JQ (2002) Impaired preprodynorphin, but not preproenkephalin, mRNA induction in the striatum of mGluR1 mutant mice in response to acute administration of the full dopamine D(1) agonist SKF-82958. Synapse 44:86–93.
- Mao L, Wang JQ (2001) Selective activation of group I metabotropic glutamate receptors upregulates preprodynorphin, substance P, and preproenkephalin mRNA expression in rat dorsal striatum. Synapse 39:82–94.
- Mao L, Wang JQ (2002) Activation of metabotropic glutamate receptor mediates upregulation of transcription factor mRNA expression in rat striatum induced by acute administration of amphetamine. Brain Res 924:167–175.
- Mao L, Wang JQ (2003a) Contribution of ionotropic glutamate receptors to acute amphetamine-stimulated preproenkephalin mRNA expression in the rat striatum in vivo. Neurosci Lett 346:17–20.
- Mao L, Wang JQ (2003b) Group I metabotropic glutamate receptormediated calcium signalling and immediate early gene expression in cultured rat striatal neurons. Eur J Neurosci 17:741–750.
- Mao L, Wang JQ (2003c) Metabotropic glutamate receptor 5-regulated Elk-1 phosphorylation and immediate early gene expression in striatal neurons. J Neurochem 85:1006–1017.
- Mao L, Yang L, Tang Q, Samdani S, Zhang G, Wang JQ (2005) The scaffold protein Homer1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons. J Neurosci 25:2741–2752.
- Mao LM, Zhang GC, Liu XY, Fibuch EE, Wang JQ (2008) Group I metabotropic glutamate receptor-mediated gene expression in striatal neurons. Neurochem Res 33:1920–1924.
- Marie-Claire C, Courtin C, Roques BP, Noble F (2004) Cytoskeletal genes regulation by chronic morphine treatment in rat striatum. Neuropsychopharmacology 29:2208–2215.
- Marshall JF, Cole BN, LaHoste GJ (1993) Dopamine D2 receptor control of pallidal fos expression: comparisons between intact and 6-hydroxydopamine-treated hemispheres. Brain Res 632:308–313.
- Marshall JF, Henry BL, Billings LM, Hoover BR (2001) The role of the globus pallidus D2 subfamily of dopamine receptors in pallidal immediate early gene expression. Neuroscience 105:365–378.
- Matus-Leibovitch N, Nevo I, Vogel Z (1997) Differential distribution of synapsin IIa and IIb mRNA in various brain structures and the effect

of chronic morphine administration on the regional expression of these isoforms. Brain Res Mol Brain Res 45:301–316.

- Mavridis M, Besson MJ (1999) Dopamine-opiate interaction in the regulation of neostriatal and pallidal neuronal activity as assessed by opioid precursor peptides and glutamate decarboxylase messenger RNA expression. Neuroscience 92:945–966.
- McCarson KE, Krause JE, McLean S (1998) Chronic non-peptide neurokinin receptor antagonist treatment alters striatal tachykinin peptide and receptor gene expression in the rat. Neurosci Lett 251:113–116.
- McFarland NR, Haber SN (2000) Convergent inputs from thalamic motor nuclei and frontal cortical areas to the dorsal striatum in the primate. J Neurosci 20:3798–3813.
- McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- McGinty JF, Shi XD, Schwendt M, Saylor A, Toda S (2008) Regulation of psychostimulant-induced signaling and gene expression in the striatum. J Neurochem 104:1440–1449.
- Merchant KM, Dorsa DM (1993) Differential induction of neurotensin and c-fos gene expression by typical versus atypical antipsychotics. Proc Natl Acad Sci USA 90:3447–3451.
- Mijnster MJ, Galis-de Graaf Y, Voorn P (1998a) Serotonergic regulation of neuropeptide and glutamic acid decarboxylase mRNA levels in the striatum and globus pallidus: studies with fluoxetine and DOI. Brain Res Mol Brain Res 54:64–73.
- Mijnster MJ, Schotte A, Docter GJ, Voorn P (1998b) Effects of risperidone and haloperidol on tachykinin and opioid precursor peptide mRNA levels in the caudate-putamen and nucleus accumbens of the rat. Synapse 28:301–312.
- Miller JC (1990) Induction of c-fos mRNA expression in rat striatum by neuroleptic drugs. J Neurochem 54:1453–1455.
- Miwa H, Fuwa T, Nishi K, Kondo T (2001) Subthalamo-pallido-striatal axis: a feedback system in the basal ganglia. Neuroreport 12:3795–3798.
- Miyachi S, Hasegawa YT, Gerfen CR (2005) Coincident stimulation of convergent cortical inputs enhances immediate early gene induction in the striatum. Neuroscience 134:1013–1022.
- Morelli M, Fenu S, Carta A, Di Chiara G (1996) Effect of MK 801 on priming of D1-dependent contralateral turning and its relationship to c-fos expression in the rat caudate-putamen. Behav Brain Res 79:93–100.
- Morelli M, Fenu S, Cozzolino A, Pinna A, Carta A, Di Chiara G (1993) Blockade of muscarinic receptors potentiates D1 dependent turning behavior and c-fos expression in 6-hydroxydopamine-lesioned rats but does not influence D2 mediated responses. Neuroscience 53:673–678.
- Morelli M, Pinna A, Fenu S, Carta A, Cozzolino A, Di Chiara G (1994) Differential effect of MK 801 and scopolamine on c-fos expression induced by 1-dopa in the striatum of 6-hydroxydopamine lesioned rats. Synapse 18:288–293.
- Morelli M, Pinna A, Wardas J, Di Chiara G (1995) Adenosine A2 receptors stimulate c-fos expression in striatal neurons of 6-hydroxydopaminelesioned rats. Neuroscience 67:49–55.
- Mulder AH, Wardeh G, Hogenboom F, Frankhuyzen AL (1984) Kappaand delta-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. Nature 308:278–280.
- Nakahara T, Kuroki T, Hashimoto K, Hondo H, Tsutsumi T, Motomura K, Ueki H, Hirano M, Uchimura H (2000) Effect of atypical antipsychotics on phencyclidine-induced expression of arc in rat brain. Neuroreport 11:551–555.

- Nakazato E, Ohno M, Watanabe S (1998) MK-801 reverses Fos expression induced by the full dopamine D1 receptor agonist SKF-82958 in the rat striatum. Eur J Pharmacol 342:209–212.
- Nisenbaum LK, Crowley WR, Kitai ST (1996) Partial striatal dopamine depletion differentially affects striatal substance P and enkephalin messenger RNA expression. Brain Res Mol Brain Res 37:209–216.
- Nisenbaum LK, Kitai ST, Crowley WR, Gerfen CR (1994) Temporal dissociation between changes in striatal enkephalin and substance P messenger RNAs following striatal dopamine depletion. Neuroscience 60:927–937.
- Noailles PA, Villegas M, Ledoux M, Lucas LR, McEwen BS, Angulo JA (1996) Acute treatment with the *N*-methyl-D-aspartate receptor antagonist MK-801: effect of concurrent administration of haloperidol or scopolamine on preproenkephalin mRNA levels of the striatum and nucleus accumbens of the rat brain. Neurosci Lett 202:165–168.
- Normand E, Popovici T, Onteniente B, Fellmann D, Piatier-Tonneau D, Auffray C, Bloch B (1988) Dopaminergic neurons of the substantia nigra modulate preproenkephalin A gene expression in rat striatal neurons. Brain Res 439:39–46.
- Ohno M, Yoshida H, Watanabe S (1994) NMDA receptor-mediated expression of Fos protein in the rat striatum following methamphetamine administration: relation to behavioral sensitization. Brain Res 665:135–140.
- Ossowska K, Konieczny J, Wardas J, Gołembiowska K, Wolfarth S, Pilc A (2002) The role of striatal metabotropic glutamate receptors in Parkinson's disease. Amino Acids 23:193–198.
- Ossowska K, Konieczny J, Wardas J, Pietraszek M, Kuter K, Wolfarth S, Pilc A (2007) An influence of ligands of metabotropic glutamate receptor subtypes on parkinsonian-like symptoms and the striatopallidal pathway in rats. Amino Acids 32:179–188.
- Ossowska K, Pietraszek M, Wardas J, Wolfarth S (2004) Potential antipsychotic and extrapyramidal effects of (R,S)-3,4-dicarboxyphenylglycine [(R,S)-3,4-DCPG], a mixed AMPA antagonist/mGluR8 agonist. Pol J Pharmacol 56:295–304.
- Ossowska K, Wardas J, Pietraszek M, Konieczny J, Wolfarth S (2003) The striopallidal pathway is involved in antiparkinsonian-like effects of the blockade of group I metabotropic glutamate receptors in rats. Neurosci Lett 342:21–24.
- Ozawa T, Nakagawa T, Shige K, Minami M, Satoh M (2001) Changes in the expression of glial glutamate transporters in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal. Brain Res 905:254–258.
- Page KJ, Everitt BJ (1993) Transsynaptic induction of c-fos in basal forebrain, diencephalic and midbrain neurons following AMPAinduced activation of the dorsal and ventral striatum. Exp Brain Res 93:399–411.
- Palmer LC, Hess US, Larson J, Rogers GA, Gall CM, Lynch G (1997) Comparison of the effects of an ampakine with those of methamphetamine on aggregate neuronal activity in cortex versus striatum. Brain Res Mol Brain Res 46:127–135.
- Pang Y, Kiba H, Jayaraman A (1993) Acute nicotine injections induce c-fos mostly in non-dopaminergic neurons of the midbrain of the rat. Brain Res Mol Brain Res 20:162–170.
- Papadia S, Hardingham G (2007) The dichotomy of NMDA receptor signaling. Neuroscientist 13:572–579.
- Parelkar NK, Wang JQ (2003) Preproenkephalin mRNA expression in rat dorsal striatum induced by selective activation of metabotropic glutamate receptor subtype-5. Synapse 47:255–261.

- Parelkar NK, Wang JQ (2004) mGluR5-dependent increases in immediate early gene expression in the rat striatum following acute administration of amphetamine. Brain Res Mol Brain Res 122:151–157.
- Paul ML, Graybiel AM, David JC, Robertson HA (1992) D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J Neurosci 12:3729–3742.
- Pei Q, Lewis L, Sprakes ME, Jones EJ, Grahame-Smith DG, Zetterstrom TS (2000) Serotonergic regulation of mRNA expression of Arc, an immediate early gene selectivity localized at neuronal dendrites. Neuropharmacology 39:463–470.
- Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401–404.
- Pillot C, Héron A, Schwartz JC, Arrang JM (2003) Ciproxifan, a histamine H3-receptor antagonist/inverse agonist, modulates the effects of methamphetamine on neuropeptide mRNA expression in rat striatum. Eur J Neurosci 17:307–314.
- Pillot C, Ortiz J, Héron A, Ridray S, Schwartz JC, Arrang JM (2002) Ciproxifan, a histamine H3-receptor antagonist/inverse agonist, potentiates neurochemical and behavioral effects of haloperidol in the rat. J Neurosci 22:7272–7280.
- Pinna A, Wardas J, Cozzolino A, Morelli M (1999) Involvement of adenosine A2A receptors in the induction of c-fos expression by clozapine and haloperidol. Neuropsychopharmacology 20:44–51.
- Pinna A, Wardas J, Cristalli G, Morelli M (1997) Adenosine A2A receptor agonists increase Fos-like immunoreactivity in mesolimbic areas. Brain Res 759:41–49.
- Polese D, de Serpis AA, Ambesi-Impiombato A, Muscettola G, de Bartolomeis A (2002) Homer 1a gene expression modulation by antipsychotic drugs: involvement of the glutamate metabotropic system and effects of D-cycloserine. Neuropsychopharmacology 27:906–913.
- Pollack AE, Angerer MR (2005) Muscarinic receptor blockade attenuates reserpine-mediated Fos induction in the rat striatopallidal pathway. Brain Res 1058:189–192.
- Pollack AE, Bird JL, Lambert EB, Florin ZP, Castellar VL (1999) Role of NMDA glutamate receptors in regulating D2 dopamine-dependent Fos induction in the rat striatopallidal pathway. Brain Res 818:543–547.
- Pollack AE, Fink JS (1995) Adenosine antagonists potentiate D2 dopamine-dependent activation of Fos in the striatopallidal pathway. Neuroscience 68:721–728.
- Pollack AE, Wooten GF (1992) D2 dopaminergic regulation of striatal preproenkephalin mRNA levels is mediated at least in part through cholinergic interneurons. Brain Res Mol Brain Res 13:35–41.
- Pompeiano M, Palacios JM, Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Brain Res Mol Brain Res 23:163–178.
- Przewłocka B, Turchan J, Laso W, Przewłocki R (1996) The effect of single and repeated morphine administration on the prodynorphin system activity in the nucleus accumbens and striatum of the rat. Neuroscience 70:749–754.
- Périer C, Marin C, Bonastre M, Tolosa E, Hirsch EC (2002) AMPA receptor antagonist LY293558 reverses preproenkephalin mRNA overexpression in the striatum of 6-OHDA-lesioned-rats treated with 1-dopa. Eur J Neurosci 16:2236–2240.
- Radulovic J, Blank T, Nijholt I, Kammermeier J, Spiess J (2000) In vivo NMDA/dopamine interaction resulting in Fos production in the

limbic system and basal ganglia of the mouse brain. Brain Res Mol Brain Res 75:271–280.

- Rajadhyaksha A, Barczak A, Macías W, Leveque JC, Lewis SE, Konradi C (1999) L-Type Ca(2+) channels are essential for glutamate-mediated CREB phosphorylation and c-fos gene expression in striatal neurons. J Neurosci 19:6348–6359.
- Rajadhyaksha A, Leveque J, Macías W, Barczak A, Konradi C (1998) Molecular components of striatal plasticity: the various routes of cyclic AMP pathways. Dev Neurosci 20:204–215.
- Rawls SM, McGinty JF (2000) Delta opioid receptors regulate calciumdependent, amphetamine-evoked glutamate levels in the rat striatum: an in vivo microdialysis study. Brain Res 861:296–304.
- Richardson PJ, Kase H, Jenner PG (1997) Adenosine A2A receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci 18:338–344.
- Rivkees SA, Price SL, Zhou FC (1995) Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. Brain Res 677:193–203.
- Robbins MJ, Critchlow HM, Lloyd A, Cilia J, Clarke JD, Bond B, Jones DN, Maycox PR (2008) Differential expression of IEG mRNA in rat brain following acute treatment with clozapine or haloperidol: a semi-quantitative RT-PCR study. J Psychopharmacol (Oxford) 22:536–542.
- Robertson GS, Vincent SR, Fibiger HC (1992) D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. Neuroscience 49:285–296.
- Robertson HA, Paul ML, Moratalla R, Graybiel AM (1991) Expression of the immediate early gene c-fos in basal ganglia: induction by dopaminergic drugs. Can J Neurol Sci 18:380–383.
- Robinson TE, Castañeda E, Whishaw IQ (1990) Compensatory changes in striatal dopamine neurons following recovery from injury induced by 6-OHDA or methamphetamine: a review of evidence from microdialysis studies. Can J Psychol 44:253–275.
- Romualdi P, Lesa G, Donatini A, Ferri S (1995) Long-term exposure to opioid antagonists up-regulates prodynorphin gene expression in rat brain. Brain Res 672:42–47.
- Romualdi P, Landuzzi D, D'Addario C, Candeletti S (2002) Modulation of proorphaninFQ/N gene expression by morphine in the rat mesocorticolimbic system. Neuroreport 13:645–648.
- Ruskin DN, Marshall JF (1995) D1 dopamine receptors influence Fos immunoreactivity in the globus pallidus and subthalamic nucleus of intact and nigrostriatal-lesioned rats. Brain Res 703:156–164.
- Ruskin DN, Marshall JF (1997) Differing influences of dopamine agonists and antagonists on Fos expression in identified populations of globus pallidus neurons. Neuroscience 81:79–92.
- Ruskin DN, Rawji SS, Walters JR (1998) Effects of full D1 dopamine receptor agonists on firing rates in the globus pallidus and substantia nigra pars compacta in vivo: tests for D1 receptor selectivity and comparisons to the partial agonist SKF 38393. J Pharmacol Exp Ther 286:272–281.
- Saklayen SS, Mabrouk OS, Pehek EA (2004) Negative feedback regulation of nigrostriatal dopamine release: mediation by striatal D1 receptors. J Pharmacol Exp Ther 311:342–348.
- Sandstrom MI, Sarter M, Bruno JP (1996) Interactions between D1 and muscarinic receptors in the induction of striatal c-fos in rats depleted of dopamine as neonates. Brain Res Dev Brain Res 96:148–158.
- Schad CA, Justice JB Jr., Holtzman SG (1996) Differential effects of delta- and mu-opioid receptor antagonists on the amphetamine-

induced increase in extracellular dopamine in striatum and nucleus accumbens. J Neurochem 67:2292–2299.

- Schiffmann SN, Dassesse D, d'Alcantara P, Ledent C, Swillens S, Zoli M (2003) A2A receptor and striatal cellular functions: regulation of gene expression, currents, and synaptic transmission. Neurology 61:S24–S29.
- Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277–292.
- Schiffmann SN, Vanderhaeghen JJ (1993) Caffeine regulates neurotensin and cholecystokinin messenger RNA expression in the rat striatum. Neuroscience 54:681–689.
- Schilström B, Rawal N, Mameli-Engvall M, Nomikos GG, Svensson TH (2003) Dual effects of nicotine on dopamine neurons mediated by different nicotinic receptor subtypes. Int J Neuropsychopharmacol 6:1–11.
- Senger B, Brog JS, Zahm DS (1993) Subsets of neurotensin-immunoreactive neurons in the rat striatal complex following antagonism of the dopamine D2 receptor: an immunohistochemical double-labeling study using antibodies against Fos. Neuroscience 57:649–660.
- Sgambato V, Abo V, Rogard M, Besson MJ, Deniau JM (1997) Effect of electrical stimulation of the cerebral cortex on the expression of the Fos protein in the basal ganglia. Neuroscience 81:93–112.
- Sgambato V, Maurice N, Besson MJ, Thierry AM, Deniau JM (1999) Effect of a functional impairment of corticostriatal transmission on cortically evoked expression of c-Fos and zif 268 in the rat basal ganglia. Neuroscience 93:1313–1321.
- Sharp FR, Liu J, Nickolenko J, Bontempi B (1995) NMDA and D1 receptors mediate induction of c-fos and junB genes in striatum following morphine administration: implications for studies of memory. Behav Brain Res 66:225–230.
- Shoda T, Fukuda K, Uga H, Mima H, Morikawa H (2001) Activation of mu-opioid receptor induces expression of c-fos and junB via mitogen-activated protein kinase cascade. Anesthesiology 95:983–989.
- Sijbesma H, Schipper J, Cornelissen JC, de Kloet ER (1991) Species differences in the distribution of central 5-HT1 binding sites: A comparative autoradiographic study between rat and guinea pig. Brain Res 555:295–304.
- Sirinathsinghji DJ, Schuligoi R, Heavens RP, Dixon A, Iversen SD, Hill RG (1994) Temporal changes in the messenger RNA levels of cellular immediate early genes and neurotransmitter/receptor genes in the rat neostriatum and substantia nigra after acute treatment with eticlopride, a dopamine D2 receptor antagonist. Neuroscience 62:407–423.
- Sivam SP, Breese GR, Napier TC, Mueller RA, Hong JS (1986) Dopaminergic regulation of proenkephalin-A gene expression in the basal ganglia. NIDA Res Monogr 75:389–392.
- Smeal RM, Keefe KA, Wilcox KS (2008) Differences in excitatory transmission between thalamic and cortical afferents to single spiny efferent neurons of rat dorsal striatum. Eur J Neurosci 28:2041–2052.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Soghomonian JJ, Chesselet MF (1992) Effects of nigrostriatal lesions on the levels of messenger RNAs encoding two isoforms of glutamate decarboxylase in the globus pallidus and entopeduncular nucleus of the rat. Synapse 11:124–133.
- Soghomonian JJ, Pedneault S, Audet G, Parent A (1994) Increased glutamate decarboxylase mRNA levels in the striatum and pallidum of MPTP-treated primates. J Neurosci 14:6256–6265.

- Spangler R, Goddard NL, Avena NM, Hoebel BG, Leibowitz SF (2003) Elevated D3 dopamine receptor mRNA in dopaminergic and dopaminoceptive regions of the rat brain in response to morphine. Brain Res Mol Brain Res 111:74–83.
- Steiner H, Gerfen CR (1995) Dynorphin opioid inhibition of cocaineinduced, D1 dopamine receptor-mediated immediate-early gene expression in the striatum. J Comp Neurol 353:200–212.
- Steiner H, Gerfen CR (1996) Dynorphin regulates D1 dopamine receptormediated responses in the striatum: relative contributions of pre- and postsynaptic mechanisms in dorsal and ventral striatum demonstrated by altered immediate-early gene induction. J Comp Neurol 376:530–541.
- Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 123:60–76.
- Steiner H, Gerfen CR (1999) Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. Neuroscience 88:795–810.
- Svenningson P, Tzavara ET, Qi H, Carruthers R, Witkin JM, Nomikos GG, Greengard P (2007) Biochemical and behavioral evidence for antidepressant-like effects of 5-HT6 receptor stimulation. J Neurosci 27:4201–4209.
- Svenningsson P, Fourreau L, Bloch B, Fredholm BB, Gonon F, Le Moine C (1999) Opposite tonic modulation of dopamine and adenosine on c-fos gene expression in striatopallidal neurons. Neuroscience 89:827–837.
- Svenningsson P, Fredholm BB (1997) Caffeine mimics the effect of a dopamine D2/3 receptor agonist on the expression of immediate early genes in globus pallidus. Neuropharmacology 36:1309–1317.
- Svenningsson P, Johansson B, Fredholm BB (1996) Caffeine-induced expression of c-fos mRNA and NGFI-A mRNA in caudate putamen and in nucleus accumbens are differentially affected by the N-methyl-D-aspartate receptor antagonist MK-801. Brain Res Mol Brain Res 35:183–189.
- Svenningsson P, Nomikos GG, Fredholm BB (1995) Biphasic changes in locomotor behavior and in expression of mRNA for NGFI-A and NGFI-B in rat striatum following acute caffeine administration. J Neurosci 15:7612–7624.
- Svenningsson P, Nomikos GG, Ongini E, Fredholm BB (1997) Antagonism of adenosine A2A receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate-putamen and nucleus accumbens. Neuroscience 79:753–764.
- Teodorov E, Modena CC, Sukikara MH, Felicio LF (2006) Preliminary study of the effects of morphine treatment on opioid receptor gene expression in brain structures of the female rat. Neuroscience 141:1225–1231.
- Thomsen MS, Mikkelsen JD, Timmermann DB, Peters D, Hay-Schmidt A, Martens H, Hansen HH (2008) The selective alpha7 nicotinic acetylcholine receptor agonist A-582941 activates immediate early genes in limbic regions of the forebrain: Differential effects in the juvenile and adult rat. Neuroscience 154:741–753.
- Tilakaratne N, Friedman E (1996) Genomic responses to 5-HT1A or 5-HT2A/2C receptor activation is differentially regulated in four regions of rat brain. Eur J Pharmacol 307:211–217.
- Tjon GH, Voorn P, Vanderschuren LJ, de Vries TJ, Michiels NH, Jonker AJ, Klop H, Netsby P, Mulder AH, Schoffelmeer AN (1997) Delayed occurrence of enhanced striatal preprodynorphin gene expression in behaviorally sensitized rats: differential long-term effects of intermittent and chronic morphine administration. Neuroscience 76:167–176.
- Tomasetti C, Dell'Aversano C, Iasevoli F, de Bartolomeis A (2007) Homer splice variants modulation within cortico-subcortical regions

by dopamine D2 antagonists, a partial agonist, and an indirect agonist: implication for glutamatergic postsynaptic density in antipsychotics action. Neuroscience 150:144–158.

- Tomitaka S, Hashimoto K, Narita N, Minabe Y, Tamura A (1995) Amantadine induces c-fos in rat striatum: reversal with dopamine D1 and NMDA receptor antagonists. Eur J Pharmacol 285:207–211.
- Torres G, Rivier C (1993) Cocaine-induced expression of striatal c-fos in the rat is inhibited by NMDA receptor antagonists. Brain Res Bull 30:173–176.
- Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci 19:4180–4188.
- Turchan J, Laso W, Budziszewska B, Przewłocka B (1997) Effects of single and repeated morphine administration on the prodynorphin, proenkephalin and dopamine D2 receptor gene expression in the mouse brain. Neuropeptides 31:24–28.
- Tzaferis JA, McGinty JF (2001) Kappa opioid receptor stimulation decreases amphetamine-induced behavior and neuropeptide mRNA expression in the striatum. Brain Res Mol Brain Res 93:27–35.
- Vaccarino FM, Hayward MD, Nestler EJ, Duman RS, Tallman JF (1992) Differential induction of immediate early genes by excitatory amino acid receptor types in primary cultures of cortical and striatal neurons. Brain Res Mol Brain Res 12:233–241.
- van de Witte SV, Drukarch B, Stoof JC, Voorn P (1998) Priming with 1-DOPA differently affects dynorphin and substance P mRNA levels in the striatum of 6-hydroxydopamine-lesioned rats after challenge with dopamine D1-receptor agonist. Brain Res Mol Brain Res 61:219–223.
- Van De Witte SV, Groenewegen HJ, Voorn P (2002) MK-801 alters the effects of priming with 1-DOPA on dopamine D1 receptor-induced changes in neuropeptide mRNA levels in the rat striatal output neurons. Synapse 43:1–11.
- Villaró MT, Cortés R, Mengod G (2005) Serotonin 5-HT4 receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. J Comp Neurol 484:418–439.
- Walker PD, Capodilupo JG, Wolf WA, Carlock LR (1996) Preprotachykinin and preproenkephalin mRNA expression within striatal subregions in response to altered serotonin transmission. Brain Res 732:25–35.
- Wang JQ (1998) Regulation of immediate early gene c-fos and zif/268 mRNA expression in rat striatum by metabotropic glutamate receptor. Brain Res Mol Brain Res 57:46–53.
- Wang JQ, Daunais JB, McGinty JF (1994a) NMDA receptors mediate amphetamine-induced upregulation of zif/268 and preprodynorphin mRNA expression in rat striatum. Synapse 18:343–353.
- Wang JQ, Daunais JB, McGinty JF (1994b) Role of kainate/AMPA receptors in induction of striatal zif/268 and preprodynorphin mRNA by a single injection of amphetamine. Brain Res Mol Brain Res 27:118–126.
- Wang JQ, McGinty JF (1996a) Acute methamphetamine-induced zif/268, preprodynorphin, and preproenkephalin mRNA expression in rat striatum depends on activation of NMDA and kainate/AMPA receptors. Brain Res Bull 39:349–357.
- Wang JQ, McGinty JF (1996b) D1 and D2 receptor regulation of preproenkephalin and preprodynorphin mRNA in rat striatum following acute injection of amphetamine or methamphetamine. Synapse 22:114–122.
- Wang JQ, McGinty JF (1996c) Intrastriatal injection of the metabotropic glutamate receptor antagonist MCPG attenuates acute amphetaminestimulated neuropeptide mRNA expression in rat striatum. Neurosci Lett 218:13–16.

- Wang JQ, McGinty JF (1996d) Muscarinic receptors regulate striatal neuropeptide gene expression in normal and amphetamine-treated rats. Neuroscience 75:43–56.
- Wang JQ, McGinty JF (1996e) Scopolamine augments c-fos and zif/268 messenger RNA expression induced by the full D(1) dopamine receptor agonist SKF-82958 in the intact rat striatum. Neuroscience 72:601–616.
- Wang JQ, McGinty JF (1997a) The full D1 dopamine receptor agonist SKF-82958 induces neuropeptide mRNA in the normosensitive striatum of rats: regulation of D1/D2 interactions by muscarinic receptors. J Pharmacol Exp Ther 281:972–982.
- Wang JQ, McGinty JF (1997b) Intrastriatal injection of a muscarinic receptor agonist and antagonist regulates striatal neuropeptide mRNA expression in normal and amphetamine-treated rats. Brain Res 748:62–70.
- Wang JQ, McGinty JF (1998) Metabotropic glutamate receptor agonist increases neuropeptide mRNA expression in rat striatum. Brain Res Mol Brain Res 54:262–269.
- Wang R, Boules M, Gollatz E, Williams K, Tiner W, Richelson E (2005) Effects of 5 daily injections of the neurotensin-mimetic NT69L on the expression of neurotensin receptors in rat brain. Brain Res Mol Brain Res 138:24–34.
- Ward RP, Dorsa DM (1999) Molecular and behavioral effects mediated by Gs-coupled adenosine A2a, but not serotonin 5-Ht4 or 5-Ht6 receptors following antipsychotic administration. Neuroscience 89:927–938.
- Wardas J, Pietraszek M, Wolfarth S, Ossowska K (2003) The role of metabotropic glutamate receptors in regulation of striatal proenkephalin expression: implications for the therapy of Parkinson's disease. Neuroscience 122:747–756.
- Werme M, Olson L, Brene S (2000) NGFI-B and nor1 mRNAs are upregulated in brain reward pathways by drugs of abuse: different effects in Fischer and Lewis rats. Brain Res Mol Brain Res 76:18–24.
- Wirtshafter D, Asin KE (1994) Interactive effects of stimulation of D1 and D2 dopamine receptors on fos-like immunoreactivity in the normosensitive rat striatum. Brain Res Bull 35:85–91.
- Wirtshafter D, Asin KE (2001) Comparative effects of scopolamine and quinpirole on the striatal fos expression induced by stimulation of D(1) dopamine receptors in the rat. Brain Res 893:202–214.

- Yamada M, Bolden-Waston C, Watson MA, Chevet T, Coleman NJ, Yamada M, Richelson E (1995) Regulation of neurotensin receptor mRNA expression by the receptor antagoinst SR 48692 in the rat midbrain dopaminergic neurons. Brain Res Mol Brain Res 33:343–346.
- Yanahashi S, Hashimoto K, Hattori K, Yuasa S, Iyo M (2004) Role of NMDA receptor subtypes in the induction of catalepsy and increase in Fos protein expression after administration of haloperidol. Brain Res 1011:84–93.
- Yang L, Mao L, Tang Q, Samdani S, Liu Z, Wang JQ (2004) A novel Ca<sup>2+</sup> -independent signaling pathway to extracellular signal-regulated protein kinase by coactivation of NMDA receptors and metabotropic glutamate receptor 5 in neurons. J Neurosci 24:10846–10857.
- Yano M, Steiner H (2007) Methylphenidate and cocaine: the same effects on gene regulation? Trends Pharmacol Sci 28:588–596.
- Yoshikawa M, Shinomiya T, Takayasu N, Tsukamoto H, Kawaguchi M, Kobayashi H, Oka T, Hashimoto A (2008) Long-term treatment with morphine increases the D-serine content in the rat brain by regulating the mRNA and protein expressions of serine racemase and D-amino acid oxidase. J Pharmacol Sci 107:270–276.
- Yukhananov RY, Handa RJ (1997) Effect of morphine on proenkephalin gene expression in the rat brain. Brain Res Bull 43:349–356.
- Zhou Y, Bendor J, Hofman L, Randesi M, Ho A, Kreek MJ (2006) Mu opioid receptor and orexin/hypocretin mRNA levels in the lateral hypothalamus and striatum are enhanced by morphine withdrawal. J Endocrinol 191:137–145.
- Ziolkowska B, Gieryk A, Bilecki W, et al. (2005) Regulation of α-synuclein expression in limbic and motor brain regions of morphine-treated mice. J Neurosci 25:4996–5003.
- Ziólkowska B, Höllt V (1993) The NMDA receptor antagonist MK-801 markedly reduces the induction of c-fos gene by haloperidol in the mouse striatum. Neurosci Lett 156:39–42.
- Ziółkowska B, Urba ski MJ, Wawrzcak-Bargieła A, Bilecki W, Przewłocki R (2005) Morphine activates Arc expession in the mouse striatum and in mouse neuroblastoma Neuro2A MOR1A cells expressing mu-opioid receptors. J Neurosci Res 82:563–570.

# D1 Dopamine Receptor Supersensitivity in the Dopamine-Depleted Striatum: Aberrant ERK1/2 Signaling

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- I. Introduction: D1 and D2 Dopamine Receptors in Direct and Indirect Striatal Projections
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# I. INTRODUCTION: D1 AND D2 DOPAMINE RECEPTORS IN DIRECT AND INDIRECT STRIATAL PROJECTIONS

A key to understanding the function of dopamine in the basal ganglia was the demonstration that D1 and D2 dopamine receptors are segregated in the direct and indirect striatal projection neurons (Fig. 28.1, Gerfen et al., 1990). Striatal medium spiny neurons, which constitute over 90-95% of the neuron population of the striatum and nucleus accumbens, are composed of two major subtypes based on their axonal projections. One subtype projects axons through the globus pallidus, making some contacts there, but extends axons to terminate in the internal segment of the globus pallidus (or entopeduncular nucleus) and substantia nigra (see also Chapter 1). These nuclei constitute the major output system of the basal ganglia; striatal neurons that project to them directly make up the so-called "direct" striatal projection pathway. The other subtype of striatal projection neurons extends its axon only to the globus pallidus. Neurons in this nucleus provide inputs to the internal segment of the globus pallidus and substantia nigra and to the subthalamic nucleus, which in turn projects to these basal ganglia output nuclei. Thus, striatal neurons that project only to the globus pallidus, are connected through multiple synaptic connections to the output of the basal ganglia, and are considered to give rise to the "indirect" striatal projection pathway. Neurons of the direct and indirect pathways are approximately equal in number and intermingled with one another in both the patch and matrix compartments (Gerfen and Young, 1988).

The functional significance of the striatal direct and indirect pathways was established by the observations that following dopamine depletion in the striatum, there are differential changes in GABA receptor binding in the globus pallidus and substantia nigra (Pan et al., 1985) and in the expression of peptides expressed by striatal direct and indirect pathway neurons (Young et al., 1986). These findings led to the hallmark theory that clinical movement disorders such as Parkinson's disease result from an imbalance in the output activity of the direct and indirect pathways (Albin et al., 1989; DeLong, 1990). This theory suggests that akinesia,



FIGURE 28.1 Circuitry involved in Parkinson's disease. (Upper diagram) Direct and indirect pathways of the basal ganglia are shown in a sagittal brain section of the mouse. The cerebral cortex and thalamus provide excitatory inputs (green arrows) to the striatum, the main input nucleus of the basal ganglia. The output of the basal ganglia originates from the internal part of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) and is directed primarily to thalamic nuclei, which project to frontal areas of the cerebral cortex. The direct pathway originates from striatal projection neurons (red) whose axons extend directly to the GPi and SNr output nuclei. The indirect pathway originates from striatopallidal neurons (blue) whose axons terminate within the external globus pallidus (GPe). Neurons in the GPe, in turn, project to the subthalamic nucleus (STN), which projects to the GPi and SNr. Thus, striatopallidal neurons are connected indirectly, through the GPe and STN, with the output of the basal ganglia. (Lower images) D1 and D2 dopamine receptors are segregated to direct- and indirect-pathway neurons, respectively. Sagittal sections from BAC transgenic mice in which these receptors are labeled with EGFP show labeling of the cell bodies in the striatum as well as their axonal projections. D2-BAC transgenic mice show labeling of the indirect-pathway neurons (these axon projections terminate in the GPe), whereas D1-BAC mice show labeling of the direct pathway, as seen by labeling of axon terminals in the GPi and SNr (Gong et al., 2003; 2007). (see Color Plate Section to view the color version of this figure)

which characterizes Parkinson's disease, is a consequence of increased functional activity in the indirect striatal pathway.

The underlying mechanism responsible for dopaminemediated differential changes in the functional activity of the direct and indirect pathways was revealed by the demonstration that D1 and D2 receptors are respectively segregated in the neurons giving rise to these pathways (Gerfen et al., 1990). Two lines of evidence were provided in this study. The first were neuroanatomical findings. In situ hybridization histochemical localization of the mRNAs encoding D1 receptors demonstrated the selective expression of this receptor in neurons that project to the substantia nigra and co-express the peptides dynorphin and substance P, markers of the direct pathway. On the other hand, D2 receptor mRNA was shown to be expressed selectively in neurons that project to the globus pallidus and co-express the peptide enkephalin, a marker of indirect pathway neurons. The second line of evidence was provided by functional studies. Following dopamine depletion of the nigrostriatal pathway, enkephalin expression increases in indirect pathway neurons, whereas substance P and dynorphin expression decreases in direct pathway neurons. These dopamine-lesion induced changes in gene expression are selectively reversed in indirect pathway neurons with D2 receptor agonist treatment and in direct pathway neurons with D1 receptor agonist treatment (Gerfen et al., 1990). This finding was somewhat controversial initially as some investigators maintained that D1 and D2 receptors are co-expressed in most striatal medium spiny neurons (Surmeier et al., 1992). However, the segregation of D1 and D2 receptors in direct and indirect pathway neurons has been confirmed by numerous studies (Le Moine et al., 1990; 1995; Hersch et al., 1995, Gong et al, 2002; 2007) such that there is now a consensus in the field, upholding the original finding.

The demonstration of a segregation of D1 and D2 receptors in direct and indirect pathway neurons, respectively (Gerfen et al., 1990), provided the basis for understanding of functional changes in movement disorders such as Parkinson's disease (Albin et al., 1988; DeLong, 1990). The central tenet of the theory of movement disorders is that they result from imbalanced activity in the direct and indirect striatal pathways. In Parkinson's disease, which is marked by akinesia, the theory suggested that there is increased activity in the indirect pathway. Neurons of this pathway express the D2 receptor, which is coupled to the inhibitory G protein, Gi. In the normal animal, dopamine stimulating the D2 receptor provides an inhibitory function (see also Chapter 6). On the other hand, the D1 receptor expressed on direct pathway neurons is coupled to stimulatory G proteins, Gs and Golf. Consequently, in Parkinson's disease, the loss of dopamine input to the striatum has

opposite affects on the direct and indirect pathways, with increased function in the indirect pathway and decreased function in the direct pathway. Surgical therapies developed to reverse this imbalance by interfering with altered function in the indirect pathway proved to have considerable clinical benefit (Bakay et al., 1992; Lozano et al., 1998).

## II. DOPAMINE RECEPTOR SUPERSENSITIVITY IN PARKINSON'S DISEASE

Treatment of Parkinson's disease with L-DOPA (Birkmayer and Hornykiewicz, 1962) remains the primary therapy. While a very effective therapy, long-term treatment invariably leads to the development of dyskinesias (Bergmann et al., 1987) (see also Chapters 36 and 39). We have proposed that L-DOPA-induced dyskinesia in the treatment of Parkinson's disease results from an aberrant switch in the linkage of the D1 receptor to signal transduction systems that activate the protein kinase, extracellular signalregulated protein kinase (ERK1/2) (Gerfen et al., 2002). As discussed, dopamine depletion of the striatum results in opposite effects on the function of D2-indirect and D1-direct pathway neurons evidenced by changes in gene expression (Gerfen et al., 1990). While, either L-DOPA or selective D2 and D1 receptor agonist treatments reverse some of the gene expression changes, the response of D1 receptor-expressing direct pathway neurons is supersensitive to these treatments, which is evident by the induction of a large number of so called immediate-early genes (IEGs) (Berke et al., 1998).

The supersensitive response of striatal neurons following lesions of the nigrostriatal dopamine system was first described by Ungerstedt (1971), who observed that animals with unilateral lesions exhibited a robust rotation contralateral to the lesioned side in response to direct dopamine receptor agonist treatment. This experimental paradigm remains the standard animal model for the study of Parkinson's disease. The reasonable explanation for why these animals display contralateral rotations following dopamine receptor agonist treatment is that striatal neurons compensate for the loss of dopamine by increasing their expression of dopamine receptors in order to increase their response to decreased levels of neurotransmitter. Thus, striatal neurons in the lesioned striatum would produce a supersensitive response relative to the dopamineintact striatum, which resulted in the behavioral rotation. However, our studies demonstrating the segregation of D1

and D2 receptors on direct and indirect striatal neurons provide a different model (Gerfen et al., 1990). Following dopamine lesions, there is an increase in D2 receptor expression in indirect pathway neurons and a decrease in D1 receptor expression in direct pathway neurons. Rather than reflecting a compensatory response of striatal neurons to decreased dopamine input, these changes in receptor expression reflect the simple consequence of the loss of dopamine function on these neurons. Thus, in indirect pathway neurons, the absence of dopamine acting on D2 receptors, coupled to the inhibitory G protein, Gi, results in increased gene expression, including the D2 receptor. On the other hand, in direct pathway neurons, the absence of dopamine acting on D1 dopamine receptors, coupled to the stimulatory G proteins, Gs and Golf, results in decreased gene expression, including the D1 receptor.

In addition to the behavioral rotational response in the unilateral dopamine lesion paradigm, an enhanced cellular response was demonstrated in the dopamine-depleted striatum, that is, marked induction of IEGs, such as c-fos, in response to dopamine agonists (Robertson et al. 1989; 1990). Significantly, the IEG response to L-DOPA or dopamine agonists such as apomorphine was found to occur exclusively in D1 receptor-expressing direct pathway neurons. This IEG response provides a cellular measure of receptor supersensitivity. What is most interesting about this IEG response in D1 receptor-expressing neurons, is that it occurs upon the first treatment with dopamine receptor agonist, when the level of D1 receptor expression is decreased compared with neurons in the dopamineintact striatum (Berke et al., 1998). This finding indicated that in the dopamine-lesioned striatum, the supersensitive response of D1 receptor-expressing neurons is not a consequence of increased D1 receptor expression, but due to a change in the coupling of this receptor to signal transduction systems.

Psychostimulants, such as cocaine and amphetamine, produce robust induction of IEGs in the normal dopamineinnervated striatum (Graybiel et al., 1990). This raises the question as to whether the D1 receptor-mediated supersensitive induction of IEGs in the dopamine-depleted striatum is due to an amplification of the normal D1 receptor coupling to signal transduction. However, psychostimulantinduced IEG expression differs in important ways from the D1 receptor response in the dopamine-depleted striatum. First, whereas the psychostimulant response is dependent on glutamate (NMDA) receptor activation (Konradi et al., 1995), the D1 receptor-mediated IEG induction in the dopamine-depleted striatum occurs independently of NMDA receptor function (Keefe and Gerfen, 1996). Second, repeated psychostimulant treatment produces an attenuated striatal IEG response (Steiner and Gerfen, 1993) (see also Chapter 29), while the response in the dopamine-depleted striatum remains elevated or even increases with extended dopamine receptor agonist treatment (Steiner and Gerfen, 1996).

Using pharmacologic treatment paradigms to compare D1 receptor-mediated signaling in the dopamine-intact and -lesioned striatum, activation of the protein kinase, extracellular signal-regulated protein kinase (ERK1/2), was demonstrated to occur exclusively in the dopaminedepleted striatum (Fig. 28.2) (Gerfen et al., 2002). In this study, pharmacologic treatments with high doses of D1 receptor agonists, or combined D1 and D2 receptor agonists, produced induction of IEGs in the dopamine-intact striatum at levels comparable to those in the dopaminedepleted striatum (Fig. 28.3). However, activation of ERK1/2 occurred only in the dopamine-depleted striatum. In the dopamine-intact striatum, dopamine agonist-induced activation of ERK1/2 was limited to the nucleus accumbens. These results suggest that depletion of dopamine in the dorsal striatum produces an aberrant coupling of the D1 receptor with activation of ERK1/2.

Some recent studies might be considered to contradict the proposal that coupling of D1 receptors with activation of ERK1/2 is dependent on dopamine depletion in the striatum. For example, psychostimulant treatments have been reported to activate ERK1/2 in the dopamine-intact striatum (Valjent et al., 2005) (see also Chapter 30). In this study, d-amphetamine treatment was shown to produce activation of ERK1/2 in D1 receptor-expressing neurons in the striatum and nucleus accumbens, and also that this activation is mediated by D1 receptors and dependent on DARPP-32. Psychostimulant activation of ERK1/2 in the nucleus accumbens is consistent with our report that D1 agonist treatment activates ERK1/2 in the dopamine-intact nucleus accumbens. However, there are significant differences between the activation of ERK1/2 produced by d-amphetamine in the dopamine-intact dorsal striatum compared with the D1 receptor-mediated activation of ERK1/2 in the dopamine-depleted striatum. Most notably, in the dopamine-intact striatum psychostimulant activation of ERK1/2 occurs in a relatively small percentage of neurons, approximately 10% of D1 neurons, compared with the D1 receptormediated activation of ERK1/2 in nearly all D1 neurons in

the dopamine-depleted striatum. In a subsequent study, we examined psychostimulant treatment (both d-amphetamine and cocaine) activation of ERK1/2 in transgenic mice with a deletion of the D1 receptor (Gerfen et al., 2008). Results demonstrated that while psychostimulant activation of ERK1/2 was reduced in the nucleus accumbens in mice with D1 receptors knocked out, it persisted in the small number of neurons in the dorsal striatum, which suggests that non-dopaminergic mechanisms are involved in some of the affects of amphetamine treatment (Fig. 28.4). This is in contrast to the result of L-DOPA treatment in mice with a genetic deletion of the D1 receptor in which there was no activation of ERK1/2 in the dopamine-depleted striatum. Moreover, this study also demonstrated that in mice with a genetic deletion of DARPP-32, L-DOPA or D1 receptor agonist treatments resulted in activation of ERK1/2 in nearly all D1 receptor-expressing neurons in the dopaminedepleted striatum, comparable to that produced in wildtype mice (Gerfen et al., 2008). These results demonstrate distinct regional differences in the normal coupling of the D1 receptor with activation of ERK1/2. In the dopamineintact ventral striatum, which includes the nucleus accumbens, activation of ERK1/2 is coupled to the D1 receptor and involves DARPP-32. However, in the dopamine-intact dorsal striatum, activation of ERK1/2 with psychostimulant treatment occurs independent of D1 receptors in a small percentage of neurons. Together these studies suggest that following dopamine depletion in the dorsal striatum there is an aberrant coupling of the D1 receptor to activation of ERK1/2, that does not involve DARPP-32.

## III. ABERRANT ACTIVATION OF ERK1/2 INVOLVING SEROTONIN 5-HT2 RECEPTORS IN THE DORSAL STRIATUM

Another example of the aberrant activation of ERK1/2 in direct pathway neurons was identified that involves serotonin receptor mechanisms (Brown and Gerfen, 2006). Serotonin input to the striatum is relatively sparse compared to dopamine input and is most robust in the ventral striatum, with little input to the dorsal striatum. However, lesions of the nigrostriatal pathway made shortly after birth result in a pronounced sprouting of serotonin fibers into the dorsal striatum (Fig. 28.5). Using this experimental paradigm, treatments with agonists for the serotonin receptor 5-HT2 revealed an aberrant coupling to activation



**FIGURE 28.2** D1 dopamine receptor-mediated phosphorylation of ERK1/2 (p-ERK1/2) in the dopamine-depleted striatum. Unilateral lesion of the nigrostriatal dopamine system is demonstrated by the loss of tyrosine hydroxylase immunoreactivity in the right lesioned striatum (A). After treatment (15 min) with the partial D1 agonist SKF38393 (2 mg/kg, i.p.), p-ERK1/2 is not evident in the dopamine-intact striatum (B) but is present in numerous neurons in the dopamine-depleted striatum (C). To determine the type of striatal neuron in which p-ERK1/2 is present, sections are processed to display both p-ERK1/2 with a green fluorescent label (D) and enkephalin mRNA with a red fluorescent label (D'). Nearly all p-ERK1/2-immunoreactive neurons (*blue arrows*) are enkephalin negative. Only a small number of enkephalin-positive neurons display p-ERK1/2 immunoreactivity (*bigger arrow*), whereas the vast majority are p-ERK1/2 negative (*orange arrows*). The graph provides quantitative data of the average number of pERK-positive/enkephalin-positive (*yellow*), and pERK-negative/enkephalin-positive (*red*) neurons in a 500  $\mu$ m<sup>2</sup> area from the lateral striatum of four animals. Enkephalin provides a marker of indirect projection neurons, with any given striatal area having an equal number of direct projecting, enkephalin-negative neurons (Gerfen and Young, 1988). Data indicate that, in the dopamine-intact striatum, there are few pERK1/2-immunoreactive neurons, whereas in the dopamine-depleted striatum, D1 agonist-induced p-ERK1/2 occurs selectively in enkephalin-negative, direct striatal projection neurons. (see Color Plate Section to view the color version of this figure)

of ERK1/2 following neonatal, but not adult lesions of the nigrostriatal dopamine system. In animals in which lesions of the nigrostriatal dopamine system were produced in adults, treatment with 5-HT2 receptor agonists produced robust c-fos induction in both the lesioned and dopamine-intact striatum, but produced little to no activation of ERK1/2 in either striatum. In these animals, serotonin innervation in both the lesioned and dopamine-intact



**FIGURE 28.3** Demonstration of distinct mechanisms of D1 dopamine receptor-mediated gene regulation in the dopamine (DA)-intact and -depleted striatum, using the full D1 agonist SKF81297 alone or combined with other drugs. A–D, In situ hybridization histochemical localization of mRNA encoding c-*fos* 45 min after different drug combinations: A, SKF81297 (0.5 mg/kg); B, SKF81297 (2.0 mg/kg); C, SKF81297 (2.0 mg/kg) combined with the muscarinic receptor antagonist scopolamine (5 mg/kg); or D, SKF81297 (2.0 mg/kg) combined with the D2 receptor agonist quinpirole (1 mg/kg) and scopolamine. The low dose of agonist alone (A) demonstrates the supersensitive response by the selective induction of c-*fos* in the dopamine-depleted striatum. Bilateral induction of c-*fos* in both the dopamine-intact and -depleted striatum follows treatment with the high dose of the full D1 agonist alone (B) or in combination with other drugs (C, D). However, when animals receiving any of these treatments are killed at 15 min, p-ERK1/2-immunoreactive neurons are evident only in the dopamine-depleted striatum and not in the dopamine-intact striatum (data not shown). The treatment combining full D1 agonist with both the D2 agonist and scopolamine produces the most robust c-Fos IEG response in the dopamine-intact striatum at 45 min (E). This treatment also results in persistent p-ERK1/2 (H) and phosphorylated c-Jun (J) in the dopamine-depleted striatum but does not activate p-ERK1/2 (G) or phosphorylated c-Jun (I) in neurons in the dopamine-intact striatum, activation of ERK1/2 occurs only in the dopamine-intact and -depleted striatum, activation of ERK1/2 occurs only in the dopamine-depleted striatum.

striatum was restricted to the ventral striatum, with only very sparse innervation in the dorsal striatum. However, in animals in which the nigrostriatal dopamine pathway was lesioned in the neonate, there was sprouting of serotonin fibers in the dopamine-lesioned dorsal striatum treatment. In these animals, 5-HT2 receptor agonists produced robust activation of ERK1/2 in the dopamine-depleted striatum (Fig. 28.6). Activation of ERK1/2 occurred in nearly all D1 receptor expressing direct pathway neurons, with little activation in indirect pathway neurons. Also, in these animals, in the dopamine-intact striatum, 5-HT2 receptor agonist activation of ERK1/2 was restricted to the nucleus accumbens, with only scattered activation in the dorsal striatum. These results demonstrate that serotonin hyperinnervation of the dorsal striatum is associated with aberrant coupling of the 5-HT2 receptor to activation of ERK1/2 in direct pathway neurons. Whether this aberrant coupling is caused by the hyperinnervation of serotonin or occurs in concert with dopamine depletion is not determined at this time. However, this finding is interesting in that it suggests that in direct pathway neurons in the dorsal striatum, activation of ERK1/2 is highly regulated and that pathophysiologic alterations in the dopamine or serotonin innervation results in an aberrant coupling of D1 or 5-HT2 receptors to activation of ERK1/2. As activation of ERK1/2 has been proposed to be involved in mechanisms underlying synaptic plasticity (Thomas and Huganir, 2004), aberrant activation of ERK1/2 may produce aberrant forms



**FIGURE 28.4** D1 receptor agonist or L-DOPA-induced activation of ERK1/2 in the dopamine (DA) depleted striatum does not involve DARPP-32. Comparison of coronal brain sections at the level of the rostral striatum from wild type and DARPP-32 knockout (KO) mice, with unilateral lesions of the nigrostriatal dopamine system and treated with a D1 agonist (SKF81298, 5 mg/kg, 1 day) or L-DOPA (20 mg/kg with 12 mg/kg benserazide, 10 days). DARPP-32 immunoreactivity (IR) labels neurons in the striatum in wild type mice, which are unlabeled in DARPP-32 KO mice. Unilateral lesion of the nigrostriatal dopamine pathway in these animals is shown by the absence of tyrosine hydroxylase (TH)-IR axonal terminals in the right striatum. Activation of ERK1/2 in response to either D1 agonist treatment (left side figures) or L-DOPA treatment (right side figures) is demonstrated by phospho-ERK1/2-IR throughout the dopamine-depleted striatum. High power images from the dorsolateral striatum (inset boxes, 100 µm wide) show few to no phospho-ERK1/2 IR neurons in the DA-intact striatum. In contrast, there are numerous phospho-ERK1/2 IR neurons in the DA-depleted striatum in both the wild type and DARPP-32 KO animal.

of synaptic plasticity altering the normal function of the circuits of the basal ganglia underlying behavior.

# IV. FUNCTIONAL SIGNIFICANCE OF ABERRANT ACTIVATION OF ERK1/2 IN DIRECT PATHWAY NEURONS

The finding that degeneration of the nigrostriatal dopamine system results in an aberrant coupling of the D1 receptor to activation of ERK1/2 in neurons of the direct pathway in the dorsal striatum has been proposed to produce functional alterations in these neurons that underlie L-DOPA dyskinesia in Parkinson's disease (Gerfen et al., 2002, Nadjar et al., 2009). This is based on the proposal that activation of ERK1/2 is involved in mechanisms of synaptic plasticity (Thomas and Huganir, 2004); aberrant activation

of ERK1/2 may produce synaptic plasticity inappropriate to normal behavior. A key feature of L-DOPA-induced dyskinesia is that these abnormal movements develop following repeated treatment with L-DOPA in animals (and humans) with degeneration of the nigrostriatal dopamine system. Thus, it is suggested that repeated L-DOPA treatment results in activation of ERK1/2 in direct pathway neurons and enhances synaptic plasticity, which over time alters activity in basal ganglia circuits to produce uncontrolled dyskinetic movements. Studies in animal models of L-DOPA-induced dyskinesia have shown that blockade of ERK1/2 activation reduces L-DOPA-induced dyskinesias (Aubert et al., 2002; Santini et al., 2007; Westin et al., 2007; Nadjar et al., 2009). These studies support the proposal that aberrant coupling of D1 receptors to activation of ERK1/2 enhancing synaptic plasticity within basal ganglia circuits, underlies L-DOPA-induced dyskinesia.



**FIGURE 28.5** Unilateral 6-OHDA lesions in adult and neonatal rat pups leads to permanent changes in both the dopaminergic and serotonergic systems within the striatum. Brightfield images show that unilateral 6-OHDA lesions lead to complete destruction of dopamine axon terminals (A, B), as shown by the lack of tyrosine hydroxylase immunoreactivity (TH-IR) within the lesioned striatum. The dopamine terminal field contralateral to the lesioned striatum remains intact. C, D: Brightfield montage images of serotonin transporter (SERT)-IR staining (marker for serotonin terminals) in sections adjacent to those stained for TH-IR. After a 6-OHDA lesion, 5-HT axonal hyperinnervation occurs only within the neonatal lesioned striatum (D). 1C-1,1C-2, 1D-1, and 1D-2 show representative high magnification darkfield images of serotonin axons within the striatum (SERT-IR), after unilateral adult and neonatal 6-OHDA lesions. Images were captured within striatal regions defined by the rectangular boxes in (C) and (D). After adult lesions, SERT-IR within the lesioned (C-1) and intact (C-2) striatum was unaltered. However, neonatal dopamine ablation resulted in 5-HT axonal hyperinnervation of the lesioned striatum (D-1) but not the intact striatum (D-2). Scale bar: 1 mm for A, B; 120 µm for C, D; 50 µm for 1C-1, 1C-2, 1D-1, and 1D-2.

An analogy between L-DOPA-induced dyskinesia in Parkinson's disease may be made with psychostimulant addiction. As we and others have shown, in the intact nucleus accumbens, psychostimulant treatment activates ERK1/2 mediated by D1 receptors. This activation of ERK1/2 has been proposed to contribute to psychostimulant addiction (Valjent et al., 2000, Hyman et al., 2006). These findings suggest that activation of ERK1/2 function in the striatum may be involved in several neuropathologies that are dependent on D1 and/or 5-HT2 receptor signaling.

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**FIGURE 28.6** 5-HT2 receptor agonist treatment (DOI, -2,5,Dimethoxy-4-iodoamphetamine hydrochloride, 5 mg/kg, i.p.) differentially induces ERK1/2 phosphorylation (pERK) in striatal neurons, following neonatal 6-OHDA lesions. Adult (A) and neonatal (B) lesioned animals were treated with DOI and processed for Fos-IR 60 minutes after drug administration, or separate groups of adult (C) and neonatal (D) lesioned animals were treated with DOI and processed for MAPK-IR 15 minutes after drug treatment. High magnification images from within the boxed striatal areas are shown below each brain section. DOI (5 mg/kg) administration produced robust Fos-IR throughout the adult lesioned (A-1) and intact (A-2) striatum as well as in the neonatal lesioned (B-1) and intact (B-2) striatum, demonstrating that DOI activates striatal neurons. Drug administration to animals lesioned as adults failed to induce ERK1/2 phosphorylation within lesioned (C-1) or intact (C-2) striatum, regardless of the dose used. In contrast, robust ERK1/2 phosphorylation was seen throughout the striatum of animals lesioned as neonates (D-1) in a distribution similar to that seen following PCA treatment. Little pERK1/2 staining was seen within the intact striatum (D-2). Scale bar 100  $\mu$ m in photomicrographs.

### REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Aubert I, Guigoni C, Håkansson K, Li Q, Dovero S, Barthe N, Bioulac BH, Gross CE, Fisone G, Bloch B, Bezard E (2005) Increased D1

dopamine receptor signaling in levodopa-induced dyskinesia. Ann Neurol 57:17–26.

- Bakay RA, DeLong MR, Vitek JL (1992) Posteroventral pallidotomy for Parkinson's disease. J Neurosurg 77:487–498.
- Bergmann KJ, Mendoza MR, Yahr MD (1987) Parkinson's disease and long-term levodopa therapy. Adv Neurol 45:463–467.

- Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR (1998) A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci 18:5301–5310.
- Birkmayer W, Hornykiewicz O (1962) The 1-dihydroxyphenylalanine (L-DOPA) effect in Parkinson's syndrome in man: On the pathogenesis and treatment of Parkinson akinesis. Arch Psychiatr Nervenkr Z Gesamte Neurol Psychiatr 203:560–574.
- Brown P, Gerfen CR (2006) Plasticity within striatal direct pathway neurons following neonatal dopamine depletion is mediated through a novel functional coupling of serotonin 5-HT2 receptors to the ERK1/2/MAP Kinase pathway. J Comp Neurol 498:415–430.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Gerfen CR, Young WS 3rd (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161–167.
- Gerfen CR (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science 246:385–388.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gerfen CR, Miyachi S, Paletzi R, Brown P (2002) D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2MAPkinase. J Neurosci 22:5042–5054.
- Gerfen CR, Paletzki R, Worley P (2008) Differences between dorsal and ventral striatum in drd1a dopamine receptor coupling of dopamineand cAMP-regulated phosphoprotein-32 to activation of extracellular signal-regulated kinase. J Neurosci 28:7113–7120.
- Gong S, Zheng C, Doughty ML, et al. (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425:917–925.
- Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci 27:9817–9823.
- Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 87:6912–6916.
- Hersch SM, Ciliax BJ, Gutekunst CA, et al. (1995) Electron microscope analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598.
- Keefe KA, Gerfen CR (1996) D1 dopamine receptor-mediated induction of zif268 and c-fos in the dopamine-depleted striatum: differential regulation and independence from NMDA receptors. J Comp Neurol 367:165–176.
- Konradi C, Leveque JC, Hyman SEJ (1996) Amphetamine and dopamineinduced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. Neuroscience 16:4231–4239.

- LeMoine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426.
- LeMoine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc Natl Acad Sci USA 87:230–234.
- Lozano AM, Lang AE, Hutchison WD, Dostrovsky JO (1998) New developments in understanding the etiology of Parkinson's disease and in its treatment. Curr Opin Neurobiol 1998:783–790.
- Nadjar A, Gerfen CR, Bezard E (2009) Priming for 1-DOPA-induced dyskinesia in Parkinson's disease: A feature inherent to the treatment or the disease?. Prog Neurobiol 87:1–9.
- Pan HS, Penney JB, Young AB (1985) Gamma-aminobutyric acid and benzodiazepine receptor changes induced by unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. J Neurochem 45:1396–1404.
- Robertson GS, Vincent SR, Fibiger HC (1990) Striatonigral projection neurons contain D1 dopamine receptor-activated c-fos. Brain Res 523:288–290.
- Robertson GS, Herrera DG, Dragunow M, Robertson HA (1989) 1-DOPA activates c-fos in the striatum ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra. Eur J Pharmacol 159:99–100.
- Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault JA, Herve D, Greengard P, Fisone G (2007) Critical involvement of cAMP/ DARPP-32 and extracellular signal-regulated protein kinase signaling in 1-DOPA-induced dyskinesia. J Neurosci 27:6995–7005.
- Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89:10178–10182.
- Steiner H, Gerfen CR (1993). Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum J Neurosci 13:5066–5081.
- Steiner H, Gerfen CR (1996) Dynorphin regulates D1 dopamine receptormediated responses in the striatum: relative contributions of pre- and postsynaptic mechanisms in dorsal and ventral striatum demonstrated by altered immediate-early gene induction. J Comp Neurol 376:530–541.
- Thomas GM, Huganir RL (2004) MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci 5:173–183.
- Ungerstedt U (1971) Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand Suppl 367:69–93.
- Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J (2000) Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J Neurosci 20:8701–8709.
- Valjent E, Pascoli V, Svenningsson P, et al. (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA 102:491–496.
- Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA (2007) Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of 1-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol Psychiatry 62:800–810.
- Young WS, Bonner TI, Brann MR (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc Natl Acad Sci USA 83:9827–9831.

# Psychostimulant-Induced Gene Regulation in Corticostriatal Circuits

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## I. INTRODUCTION

Human imaging studies show that repeated exposure to psychostimulants such as cocaine and similar compounds produces functional changes in various brain regions, and these changes are especially prominent in areas of the cerebral cortex and the basal ganglia (e.g., London et al., 1990; Breiter et al., 1997; Beveridge et al., 2006; Porrino et al., 2007). Interactions between the cortex and the basal ganglia are critical for the organization of normal motivated behavior (Albin et al., 1989; DeLong, 1990; Robbins et al., 1998). These interactions are mediated by distinct anatomical loops that arise in all parts of the cortex, project in a topographical manner to specific functional domains of the striatum, and, from there, via basal ganglia output nuclei and the thalamus, back to the cortex (Alexander et al., 1986; Alexander et al., 1990; Groenewegen et al., 1990) (see Chapter 1). Drug-induced functional changes in these circuits are thought to reflect neuroplastic changes associated with drug taking and addiction. Identification of the affected circuits and their neuronal processes will further our understanding of the addiction process, which will inform treatment approaches.

Psychostimulants such as cocaine, amphetamine and methylphenidate act by facilitating release and/or blocking reuptake of monoamines, thereby creating increased extracellular levels of these amines and excessive receptor stimulation. Cocaine and amphetamine produce dopamine, norepinephrine and serotonin overflow, whereas methylphenidate only affects dopamine and norepinephrine, but not serotonin (Di Chiara and Imperato, 1988b; Hurd and Ungerstedt, 1989; Ritz et al., 1990; Gatley et al., 1996; Kuczenski and Segal, 1997; Gerasimov et al., 2000; Kuczenski and Segal, 2001; for review, see Yano and Steiner, 2007). It is the potentiation of the dopamine transmission (Di Chiara and Imperato, 1988b) in the basal ganglia that is considered to be of critical importance for the addiction process, whereas serotonin and norepinephrine play modulatory roles (Berke and Hyman, 2000; Nestler, 2001).

Over the last two decades, a wealth of studies have characterized psychostimulant-induced molecular changes in the basal ganglia, especially in the striatum (e.g., Hyman and Nestler, 1996; Harlan and Garcia, 1998; Torres and Horowitz, 1999; Berke and Hyman, 2000; Nestler, 2001; Kelley, 2004; Yano and Steiner, 2007). Among the many drug-induced molecular effects discovered, it is changes in gene regulation that are thought to meditate the longterm behavioral changes, by altering cortico-basal ganglia circuits and their functional properties. A good part of this work has focused on the limbic (ventral) striatum, which mediates motivational processes (Pierce and Kalivas, 1997) and is thus considered of central importance in early addiction stages. However, recent imaging studies indicate that, as the disease progresses, associational and sensorimotor (dorsolateral) domains of the striatum are increasingly affected (Porrino et al., 2007). These domains are implicated in habitual and compulsive aspects of drug taking (Berke and Hyman, 2000; Everitt and Robbins, 2005), and indeed display particularly robust gene regulation effects after acute (Fig. 29.1) and repeated treatment with various psychostimulants (e.g., Steiner and Gerfen, 1993; Badiani et al., 1998; Willuhn et al., 2003; Yano and Steiner, 2005b).

The present review summarizes the work on psychostimulant-induced gene regulation in these circuits and addresses the potential functional significance of such molecular changes. The vast majority of the studies performed to date, and thus the focus of this review, is on gene regulation of neuropeptide transmitters and so-called immediate-early genes (IEGs). Neuropeptides are often selectively expressed by specific neuronal types and thus serve as cell type markers, but they also modulate basal ganglia functions on several levels (see Box 29.2, below). IEGs are frequently used as markers of cell activation due to their rapid and transient induction by neuronal activity (Sharp et al., 1993; Chaudhuri, 1997; Melzer and Steiner, 1997) and drug treatments (Harlan and Garcia, 1998; Torres and Horowitz, 1999). However,



**FIGURE 29.1** Induction of *c-fos* by cocaine. Illustrations of film autoradiograms depict *c-fos* expression in coronal sections from rostral (top), middle (center) and caudal striatal levels (bottom) in rats that received a vehicle injection (left halfbrains) or a cocaine injection (25 mg/kg; right halfbrains) and were killed 30 min later (Brandon and Steiner, 2003). The striatum is outlined in the cocaine-treated animals. Note the considerable regional differences in the *c-fos* response, which peaks in the middle-to-caudal sensorimotor striatum. Repeated psychostimulant treatment produces neuroadaptations in the same striatal regions/neurons that show acute gene induction (see text); acute gene induction thus serves as a marker for striatal regions susceptible for such drug-induced neuroplasticity.

IEGs are also of interest because of their direct involvement in neuroplasticity. Many IEGs encode transcription factors that regulate the expression of other genes (e.g., c-fos, zif 268; Knapska and Kaczmarek, 2004). Others code for members of a family of scaffolding proteins (e.g., *ania*, *homer*) that anchor receptors to the postsynaptic density and play a role in receptor trafficking, spine formation and other processes of synaptic plasticity (homer 1a; Xiao et al., 2000; Thomas, 2002). These latter may be involved in abnormal spine formation in striatal neurons found after psychostimulant treatment (Robinson and Kolb, 1997; Kolb et al., 2003; Ferrario et al., 2005; Jedynak et al., 2007). In addition, psychostimulants regulate the expression of a multitude of other molecules in cortico-basal ganglia circuits (e.g., Berke et al., 1998; McClung and Nestler, 2003; Yuferov et al., 2003; Konradi et al., 2004; Black et al., 2006). The interested reader is referred to several other recent reviews on this topic (e.g., Harlan and Garcia, 1998; Torres and Horowitz, 1999; Berke and Hyman, 2000; McClung et al., 2004; Yuferov et al., 2005; Yano and Steiner, 2007).

# II. GENE REGULATION IN THE STRIATUM OCCURS MOSTLY IN DIRECT PATHWAY NEURONS AND IS MEDIATED BY D1 DOPAMINE RECEPTORS

Chapter 27 by Keefe and Horner in this volume describes in great detail how the different neurotransmitter systems in the striatum affect gene regulation. Given the various neurochemical effects of psychostimulants (see Section I), it is not surprising that these drugs powerfully alter the expression of many genes. By far best-studied are psychostimulant effects on the expression of IEGs and neuropeptides in striatal projection neurons (Harlan and Garcia, 1998; Torres and Horowitz, 1999; Berke and Hyman, 2000; Yano and Steiner, 2007). The affected IEGs include c-fos (Fig. 29.1), fosB, zif 268 (ngfi-A, egr1) (Fig. 29.5), arc, jun-B, and homer 1a; neuropeptides include substance P (ppt), dynorphin (ppd) and enkephalin (ppe). It is worth noting that drug-induced regulation is typically correlated between different genes in a given striatal region/neuron (Steiner and Gerfen, 1993; Willuhn et al., 2003; Yano and Steiner, 2005a, 2005b; Unal et al., 2009); that is, if genes respond, they tend to respond in a coordinated manner. This is not surprising as many of these genes are regulated by shared second messenger signaling mechanisms (see Chapter 26).

Given the differential functional roles of the two subtypes of striatal projection neurons, direct pathway (striatonigral) neurons and indirect pathway (striatopallidal) neurons (Fig. 29.2; see Chapter 1), it was of considerable interest to determine whether psychostimulants alter gene regulation in both types or whether one is preferentially affected. Early clues were obtained from drug effects on the expression of neuropeptides that are differentially expressed by these neurons. Substance P and dynorphin are predominantly contained in striatonigral neurons, whereas enkephalin is predominantly expressed in striatopallidal neurons (see Reiner and Anderson, 1990; Steiner and Gerfen, 1998, for reviews) (Fig. 29.2). Many studies showed that cocaine and amphetamine robustly induce expression of substance P and dynorphin (e.g., Hurd and Herkenham, 1992; Steiner and Gerfen, 1993; Daunais and McGinty, 1994; Wang et al., 1995; Drago et al., 1996; Adams et al., 2001; Brandon and Steiner, 2003), while enkephalin expression is only modestly affected by psychostimulants (Steiner and Gerfen, 1993; Wang and McGinty, 1996a; Spangler et al., 1997; Mathieu-Kia and Besson, 1998; Brandon and Steiner, 2003). [It is important to note that this is not due to inert enkephalin regulation. Enkephalin expression is readily induced, for example, by D2 receptor antagonists



**FIGURE 29.2** Schematic illustration of cortico-basal ganglia-thalamocortical circuits. The direct and indirect pathways from the striatum to the basal ganglia output nuclei are highlighted. Direct pathway (striatonigral) neurons contain mainly D1 dopamine receptors and the neuropeptides substance P (SP) and dynorphin (DYN), while neurons that give rise to the indirect pathway (striatopallidal neurons) express mostly D2 receptors and the opioid peptide enkephalin (ENK), in addition to their main transmitter GABA. GLU, glutamate; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus.

or dopamine depletion (e.g., Young et al., 1986; Voorn et al., 1987; Gerfen et al., 1990; Steiner and Gerfen, 1999; see Steiner and Gerfen, 1998, for review).] These findings thus indicated that psychostimulant-induced gene regulation occurs preferentially (but not exclusively) in striatonigral neurons.

Double-labeling studies employing neuropeptide mRNAs or tract tracers as markers confirmed this differential gene regulation also for IEGs. Cocaine and amphetamine were found to induce IEGs predominantly in striatonigral neurons (Berretta et al., 1992; Cenci et al., 1992; Johansson et al., 1994; Kosofsky et al., 1995; Badiani et al., 1999). However, depending on the treatment conditions (e.g., cortical activation; see Box 29.1), some IEG induction also occurs in striatopallidal neurons (e.g., Jaber et al., 1995; Badiani et al., 1999; Uslaner et al., 2001b).

The above described differential effects on striatonigral vs. striatopallidal neurons are also consistent with the distribution of dopamine receptor subtypes that mediate such gene regulation. Dopamine receptors are largely segregated between the two subtypes of projection neurons (see Chapter 1). Striatonigral neurons predominantly contain D1 receptors, while striatopallidal neurons mostly express D2 receptors (Fig. 29.2), and only a minor portion of neurons contains both receptor subtypes at comparable levels (Gerfen et al., 1990; Le Moine et al., 1990; Le Moine et al., 1991; Curran and Watson, 1995; Hersch et al., 1996). [Note

#### Box 29.1 Role of Cortical Activity in Striatal Gene Regulation – Effect of Context

Cortical input is critical for gene regulation in striatal neurons (see main text). For example, eliminating cortical input or blocking glutamate receptors attenuates psychostimulant-induced gene regulation (e.g., Cenci and Björklund, 1993; Torres and Rivier, 1993; Wang et al., 1994b; Vargo and Marshall, 1995). Importantly, such input to some degree also determines the relative distribution of molecular changes between direct and indirect pathway neurons. First, studies showed that stimulation (disinhibition) or lesion of the cortex tend to affect gene regulation preferentially in indirect (striatopallidal) neurons (e.g., Uhl et al., 1988; Berretta et al., 1997; Ferguson and Robinson, 2004), for reasons that are not entirely clear. Recent evidence indicates that this may be due to differential glutamate release from indirect vs. direct pathway-targeting corticostriatal terminals or other differential features between these inputs (Lei et al., 2004; see Chapter 18).

Second, as discussed in the main text, selective D1 and non-selective D1/D2 receptor agonists, as well as dopamine reuptake blockers (psychostimulants) predominantly enhance gene regulation in direct pathway (striatonigral) neurons (e.g., Robertson et al., 1990; Cenci et al., 1992; Robertson et al., 1992; Johansson et al., 1994; Badiani et al., 1999), while selective D2 receptor agonists suppress gene expression in striatopallidal neurons (e.g., Gerfen et al., 1990; Le Moine et al., 1997; Pinna et al., 1997). Therefore, to what degree psychostimulants enhance gene expression (IEGs, enkephalin) also in striatopallidal neurons (see main text) seems to depend on the balance between (stimulatory) cortical input vs. (inhibitory) D2 receptor activation in these neurons (see Fig. 29.3).

This is important because conditions that activate the cortex will thus tend to produce molecular changes also in the indirect (striatopallidal) pathway, as shown by a recent series of studies. These studies demonstrated that, depending on whether psychostimulants were administered in a familiar environment (home cage) or an unfamiliar (novel) environment, IEG induction was practically restricted to striatonigral neurons or was also present in a portion of striatopallidal neurons, respectively (Badiani et al., 1999; Uslaner et al., 2001b; Uslaner et al., 2003b; Ferguson and Robinson, 2004). Consistent with a glutamate-dopamine balance (see above), this novelty effect was seen with low-to-moderate, but not high, doses of psychostimulants (Uslaner et al., 2003b), which presumably produced low-to-moderate stimulation of inhibitory D2 receptors. Besides gene regulation in striatopallidal neurons, potentiated gene induction was also found in the subthalamic nucleus under "novel" conditions (Uslaner et al., 2001b; Uslaner et al., 2003a; Uslaner et al., 2003b), indicating that other nodes of the indirect pathway are also affected.

Administration of the psychostimulant in the novel environment also produces greater IEG induction in the cortex (mostly sensorimotor; e.g., Badiani et al., 1998; Uslaner et al., 2001a), presumably indicating enhanced cortical activity, and results in more robust behavioral activation (e.g., Badiani et al., 1998) than drug treatment "at home" (see Badiani and Robinson, 2004, for review). It is unclear whether the enhanced gene regulation in the basal ganglia induced by novelty is secondary to the enhanced behavioral activation (increased sensorimotor feedback to the cortex), or whether it reflects generally enhanced cortical activation related to arousal or stress, or other factors. However, recent findings (Yano and Steiner, 2005a; Conversi et al., 2006; Unal et al., 2009) indicate that arousal/stress-related activities are important determinants of psychostimulant-induced gene regulation in the cortex.

Together, these findings demonstrate that environmental (contextual) variables play an important role in psychostimulant-induced neuroplasticity in cortico-basal ganglia-cortical circuits, especially in determining gene regulation in the indirect pathway. Future studies will have to elucidate the clinical significance of this effect.

that the expression patterns in the ventral striatum are somewhat more complex, as some neurons also feature other dopamine receptors (D3) or neuropeptide combinations (Curran and Watson, 1995; Le Moine and Bloch, 1995, 1996).]

A number of studies demonstrated that IEG expression induced by the psychostimulants cocaine, amphetamine and methylphenidate is blocked by systemic (Graybiel et al., 1990; Young et al., 1991; Cole et al., 1992; Moratalla et al., 1992) or intrastriatal administration (Steiner and Gerfen, 1995; Yano et al., 2006) of the selective D1 receptor antagonist SCH-23390. Confirming the critical importance of the D1 receptor, psychostimulant-induced IEG expression is also eliminated by targeted deletion of the D1 receptor (D1 receptor knockouts) (Drago et al., 1996; Moratalla et al., 1996b; Zhang et al., 2004).

D2 receptor activation inhibits gene expression in striatopallidal neurons (e.g., Gerfen et al., 1990; Le Moine et al., 1997; Pinna et al., 1997). However, concurrent stimulation of D2 receptors together with D1 receptors potentiates D1 receptor-mediated gene expression in striatonigral neurons (D1–D2 receptor synergy; e.g., Paul et al., 1992; LaHoste et al., 1993; Gerfen et al., 1995; Le Moine et al., 1997). Similarly, a full gene response to psychostimulants requires combined stimulation of D1 and D2 receptors (Ruskin and Marshall, 1994). This facilitating effect of D2 receptors is thought to be mediated by cholinergic interneurons (via D2 receptor-induced inhibition of acetylcholine release; Wang and McGinty, 1996b) and/or direct interactions between striatopallidal and striatonigral neurons (Gerfen et al., 1995) (but see LaHoste et al., 2000).

Glutamate input is critical for most processes in striatal neurons. This is also true for psychostimulant-induced gene regulation (for reviews, see Hyman et al., 1996; Wang and McGinty, 1996b). Thus, blocking glutamate (NMDA) receptors (e.g., Johnson et al., 1991; Torres and Rivier, 1993; Wang et al., 1994b, 1994a; Hanson et al., 1995) or elimination of corticostriatal afferents (Cenci and Björklund, 1993; Vargo and Marshall, 1995; Ferguson and Robinson, 2004) attenuates psychostimulant-induced gene expression in striatal neurons. While these latter findings clearly demonstrate an important role for cortical input (see Box 29.1), other evidence indicates that glutamate input from the thalamus may also participate in such gene regulation (Giorgi et al., 2001; Bacci et al., 2004). One of our recent studies indicated that indeed re-entrant activity via the thalamus facilitates striatal gene expression induced by psychostimulants (methylphenidate; Yano et al., 2006).

In conclusion, the above findings show that psychostimulants preferentially alter gene regulation in direct pathway (striatonigral) neurons, an effect that is mediated by D1 receptors, modulated by D2 receptors and dependent on glutamate input.

# III. NEUROADAPTATIONS AFTER REPEATED PSYCHOSTIMULANT TREATMENTS

Psychostimulant exposure produces a variety of neuroadaptations and other neuronal changes in the basal ganglia. A comprehensive review of these changes is beyond the scope of the present chapter, and the reader is referred to several excellent recent reviews on this topic (e.g., Hyman and Nestler, 1996; Kuhar and Pilotte, 1996; Berke and Hyman, 2000; Nestler, 2001; Kelley, 2004; Hyman, 2005). Examples of three types of neuroadaptations associated with altered gene regulation will be addressed here: (1) changes in neuropeptide function (dynorphin); (2) altered gene inducibility; and (3) alternative splicing (deltaFosB). These neuroadaptations after repeated psychostimulant treatment occur in the same striatal regions/neurons that show acute gene induction (Steiner and Gerfen, 1993; Willuhn et al., 2003; Unal et al., 2009; see following Sections); acute gene induction thus serves as a marker for striatal regions susceptible for such drug-induced neuroplasticity (Fig. 29.1).

### A. Increased Dynorphin Expression

Increased dynorphin expression in the striatonigral pathway is among the best-established molecular changes after repeated cocaine and amphetamine treatments. Both elevated mRNA (e.g., Hurd and Herkenham, 1992; Spangler et al., 1993; Steiner and Gerfen, 1993; Daunais and McGinty, 1994; Wang et al., 1994b; Spangler et al., 1996; Adams et al., 2003; Willuhn et al., 2003; Horner et al., 2005) and peptide levels (e.g., Hanson et al., 1987; Li et al., 1988; Sivam, 1989; Smiley et al., 1990) after repeated psychostimulant treatment have been shown by many labs. Notably, increased dynorphin expression has also been demonstrated in human cocaine addicts (Hurd and Herkenham, 1993).

After acute cocaine and amphetamine administration, elevated mRNA levels can be seen within 30 min of drug injection (Brandon and Steiner, 2003; Willuhn et al., 2003), are prominent at 2–3 hours (Hurd and Herkenham, 1992; Smith and McGinty, 1994; Wang and McGinty, 1995; Adams et al., 2003) and have been found to last for at least 18–30 hours (Smith and McGinty, 1994; Wang and McGinty, 1995). Because of the long half-life of dynorphin mRNA, levels accumulate with daily repeated drug treatment. Indeed, after repeated treatment with the dopamine precursor L-DOPA (in an animal model of Parkinson's disease), elevated dynorphin mRNA levels in the striatum were found to last for several weeks past cessation of the treatment (Andersson et al., 2003; see Chapter 36).

What is the functional significance of increased dynorphin expression in the striatum? Findings indicate that opioid peptides such as dynorphin act, at least in part, as negative feedback systems (Steiner and Gerfen, 1998) to limit dopamine and glutamate input to striatal neurons (see Box 29.2). Repeated, excessive activation of these neurons by pharmacological treatments (or other experimental manipulations; Box 29.2) is thought to trigger compensatory upregulation of opioid peptide function which acts as a "brake" to maintain systems homeostasis (Hyman and Nestler, 1996).

In the case of upregulated dynorphin function after repeated psychostimulant exposure, it is thus to be expected that during early withdrawal from drug use the "brake" is still on for some time due to the relatively long half-life of these peptides (see above). Such increased dynorphin signaling would thus excessively inhibit inputs to the striatum (Hyman and Nestler, 1996; Steiner and Gerfen, 1998; Shippenberg et al., 2007). There is good

#### Box 29.2 Opioid Peptides as Negative Feedback Mechanisms that Regulate Striatal Output

As mentioned above, the opioid peptides dynorphin and enkephalin are selectively expressed by direct pathway (striatonigral) and indirect pathway (striatopallidal) neurons, respectively (Brownstein et al., 1977; Vincent et al., 1982; Beckstead and Kersey, 1985; Gerfen and Young, 1988; see Reiner and Anderson, 1990, for review) (Fig. 29.2). A wealth of studies showed that repeated treatment with psychostimulants (see main text), direct dopamine receptor agonists (e.g., Gerfen et al., 1990; Steiner and Gerfen, 1996; see Steiner and Gerfen, 1998, for review), or L-DOPA (e.g., Engber et al., 1991; Andersson et al., 1999; Cenci, 2002; see Chapter 36) produce robust increases in dynorphin expression in striatonigral neurons. On the other hand, both repeated D2 receptor antagonist treatment or dopamine depletion result in dramatically increased enkephalin expression in striatopallidal neurons (see Steiner and Gerfen, 1998, for review). The increase in dynorphin expression is thought to be the result of repeated over-activation of striatonigral neurons due to excessive D1 receptor stimulation, whereas the increase in enkephalin expression is related to chronic disinhibition of striatopallidal neurons, due to loss of D2 receptor-mediated inhibition (Steiner and Gerfen, 1998).

There is good evidence to indicate that the changes in both dynorphin and enkephalin expression reflect neuroadaptive responses to maintain a "systems equilibrium". Thus, repeated/ chronic over-activation is thought to produce a compensatory upregulation of these peptides in order to counteract such activation (act as a "brake"; Hyman and Nestler, 1996), for example, by inhibiting facilitatory inputs to these neurons. This is best established for dynorphin. There are several mechanisms by which dynorphin can inhibit dopamine and glutamate input in the striatum (Fig. 29.3) (for reviews, see Steiner and Gerfen, 1998; Shippenberg et al., 2007). (1) Dynorphin by stimulating kappa opioid receptors on dopamine neurons (Meng et al., 1993; Minami et al., 1993; Mansour et al., 1994) inhibits striatal dopamine release (Di Chiara and Imperato, 1988a; Spanagel et al., 1992; Marinelli et al., 1998) via at least two mechanisms. First, dynorphin locally released in the striatum (You et al., 1994a) from striatonigral neuron axon collaterals (Wilson and Groves, 1980), or perhaps even from their dendrites (Drake et al., 1994; Simmons et al., 1995), stimulates presynaptic kappa receptors on dopamine terminals to attenuate release (e.g., Mulder et al., 1984). Second, dynorphin released by striatonigral neurons in the substantia nigra (You et al., 1994a) inhibits striatal dopamine release via stimulation of kappa receptors on dendrites or cell bodies of dopamine neurons (Reid et al., 1988). (2) Kappa receptors are also expressed by neurons in some cortical regions (mostly deep layers of secondary somatosensory and insular cortex as well as claustrum; Meng et al., 1993; Minami et al., 1993; Mansour et al., 1994). There is evidence that striatal kappa receptors, presumably on terminals of corticostriatal neurons, inhibit glutamate release (Gray et al., 1999). (3) Furthermore, in some striatal areas, kappa receptors on striatonigral neurons (Meng et al., 1993; Minami et al., 1993; Mansour et al.,



**FIGURE 29.3** Schematic illustration of negative feedback mechanisms mediated by opioid peptides in direct and indirect pathways. Dynorphin (DYN) released from direct pathway (striatonigral) neurons in the striatum (local axon collaterals) and in the midbrain acts on kappa opioid receptors to inhibit dopamine (DA) input from the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), as well as some glutamate (GLU) input from the cortex. Similarly, there is evidence that enkephalin (ENK) acting on delta and mu opioid receptors in the striatum inhibits excitatory responses in striatopallidal neurons (exact mechanisms unclear, see text). GPe, globus pallidus external segment; GPi, globus pallidus internal segment; SNr, substantia nigra pars reticulata.

1994) can limit effects of D1 receptor stimulation postsynaptically in these neurons (Steiner and Gerfen, 1996).

One consequence of increased dynorphin/kappa receptor action in the striatum is attenuation of IEG induction by psychostimulants (Steiner and Gerfen, 1995; see main text) or selective D1 receptor agonists (Steiner and Gerfen, 1996). Similar functional evidence exists for the role of enkephalin as a negative feedback mechanism to inhibit striatopallidal neurons (Fig. 29.3). Using IEG markers, we showed that, similar to dynorphin (kappa) agonists in striatonigral neurons (Steiner and Gerfen, 1995), stimulation of enkephalin (mu, delta) receptors in the striatum inhibits IEG induction (by glutamate, due to blockade of inhibitory D2 receptors) in striatopallidal neurons (Steiner and Gerfen, 1999). In addition, there is evidence for enkephalin-mediated inhibition of GABA release from striatopallidal terminals (Dewar et al., 1987; Maneuf et al., 1994). As mentioned above, enkephalin expression is robustly increased by experimental manipulations that disinhibit these neurons (e.g., loss of D2 receptor stimulation). Evidence indicates that increased enkephalin function, by "normalizing" striatopallidal function after chronic D2 receptor blockade, acts as a compensatory mechanism to allow some behavioral recovery (Steiner and Gerfen, 1999).

The above reviewed findings indicate that these opioid peptides serve as negative feedback systems to regulate the pathways they are contained in. [Note that this is likely not the only function of these peptides in the basal ganglia, as several other opioid effects have also been demonstrated, including an enkephalin (delta) regulation of striatal acetylcholine release (Mulder et al., 1984; Schoffelmeer et al., 1988), as well as dynorphin (kappa) effects on substantia nigra output neurons (e.g., Thompson and Walker, 1990).] However, it is interesting to note that a number of experimental manipulations that excessively activate other neuronal systems have also been found to produce increased opioid peptide expression in those systems. These include increased dynorphin expression in the spinal cord in response to nociceptive stimuli produced by peripheral inflammation and chronic arthritis (Ruda et al., 1995); in the hypothalamus by hyperosmotic treatment (salt loading; e.g., Lightman and Young, 1987); as well as in the hippocampus by seizures, where dynorphin is known to have anticonvulsant activity (e.g., Simmons and Chavkin, 1996; Simonato and Romualdi, 1997; Loacker et al., 2007). Therefore, opioid peptides may represent a general gain control mechanism that responds to perturbations of inputs with compensatory upregulation to maintain "healthy" activity levels in these neurons. Behavioral consequences of such gain control seem to range, for example, from anhedonia and depression in psychostimulant addiction (see main text) to pain control (analgesic effects of opioid receptor agonists), depending on the brain systems affected.

evidence to suggest that increased dynorphin function contributes to somatic signs of withdrawal, such as dysphoria, anxiety, anhedonia and depression, after discontinuation of drug use (Nestler and Carlezon, 2006). These effects are thought to contribute to maintenance of drug use or relapse during abstinence (for an excellent recent review on dynorphin function, see Shippenberg et al., 2007).

As mentioned earlier, in contrast to cocaine and amphetamine, only modest or no changes in dynorphin expression were seen after acute (Yano and Steiner, 2005b) and repeated methylphenidate treatments (Brandon and Steiner, 2003; Adriani et al., 2006) [treatments that, however, were sufficient to produce a number of other neuroadaptations (see Yano and Steiner, 2007, for review)]. Potential mechanisms underlying this differential effect and possible clinical relevance are discussed in Section V. Based on the above considerations, one would thus predict that lesser effects on dynorphin expression would indicate a lower addiction liability for this psychostimulant compared with cocaine and amphetamine.

## B. Blunted Gene Inducibility

A second well-established molecular consequence of repeated psychostimulant treatment is blunting of gene inducibility. That is, while genes are still induced after repeated drug treatment, this induction is often significantly reduced compared with induction by an acute drug administration. For example, early studies demonstrated blunted induction for several transcription factor IEGs (c-*fos*, *zif 268* etc.) in the striatum after repeated cocaine and amphetamine treatment (e.g., Hope et al., 1992; Persico et al., 1993; Steiner and Gerfen, 1993; Daunais and McGinty, 1994; Hope et al., 1994; Moratalla et al., 1996a). However, further studies showed that other genes are similarly affected, including, for example, the "effector" IEGs *arc* (Chase et al., 2007) and *homer 1a* (Unal et al., 2009), and the neuropeptide substance P (Steiner and Gerfen, 1993). Importantly, in contrast to the relative lack of effects on dynorphin expression (see above), repeated methylphenidate treatment also produces significant blunting of gene induction. This has been shown, for example, for c-*fos, zif 268, arc* and substance P (Brandon and Steiner, 2003; Chase et al., 2003; Hawken et al., 2004; Chase et al., 2007; Cotterly et al., 2007). On the other hand, *homer 1a* induction seems to be minimally affected by repeated methylphenidate treatment (Cotterly et al., 2007).

Blunting of gene inducibility is long-lasting. Thus, a recent study showed that even 3 weeks after a 5-day repeated cocaine treatment, *zif 268* and *homer 1a* induction by a cocaine challenge was still markedly blunted (Unal et al., 2009). Consistent with a compensatory neuroadaptation, the degree of blunting is directly correlated with the magnitude of the initial (acute) gene induction in a given striatal region (the greater the induction after the first drug administration, the more blunted the induction after chronic treatment; Cotterly et al., 2007; Unal et al., 2009).

Interestingly, there seems to be differential blunting in striatal patch (striosome) vs. matrix compartments (see Chapter 1) in some (but not other) striatal regions. Several studies showed that blunting is more pronounced in the matrix and thus results in a striosome-dominant gene expression pattern (Graybiel et al., 2000). The functional significance of this differential effect is not known, but has been interpreted as indicating relatively greater dampening of transstriatal activity through the matrix (sensorimotor activity) versus activity through the striosomes (projecting to the dopamine cell regions) (Graybiel et al., 2000). This phenomenon has been associated with the appearance of behavioral stereotypies across various drug treatments (Canales and Graybiel, 2000; Graybiel et al., 2000; Saka et al., 2004; see below), although it is presently unclear whether there is a causal relationship between the two (Canales, 2005), or whether they represent epiphenomena.

Several mechanisms may contribute to blunting of gene induction after repeated drug treatment, some shorterlasting, some long-lasting. Systems-level neuroadaptations as well as intracellular (epigenetic) adaptations have been proposed:

- 1. Given the importance of excitatory inputs for striatal gene regulation (see above), blunted gene induction may, at least in part, reflect dampened inputs from the cortex (and/or thalamus), perhaps involving long-term depression (LTD)-like synapse modifications (see Graybiel et al., 2000; Unal et al., 2009, for discussion).
- 2. Local striatal dynorphin levels appear to inhibit gene induction via direct and indirect mechanisms (dopamine, glutamate release; see Box 29.2). For one, acute IEG induction by cocaine is negatively correlated with the local levels of dynorphin mRNA expression (Steiner and Gerfen, 1993) and dynorphin (kappa opioid) receptor mRNA and binding (Mansour et al., 1994). Moreover, kappa receptor stimulation in the striatum inhibits cocaine- and D1 receptor-mediated IEG expression (Steiner and Gerfen, 1995, 1996). Increased dynorphin levels after repeated treatments may thus contribute to blunting of gene induction (see Steiner and Gerfen, 1998, for review).
- **3.** A mechanism that may best explain the endurance of psychostimulant effects such as blunted gene induction is epigenetic modification of gene regulation (e.g., Renthal et al., 2008; see Renthal and Nestler, 2008, for review). This includes chromatin remodeling, involving mechanisms such as histone acetylation and phosphorylation. These mechanisms are reviewed in detail in Chapter 30.

The exact consequences of blunted gene induction for basal ganglia function are presently unknown. However, the functional integrity of neurons depends on balanced regulation of gene expression as most cellular components have limited half-lives and need to be replenished. It is assumed that disruption of such homeostatic regulation by psychostimulants results in deficient neuronal function that contributes to behavioral manifestations of psychostimulant addiction (e.g., Hyman and Nestler, 1996; Nestler, 2001) (see also Section V).

# C. Alternative Splicing: Accumulation of deltaFosB

Another often described molecular change induced by psychostimulants is accumulation of the transcription factor deltaFosB in striatonigral neurons (McClung et al., 2004). DeltaFosB is a truncated isoform of FosB (member of AP-1 family of transcription factors), which is produced by alternative splicing (Nakabeppu and Nathans, 1991). This truncation renders this molecule highly stable. Therefore, with daily repeated drug treatments, deltaFosB accumulates in cells and displaces other members of the AP-1 family from the AP-1 transcriptional complex, thus altering the function of this complex (Nakabeppu and Nathans, 1991; McClung et al., 2004).

deltaFosB Psychostimulant-induced accumulation occurs in striatonigral neurons and is well-established for repeated cocaine and amphetamine treatments (Hope et al., 1994; Nye et al., 1995; Chen et al., 1997; Renthal et al., 2008). There is also evidence for increased deltaFosB levels in the striatum after repeated methylphenidate treatment (Chase et al., 2005a, 2005b). However, deltaFosB is not only induced by psychostimulants, but appears to be produced by many manipulations that involve excessive neuronal activation (McClung et al., 2004). In the basal ganglia, deltaFosB was first described after dopamine depletion (in disinhibited striatopallidal neurons) (Jian et al., 1993; Doucet et al., 1996). Similarly, deltaFosB accumulates in striatopallidal neurons during chronic treatment with antipsychotic drugs (D2 receptor blockers) (Hiroi and Graybiel, 1996; Atkins et al., 1999). Moreover, repeated L-DOPA treatment produces massive deltaFosB accumulation mostly in striatonigral neurons (Andersson et al., 1999; Andersson et al., 2003; see Chapter 36). Therefore, deltaFosB accumulation occurs in the same neurons that show other changes in gene regulation after repeated drug treatments.

Findings indicate that many genes are affected by this transcription factor (those with AP-1 and CRE binding sites in their promoter); some are activated, some are repressed, depending in part also on the length of the drug treatment (McClung and Nestler, 2003; McClung et al., 2004). One example is the dynorphin gene, which seems to be upregulated by deltaFosB action (Andersson et al., 1999; but see McClung et al., 2004).

What are the behavioral consequences of deltaFosB accumulation? One approach used to investigate the function of such transcription factors and their targets is overexpression (or silencing) of their genes with transgenic techniques (e.g., McClung and Nestler, 2003; McClung et al., 2004). While this is a successful approach to assess their molecular actions, it has been pointed out that, as these processes are tightly regulated by negative feedback mechanisms, it is sometimes difficult to differentiate between direct cellular effects of the transgenic manipulation and indirect, compensatory effects that occur as a response to the abnormal, prolonged perturbation of the normal expression of these genes (McClung et al., 2004). This caveat is even more important for the interpretation of behavioral effects of transgenic manipulations, as behavior is likely also affected by further compensatory adaptations at the systems level.

Reported behavioral consequences of upregulated deltaFosB function in the basal ganglia range from changes in the "behavioral sensitivity" to drugs in the case of psychostimulants (e.g., locomotor activation; McClung et al., 2004), to tardive dyskinesia after antipsychotic treatment (Atkins et al., 1999), to dyskinetic behavior in the case of L-DOPA (Andersson et al., 1999; Cenci, 2002; see Chapter 36), depending on which neurons and cortico-basal ganglia circuits are affected by these treatments.

# IV. TOPOGRAPHY OF PSYCHOSTIMULANT-INDUCED GENE REGULATION: SENSORIMOTOR CORTICOSTRIATAL CIRCUITS ARE MOSTLY AFFECTED

As mentioned above, the behavioral consequences of psychostimulant-induced molecular changes depend on the particular circuits altered by these drugs. Which corticostriatal circuits are affected? From the very first studies that assessed the distribution of psychostimulant-induced gene regulation in the striatum, it was clear that these effects differ dramatically between different striatal regions. This is true for cocaine (Fig. 29.1) (e.g., Moratalla et al., 1992; Steiner and Gerfen, 1993; Adams et al., 2001; Willuhn et al., 2003), amphetamine (Moratalla et al., 1992; Badiani et al., 1998; Adams et al., 2001; Uslaner et al., 2001a) as well as methylphenidate (Fig. 29.5) (Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007).

#### A. Mapping of Striatal Gene Regulation

In a series of studies, we investigated the corticostriatal circuits/functional domains affected by cocaine and methylphenidate. These studies employed a novel mapping approach with striatal sampling areas (sectors) that are based on their predominant cortical inputs (see Box 29.3). Using this approach, we showed that cocaine (Willuhn et al., 2003; Unal et al., 2009) and methylphenidate (Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007) affect very similar corticostriatal circuits. Moreover, as mentioned above, changes in gene regulation after acute and repeated psychostimulant treatment show a similar regional distribution. Such regionally correlated effects were demonstrated for acute IEG induction vs. increased dynorphin expression or blunted gene induction after repeated treatments (Brandon and Steiner, 2003; Willuhn et al., 2003; Cotterly et al., 2007; Unal et al., 2009).

Based on our studies (Steiner and Gerfen, 1993; Willuhn et al., 2003; Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007; Unal et al., 2009), the regional distribution of such gene regulation is summarized as follows:

- Both cocaine (Fig. 29.1) and methylphenidate (Fig. 29.5) produce the most robust changes in gene regulation in sensorimotor sectors of the middle and caudal striatum (e.g., Steiner and Gerfen, 1993; Willuhn et al., 2003; Yano and Steiner, 2005a, 2005b; Unal et al., 2009). A similar regional distribution has also been shown for amphetamine (e.g., Badiani et al., 1998).
- 2. In the sensorimotor striatum, maximal changes are present in the dorsal sectors (approximately the dorsal one-third). These sectors are unique in that they receive the densest input from the medial agranular cortex (M2, Fig. 29.4) (Reep et al., 1987; Berendse et al., 1992; Cowan and Wilson, 1994; Kincaid and Wilson, 1996; Reep et al., 2003), in addition to convergent inputs from the somatosensory (or visual) and primary motor cortex (e.g., Reep et al., 1987; McGeorge and Faull, 1989; Brown and Sharp, 1995; Alloway et al., 1999; Reep et al., 2003). Surrounding sectors that are, to some lesser extent, also targets of medial agranular projections (Reep et al., 1987; Berendse et al., 1992; Kincaid and Wilson, 1996; Zheng and Wilson, 2002; Reep et al., 2003), also consistently show changes in gene expression. The medial agranular cortex in the rat has mixed prefrontal/premotor features [e.g., connections with the mediodorsal as well as the ventral lateral and ventromedial thalamic nuclei; neurons that project to brain stem and spinal cord (Donoghue and Wise, 1982; Reep et al., 1987; Passingham et al., 1988; Berendse et al., 1992; Cowan and Wilson, 1994; Reep et al., 2003; see also Preuss, 1995; Uylings et al., 2003)], and

#### Box 29.3 Mapping of Affected Functional Domains in the Striatum

In a recent series of studies, we mapped the topography of gene regulation induced by acute and repeated treatments with cocaine (Willuhn et al., 2003; Willuhn and Steiner, 2006; Unal et al., 2009) and methylphenidate (Yano and Steiner, 2005a, 2005b; Yano et al., 2006; Cotterly et al., 2007) in the rat striatum (results described in the main text). In order to determine the corticostriatal circuits altered by these psychostimulants, we developed a mapping approach that uses striatal sampling areas (sectors) that are mostly defined by their predominant cortical inputs (Fig. 29.4), based on an extensive literature of tracttracing and functional mapping studies (see Willuhn et al., 2003). Thus, gene expression was measured in a total of 23 striatal sectors, 18 representing the caudate-putamen and 5 the nucleus accumbens (Fig. 29.4), in coronal sections from three standard rostrocaudal levels, rostral (at a midnucleus accumbens level), middle and caudal (at the level of the rostral globus pallidus).

While this dissection of the striatum into sectors with designated cortical input regions does reflect the general topographical organization of the corticostriatal projections (e.g., Webster, 1961; McGeorge and Faull, 1989; Berendse et al., 1992), these maps (Fig. 29.4) represent a gross

oversimplification of cortical inputs. For one, in order to facilitate reliable sampling, we designed simple sectors of a certain minimal size. Their outlines are sometimes adapted from published schematic illustrations of principal terminal fields (e.g., "the most densely labeled regions"; Berendse et al., 1992); however, the borders of these sectors are also to a variable degree arbitrary and do not describe exact or exclusive target areas of the connections shown (Fig. 29.4). Other cortical inputs are neglected altogether, for example, minor inputs from the visual and auditory cortex to dorsal/medial striatal sectors on these levels (Webster, 1961; Donoghue and Herkenham, 1986; Faull et al., 1986; McGeorge and Faull, 1989), and inputs from piriform and entorhinal cortex to ventral and medial striatal areas (e.g., McGeorge and Faull, 1989; Brog et al., 1993). Furthermore, while some corticostriatal neurons have relatively restricted terminal fields in specific striatal regions, other types have fairly widespread (but less dense) projections (e.g., Cowan and Wilson, 1994; Kincaid and Wilson, 1996; Wright et al., 1999; see for example Chapters 1 and 20). The chosen rostrocaudal levels appear to be far enough apart to separate major corticostriatal inputs, but many striatal sectors receive convergent inputs from functionally related



**FIGURE 29.4** Schematic illustration of the striatal sectors used for mapping gene expression and their main cortical inputs in the rat. Gene expression was measured in sections from four rostrocaudal levels, frontal ( $\pm 2.7$  mm rostral to bregma), rostral ( $\pm 1.6$ ), middle ( $\pm 0.4$ ) and caudal (-0.8) (for more details, see Willuhn et al., 2003; Yano and Steiner, 2005a, 2005b). The scatterplot (inset lower left) shows the relationship between methyl-phenidate-induced *zif 268* expression in individual striatal sectors and in their indicated cortical input regions (values averaged if more than one). Values are differences in gene expressed as the percentage of maximal increase in the striatum (see Yano and Steiner, 2005a). \*\*\*P < 0.001.

cortical areas distributed over a considerable rostrocaudal extent (e.g., McGeorge and Faull, 1989) and some overlap is thus likely still present [for simplicity, the arrows in these schemes (Fig. 29.4) often only connect cortical and striatal areas within a given rostrocaudal level].

In the face of the above limitations, how reliable do these sectors reflect their denoted cortical inputs? Psychostimulants also produce robust IEG induction in the cortex (Fig. 29.5) (e.g., Dilts et al., 1993; Johansson et al., 1994; Badiani et al., 1998; Yano and Steiner, 2005a). In order to functionally validate the above striatal dissection, we measured IEG induction by acute methylphenidate administration in 22 cortical areas on four rostrocaudal levels (frontal, rostral, middle, caudal; Fig. 29.4) and correlated the changes in these cortical areas with those in their designated striatal target sectors (areas connected by arrows in Fig. 29.4). Notably, dopamine

can thus be considered a prefrontal/motor interface. Our findings thus indicate that sensorimotor corticostriatal circuits under the influence of medial agranular (premotor) input are particularly prone to psychostimulant-induced neuroplasticity.

- **3.** Consistently also affected, albeit to a lesser degree, were medial and rostral striatal sectors ("associational" sectors). These receive inputs from several prefrontal regions including the cingulate, prelimbic and orbital cortex and others (e.g., Berendse et al., 1992; see also Chapter 20).
- **4.** Generally, small or no changes in gene regulation were seen in ventral striatal sectors that receive inputs mostly from the dorsal agranular insular cortex (e.g., Berendse et al., 1992), on all three rostrocaudal levels.
- 5. Well-appreciated are psychostimulant-induced molecular changes in the nucleus accumbens (e.g., Graybiel et al., 1990; Hope et al., 1992; Hope et al., 1994; for reviews, see Berke and Hyman, 2000; Nestler, 2001; Chapter 33), as these may result in altered motivational (reward) processes (Pierce and Kalivas, 1997). Such changes are likely of special importance for the initial stages of addiction (see Chapter 33), but probably also contribute to later events such as relapse to drug taking after abstinence (e.g., Conrad et al., 2008). However, while we consistently also observed gene regulation effects of cocaine (Willuhn et al., 2003; Unal et al., 2009) and methylphenidate (Brandon and Steiner, 2003; Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007) in the nucleus accumbens, these effects were typically very modest compared with those in the sensorimotor

agonists and psychostimulants increase gene expression across the whole cortical column (e.g., Steiner and Gerfen, 1994; LaHoste et al., 1996; Steiner and Kitai, 2000; Yano and Steiner, 2005a; Unal et al., 2009). Cortical gene expression was thus measured and averaged across all layers.

Our results show that the increases in IEG expression in our striatal sectors were indeed positively correlated with the increases in their designated cortical input regions (Fig. 29.4) (Yano and Steiner, 2005a; Cotterly et al., 2007). Similarly, methylphenidate-induced increases in substance P expression in these striatal sectors were positively correlated with IEG induction in the cortical input regions (Yano and Steiner, 2005a). These findings indicate that psychostimulant-induced gene regulation in cortical and striatal nodes of corticostriatal circuits is coordinated. Moreover, these results provide a functional validation of our dissection of the striatum.

striatum. Interestingly, the most robust effects were consistently seen in the lateral part of the shell (Fig. 29.5) (Brandon and Steiner, 2003; Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007; Unal et al., 2009), which also receives medial agranular input (Reep et al., 1987), in addition to inputs from the ventral agranular insular cortex (Berendse et al., 1992) and other limbic areas (e.g., McGeorge and Faull, 1989; Brog et al., 1993; Wright and Groenewegen, 1996). The functional significance of these lateral shell effects is currently unknown, but it is of interest to note that the insular cortex, one of the input regions of that part of the nucleus accumbens, is associated with craving in drug addiction (Naqvi et al., 2007), which often drives relapse.

While, as noted above, effects of cocaine and methylphenidate overall displayed a similar topography, some differences in their rostrocaudal distribution were observed. Cocaine effects (Fig. 29.1) peak in the middle-to-caudal (postcommissural) striatum (Willuhn et al., 2003; Unal et al., 2009), corresponding to the putamen, while those of methylphenidate (Fig. 29.5) tend to peak further rostrally (Yano and Steiner, 2005a, 2005b; Cotterly et al., 2007). Methylphenidate effects also tended to spread more into rostral and medial cortical areas (Fig. 29.5; Yano and Steiner, 2005a; Cotterly et al., 2007) than those of cocaine (Unal et al., 2009).

The bases for these marked differential effects between striatal regions, or the more subtle differences between cocaine and methylphenidate, are unclear. The latter are likely related to the differential neurochemical effects of these psychostimulants (see Yano and Steiner, 2007).



**FIGURE 29.5** Topography of *zif 268* induction by methylphenidate in corticostriatal circuits. On the left, illustrations of film autoradiograms depict *zif 268* expression in coronal sections from rostral (top), middle (center) and caudal striatal levels (bottom) at 0min (control; left half-brains) and 60min after injection of methylphenidate (MP, 5 mg/kg, i.p.; right halfbrains). On the right, maps show the increase in *zif 268* expression at 60min after methylphenidate injection (over 0min controls) in the different cortical areas and striatal sectors. The increases (P < 0.05 vs. 0min) are expressed as the percentage of the maximal increase (% of max.) in the striatum and are coded as indicated (modified from Yano and Steiner, 2005a).

Regarding the regional differences, given the importance of cortical (or thalamic) inputs for such gene regulation (see Box 29.1), it can be speculated that activity levels or patterns differ between different corticostriatal (or thalamostriatal) projections (see also following section). In addition, a number of local striatal factors likely also play a role. For example, as discussed above, local dynorphin action regulates such striatal responses. Moreover, striatal areas with moderate to low gene responses also display high levels of D3 receptor expression. D3 receptors are partly co-expressed with D1 receptors in direct pathway neurons (Le Moine and Bloch, 1996; Schwartz et al., 1998), but have opposite effects on second messenger signaling (Zhang et al., 2004), and thus dampen gene regulation effects of D1 receptor stimulation (Carta et al., 2000; Zhang et al., 2004). Future studies will have to elucidate the different mechanisms that govern such molecular effects - and thus the potential for neuroplasticity - in the different parts of the striatum.

# **B.** Relationship Between Cortical and Striatal Gene Regulation

Psychostimulants induce gene (IEG) expression also in the cortex (Fig. 29.5) (e.g., Graybiel et al., 1990; Dilts et al., 1993; Johansson et al., 1994; Steiner and Gerfen, 1994; Curran et al., 1996; Badiani et al., 1998; Yano and Steiner, 2005a; Unal et al., 2009). This IEG response presumably reflects increased synaptic activity (Chaudhuri, 1997), as it is at least in part the result of altered basal ganglia output (see Steiner, 2007, for review). For example, studies using striatal dopamine receptor antagonism showed that such cortical IEG induction is dependent on psychostimulant-mediated activation of the D1 receptor-regulated direct striatal output pathway and resulting facilitation of cortical activation (Steiner and Kitai, 2000; Yano et al., 2006; Gross and Marshall, 2009).

In order to determine whether or not cortical and striatal gene regulation is related (and to functionally validate our striatal dissection), we measured psychostimulant-induced IEG expression throughout the major functional subdivisions of the cortex (22 areas on four rostrocaudal levels; Fig. 29.4) and compared the effects in these cortical regions with those in their striatal target sectors (see Box 29.3). Our findings demonstrate that IEG induction (zif 268, *homer 1a*) by acute methylphenidate was indeed positively correlated between cortical areas and their striatal target sectors (Fig. 29.4; Yano and Steiner, 2005a; Cotterly et al., 2007). Moreover, IEG induction in these cortical areas was also correlated with increases in substance P and dynorphin expression (direct pathway), but not with enkephalin expression (indirect pathway), in their striatal target sectors (Yano and Steiner, 2005a). Interestingly, no correlation between cortical and striatal gene induction was found after repeated methylphenidate treatment (Cotterly et al., 2007).

These findings demonstrate that:

- 1. Acute psychostimulants alter gene regulation in specific corticostriatal circuits; molecular changes in cortical areas and their striatal target sectors are coordinated.
- **2.** Despite the fact that (some) corticostriatal projections are fairly widespread (see Box 29.3), these results indicate that corticostriatal projections do respect general functional domains (e.g., sensorimotor), as delineated by the early tract-tracing studies (see Box 29.3).
- **3.** Repeated psychostimulant treatments appear to disrupt such coordinated cellular responses between the cortex and the striatum (Cotterly et al., 2007). It remains to be
seen whether this latter dysregulation reflects a dissociation of neuronal activity and/or of molecular responses between cortical and striatal nodes of corticostriatal circuits (see Cotterly et al., 2007, for discussion). Future studies will have to determine the functional significance of this corticostriatal dysregulation after repeated psychostimulant treatment.

# V. FUNCTIONAL CONSEQUENCES OF PSYCHOSTIMULANT-INDUCED MOLECULAR CHANGES IN THE STRIATUM

What are the behavioral consequences of psychostimulantinduced molecular changes in corticostriatal circuits? It is now accepted that cellular and molecular changes in these circuits are critical for psychostimulant addiction. The various brain functions and dysfunctions involved in addiction (e.g., reward processes, habit formation, compulsion) all depend on specific trans-striatal circuits. How are the psychostimulant-induced molecular changes in these circuits related to addiction? Psychostimulant effects on motivational (reward) processes are addressed in Chapter 33, as are effects on habit formation. The following sections present two examples of behavioral changes that are most clearly linked to neuronal changes in the sensorimotor striatal circuits that display the most robust gene regulation effects. These are, first, behavioral stereotypies, a longknown consequence of such drug treatments, which may be related to compulsions, and second, altered procedural learning, an effect that has received more recent attention because of its potential relevance for habit formation in drug addiction (see Chapter 33). The last section presents, as an example for possible clinical relevance, implications on the addiction liability of certain prescription medications, based on their effects on gene regulation.

# A. Behavioral Stereotypies

The most obvious behavioral changes associated with the changes in striatal gene regulation after repeated cocaine (Unal et al., 2009) and methylphenidate treatment (Cotterly et al., 2007) that we observed were increased behavioral "stereotypies". In rodents, the best-established behavioral effects of repeated dopamine agonist or psychostimulant treatments include increased locomotor activity and, especially with higher doses, appearance of highly repetitive behaviors, termed behavioral stereotypies (e.g., Ellinwood and Balster, 1974; Kalivas and Stewart, 1991; Kuczenski

and Segal, 1999). Stereotypies denotes compulsive repetition of specific behavioral elements without apparent purpose. In our studies (Cotterly et al., 2007; Unal et al., 2009), the stereotypies mostly involved episodes of focused, repetitive whisking/sniffing combined with head movements (head bobbing), interspersed with stereotypical locomotion patterns (ambulation along the wall of the activity chamber without interruption for extended periods of time). [Increased locomotor counts after repeated psychostimulant treatments are most often described as "behavioral sensitization". However, as the emitted locomotor patterns are different from normal locomotion (i.e., more repetitive, "stereotypical"), they are perhaps better understood, functionally, as another form of stereotypies.]

Several lines of evidence have implicated sensorimotor striatal circuits in behavioral stereotypies. For one, previous work demonstrated that the mid- to caudal sensorimotor regions of the striatum that showed the pronounced changes in gene regulation in our studies (see above) display neck/trunk, limb, and whisker-related neuronal activities (e.g., West et al., 1990; Carelli and West, 1991; Brown, 1992; Brown and Sharp, 1995; Brown et al., 1998). Moreover, it has also been shown that such striatal responses correlate with psychostimulant-induced focused stereotypies (e.g., Rebec et al., 1997; White et al., 1998). While it is likely that multiple brain areas are involved in the generation/execution of such stereotypical behaviors, a critical role for basal ganglia systems is demonstrated by the finding that stereotypies can be induced or inhibited by drugs locally administered into basal ganglia nuclei (e.g., Newman et al., 1997; Canales et al., 2000; Steiner and Kitai, 2000).

Molecular changes in sensorimotor striatal regions have been directly related to such behavioral deficits. Thus, as mentioned above, a strong association between behavioral stereotypies and blunting of gene induction preferentially in the matrix has been demonstrated (Canales and Graybiel, 2000; Graybiel et al., 2000; Saka et al., 2004). This has been taken to indicate that "dampened" sensorimotor corticostriatal activity and/or striatal output through the matrix after repeated psychostimulant treatments (see Graybiel et al., 2000; Cotterly et al., 2007, for discussion) might underlie this behavioral dysfunction.

What basal ganglia dysfunction do behavioral stereotypies reflect? Cortico-basal ganglia-cortical loops play a critical role in selection and switching of motor actions (and thoughts) (e.g., Mink, 1996; Redgrave et al., 1999; see Chapter 31), and behavioral stereotypies can be interpreted as a switching dysfunction (Redgrave and Gurney, 2006). It has also been noted that such stereotypies share both phenomenological characteristics and neuronal substrates with motor compulsions in humans (Graybiel and Rauch, 2000). If not mechanistically related to compulsions, stereotypies may thus reflect similar basal ganglia output deficiencies. Future studies will have to clarify the exact relationship between psychostimulant-induced stereotypies and compulsive behavior and their underlying neuronal dysfunctions.

# **B.** Effects of Psychostimulants on Procedural Learning – Role in Addiction?

Recent interest in psychostimulant-induced molecular changes in the striatum was sparked by the proposal that addiction to psychostimulants, which entails habitual or compulsive drug taking, may involve aberrant procedural (habit) learning, as a consequence of excessive dopamine receptor stimulation and ensuing molecular adaptations in sensorimotor striatal neurons (White, 1996; Robbins and Everitt, 1999; Berke and Hyman, 2000; Everitt et al., 2001) (see Chapter 33). This notion is based in part on the finding that psychostimulants often affect the same molecular mechanisms that are known to mediate learning and memory (Berke and Hyman, 2000; Nestler, 2001; Kelley, 2004). For example, IEGs such as c-fos and zif 268 are also involved in processes of learning and memory consolidation (e.g., Stork and Welzl, 1999; Davis et al., 2003; Kelley, 2004; Izquierdo et al., 2006), while synaptic scaffolding proteins such as homer are thought to directly regulate synaptic efficacy (Xiao et al., 2000; Thomas, 2002), presumably a mechanism of long-term memory formation.

The sensorimotor striatum has long been implicated in procedural learning, including habit (stimulus-response, S-R) learning and motor-skill learning (e.g., Mishkin et al., 1984; Graybiel, 1995; Knowlton et al., 1996; Packard and Knowlton, 2002; see Chapter 32). For example, lesions or transient inactivation of the sensorimotor striatum have been shown to disrupt instrumental learning in maze and lever-press tasks (e.g., Packard and McGaugh, 1992; Yin et al., 2004; Featherstone and McDonald, 2005). Similarly, motor-skill learning, another form of procedural learning (Squire, 1987), is also dependent on normal function of the sensorimotor striatum (e.g., Ogura et al., 2005; Akita et al., 2006; Dang et al., 2006). The cellular and molecular processes underlying such learning, however, remain poorly understood. Evidence indicates that striatal dopamine regulates such processes. Thus, dopamine receptor agonists

infused into the striatum modified procedural maze learning (Packard and White, 1991; Packard et al., 1994). Conversely, moderate striatal dopamine depletion impaired skill learning in a rotarod task (Ogura et al., 2005; Akita et al., 2006).

What molecular mechanisms in the striatum mediate procedural learning and where do they occur? How are these molecular processes affected by psychostimulants? We have recently used the IEGs c-fos and homer 1a as markers to map motor-skill learning-associated molecular changes throughout the striatum, and we determined their modification by cocaine (Willuhn et al., 2003; Willuhn and Steiner, 2005, 2006). In these studies, we employed a novel motor-skill learning paradigm in which rats learn to run on a running wheel, a motor skill that they acquire within 1-2 trials and that lasts for weeks to months (Willuhn and Steiner, 2006, 2008; see Box 29.4). Our results show that this wheel-skill learning is associated with transiently increased gene expression (IEGs, substance P) in parts of the sensorimotor striatum, and that this gene regulation is abnormally enhanced when rats train under the influence of cocaine (Willuhn et al., 2003; Willuhn and Steiner, 2005, 2006). Further studies showed that, consistent with the known mechanisms of procedural learning and psychostimulant-induced gene regulation (see above), both wheel-skill learning (Fig. 29.6) and associated molecular changes are mediated by dopamine tone at the D1 receptor in the striatum and are modified by cocaine (see Box 29.4). Especially, long-term consolidation of this skill memory was critically dependent on D1 receptor-mediated processes (Willuhn and Steiner, 2008). Furthermore, as is the case for other memory consolidation (cf. Willuhn and Steiner, 2009), our findings also showed that post-training processes in the striatum are necessary for such skill-memory consolidation to occur, and that these processes are also regulated by cocaine (Willuhn and Steiner, 2009). Taken together, these findings indicate that, similar to other forms of learning and memory formation (Stork and Welzl, 1999; Izquierdo et al., 2006), such procedural learning involves altered gene expression, probably for synapse modification or other cellular plasticity in the sensorimotor striatum, and that this gene regulation is mediated by dopamine (D1 receptor) and can thus be modified by psychostimulants.

In summary, our findings indicate that the effects of psychostimulants such as cocaine on procedural learning are complex, as they interfere with multiple processes of learning and memory consolidation. Future studies will have to elucidate if and how such mechanisms of motor learning contribute to the progression from casual to habitual/compulsive drug use.

#### Box 29.4 Effects of Cocaine on Procedural Learning in a Motor-Skill Paradigm

It has been proposed that psychostimulant-induced "aberrant" procedural learning plays a role in psychostimulant addiction (see main text). We have investigated how psychostimulants (cocaine) affect processes of procedural learning in a striatum-dependent learning paradigm – wheel-skill learning in rats – which offers a number of advantages for investigation of drug effects on procedural learning (see below).

What is learned in this task? Naïve rats are unable to run on a running wheel with an appropriate speed such as to remain at the bottom of the wheel; running thus causes the wheel to swing (Fig. 29.6). During training rats learn to control/balance the wheel in order to avoid such swinging (wheel skill) (Willuhn and Steiner, 2006, 2008). As is typical for skill learning (cf., Willuhn and Steiner, 2008), this motor skill is acquired fast; even 1–2 training sessions are sufficient to produce long-term skill memory that lasts for weeks to months (Fig. 29.6; Willuhn and Steiner, 2008, 2009). In this task, the effects of drugs (given before, during or after the training) on learning are assessed by comparing the motor response after the training with the pre-training response.

Advantages of this task over other paradigms used to investigate processes of procedural learning include the following. First, in contrast to other learning tasks, wheel-skill learning is not dependent on motivational manipulations such as food deprivation (e.g., used in instrumental learning), electric shocks (e.g., in avoidance learning), or forced locomotion (e.g., skill learning on rotarod), because rats voluntarily



**FIGURE 29.6** Motor-skill learning in a running-wheel paradigm. A, Video stills show a rat committing a running error (left) and a rat running at the bottom of the wheel after some training (right). Running errors are measured by counting the number of interruptions by the rat's body of an imaginary horizontal plane at one third of the wheel diameter above the bottom (arrow; see Willuhn and Steiner, 2008, for details). B, The wheel skill is long-lasting. The number (mean  $\pm$  SEM) of running errors (in percent of pretraining values) is depicted for rats tested before (pre) and repeatedly after (post) a 2-day training (60-min sessions). The rats were retrained on days 130 and 131 after the initial training. C, Effects of systemic blockade of D1 receptors with cocaine (bottom) or without cocaine (top) during a 2-day training on running-wheel learning. Rats received a systemic injection of vehicle (SCH0),  $3\mu g/kg$  (SCH3) or  $10\mu g/kg$  (SCH10) of the D1 receptor antagonist SCH-23390 either alone or followed by an injection of cocaine (25 mg/kg) before each 40-min training session and were tested on days 1, 6, 18 and 26 after the training. D, Effects of intrastriatal blockade of D1 receptors plus systemic cocaine during the training on running-wheel learning. Rats received an intrastriatal (is) infusion of the D1 receptor antagonist SCH-23390 (0, 0.3 or  $1\mu g$ ) followed by cocaine (25 mg/kg) before each training session and were tested on days 1, 13 and 27 after the training. The p values for the overall training effect (Friedman test) in individual groups is also shown \*\**P* < 0.01, \**P* < 0.05 vs. pretraining values. Modified from Willuhn and Steiner, 2008.

run on wheels (Sherwin, 1998). This minimizes potential confounds by factors such as stress. Second, as mentioned above, long training periods are unnecessary for robust motor memory formation (Fig. 29.6). This facilitates dissociation of molecular changes related to learning from those produced by repeated drug exposure (during the training). Third, the motor response after the training can be assessed in a drug-free state, thus avoiding potential confounds by acute drug effects on behavior.

Using this task, we found that this wheel-skill learning is associated with gene regulation predominantly in parts of the sensorimotor striatum that display neuronal responses during body/leg movements (see main text). The affected genes included c-fos, zif 268, homer 1a and substance P, but not dynorphin and enkephalin (Willuhn et al., 2003; Willuhn and Steiner, 2005, 2006). "Basal" expression of some of these genes was transiently increased (for at least 24h) after the training. Moreover, these learning-related molecular changes were abnormally enhanced when the wheel-skill training occurred under the influence of cocaine (Willuhn et al., 2003; Willuhn and Steiner, 2005, 2006). Thus, one day after training under the influence of cocaine, gene induction by a cocaine challenge was greatly potentiated, as compared to cocaine-treated, untrained controls (Willuhn et al., 2003; Willuhn and Steiner, 2005, 2006). This differential gene response was maximal at the beginning of the training and disappeared by day 8 of the training; it was thus associated with the learning phase (see above) and not with running (exercising) per se (Willuhn and Steiner, 2005, 2006). Besides enhancing learning-related gene regulation in specific striatal regions, cocaine also produced a more widespread distribution of such learning-associated molecular changes (Willuhn and Steiner, 2005, 2006). Together, these findings indicate that such procedural learning involves gene regulation in striatal neurons similar to learning-related gene regulation in other brain systems (e.g., Stork and Welzl, 1999; Izquierdo et al., 2006), and that cocaine enhances such gene regulation, but also abnormally widens it. These findings are consistent with the notion that psychostimulants modify processes of procedural learning to produce aberrant learning (see Chapter 33).

What cellular mechanisms are affected? In a series of followup studies, we investigated the processes that mediate this form of procedural learning. Our findings are summarized as follows: (1) This skill learning is dependent on optimal stimulation of D1 receptors (inverted U function) (Fig. 29.6). Thus, either decreasing dopamine tone at the D1 receptor (by low doses of the D1 receptor antagonist SCH-23390, which did not inhibit the amount of running), or abnormally increasing dopamine tone (by cocaine) during the training tended to impair wheel-skill learning (Willuhn and Steiner, 2008). In contrast, combining cocaine and a low dose of the D1 receptor antagonist restored normal learning of this skill. (2) Intrastriatal administration of the D1 receptor antagonist blocked long-term skill-memory consolidation (Fig. 29.6; Willuhn and Steiner, 2008), as well as the associated gene regulation (see above), demonstrating the critical role for D1 receptor-mediated molecular changes in the striatum in cellular plasticity subserving long-term consolidation. (3) Further studies showed that not only neuronal activity during the training, but also post-training processing in the sensorimotor striatum is critical for skill-memory formation. Consolidation of long-term memory was also blocked by post-trial intrastriatal interference (lidocaine infusion) (Willuhn and Steiner, 2009). (4) Interestingly, cocaine prevented this post-trial disruption of consolidation (i.e., stabilized consolidation) (Willuhn and Steiner, 2009), possibly by enhancing neuronal processes and rendering them less vulnerable to interference (see Willuhn and Steiner, 2009, for discussion).

Taken together, these findings indicate that cocaine abnormally enhances D1 receptor-mediated cellular processes of procedural learning in striatal neurons. These processes include a transient enhancement of gene inducibility, which seems to enable consolidation of long-term memory by facilitating gene expression related to synapse modifications and/ or other forms of cellular plasticity (see Willuhn and Steiner, 2006, for discussion). It remains to be seen whether similar psychostimulant-induced cellular changes are important for aberrant procedural learning in addiction.

# C. Facilitatory Role of Serotonin in Striatal Gene Regulation: Possible Clinical Implications

For cocaine and amphetamine, there is little doubt that changes in gene regulation produced by these drugs underlie addiction. Only such changes endure long enough to mediate behavioral pathologies that can last for a lifetime, such as addiction (Renthal and Nestler, 2008) (see also Chapter 30). However, other psychostimulants are commonly used as medications, and their potential for addiction therefore most likely also depends on their propensity to induced gene regulation. As discussed earlier, the likelihood for gene regulation effects rests on the particular neurochemical effects of a given drug. This section presents an example for the assessment of the addiction liability of prescription medications based on their neurochemical actions.

As described in Chapter 27 by Keefe and Horner, several neurotransmitters interact to regulate striatal gene expression. Recent evidence indicates that, in addition to dopamine and glutamate (see Section II), the role of serotonin is of special interest regarding the clinical implications of psychostimulant-induced gene regulation. Previous studies showed that serotonin contributes significantly to various behavioral effects of cocaine (for reviews, see Filip et al., 2005; Muller and Huston, 2006; Carey et al., 2008). Similarly, while dopamine is critical for cocaine-induced gene regulation in the striatum (see above), serotonin facilitates such effects (Bhat and Baraban, 1993). Thus, attenuation of the serotonin transmission by transmitter depletion (Bhat and Baraban, 1993), receptor antagonism (Lucas et al., 1997; Castanon et al., 2000), or receptor deletion (Lucas et al., 1997) reduces IEG induction by cocaine in the striatum. Conversely, direct and indirect serotonin receptor agonists increase striatal IEG expression (Li and Rowland, 1993; Torres and Rivier, 1994; Wirtshafter and Cook, 1998; Gardier et al., 2000). Serotonin also regulates the expression of the opioid peptides dynorphin and enkephalin (e.g., Morris et al., 1988; Walker et al., 1996; Mijnster et al., 1998), and contributes significantly to cocaine-induced dynorphin expression in the striatum (Horner et al., 2005).

The role of serotonin in gene regulation appears to be important for understanding the potential of methylphenidate (Ritalin) - a psychostimulant with wide medical use (see below) - to induce molecular changes, and thus its addiction liability. Methylphenidate differs from cocaine in that it has a very low affinity for the serotonin transporter (Schweri et al., 1985; Gatley et al., 1996), and, in contrast to cocaine, it does not produce serotonin overflow (Kuczenski and Segal, 1997; Kankaanpaa et al., 2002; Borycz et al., 2008; for review, see Yano and Steiner, 2007). Recent studies show that, while sufficient to produce robust changes in the expression of IEGs (Lin et al., 1996; Penner et al., 2002; Brandon and Steiner, 2003; Chase et al., 2003; Yano and Steiner, 2005a, 2005b) and substance P (Brandon and Steiner, 2003; Yano and Steiner, 2005b) in the striatum, methylphenidate had only modest or no effects on dynorphin and enkephalin expression (Brandon and Steiner, 2003; Yano and Steiner, 2005b; Adriani et al., 2006). This latter effect differs markedly from those of cocaine and amphetamine, which produce significant changes in opioid peptide expression (see above; Yano and Steiner, 2007). This finding may indicate a reduced addiction liability for methylphenidate as compared with these other psychostimulants.

Is this differential gene regulation due to the lack of methylphenidate effects on the serotonin transmission? Would enhancing the serotonin transmission in conjunction with methylphenidate enhance its molecular effects and make them more "cocaine-like"? Recent findings indicate that this is indeed the case. Thus, adding a serotonin-enhancing drug – the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Prozac) – to methylphenidate treatment potentiated both methylphenidate-induced behavioral activation (Borycz et al., 2008) and gene regulation in the striatum (Steiner et al., 2010).

The potential significance of these findings relates to the medical use of methylphenidate and fluoxetine. Methylphenidate is an effective and often prescribed medication to control the symptoms of attention-deficit hyperactivity disorder (ADHD) (Biederman et al., 2007; Kollins, 2008; Swanson and Volkow, 2008), but this psychostimulant is also abused for recreational purposes (Kollins, 2008; Swanson and Volkow, 2008; Wilens et al., 2008) and sometimes promoted as a so-called "cognitive enhancer" (Greely et al., 2008). SSRIs such as fluoxetine, on the other hand, are among the first-line treatments for depressive and anxiety disorders and others (Petersen et al., 2002) and are given to millions of patients in the US alone every year. There is concern that spread of methylphenidate and SSRI use will lead to increased accidental co-exposure to these drugs, which would be expected to enhance the likelihood for drug-induced molecular changes (see above). While evidence is lacking that proper medical use (oral administration of low doses) of methylphenidate alone facilitates subsequent substance use disorder/psychostimulant addiction (Barkley et al., 2003; Wilens et al., 2003; but see Volkow and Insel, 2003; Kollins, 2008), potentiated molecular changes due to co-exposure with SSRIs may increase the addiction liability. Future studies will have to assess the risk for substance use disorder produced by psychostimulant/SSRI co-exposure.

# VI. SUMMARY AND CONCLUSIONS

The studies summarized in this review demonstrate that psychostimulants alter the expression of perhaps hundreds of genes in the cortex and the basal ganglia (Berke et al., 1998; McClung and Nestler, 2003; Yuferov et al., 2003; Konradi et al., 2004; Black et al., 2006). These include effector genes such as those encoding neuropeptide transmitters that modulate basal ganglia circuit activity in several nuclei, but also transcription factors that regulate the expression of other genes, as well as a variety of other neuroplasticity-related molecules. The findings of numerous studies show that, in the striatum, psychostimulants affect gene regulation predominantly in neurons that give rise to the direct striatal output pathway, with lesser to minimal molecular effects in the indirect pathway. Importantly, the relative impact on these two pathways is to some degree dependent on cortex-activating contextual variables (e.g., arousal). For the last two decades, much effort in addiction research has been devoted to understanding the significance of molecular changes in motivation-related (limbic) striatal domains. However, molecular imaging studies demonstrate that psychostimulant-induced gene regulation is considerably more pronounced in associational and, especially, sensorimotor corticostriatal domains. Future work will have to determine the functional consequences of such changes in these latter domains. However, studies indicate that these molecular changes occur in striatal areas associated with switching functions (for motor acts, thoughts), procedural learning and compulsion. They may thus underlie aberrant habit formation and compulsive behavior that signify drug addiction.

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# REFERENCES

- Adams DH, Hanson GR, Keefe KA (2001) Differential effects of cocaine and methamphetamine on neurotensin/neuromedin N and preprotachykinin messenger RNA expression in unique regions of the striatum. Neuroscience 102:843–851.
- Adams DH, Hanson GR, Keefe KA (2003) Distinct effects of methamphetamine and cocaine on preprodynorphin messenger RNA in rat striatal patch and matrix. J Neurochem 84:87–93.
- Adriani W, Leo D, Greco D, Rea M, di Porzio U, Laviola G, Perrone-Capano C (2006) Methylphenidate administration to adolescent rats determines plastic changes in reward-related behavior and striatal gene expression. Neuropsychopharmacology 31:1946–1956.
- Akita H, Ogata M, Jitsuki S, Ogura T, Oh-Nishi A, Hoka S, Saji M (2006) Nigral injection of antisense oligonucleotides to synaptotagmin I using HVJ-liposome vectors causes disruption of dopamine release in the striatum and impaired skill learning. Brain Res 1095:178–189.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381.
- Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119–146.
- Alloway KD, Crist J, Mutic JJ, Roy SA (1999) Corticostriatal projections from rat barrel cortex have an anisotropic organization that correlates with vibrissal whisking behavior. J Neurosci 19:10908–10922.
- Andersson M, Hilbertson A, Cenci MA (1999) Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis 6:461–474.

- Andersson M, Westin JE, Cenci MA (2003) Time course of striatal DeltaFosB-like immunoreactivity and prodynorphin mRNA levels after discontinuation of chronic dopaminomimetic treatment. Eur J Neurosci 17:661–666.
- Atkins JB, Chlan-Fourney J, Nye HE, Hiroi N, Carlezon WAJ, Nestler EJ (1999) Region-specific induction of deltaFosB by repeated administration of typical versus atypical antipsychotic drugs. Synapse 33:118–128.
- Bacci JJ, Kachidian P, Kerkerian-Le Goff L, Salin P (2004) Intralaminar thalamic nuclei lesions: widespread impact on dopamine denervationmediated cellular defects in the rat basal ganglia. J Neuropathol Exp Neurol 63:20–31.
- Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol 15:327–339.
- Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1998) Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: modulation by environmental novelty. J Neurosci 18:10579–10593.
- Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1999) Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. Behav Brain Res 103:203–209.
- Barkley RA, Fischer M, Smallish L, Fletcher K (2003) Does the treatment of attention-deficit/hyperactivity disorder with stimulants contribute to drug use/abuse? A 13-year prospective study. Pediatrics 111:97–109.
- Beckstead RM, Kersey KS (1985) Immunohistochemical demonstration of differential substance P-, Met-enkephalin-, and glutamic acid decarboxylase-containing cell and axon distributions in the corpus striatum of the cat. J Comp Neurol 232:481–498.
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314–347.
- Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25:515–532.
- Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR (1998) A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci 18:5301–5310.
- Berretta S, Robertson HA, Graybiel AM (1992) Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. J Neurophysiol 68:767–777.
- Berretta S, Parthasarathy HB, Graybiel AM (1997) Local release of GABAergic inhibition in the motor cortex induces immediateearly gene expression in indirect pathway neurons of the striatum. J Neurosci 17:4752–4763.
- Beveridge TJ, Smith HR, Daunais JB, Nader MA, Porrino LJ (2006) Chronic cocaine self-administration is associated with altered functional activity in the temporal lobes of non human primates. Eur J Neurosci 23:3109–3118.
- Bhat RV, Baraban JM (1993) Activation of transcription factor genes in striatum by cocaine: role of both serotonin and dopamine systems. J Pharmacol Exp Ther 267:496–505.
- Biederman J, Wilens TE, Spencer TJ, Adler LA (2007) Diagnosis and treatment of adults with attention-deficit/hyperactivity disorder. CNS Spectr 12:1–15.
- Black YD, Maclaren FR, Naydenov AV, Carlezon WAJ, Baxter MG, Konradi C (2006) Altered attention and prefrontal cortex gene expression in rats after binge-like exposure to cocaine during adolescence. J Neurosci 26:9656–9665.

- Borycz J, Zapata A, Quiroz C, Volkow ND, Ferre S (2008) 5-HT(1B) receptormediated serotoninergic modulation of methylphenidate-induced locomotor activation in rats. Neuropsychopharmacology 33:619–626.
- Brandon CL, Steiner H (2003) Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. Eur J Neurosci 18:1584–1592.
- Breiter HC, Gollub RL, Weisskoff RM, et al. (1997) Acute effects of cocaine on human brain activity and emotion. Neuron 19:591–611.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol 338:255–278.
- Brown LL (1992) Somatotopic organization in rat striatum: evidence for a combinational map. Proc Natl Acad Sci USA 89:7403–7407.
- Brown LL, Sharp FR (1995) Metabolic mapping of rat striatum: somatotopic organization of sensorimotor activity. Brain Res 686:207–222.
- Brown LL, Smith DM, Goldbloom LM (1998) Organizing principles of cortical integration in the rat neostriatum: corticostriate map of the body surface is an ordered lattice of curved laminae and radial points. J Comp Neurol 392:468–488.
- Brownstein MJ, Mroz EA, Tappaz ML, Leeman SE (1977) On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res 135:315–323.
- Canales JJ (2005) Intermittent cortical stimulation evokes sensitization to cocaine and enduring changes in matrix and striosome neuron responsiveness. Synapse 57:56–60.
- Canales JJ, Graybiel AM (2000) A measure of striatal function predicts motor stereotypy. Nat Neurosci 3:377–383.
- Canales JJ, Gilmour G, Iversen SD (2000) The role of nigral and thalamic output pathways in the expression of oral stereotypies induced by amphetamine injections into the striatum. Brain Res 856:176–183.
- Carelli RM, West MO (1991) Representation of the body by single neurons in the dorsolateral striatum of the awake, unrestrained rat. J Comp Neurol 309:231–249.
- Carey RJ, Huston JP, Müller CP (2008) Pharmacological inhibition of dopamine and serotonin activity blocks spontaneous and cocaineactivated behaviour. Prog Brain Res 172:347–360.
- Carta AR, Gerfen CR, Steiner H (2000) Cocaine effects on gene regulation in the striatum and behavior: increased sensitivity in D3 dopamine receptor-deficient mice. Neuroreport 11:2395–2399.
- Castanon N, Scearce-Levie K, Lucas JJ, Rocha B, Hen R (2000) Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists. Pharmacol Biochem Behav 67:559–566.
- Cenci MA (2002) Transcription factors involved in the pathogenesis of L -DOPA-induced dyskinesia in a rat model of Parkinson's disease. Amino Acids 23:105–109.
- Cenci MA, Björklund A (1993) Transection of corticostriatal afferents reduces amphetamine- and apomorphine-induced striatal Fos expression and turning behaviour in unilaterally 6-hydroxydopaminelesioned rats. Eur J Neurosci 5:1062–1070.
- Cenci MA, Campbell K, Wictorin K, Björklund A (1992) Striatal c-*fos* induction by cocaine or apomorphine occurs preferentially in output neurons projecting to the substantia nigra in the rat. Eur J Neurosci 4:376–380.
- Chase T, Carrey N, Soo E, Wilkinson M (2007) Methylphenidate regulates activity regulated cytoskeletal associated but not brain-derived neurotrophic factor gene expression in the developing rat striatum. Neuroscience 144:969–984.

- Chase TD, Brown RE, Carrey N, Wilkinson M (2003) Daily methylphenidate administration attenuates c-fos expression in the striatum of prepubertal rats. Neuroreport 14:769–772.
- Chase TD, Carrey N, Brown RE, Wilkinson M (2005a) Methylphenidate differentially regulates c-fos and fosB expression in the developing rat striatum. Dev Brain Res 157:181–191.
- Chase TD, Carrey N, Brown RE, Wilkinson M (2005b) Methylphenidate regulates c-fos and fosB expression in multiple regions of the immature rat brain. Dev Brain Res 156:1–12.
- Chaudhuri A (1997) Neural activity mapping with inducible transcription factors. Neuroreport 8:v-ix.
- Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: Stable variants of deltaFosB Induced in brain by chronic treatments. J Neurosci 17:4933–4941.
- Cole AJ, Bhat RV, Patt C, Worley PF, Baraban JM (1992) D<sub>1</sub> dopamine receptor activation of multiple transcription factor genes in rat striatum. J Neurochem 58:1420–1426.
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118–121.
- Conversi D, Bonito-Oliva A, Orsini C, Cabib S (2006) Habituation to the test cage influences amphetamine-induced locomotion and Fos expression and increases FosB/DeltaFosB-like immunoreactivity in mice. Neuroscience 141:597–605.
- Cotterly L, Beverley JA, Yano M, Steiner H (2007) Dysregulation of gene induction in corticostriatal circuits after repeated methylphenidate treatment in adolescent rats: Differential effects on zif 268 and homer 1a. Eur J Neurosci 25:3617–3628.
- Cowan RL, Wilson CJ (1994) Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. J Neurophysiol 71:17–32.
- Curran EJ, Watson SJ (1995) Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: a double in situ hybridization study. J Comp Neurol 361:57–76.
- Curran EJ, Akil H, Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. Neurochem Res 21:1425–1435.
- Dang MT, Yokoi F, Yin HH, Lovinger DM, Wang Y, Li Y (2006) Disrupted motor learning and long-term synaptic plasticity in mice lacking NMDAR1 in the striatum.. Proc Natl Acad Sci USA 103:15254–15259.
- Daunais JB, McGinty JF (1994) Acute and chronic cocaine administration differentially alters striatal opioid and nuclear transcription factor mRNAs. Synapse 18:35–45.
- Davis S, Bozon B, Laroche S (2003) How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? Behav Brain Res 142:17–30.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Dewar D, Jenner P, Marsden CD (1987) Effects of opioid agonist drugs on the in vitro release of 3H-GABA, 3H-dopamine and 3H-5HT from slices of rat globus pallidus. Biochem Pharmacol 36:1738–1741.
- Di Chiara G, Imperato A (1988a) Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J Pharmacol Exp Ther 244:1067–1080.

- Di Chiara G, Imperato A (1988b) Drugs abused by humans preferen tially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85:5274–5278.
- Dilts RPJ, Helton TE, McGinty JF (1993) Selective induction of Fos and FRA immunoreactivity within the mesolimbic and mesostriatal dopamine terminal fields. Synapse 13:251–263.
- Donoghue JP, Wise SP (1982) The motor cortex of the rat: cytoarchitecture and microstimulation mapping. J Comp Neurol 212:76–88.
- Donoghue JP, Herkenham M (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. Brain Res 365:397–403.
- Doucet JP, Nakabeppu Y, Bedard PJ, et al. (1996) Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. Eur J Neurosci 8:365–381.
- Drago J, Gerfen CR, Westphal H, Steiner H (1996) D1 dopamine receptor-deficient mouse: Cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. Neuroscience 74:813–823.
- Drake CT, Terman GW, Simmons ML, Milner TA, Kunkel DD, Schwartzkroin PA, Chavkin C (1994) Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. J Neurosci 14:3736–3750.
- Ellinwood EH Jr., Balster RL (1974) Rating the behavioral effects of amphetamine. Eur J Pharmacol 28:35–41.
- Engber TM, Susel Z, Kuo S, Gerfen CR, Chase TN (1991) Levodopa replacement therapy alters enzyme activities in and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats. Brain Res 552:113–118.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Rev 36:129–138.
- Faull RLM, Nauta WJH, Domesick VB (1986) The visual cortico-striatonigral pathway in the rat. Neuroscience 19:1119–1132.
- Featherstone RE, McDonald RJ (2005) Lesions of the dorsolateral striatum impair the acquisition of a simplified stimulus-response dependent conditional discrimination task. Neuroscience 136:387–395.
- Ferguson SM, Robinson TE (2004) Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade. J Neurochem 91:337–348.
- Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751–759.
- Filip M, Frankowska M, Zaniewska M, Golda A, Przegalinski E (2005) The serotonergic system and its role in cocaine addiction. Pharmacol Rep 57:685–700.
- Gardier AM, Moratalla R, Cuellar B, Sacerdote M, Guibert B, Lebrec H, Graybiel AM (2000) Interaction between the serotoninergic and dopaminergic systems in d-fenfluramine-induced activation of c-fos and jun B genes in rat striatal neurons. J Neurochem 74:1363–1373.
- Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS (1996) Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 58:231–239.
- Gerasimov MR, Franceschi M, Volkow ND, et al. (2000) Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. J Pharmacol Exp Ther 295:51–57.

- Gerfen CR, Young WS III (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161–167.
- Gerfen CR, Keefe KA, Gauda EB (1995) D1 and D2 dopamine receptor function in the striatum: coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate-early gene response in D1-containing neurons. J Neurosci 15:8167–8176.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Giorgi S, Rimoldi M, Consolo S (2001) Parafascicular thalamic nucleus deafferentation reduces c-fos expression induced by dopamine D-1 receptor stimulation in rat striatum. Neuroscience 103:653–661.
- Gray AM, Rawls SM, Shippenberg TS, McGinty JF (1999) The kappaopioid agonist, U-69593, decreases acute amphetamine-evoked behaviors and calcium-dependent dialysate levels of dopamine and glutamate in the ventral striatum. J Neurochem 73:1066–1074.
- Graybiel AM (1995) Building action repertoires: memory and learning functions of the basal ganglia. Curr Opin Neurobiol 5:733–741.
- Graybiel AM, Rauch SL (2000) Toward a neurobiology of obsessive-compulsive disorder. Neuron 28:343–347.
- Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the *c-fos* gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 87:6912–6916.
- Graybiel AM, Canales JJ, Capper-Loup C (2000) Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. Trends Neurosci 23:S71–S77.
- Greely H, Sahakian B, Harris J, Kessler RC, Gazzaniga M, Campbell P, Farah MJ (2008) Towards responsible use of cognitive-enhancing drugs by the healthy. Nature 456:702–705.
- Groenewegen HJ, Berendse HW, Wolters JG, Lohman AH (1990) The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. Prog Brain Res 85:95–116.
- Gross NB, Marshall JF (2009) Striatal dopamine and glutamate receptors modulate methamphetamine-induced cortical Fos expression. Neuroscience 161:1114–1125.
- Hanson GR, Merchant KM, Letter AA, Bush L, Gibb JW (1987) Methamphetamine-induced changes in the striatal-nigral dynorphin system: role of D-1 and D-2 receptors. Eur J Pharmacol 144:245–246.
- Hanson GR, Singh N, Merchant K, Johnson M, Gibb JW (1995) The role of NMDA receptor systems in neuropeptide responses to stimulants of abuse. Drug Alcohol Depend 37:107–110.
- Harlan RE, Garcia MM (1998) Drugs of abuse and immediate-early genes in the forebrain. Mol Neurobiol 16:221–267.
- Hawken CM, Brown RE, Carrey N, Wilkinson M (2004) Long-term methylphenidate treatment down-regulates c-fos in the striatum of male CD-1 mice. Neuroreport 15:1045–1048.
- Hersch SM, Ciliax BJ, Gutekunst CA, et al. (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222–5237.
- Hiroi N, Graybiel AM (1996) Atypical and typical neuroleptic treatments induce distinct programs of transcription factor expression in the striatum. J Comp Neurol 374:70–83.

- Hope B, Kosofsky B, Hyman SE, Nestler EJ (1992) Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci USA 89:5764–5768.
- Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 13:1235–1244.
- Horner KA, Adams DH, Hanson GR, Keefe KA (2005) Blockade of stimulant-induced preprodynorphin mRNA expression in the striatal matrix by serotonin depletion. Neuroscience 131:67–77.
- Hurd YL, Ungerstedt U (1989) In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. Eur J Pharmacol 166:251–260.
- Hurd YL, Herkenham M (1992) Influence of a single injection of cocaine, amphetamine or GBR 12909 on mRNA expression of striatal neuropeptides. Mol Brain Res 16:97–104.
- Hurd YL, Herkenham M (1993) Molecular alterations in the neostriatum of human cocaine addicts. Synapse 13:357–369.
- Hyman SE (2005) Addiction: a disease of learning and memory. Am J Psychiatry 162:1414–1422.
- Hyman SE, Nestler EJ (1996) Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am J Psychiatry 153:151–162.
- Hyman SE, Cole RL, Schwarzschild M, Cole D, Hope B, Konradi C (1996) Molecular mechanisms of striatal gene regulation: a critical role for glutamate in dopamine-mediated gene induction.
  In: Pharmacological Regulation of Gene Expression in the CNS (KM Merchant Ed.), pp. 115–131. Boca Raton: CRC.
- Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M (2006) Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci 29:496–505.
- Jaber M, Cador M, Dumartin B, Normand E, Stinus L, Bloch B (1995) Acute and chronic amphetamine treatments differently regulate neuropeptide messenger RNA levels and Fos immunoreactivity in rat striatal neurons. Neuroscience 65:1041–1050.
- Jedynak JP, Uslaner JM, Esteban JA, Robinson TE (2007) Methamphetamine-induced structural plasticity in the dorsal striatum. Eur J Neurosci 25:847–853.
- Jian M, Staines WA, Iadarola MJ, Robertson GS (1993) Destruction of the nigrostriatal pathway increases Fos-like immunoreactivity predominantly in striatopallidal neurons. Mol Brain Res 19:156–160.
- Johansson B, Lindström K, Fredholm BB (1994) Differences in the regional and cellular localization of c-fos messenger RNA induced by amphetamine, cocaine and caffeine in the rat. Neuroscience 59:837–849.
- Johnson M, Bush LG, Gibb JW, Hanson GR (1991) Role of N-methyl-Daspartate (NMDA) receptors in the response of extrapyramidal neurotensin and dynorphin A systems to cocaine and GBR 12909. Biochem Pharmacol 41:649–652.
- Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Rev 16:223–244.
- Kankaanpaa A, Meririnne E, Seppala T (2002) 5-HT3 receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. Psychopharmacology 159:341–350.
- Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161–179.
- Kincaid AE, Wilson CJ (1996) Corticostriatal innervation of the patch and matrix in the rat neostriatum. J Comp Neurol 374:578–592.

- Knapska E, Kaczmarek L (2004) A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? Prog Neurobiol 74:183–211.
- Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Science 273:1399–1402.
- Kolb B, Gorny G, Li Y, Samaha AN, Robinson TE (2003) Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. Proc Natl Acad Sci USA 100:10523–10528.
- Kollins SH (2008) ADHD, substance use disorders, and psychostimulant treatment: current literature and treatment guidelines. J Atten Disord 12:115–125.
- Konradi C, Westin JE, Carta M, Eaton ME, Kuter K, Dekundy A, Lundblad M, Cenci MA (2004) Transcriptome analysis in a rat model of L-DOPA-induced dyskinesia. Neurobiol Dis 17:219–236.
- Kosofsky BE, Genova LM, Hyman SE (1995) Substance P phenotype defines specificity of c-*fos* induction by cocaine in developing rat striatum. J Comp Neurol 351:41–50.
- Kuczenski R, Segal DS (1997) Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem 68:2032–2037.
- Kuczenski R, Segal DS (1999) Sensitization of amphetamine-induced stereotyped behaviors during the acute response. J Pharmacol Exp Ther 288:699–709.
- Kuczenski R, Segal DS (2001) Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. J Pharmacol Exp Ther 296:876–883.
- Kuhar MJ, Pilotte NS (1996) Neurochemical changes in cocaine withdrawal. Trends Pharmacol Sci 17:260–264.
- LaHoste GJ, Yu J, Marshall JF (1993) Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci USA 90:7451–7455.
- LaHoste GJ, Ruskin DN, Marshall JF (1996) Cerebrocortical Fos expression following dopaminergic stimulation: D1/D2 synergism and its breakdown. Brain Res 728:97–104.
- LaHoste GJ, Henry BL, Marshall JF (2000) Dopamine D1 receptors synergize with D2, but not D3 or D4, receptors in the striatum without the involvement of action potentials. J Neurosci 20:6666–6671.
- Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418–426.
- Le Moine C, Bloch B (1996) Expression of the D3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D1 and D2 dopamine receptors. Neuroscience 73:131–143.
- Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.
- Le Moine C, Svenningsson P, Fredholm BB, Bloch B (1997) Dopamineadenosine interactions in the striatum and the globus pallidus: inhibition of striatopallidal neurons through either D2 or A2A receptors enhances D1 receptor-mediated effects on c-fos expression. J Neurosci 17:8038–8048.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990) Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc Natl Acad Sci USA 87:230–234.
- Lei W, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.

- Li BH, Rowland NE (1993) Dexfenfluramine induces Fos-like immunoreactivity in discrete brain regions in rats. Brain Res Bull 31:43–48.
- Li SJ, Sivam SP, McGinty JF, Jiang HK, Douglass J, Calavetta L, Hong JS (1988) Regulation of the metabolism of striatal dynorphin by the dopaminergic system. J Pharmacol Exp Ther 246:403–408.
- Lightman SL, Young WS 3rd (1987) Vasopressin, oxytocin, dynorphin, enkephalin and corticotrophin-releasing factor mRNA stimulation in the rat. J Physiol 394:23–39.
- Lin JS, Hou Y, Jouvet M (1996) Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-fos immunocytochemistry in the cat. Proc Natl Acad Sci USA 93:14128–14133.
- Loacker S, Sayyah M, Wittmann W, Herzog H, Schwarzer C (2007) Endogenous dynorphin in epileptogenesis and epilepsy: anticonvulsant net effect via kappa opioid receptors. Brain 130:1017–1028.
- London ED, Cascella NG, Wong DF, et al. (1990) Cocaine-induced reduction of glucose utilization in human brain. A study using positron emission tomography and [fluorine 18]-fluorodeoxyglucose. Arch Gen Psychiatry 47:567–574.
- Lucas JJ, Segu L, Hen R (1997) 5-Hydroxytryptamine1B receptors modulate the effect of cocaine on c-fos expression: converging evidence using 5hydroxytryptamine1B knockout mice and the 5-hydroxytryptamine1B/1D antagonist GR127935. Mol Pharmacol 51:755–763.
- Maneuf YP, Mitchell IJ, Crossman AR, Brotchie JM (1994) On the role of enkephalin cotransmission in the GABAergic striatal efferents to the globus pallidus. Exp Neurol 125:65–71.
- Mansour A, Fox CA, Meng F, Akil H, Watson SJ (1994) Kappa<sub>1</sub> receptor mRNA distribution in the rat CNS: comparison to kappa receptor binding and prodynorphin mRNA. Mol Cell Neurosci 5:124–144.
- Marinelli M, Barrot M, Simon H, Oberlander C, Dekeyne A, Le Moal M, Piazza PV (1998) Pharmacological stimuli decreasing nucleus accumbens dopamine can act as positive reinforcers but have a low addictive potential. Eur J Neurosci 10:3269–3275.
- Mathieu-Kia AM, Besson MJ (1998) Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Mol Brain Res 54:141–151.
- McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and DeltaFosB. Nat Neurosci 6:1208–1215.
- McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ (2004) DeltaFosB: a molecular switch for long-term adaptation in the brain. Mol Brain Res 132:146–154.
- McGeorge AJ, Faull RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537.
- Melzer P, Steiner H (1997) Stimulus-dependent expression of immediateearly genes in rat somatosensory cortex. J Comp Neurol 380:145–153.
- Meng F, Xie G-X, Thompson RC, Mansour A, Goldstein A, Watson SJ, Akil H (1993) Cloning and pharmacological characterization of a rat *k*-opioid receptor. Proc Natl Acad Sci USA 90:9954–9958.
- Mijnster MJ, Galis-de Graaf Y, Voorn P (1998) Serotonergic regulation of neuropeptide and glutamic acid decarboxylase mRNA levels in the rat striatum and globus pallidus: studies with fluoxetine and DOI. Mol Brain Res 54:64–73.
- Minami M, Hosoi Y, Toya T, Katao Y, Maekawa K, Katsumata S, Yabuuchi K, Onogi T, Satoh M (1993) In situ hybridization study of *k*-opioid receptor mRNA in the rat brain. Neurosci Lett 162:161–164.
- Mink JW (1996) The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.

- Mishkin M, Malamut B, Bachevalier J (1984) Memories and habits: two neural systems. In: Neurobiology of Human Learning and Memory (McGaugh JL, Weinberger NM, Eds.), pp. 65–87. New York: Guilford Press.
- Moratalla R, Robertson HA, Graybiel AM (1992) Dynamic regulation of NGFI-A (zif268, egr1) gene expression in the striatum. J Neurosci 12:2609–2622.
- Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996a) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147–156.
- Moratalla R, Xu M, Tonegawa S, Graybiel AM (1996b) Cellular responses to psychomotor stimulant and neuroleptic drugs are abnormal in mice lacking the D1 dopamine receptor. Proc Natl Acad Sci USA 93:14928–14933.
- Morris BJ, Reimer S, Hollt V, Herz A (1988) Regulation of striatal prodynorphin mRNA levels by the raphe-striatal pathway. Brain Res 464:15–22.
- Mulder AH, Wardeh G, Hogenboom F, Frankhuyzen AL (1984) Kappaand delta-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. Nature 308:278–280.
- Muller CP, Huston JP (2006) Determining the region-specific contributions of 5-HT receptors to the psychostimulant effects of cocaine. Trends Pharmacol Sci 27:105–112.
- Nakabeppu Y, Nathans D (1991) A naturally occurring truncated form of FosB that inhibits Fos/Jun transcriptional activity. Cell 64:751–759.
- Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007) Damage to the insula disrupts addiction to cigarette smoking. Science 315:531–534.
- Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128.
- Nestler EJ, Carlezon WAJ (2006) The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 59:1151–1159.
- Newman DD, Rajakumar N, Flumerfelt BA, Stoessl AJ (1997) A kappa opioid antagonist blocks sensitization in a rodent model of Parkinson's disease. Neuroreport 8:669–672.
- Nye HE, Hope BT, Kelz MB, Iadarola M, Nestler EJ (1995) Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. J Pharmacol Exp Ther 275:1671–1680.
- Ogura T, Ogata M, Akita H, Jitsuki S, Akiba L, Noda K, Hoka S, Saji M (2005) Impaired acquisition of skilled behavior in rotarod task by moderate depletion of striatal dopamine in a pre-symptomatic stage model of Parkinson's disease. Neurosci Res 51:299–308.
- Packard MG, White NM (1991) Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. Behav Neurosci 105:295–306.
- Packard MG, McGaugh JL (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks: further evidence for multiple memory systems. Behav Neurosci 106:439–446.
- Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Annu Rev Neurosci 25:563–593.
- Packard MG, Cahill L, McGaugh JL (1994) Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc Natl Acad Sci USA 91:8477–8481.
- Passingham RE, Myers C, Rawlins N, Lightfoot V, Fearn S (1988) Premotor cortex in the rat. Behav Neurosci 102:101–109.
- Paul ML, Graybiel AM, David J-C, Robertson HA (1992) D1-like and D2-like dopamine receptors synergistically activate rotation and *c-fos* expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J Neurosci 12:3729–3742.

- Penner MR, McFadyen MP, Pinaud R, Carrey N, Robertson HA, Brown RE (2002) Age-related distribution of c-fos expression in the striatum of CD-1 mice after acute methylphenidate administration. Dev Brain Res 135:71–77.
- Persico AM, Schindler CW, Brannock MT, Gonzalez AM, Surratt CK, Uhl GR (1993) Dopaminergic gene expression during amphetamine withdrawal. Neuroreport 4:41–44.
- Petersen T, Dording C, Neault NB, Kornbluh R, Alpert JE, Nierenberg AA, Rosenbaum JF, Fava M (2002) A survey of prescribing practices in the treatment of depression. Prog Neuropsychopharmacol Biol Psychiatry 26:177–187.
- Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Rev 25:192–216.
- Pinna A, Wardas J, Cristalli G, Morelli M (1997) Adenosine A2A receptor agonists increase Fos-like immunoreactivity in mesolimbic areas. Brain Res 759:41–49.
- Porrino LJ, Smith HR, Nader MA, Beveridge TJ (2007) The effects of cocaine: a shifting target over the course of addiction. Prog Neuropsychopharmacol Biol Psychiatry 31:1593–1600.
- Preuss TM (1995) Do rats have prefrontal cortex? The Rose-Woolsey-Akert program reconsidered. J Cogn Neurosci 7:1–24.
- Rebec GV, White IM, Puotz JK (1997) Responses of neurons in dorsal striatum during amphetamine-induced focused stereotypy. Psychopharmacology 1997:343–351.
- Redgrave P, Gurney K (2006) The short-latency dopamine signal: a role in discovering novel actions? Nat Rev Neurosci 7:967–975.
- Redgrave P, Prescott TJ, Gurney K (1999) The basal ganglia: a vertebrate solution to the selection problem? Neuroscience 89:1009–1023.
- Reep RL, Cheatwood JL, Corwin JV (2003) The associative striatum: organization of cortical projections to the dorsocentral striatum in rats. J Comp Neurol 467:271–292.
- Reep RL, Corwin JV, Hashimoto A, Watson RT (1987) Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Res Bull 19:203–221.
- Reid M, Herrera-Marschitz M, Hökfelt T, Terenius L, Ungerstedt U (1988) Differential modulation of striatal dopamine release by intranigral injection of g-aminobutyric acid (GABA), dynorphin A and substance P. Eur J Pharmacol 147:411–420.
- Reiner A, Anderson KD (1990) The patterns of neurotransmitter and neuropeptide cooccurrence among striatal projection neurons: conclusions based on recent findings. Brain Res Rev 15:251–265.
- Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. Trends Mol Med 14:341–350.
- Renthal W, Carle TL, Maze I, et al. (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J Neurosci 28:7344–7349.
- Ritz MC, Cone EJ, Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structureactivity study. Life Sci 46:635–645.
- Robbins TW, Everitt BJ (1999) Drug addiction: bad habits add up. Nature 398:567–570.
- Robbins TW, Granon S, Muir JL, Durantou F, Harrison A, Everitt BJ (1998) Neural systems underlying arousal and attention. Implications for drug abuse. Ann NY Acad Sci 846:222–237.
- Robertson GS, Vincent SR, Fibiger HC (1990) Striatonigral projection neurons contain D1 dopamine receptor-activated c-fos. Brain Res 523:288–290.

- Robertson GS, Vincent SR, Fibiger HC (1992) D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. Neuroscience 49:285–296.
- Robinson TE, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491–8497.
- Ruda MA, Ren K, Besse D (1995) Regulation of spinal neuropeptide genes in a rat model of peripheral inflammation and hyperalgesia. Prog Brain Res 104:349–365.
- Ruskin DN, Marshall JF (1994) Amphetamine- and cocaine-induced fos in the rat striatum depends on D2 dopamine receptor activation. Synapse 18:233–240.
- Saka E, Goodrich C, Harlan P, Madras BK, Graybiel AM (2004) Repetitive behaviors in monkeys are linked to specific striatal activation patterns. J Neurosci 24:7557–7565.
- Schoffelmeer ANM, Rice KC, Jacobson AE, Van Gelderen JG, Hogenboom F, Heijna MH, Mulder AH (1988) Mu-, delta- and kappa-opioid receptor-mediated inhibition of neurotransmitter release and adenylate cyclase activity in rat brain slices: studies with fentanyl isothiocyanate. Eur J Pharmacol 154:169–178.
- Schwartz J-C, Diaz J, Bordet R, Griffon N, Perachon S, Pilon C, Ridray S, Sokoloff P (1998) Functional implications of multiple dopamine receptor subtypes: the D1/D3 receptor coexistence. Brain Res Rev 26:236–242.
- Schweri MM, Skolnick P, Rafferty MF, Rice KC, Janowsky AJ, Paul SM (1985) [3H]Threo-(+/-)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. J Neurochem 45:1062–1070.
- Sharp FR, Sagar SM, Swanson RA (1993) Metabolic mapping with cellular resolution: c-fos vs. 2-deoxyglucose. Crit Rev Neurobiol 7:205–228.
- Sherwin CM (1998) Voluntary wheel running: a review and novel interpretation. Anim Behav 56:11–27.
- Shippenberg TS, Zapata A, Chefer VI (2007) Dynorphin and the pathophysiology of drug addiction. Pharmacol Ther 116:306–321.
- Simmons ML, Chavkin C (1996) Endogenous opioid regulation of hippocampal function. Int Rev Neurobiol 39:145–196.
- Simmons ML, Terman GW, Gibbs SM, Chavkin C (1995) L-type calcium channels mediate dynorphin neuropeptide release from dendrites but not axons of hippocampal granule cells. Neuron 14:1265–1272.
- Simonato M, Romualdi P (1997) Dynorphin and epilepsy. Prog Neurobiol 50:557–583.
- Sivam SP (1989) Cocaine selectively increases striatonigral dynorphin levels by a dopaminergic mechanism. J Pharmacol Exp Ther 250:818–824.
- Smiley PL, Johnson M, Bush L, Gibb JW, Hanson GR (1990) Effects of cocaine on extrapyramidal and limbic dynorphin systems. J Pharmacol Exp Ther 253:938–943.
- Smith AJW, McGinty JF (1994) Acute amphetamine or methamphetamine alters opioid peptide mRNA expression in rat striatum. Mol Brain Res 21:359–362.
- Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 89:2046–2050.
- Spangler R, Unterwald EM, Kreek MJ (1993) "Binge" cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. Mol Brain Res 19:323–327.

- Spangler R, Ho A, Zhou Y, Maggos CE, Yuferov V, Kreek MJ (1996) Regulation of kappa opioid receptor mRNA in the rat brain by "binge" pattern cocaine administration and correlation with preprodynorphin mRNA. Mol Brain Res 38:71–76.
- Spangler R, Zhou Y, Maggos CE, Schlussman SD, Ho A, Kreek MJ (1997) Prodynorphin, proenkephalin and kappa opioid receptor mRNA responses to acute "binge" cocaine. Mol Brain Res 44:139–142.

Squire LR (1987) Memory and Brain. Oxford: Oxford University Press.

- Steiner H (2007) Basal ganglia cortex interactions: Regulation of cortical function by D1 dopamine receptors in the striatum. In: Monoaminergic Modulation of Cortical Excitability (Tseng KY, Atzori M, Eds.), pp. 265–285. Berlin: Springer.
- Steiner H, Gerfen CR (1993) Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci 13:5066–5081.
- Steiner H, Gerfen CR (1994) Tactile sensory input regulates basal and apomorphine-induced immediate-early gene expression in rat barrel cortex. J Comp Neurol 344:297–304.
- Steiner H, Gerfen CR (1995) Dynorphin opioid inhibition of cocaineinduced, D1 dopamine receptor-mediated immediate-early gene expression in the striatum. J Comp Neurol 353:200–212.
- Steiner H, Gerfen CR (1996) Dynorphin regulates D1 dopamine receptor-mediated responses in the striatum: relative contributions of pre- and postsynaptic mechanisms in dorsal and ventral striatum demonstrated by altered immediate-early gene induction. J Comp Neurol 376:530–541.
- Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 123:60–76.
- Steiner H, Gerfen CR (1999) Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. Neuroscience 88:795–810.
- Steiner H, Kitai ST (2000) Regulation of rat cortex function by D1 dopamine receptors in the striatum. J Neurosci 20:5449–5460.
- Steiner H, Van Waes V, Marinelli M (2010) Fluoxetine potentiates methylphenidate-induced gene regulation in addiction-related brain regions: Concerns for use of cognitive enhancers? Biol Psychiatry in press.
- Stork O, Welzl H (1999) Memory formation and the regulation of gene expression. Cell Mol Life Sci 55:575–592.
- Surmeier DJ, Song W-J, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
- Swanson JM, Volkow ND (2008) Increasing use of stimulants warns of potential abuse. Nature 453:586.
- Thomas U (2002) Modulation of synaptic signalling complexes by Homer proteins. J Neurochem 81:407–413.
- Thompson LA, Walker JM (1990) Inhibitory effects of the k opiate U50,488 in the substantia nigra pars reticulata. Brain Res 517:81–87.
- Torres G, Rivier C (1993) Cocaine-induced expression of striatal c-fos in the rat is inhibited by NMDA receptor antagonists. Brain Res Bull 30:173–176.
- Torres G, Rivier C (1994) Induction of c-fos in rat brain by acute cocaine and fenfluramine exposure: a comparison study. Brain Res 647:1–9.
- Torres G, Horowitz JM (1999) Drugs of abuse and brain gene expression. Psychosom Med 61:630–650.
- Uhl GR, Navia B, Douglas J (1988) Differential expression of preproenkephalin and preprodynorphin mRNAs in striatal neurons: High levels of preproenkephalin expression depend on cerebral cortical afferents. J Neurosci 8:4755–4764.

- Unal CT, Beverley JA, Willuhn I, Steiner H (2009) Long-lasting dysregulation of gene expression in corticostriatal circuits after repeated cocaine treatment in adult rats: Effects on zif 268 and homer 1a. Eur J Neurosci 29:1615–1626.
- Uslaner J, Badiani A, Day HE, Watson SJ, Akil H, Robinson TE (2001a) Environmental context modulates the ability of cocaine and amphetamine to induce c-fos mRNA expression in the neocortex, caudate nucleus, and nucleus accumbens. Brain Res 920:106–116.
- Uslaner J, Badiani A, Norton CS, Day HE, Watson SJ, Akil H, Robinson TE (2001b) Amphetamine and cocaine induce different patterns of c-fos mRNA expression in the striatum and subthalamic nucleus depending on environmental context. Eur J Neurosci 13:1977–1983.
- Uslaner JM, Crombag HS, Ferguson SM, Robinson TE (2003a) Cocaineinduced psychomotor activity is associated with its ability to induce c-fos mRNA expression in the subthalamic nucleus: effects of dose and repeated treatment. Eur J Neurosci 17:2180–2186.
- Uslaner JM, Norton CS, Watson SJ, Akil H, Robinson TE (2003b) Amphetamine-induced c-fos mRNA expression in the caudateputamen and subthalamic nucleus: interactions between dose, environment, and neuronal phenotype. J Neurochem 85:105–114.
- Uylings HB, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behav Brain Res 146:3–17.
- Vargo JM, Marshall JF (1995) Time-dependent changes in dopamine agonist-induced striatal Fos immunoreactivity are related to sensory neglect and its recovery after unilateral prefrontal cortex injury. Synapse 20:305–315.
- Vincent SR, Hökfelt T, Christensson I, Terenius L (1982) Immunohistochemical evidence for a dynorphin immunoreactive striatonigral pathway. Eur J Pharmacol 85:251–252.
- Volkow ND, Insel TR (2003) What are the long-term effects of methylphenidate treatment? Biol Psychiatry 54:1307–1309.
- Voorn P, Roest G, Groenewegen HJ (1987) Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. Brain Res 412:391–396.
- Walker PD, Capodilupo JG, Wolf WA, Carlock LR (1996) Preprotachykinin and preproenkephalin mRNA expression within striatal subregions in response to altered serotonin transmission. Brain Res 732:25–35.
- Wang JQ, McGinty JF (1995) Alterations in striatal zif/268, preprodynorphin and preproenkephalin mRNA expression induced by repeated amphetamine administration in rats. Brain Res 673:262–274.
- Wang JQ, McGinty JF (1996a) D1 and D2 receptor regulation of preproenkephalin and preprodynorphin mRNA in rat striatum following acute injection of amphetamine or methamphetamine. Synapse 22:114–122.
- Wang JQ, McGinty JF (1996b) Glutamatergic and cholinergic regulation of immediate-early gene and neuropeptide gene expression in the striatum. In: Pharmacological Regulation of Gene Expression in the CNS (Merchant KM Ed.), pp. 81–113. Boca Raton: CRC.
- Wang JQ, Daunais JB, McGinty JF (1994a) Role of kainate/AMPA receptors in induction of striatal zif/268 and preprodynorphin mRNA by a single injection of amphetamine. Mol Brain Res 27:118–126.
- Wang JQ, Daunais JB, McGinty JF (1994b) NMDA receptors mediate amphetamine-induced upregulation of zif/268 and preprodynorphin mRNA expression in rat striatum. Synapse 18:343–353.
- Wang JQ, Smith AJW, McGinty JF (1995) A single injection of amphetamine or methamphetamine induces dynamic alterations in c-fos,

zif/268 and preprodynorphin messenger RNA expression in rat forebrain. Neuroscience 68:83–95.

- Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95:532–545.
- West MO, Carelli RM, Pomerantz M, Cohen SM, Gardner JP, Chapin JK, Woodward DJ (1990) A region in the dorsolateral striatum of the rat exhibiting single-unit correlations with specific locomotor limb movements. J Neurophysiol 64:1233–1246.
- White IM, Doubles L, Rebec GV (1998) Cocaine-induced activation of striatal neurons during focused stereotypy in rats. Brain Res 810:146–152.
- White NM (1996) Addictive drugs as reinforcers: multiple partial actions on memory systems. Addiction 91:921–949.
- Wilens TE, Faraone SV, Biederman J, Gunawardene S (2003) Does stimulant therapy of attention-deficit/hyperactivity disorder beget later substance abuse? A meta-analytic review of the literature. Pediatrics 111:179–185.
- Wilens TE, Adler LA, Adams J, Sgambati S, Rotrosen J, Sawtelle R, Utzinger L, Fusillo S (2008) Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. J Am Acad Child Adolesc Psychiatry 47:21–31.
- Willuhn I, Steiner H (2005) Motor learning-related gene regulation in the striatum: Effects of cocaine. In: The Basal Ganglia VIII (Bolam JP, Ingham CA, Magill PJ, Eds.), pp. 197–207. New York: Plenum Press.
- Willuhn I, Steiner H (2006) Motor-skill learning-associated gene regulation in the striatum: Effects of cocaine. Neuropsychopharmacology 31:2669–2682.
- Willuhn I, Steiner H (2008) Motor-skill learning in a novel runningwheel task is dependent on D1 dopamine receptors in the striatum. Neuroscience 153:249–258.
- Willuhn I, Steiner H (2009) Skill-memory consolidation in the striatum: Critical for late but not early long-term memory and stabilized by cocaine. Behav Brain Res 199:103–107.
- Willuhn I, Sun W, Steiner H (2003) Topography of cocaine-induced gene regulation in the rat striatum: Relationship to cortical inputs and role of behavioural context. Eur J Neurosci 17:1053–1066.
- Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615.
- Wirtshafter D, Cook DF (1998) Serotonin-1B agonists induce compartmentally organized striatal Fos expression in rats. Neuroreport 9:1217–1221.
- Wright AK, Norrie L, Ingham CA, Hutton EA, Arbuthnott GW (1999) Double anterograde tracing of outputs from adjacent "barrel columns" of rat somatosensory cortex. Neostriatal projection patterns and terminal ultrastructure. Neuroscience 88:119–133.

- Wright CI, Groenewegen HJ (1996) Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. Neuroscience 73:359–373.
- Xiao B, Tu JC, Worley PF (2000) Homer: a link between neural activity and glutamate receptor function. Curr Opin Neurobiol 10:370–374.
- Yano M, Steiner H (2005a) Methylphenidate (Ritalin) induces Homer 1a and zif 268 expression in specific corticostriatal circuits. Neuroscience 132:855–865.
- Yano M, Steiner H (2005b) Topography of methylphenidate (Ritalin)induced gene regulation in the striatum: differential effects on cfos, substance P and opioid peptides. Neuropsychopharmacology 30:901–915.
- Yano M, Steiner H (2007) Methylphenidate and cocaine: the same effects on gene regulation? Trends Pharmacol Sci 28:588–596.
- Yano M, Beverley JA, Steiner H (2006) Inhibition of methylphenidate-induced gene expression in the striatum by local blockade of D1 dopamine receptors: Interhemispheric effects. Neuroscience 140:699–709.
- Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.
- You Z-B, Herrera-Marschitz M, Nylander I, Goiny M, O'Connor WT, Ungerstedt U, Terenius L (1994a) The striatonigral dynorphin pathway of the rat studied with in vivo microdialysis-II. Effects of dopamine D1 and D2 receptor agonists. Neuroscience 63:427–434.
- Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-Fosimmunoreactive proteins via dopaminergic D<sub>1</sub> receptors. Proc Natl Acad Sci USA 88:1291–1295.
- Young WS III, Bonner TI, Brann MR (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc Natl Acad Sci USA 83:9827–9831.
- Yuferov V, Nielsen D, Butelman E, Kreek MJ (2005) Microarray studies of psychostimulant-induced changes in gene expression. Addict Biol 10:101–118.
- Yuferov V, Kroslak T, Laforge KS, Zhou Y, Ho A, Kreek MJ (2003) Differential gene expression in the rat caudate putamen after "binge" cocaine administration: advantage of triplicate microarray analysis. Synapse 48:157–169.
- Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, Zhang J, Xu M (2004) Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. J Neurosci 24:3344–3354.
- Zheng T, Wilson CJ (2002) Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 87:1007–1017.

# Chromatin Remodeling: Role in Neuropathologies of the Basal Ganglia

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# I. INTRODUCTION

New concepts have recently emerged about the role of epigenetic modifications in the etiology of neurodegenerative and psychiatric disorders. Epigenetic mechanisms are key cellular processes that integrate diverse environmental stimuli to exert potent and long lasting changes in gene expression. These mechanisms were initially described in differentiating cells, where a clonal population of cells must decipher the correct transcriptional program from parent to daughter cells (Grewal and Moazed, 2003). It is now well established that the control of chromatin structure is largely involved in this "above the genome" cellular process. Chromatin remodeling is a dynamic, cell- and environmentspecific process that permits the control of DNA packaging and hence the access to the transcriptional machinery at specific loci. It critically depends on post-translational modifications of histones and integrates diverse external stimuli to exert potent and long lasting changes in gene expression. A large body of evidence now implicate these processes

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. in the control of gene regulation that underlies long lasting effects of environmental stimuli in mature, post-mitotic neurons. These include plasticity induced by experience and environment (Huang et al., 2002; Tsankova et al., 2004; Weaver et al., 2004; Kumar et al., 2005; Sng et al., 2006; Putignano et al., 2007; Stipanovich et al., 2008) and underlying learning and long-term memory (Alarcon et al., 2004; Korzus et al., 2004; Levenson et al., 2004; Chwang et al., 2007; Vecsey et al., 2007; Chandramohan et al., 2008; Stipanovich et al., 2008). Epigenetic dysregulation is also a common theme in disorders of synaptic plasticity and cognition associated with dysfunctions of basal ganglia, including neurodegenerative disorders such as Huntington's disease (HD) (Stack et al., 2007; Kim et al., 2008a; Roze et al., 2008a), L-DOPA-induced dyskinesia (Nicholas et al., 2008) as well as drugs of abuse (Renthal and Nestler, 2008).

**IV.** Conclusions

References

During the past few years, much attention has been paid to the role of histone acetylation in chromatin remodeling and gene regulation in neurons. It has been proposed, in various experimental paradigms, that histone deacetylase (HDAC) inhibitors, which induce hyperacetylation of histones, may improve not only learning and memory but also neuronal survival (Graff and Mansuy, 2008; Mehler, 2008). Much less is known about histone phosphorylation, a post-translational event that occurs prominently on histone H3 and seems to be critical at some specific loci, including the promoter of the immediate-early genes (IEGs) c-*fos* and c-*jun* (Crosio et al., 2003; Brami-Cherrier et al., 2007).

The first part of this review summarizes the general concepts of chromatin remodeling, histone modifications and nucleosomal response, with a particular emphasis on histone acetylation, phosphorylation and methylation, and their dynamic control. We then review how chromatin remodeling can occur in striatal neurons in responses to drugs of abuse but also in neurological diseases, including HD, Dentatorubral-pallidoluysian atrophy (DRPLA) and L-DOPA-induced dyskinesia. We conclude about the importance of better understanding the mechanisms that underlie chromatin remodeling to reveal novel drug targets for the development of improved pharmaceutical interventions.

# II. CHROMATIN REMODELING AND HISTONE MODIFICATIONS

Within the cell nucleus, DNA is packaged into chromatin, a DNA-protein complex, comprising histones and other architectural proteins (Fischle et al., 2003). Histones are highly basic proteins that act as molecular spools by coiling the DNA strands into bead-like units, referred as nucleosomes. Nucleosomes are the repeated primary units of chromatin and are composed of two copies of each of the four core histones (H2A, H2B, H3, H4), around which 147 base pairs of DNA are wrapped. Each nucleosome is connected to the neighbouring ones by stretches of 20 to 60 base pairs of DNA bound by the linker histone H1. Each histone has a specific function and contains a trihelical histone fold that mediates histone-histone and histone-DNA interaction.

Histones have a globular structure except for their highly conserved N-terminal tail domains (Luger and Richmond, 1998; Berger, 2002). These domains can undergo a wide variety of post-translational modifications including acetylation (Kuo and Allis, 1998; Sterner and Berger, 2000), phosphorylation (Cheung et al., 2000; Nowak and Corces, 2004), methylation (Zhang and Reinberg, 2001; Kouzarides, 2002), sumoylation (Nathan et al., 2003; Shiio and Eisenman, 2003), ubiquitination (Robzyk et al., 2000; Shilatifard, 2006), deimination (Cuthbert et al., 2004), proline isomerization (Nelson et al., 2006), and ADP-ribosylation



FIGURE 30.1 Regulation of chromatin remodelling by histone H3 and H4 post-translational modifications. Nucleosome is constituted by two copies of the four core histones (H2A, H2B, H3, H4) around 147 base pairs of DNA. (A) In inactive promoter, DNA is compacted into silent condensed heterochromatin. (B) After stimuli that induce transcription, histone H3 kinase phosphorylates H3 on Ser10 and histone acetyl transferases (HAT) acetylates histone H3 on Lys9/14 and H4 on Lys5 for example. These post-translational modifications of histone tails alter the DNA-histone interactions and promote chromatin decompaction. In this state the promoter is in an active state, also called euchromatin. (C) Note that these events are gene-dependent and only occur at restricted loci. The reverse process occurs via histone H3 phosphatases, for dephosphorylation, and histone deacetylases (HDAC), for deacetylation and thus results in chromatin condensation. In this state, chromatin is called heterochromatin.

(Hassa et al., 2006). These modifications can occur at numerous, but specific residues depending on the signalling conditions within the cells. They are mediated by histones modifying enzymes with various degrees of specificity (Kouzarides, 2007). For transcription to occur, decompaction and change into active decondensed euchromatin is required (Fig. 30.1). It allows the action of the core transcription machinery and transcription factors and hence the control of gene expression by gating access of the transcriptional machinery to DNA (Felsenfeld and Groudine, 2003; Li et al., 2007). The modifications of the compaction status are controlled by enzymes altering the histone-DNA link via the aforementioned covalent modifications of histones tails at specific residues. The position-specific combination of these post-translational modifications regulates the histone charge, and hence the compaction state of DNA (Cheung et al., 2000; Strahl and Allis, 2000). For example, histone acetylation at a promoter generally increases transcription, in particular acetylation of Lys9 and Lys14 of histone H3, or acetylation of Lys5 of histone H4 (Liu et al., 2005; Berger, 2007). The enzymes that catalyze these reactions are histone acetyltransferases (HATs) and several transcriptional activators, including CREB Binding Protein (CBP), are known to contain intrinsic HAT activity (Ogryzko et al., 1996). By contrast, histone deacetylases (HDACs) catalyze

deacetylation. These enzymes are often associated with transcriptional repressors to further repress chromatin remodeling. Phosphorylation on Ser10 of histone H3 also increases transcription (Nowak and Corces, 2004; Berger, 2007). By contrast, histone methylation often represses transcription, in particular di or tri-methylation of Lys9 of H3. These latter modifications are not independent since methylation of the Lys9 of H3 plays an antagonist role on Ser10 (Ser10-H3) phosphorylation and Lys9 and 14 (Lys9-14-H3) acetylation of histone H3 (Santos-Rosa and Caldas, 2005).

## A. Histone Acetylation

One of the first studied histone modifications was acetylation. It was initially linked to transcriptional activation (Brownell and Allis, 1996) and subsequently to diverse molecular processes, including gene silencing, DNA repair and cell cycle progression (Carrozza et al., 2003). Using acetyl-CoA as the acetyl donor, HATs acetylate the e-amine of lysine residues and thus remove the positive charge of lysine. This post-translational modification is thought to neutralize the positive charge of the histone tails and relax the interaction between histones and negatively charged DNA. This molecular event renders the target gene promoter more accessible. Acetylation of histones also weakens the internucleosomal interactions and destabilizes higher order chromatin structure (Garcia-Ramirez et al., 1995; Tse et al., 1998a; Tse et al., 1998b). Finally, acetylation of histones may promote the processivity of RNA polymerase through nucleosome arrays (Ura et al., 1997; Nightingale et al., 1998).

Functional characterization of yeast HAT Gcn5 mutants revealed a direct correlation between the ability of the protein to acetylate histones and to activate transcription (Kuo et al., 1998; Wang et al., 1998). In parallel with the discovery of HAT enzymes, came the identification of several HDAC enzymes, whose activities are correlated with transcriptional repression (Cress and Seto, 2000). The dynamic interplay between HATs and HDACs dictates the ultimate state of acetylation. Of importance, the amino acid sequences of histones are some of the most highly conserved among eukaryotes, and this high sequence conservation underscores the crucial nature of histone acetylation in the genome regulation.

## 1. Histone Acetyltransferases

Nuclear or A-type HAT proteins can be distinguished from the cytoplasmic B-type HATs. Within this A group, at least three families can be found: Gcn5/PCAF (for its founding member yeast Gcn5 and PCAF for p300/CBP associated factor) also called GNAT family (for Gcn5 related N-acetyltransferase), MYST (for the founding members *M*OZ, Ybf2/Sas3, Sas2 and Tip60), p300/CBP (for the two human paralogs p300 and CBP) (Marmorstein, 2001; Hodawadekar and Marmorstein, 2007). Although other nuclear HAT families have been identified, such as the steroid receptor coactivators (ACTR/AIB1, SRC1), TAF250, or ATF-2, their HAT activities have not been studied in detail. It is important to note that many of these enzymes can acetylate non-histone proteins, and could be more generally termed as protein lysine acetyltransferases.

#### **Expression patterns of HATs**

Although ubiquitously expressed, PCAF is poorly expressed in the brain when compared to other tissues such as lung or liver (Yamauchi et al., 2000). Gcn5 is also ubiquitously expressed, but Gcn5 Null mice or Gcn5 mice mutated in their HAT domain show a defect in neural tube closure as well as exencephaly in embryos (Bu et al., 2007). These data clearly implicate PCAF in neurodevelopment. In vitro, recombinant Gcn5 can acetylate free histones, but also histones on nucleosomes, with a preference for histone H3 (Lys14) when compared to H4. Very few studies have investigated the role of the MYST family of HATs in neuronal cells. One member of this family, Querkopf (Qkf, Myst4, Morf), is required for normal cerebral cortex development and adult neurogenesis (Thomas et al., 2000; Merson et al., 2006). In vitro acetylation assays suggest that some members of this family can also acetylate free histones, with a preferential activity towards H3. CBP/p300, the most studied HATs in the context of neuronal disorders, are ubiquitously expressed. Null and heterozygous mice for CBP and p300 show similar embryonic lethal phenotypes, along with defects in growth and neural tube closure (Yao et al., 1998). Unlike other HATs, recombinant p300/CBP are able to acetylate all four histones within nucleosomes as well as in their free-histone form.

#### HAT regulation

Various studies have mapped and characterized the functional domains of Gcn5/PCAF family including a C-terminal bromodomain, an Ada2 interaction domain, and the HAT domain which is required in vivo for adaptormediated transcriptional activation (Candau et al., 1997). The acetyltransferase homology region of the MYST family proteins is different from the Gcn5/PCAF family.



**FIGURE 30.2** Model of histones modification via  $Ca^{2+}$  signaling pathway. Increases in intracellular calcium signalling levels can participate in calcium remodeling via multiple pathways. In the cytoplasm calcium induces activation of CamKII and ERK, which in turn translocate to the nucleus and leads to phosphorylation of CREB. Note that ERK-induced phosphorylation of CREB is indirect, and tightly linked to MSK1 activation within the nucleus. MSK1 exerts a dual role on gene regulation, via its kinase activity towards histone H3 (P). CREB phosphorylation allows recruitment of p300/CBP to the promoter, which in turn can exerts its HAT activity towards histones (Ac). Moreover, activated CamKII phosphorylates HDAC5 leading to their nuclear export and as a consequence to an inhibition of transcription repression. Altogether, these calcium-dependent events allow chromatin remodeling, DNA decompaction and recruitment of the basal transcriptional machinery necessary for gene regulation at specific loci.

Furthermore, members of this family are involved in a wide range of functions.

CBP was first identified through its ability to coimmunoprecipitate with the phosphorylated form of CREB (Chrivia et al., 1993). CREB can be activated, phosphorylated on a critical residue, Ser133, by various protein kinases, including CaMKII (Shaywitz and Greenberg, 1999) and MSK1 (Arthur, 2008). In turn, activated, phosphorylated, CREB can recruit CBP to DNA promoter region (Fig. 30.2), and allows its HAT activity towards histone (McManus and Hendzel, 2001). CBP contains several functional domains including the binding site for the CREB transcription factor, referred to as the KIX domain, the bromodomain, two zinc finger motifs, and the HAT domain. P300 is closely related to CBP: the proteins share 63% identity at the amino-acid level and posses the same domains. CBP and p300 can interact with the basal transcription factor TATA-binding protein (TBP) and TFIIB and/or form a complex with RNA polymerase II. Moreover, as a result of many identified protein interacting domains, CBP/p300 have been proposed to function as molecular scaffolds and signal integrators through their associations with at least 45 different molecules including transcription factors such as

Jun, CREB, E1A, E2F, NF-kB, signaling molecules, and nuclear hormone receptors (Giles et al., 1998). In addition to functioning as a bridge between transcription factors and the basal transcription machinery (Goodman and Smolik, 2000), CBP/p300 are able to integrate a broad array of signaling informations. CBP/p300 are phosphorylated by the Calcium/calmodulin (CaM)-dependent protein kinase CaMKIV (Chawla et al., 1998), aphosphorylation event that may be required for their role in transcription (Hardingham et al., 1999; Hu et al., 1999). Phosphorylation of CBP by MAPK/ERK has also been reported (Janknecht and Nordheim, 1996), and was proposed to augment the ability of CBP to mediate the transcriptional activity of Elk-1. The precise phosphorylation site for ERK in CBP remains unidentified but was reported to be localized in its C-terminal region. Another kinase for CBP can be Rsk (for ribosomal S6 kinase), downstream the Ras/ERK signalling pathway. In response to NGF, activation of the Ras/ERK pathway promotes phosphorylation of Rsk and its association with p300/CBP. Interestingly this molecular interaction between Rsk and p300/CBP prevents from CREB-dependent transcription (Nakajima et al., 1996). These observations underscore the key role of intracellular signalling, and partners for CBP phosphorylation, which will guide CBP transcriptional properties. Importantly, CBP/p300 play a dual role in both histone acetylation and recruitment of the basal transcriptional machinery. This may explain the specificity of chromatin remodeling at a subset of loci. Furthermore, targeting CBP/p300 expression may be a more efficient therapy than global HDAC inhibitors - which only counteract the loss of histone acetylation - for regulating gene expression in neurological disorders.

### 2. Histone Deacetylases

Enzymes which are responsible for removing the acetyl moiety from N-e-acetylated lysine residues in histones belong to the super-family of HDACs. According to their size and structure, at least 18 members have been identified in the HDACs super-family. They are grouped into four main subtypes: class I, IIa and IIb, III and IV. Class I (HDAC1,2,3 and 8), IIa (4,5, 7 and 9), IIb (6 and 10) and IV (HDAC11) are referred as "classical" HDACs, whereas class III members are named sirtuins (SIRT1-7) (Gregoretti et al., 2004). Classical HDACs and sirtuins differ in their catalytic mechanisms. Classical HDACs are  $Zn^{2+}$  – dependent enzymes harboring a catalytic pocket with a  $Zn^{2+}$  ion at its base. Thus HDACs activity can be inhibited by

 $Zn^{2+}$  chelating compounds. HDAC6, which belongs to class IIb, is unusual when compared to the other classical DHACs, because it contains two independent catalytic domains. In contrast, sirtuins of class III require NAD<sup>+</sup> as an essential cofactor, and are not inactivated by  $Zn^{2+}$ chelating compounds.

#### Expression patterns of HDACs

Depending on their class, HDACs are either widely expressed or distributed in a tissue-specific manner. Class I HDACs display ubiquitous tissue expression, whereas class II and IV HDACs expression is tissue-restricted. All seven sirtuins (Class III) are ubiquitously expressed in human tissues, although higher levels of mRNA expression for most sirtuins are detected in the brain and testis (Michishita et al., 2005). Related to HDACs brain expression, a comprehensive gene-expression mapping of the 11 HDAC isoforms of class I, II, and IV throughout the rat brain has been conducted using high resolution in situ hybridization and imaging technology (Broide et al., 2007). This first extensive characterization of the 11 HDACs in the CNS showed the heterogeneity of HDAC expression patterns through >50 brain regions. Interestingly, HDACs are expressed primarily in neurons, although a subset can be found in oligodendrocytes but not in astrocytes nor in vessel endothelial cells. According to the score attributed from low to high expression throughout the brain, HDAC11, 3, 5 have the highest expression and HDAC10, 9 and 7 the lowest one. In the caudate-putamen and nucleus accumbens brain regions, HDAC11 is the predominant isoform followed by HDAC5, 3, 2 and 4. Low expression of HDAC1, 6, 7 is observed in these nuclei, whereas HDAC8, 9, 10 are not detected at all in these CNS structures. However, the selective contribution of each HDAC isoform in chromatin remodeling and transcriptional regulation within the striatum remains unknown.

Most of classical HDACs and Sirtuins have a nuclear localization, where they control acetylation of histones. Some of them shuttle between the nucleus and the cytoplasm where they deacetylate their non-histone targets. For example, HDAC6 and SIRT2 are localized within the cytoplasm where they deacetylate  $\alpha$ -tubulin and alter micro-tubule stability (Hubbert et al., 2002; North et al., 2003). SIRT3-5 are mitochondrial proteins involved in the regulation of energetic metabolism (Michishita et al., 2005).

In general, HDACs do not act autonomously but as components of a large protein complex that comprises transcription factors. CoREST, the neuron-specific corepressor of REST of (RE1 silencing transcription factor/neural restrictive silencing factor), binds to both HDAC 1 and 2 (You et al., 2001), or MEF-2 (myocyte-enhancing factor 2) a muscle-specific transcription factor highly expressed in the brain, which interacts with HDAC4 and 5 (Lu et al., 2000).

### HDAC regulation

HDACs are central organizers of histones post-translational modifications, and they are themselves regulated by posttranslational modifications: phosphorylation, ubiquitylation, acetylation and sumoylation (Brandl et al., 2009). HDACs enzymatic activity and cellular localization are critically controlled by these various modifications. Several protein kinases responsible for the phosphorylation of HDACs have been identified. Identification of the calcium/calmodulin-dependent kinase (CaMK) as a protein kinase for HDAC has allowed the identification of calcium signaling as an important mediator of HDACs regulation. CAMKV phosphorylates HDAC class II (HDAC4, 5 and 7) on two serines, leading to their nuclear export and as a consequence to an inhibition of transcription repression (Fig. 30.2) (McKinsey et al., 2000). In hippocampal neurons, nuclear translocation and export of HDAC4 and HDAC5 are dependent on synaptic activity and calcium influx and are sensitive to CAMK regulation (Chawla et al., 2003; Belfield et al., 2006). Low potassium, excitotoxic glutamate conditions or treatment with a CAMK inhibitor that induces cerebellar granule neurons death, promote HDAC4 translocation into the nucleus. By contrast, neuronal survival factor BDNF suppresses HDAC4 nuclear translocation (Bolger and Yao, 2005). Ex vivo experiments performed on punches of the nucleus accumbens showed that CamKII was necessary for depolarization-induced HDAC5 phosphorylation on Ser259 and its shuttling to the cytoplasm (Renthal et al., 2007).

#### **HDAC** inhibitors

Because of their widespread substrates, HDACs modulate many biological pathways, not only chromatin remodeling but also inflammation, metabolism, microtubule transport, protein aggregation. As a consequence, inhibitors of these enzymes have been recognized as important therapeutic issues for a broad range of human disorders, including in disorders related to basal ganglia dysfunctions.

Molecules that can act as HDAC inhibitors (HDACi), and thus can increase histones acetylation, have been designed. They have a standard construction and share structural similarities with their HDAC acetyl-lysine substrate. HDACis consist in a metal-binding moiety, that binds to the catalytic metal  $Zn^{2+}$  atom within the HDAC active site, and a capping group that interacts with the amino acid residues at the entrance of the active site (Bieliauskas and Pflum, 2008). HDACis can be classified into four main chemical families, the short-chain fatty acids (sodium butyrate, phenylbutyrate and valproic acid), the hyroxamic acids (Trichostatin A (TSA), and suberoylanilide hydroxamic acid-(SAHA)), the epoxytones (trapoxin) and the benzamidines.

The majority of HDACis inhibit non-specifically all HDAC isoforms. SAHA, sodium butyrate and TSA are the canonical HDACis and influence the activity of HDAC of Class I and II with roughly equivalent potency. These HDACis have been the first used in cellular or animal model of HD, drug addiction and psychiatric disorders. Sodium butyrate and phenylbutyrate attenuate neuronal loss, increase motor function and extend survival in R6/2 mice, a transgenic model of HD (Ferrante et al., 2003; Hockly et al., 2003). Modulating HDAC activity with sodium butyrate and TSA enhanced locomotor-activating effects of cocaine in mice (Kumar et al., 2005), whereas TSA and phenylbutyrate dose-dependently reduced cocaine self administration in rats (Romieu et al., 2008). Moreover, chronic injection of valproic acid into the NAc, attenuated amphetamine-induced locomotor activity in rats (Kim et al., 2008b).

A recent focus of intensive investigation has been to develop class-specific and isoform-specific HDACis. Class I- and Class-II selective HDACis have been reported (Bieliauskas and Pflum, 2008). In cellular assays, selective Class-I HDACis increase acetylation of histones but not  $\alpha$ -tubulin (Arts et al., 2007). By contrast, tubacin, a classIIb-HDAC6-specific inhibitor increases  $\alpha$ -tubulin acetylation, the known substrate of HDAC6, without any effect on histones acetylation (Haggarty et al., 2003). Tubacin is structurally related to SAHA but contains a very large capping group that putatively mimics acetylated  $\alpha$ -tubulin. However, these specific HDACis, tested in non-neuronal cellular models, need to be investigated in brain studies both in vitro and in vivo.

Brain permeability and non-specific side effects remain major limitation for drug treatment of CNS disorders. It has been demonstrated in mice that SAHA, sodium butyrate, phenylbutyrate or valproic acid present a good brain penetration but the use of the compounds might be limited by toxicity (Drummond et al., 2005). Nevertheless, some HDACis are currently in different phases of human clinical trials for CNS disorders related to striatal dysfunctions, such as HD. Now, efforts are made to design both potent and isofom-selective HDACis able to inactivate specific intracellular targets with minimal non-specific side effects.

## **B.** Histone Phosphorylation

Similarly to acetylation, histone phosphorylation is associated with decreased histone-DNA link and hence increased transcription. The most exemplified is Histone H3 at Ser10 that occurs concomitantly with IEG induction by a variety of extracellular stimuli. In 1999, this event was first described by Mahadevan's group (Thomson et al., 1999) to be mediated by the MAP kinases of ERK and p38 family, although not directly because amino acids surrounding Ser10 of H3 are not a consensus sequence for MAPK/ERK and p38. The same year, the expression of Ras, one of the activators of the MAPK/ERK signaling pathway, into fibroblasts was shown to result in a rapid phosphorylation of H3 (Chadee et al., 1999). Initially, it was thought that Lys14 acetylation and Ser10 phosphorylation of H3 played a synergistic role in the nucleosomal response at the promoter of these IEGs (Clayton and Mahadevan, 2003; Nowak and Corces, 2004). In this "synergistic model", phosphorylation is a priming event preceding acetylation, which leads, in turn, to IEG transcription. This synergistic model is supported by in vitro experiments showing that the yeast acetyltransferase GCN5 activity is dependent on the prior phosphorylation of H3 on Ser10 (Cheung et al., 2000; Lo et al., 2000; Lo et al., 2001). An alternative "parallel model" of activation has been proposed, in which phosphorylation occurs independently on pre-acetylated histone H3 to induce IEGs transcription. This model is supported by in vitro experiments of c-jun induction showing that phosphorylation of histone H3 does not necessarily favour the acetylation of Lys-14 and that these modifications occur independently (Thomson et al., 2001). In addition, the blockade of Ser10 H3 phosphorylation does not alter the Lys-14 H3 acetylation level at the promoter of c-jun in response to 12-O-tetradecanoylphorbol-13-acetate (TPA) or anisomycin, in vitro (Soloaga et al., 2003). In neurons, a body of evidence support the "parallel model". We and others demonstrated (using specific antibodies for phosphorylated-Ser10 H3, and acetylated-Lys14 antibodies, respectively) that phospho-Ser10-H3 immunoreactivity increases in vitro in response to glutamate, dopamine or acetylcholine in the striatum and hippocampus, independently of hyperacetylation of Lys14-H3 (Crosio et al., 2003; Brami-Cherrier et al., 2007). Importantly, this phosphorylation event occurs specifically at the promoter of c-fos and

c-*jun*, as demonstrated by ChiP assays (Crosio et al., 2003; Brami-Cherrier et al., 2005). Similar data were found in vivo, in striatal neurons in response to cocaine (Kumar et al., 2005). Thus, altogether, these results provide evidence that phosphorylation of H3 is necessary and sufficient for the nucleosomal response at some specific loci, including the promoters of c-*fos* and c-*jun*, at least in neuronal cells (Brami-Cherrier et al., 2009).

### 1. Histone H3 Kinases

The kinases responsible for H3 phosphorylation remain controversial, and two main families of proteins kinases have been proposed: ribosomal subunit protein S6 kinases (RSKs) and mitogen and stress activated protein kinases (MSKs). These two families of protein kinases have a similar structure and have been shown, using in vitro kinase assays, to directly phosphorylate H3 on Ser10. However, they show differences in their mode of activation (i.e. different upstream kinases that are responsible for their activity), as well as subcellular and regional distribution, which may account for their specific role in a physiological function. The main properties and physiological functions of these two families of protein kinases are described below.

The RSK family comprises 4 members, RSK1, RSK2, RSK3 and RSK4 encoded by 4 different genes (Roux and Blenis, 2004). RSKs are serine/threonine protein kinases with two kinase domains, a linker region and short N-terminal and C-terminal tails. Among these proteins, only RSK3 contains a nuclear localization signal (NLS) located in its N-terminal region. The N-terminal kinase domain (NTKD) of the RSKs belongs to the AGC family of kinases, which also includes protein kinase A, protein kinase C, protein kinase B (also called Akt), and the p70 ribosomal S6 kinases 1 and 2 (S6K1 and S6K2). The C-terminal tail contains a short docking motif, which is responsible for the specific association of RSK with ERK (Zhao et al., 1996; Smith et al., 1999). Upon mitogen stimulation, ERK1/2 activates the C-terminal kinase domain (CTKD) by phosphorylating Thr574, which in turn leads to phosphorylation of Ser381 and Ser364 in the hydrophobic pocket. This creates a docking site for PDK1 (phosphoinositide dependent protein kinase 1), the enzyme that phosphorylates Ser221 located in the activation loop of the NTKD, a reaction necessary for the catalytic activity of RSKs (Frodin et al., 2000; Frodin et al., 2002; Collins et al., 2003). Thus, both ERK and PDK1 are necessary for RSKs activation. Following their activation RSKs have, besides histone H3, multiple nuclear and cytosolic substrates including filamin A, SOS, GSK3beta, IKBalpha, Bad, ER81, CREB, CBP, and c-Fos (Roux and Blenis, 2004).

MSKs are nuclear serine/threonine protein kinases that were initially characterized as kinases activated by ERK1/2 or p38MAPK (see below) (Deak et al., 1998). MSKs are coded by two genes corresponding to two proteins, MSK1 and MSK2, which are highly conserved (90% identity between mouse and human MSK1 and MSK2) and share 75% of identical amino acids. MSK proteins share with RSKs 40% identity and the same overall structure. Both MSK1 and MSK2 contain a MAPK docking domain and a NLS sequence within their CTKD. Similarly to RSKs, MSKs are activated via sequential phosphorylation. Binding of MAPK/ERK or p38 occurs via the MAPK docking site and triggers phosphorylation of Thr700, Thr581 in the CTKD and of Ser360 in the linker domain of MSK1 (Arthur, 2008). The phosphorylation of Thr700 alleviates the inhibitory role of the C-terminal auto-inhibitory sequence and helps to protect Thr581 from dephosphorylation. Then, the CTKD phosphorylates two sites in the linker region Ser376 and 381 as well as the activation loop in the NTKD (Ser212). This results in the activation of the NTKD, and catalytic activity of MSK1. Other phosphorylation sites on Thr and Ser (Ser750, 752, 758) of MSK1 have been identified that could participate in its catalytic activity (McCoy et al., 2005; McCoy et al., 2007). Importantly, Thr581 in the CTKD is essential for the activity of MSK1 as its mutation to alanine results in an inactive kinase. It must be emphasized that, contrasting with RSKs family proteins, MSKs activation is independent of PDK1 (Frodin et al., 2000; Williams et al., 2000). Of interest, two important substrates of RSKs and MSKs are identical: the transcription factor CREB and histone H3. MSKs proteins also phosphorylate ATF1, HMGN1 and NFkappaB (Roux and Blenis, 2004).

Sassone-Corsi and collaborators (1999) first proposed that RSK2 was required for epidermal growth factor (EGF)-induced H3 phosphorylation on Ser10 (Sassone-Corsi et al., 1999). For this demonstration, they used fibroblasts of patients suffering from Coffin Lowry syndrome (CLS), an X-linked disease associated with mutations of the gene encoding RSK2, and characterized by a combination of complex bone malformations and mental retardation. In these cells, the phosphorylation of H3 induced by EGF was strongly reduced. By contrast H3 phosphorylation occurring during mitosis remained intact. Introducing wild type RSK2 gene restored EGF-stimulated phosphorylation of H3 in CLS cells. In addition, disruption of RSK2 gene by homologous recombination abolished H3 phosphorylation induced by EGF in fibroblasts.

In parallel, using in vitro kinase assays with an H3 peptide as substrate, Mahadevan and coll. proposed that MSK1 was a better kinase for Ser10 histone H3 than RSK1 or RSK2 (Thomson et al., 1999). This group also showed that MSK1 activation was produced by various stimuli, including EGF, serum, phorbol ester (TPA), stress stimuli such as UV radiation, oxidative stress and anisomycin (Deak et al., 1998; Pierrat et al., 1998), and identified ERK1/2 and stress activated protein kinase 2a (SAPK2a, also known as p38 MAPK) as the kinases upstream from MSKs (Deak et al., 1998; New et al., 1999; Wiggin et al., 2002). Albeit not specific, H89, which inhibits more potently MSK1 than RSKs, also diminished TPA-, EGF- and anisomycin-induced H3 phosphorylation (Thomson et al., 1999). Later, Soloaga et al. showed that the level of histone H3 phosphorylation was normal in CLS fibroblasts in response to stress and mitogenic stimuli (anisomycin and TPA) (Soloaga et al., 2003). However, they did not analyze EGF-induced H3 phosphorylation in CLS fibroblasts. Furthermore, they showed that histone H3 phosphorylation induced by TPA or anisomycin, was totally abolished in double knock-out mice for MSK1 and/ MSK2. These latter data firmly implicated MSKs proteins in H3 phosphorylation downstream from ERK and p38 MAPK activation. There is yet no explanation for the apparent contradiction between the data obtained by Sassone Corsi's and Mahadevan's groups. They cannot be explained by differences in cell lines used (CLS fibroblasts), but rather by the external stimuli and signaling pathways activated downstream. Indeed, one of the key differences between EGF and TPA-induced phosphorylation of H3 could be PDK1, which is known to be activated by the PI3-kinase pathway downstream from EGF-R stimulation (Corbit et al., 2000).

#### Expression patterns of MSK and RSK

In mice and human, global expression profiles indicate that both MSKs and RSKs mRNAs are ubiquitously expressed (Alcorta et al., 1989; Moller et al., 1994; Deak et al., 1998). The strongest expression of the *Rsk2* and *Rsk3* gene transcripts was found in skeletal muscle, heart and pancreas, whereas low levels were observed in the brain. *Rsk1* was mainly expressed in kidney, lung and pancreas, with also a low level in brain (Zeniou et al., 2002). By contrast both *Msk1* and *Msk2* mRNAs are enriched in the brain, and also strongly expressed in the placenta and pancreas (Deak et al., 1998). More recently, the expression pattern and subcellular localization of MSK1 and RSK1 proteins were described in the adult mouse brain by immunohistochemistry (Heffron and Mandell, 2005). MSK1 protein expression is especially high in the striatum, olfactory tubercle neurons, whereas RSK1 shows the highest expression level in the cerebellum. MSK1 is also strongly enriched in the amygdala and some neurons in the hippocampus (personal observations) and to a lesser degree in cerebellar Purkinje cells, whereas its expression is very low in the cortex and hippocampus (Heffron and Mandell, 2005). MSK1 is mainly expressed in neurons, whereas RSK1 is also found in microglia throughout the brain. At the subcellular level, MSK1 is confined to the nucleus of all expressing neurons. In contrast, intense immunoreactivity of RSK1 is found in cell bodies of granular neurons in cerebellum and within the neuropil of the molecular layer. The nuclear restriction of MSK1 in neurons, which has been already described in other cell types (Deak et al., 1998; Pierrat et al., 1998), is consistent with its role as a direct kinase for histones and transcription factors. Thus, the comparative expression pattern of RSK1 and MSK1 in neuronal cell bodies and neuropil suggests that they have different physiological roles in neurons.

MSK1 is particularly enriched in the striatum, where it has been reported to be present in  $\sim 60\%$  of the neurons, likely to be projection neurons, the GABAergic mediumsize spiny neurons (MSNs) (Heffron and Mandell, 2005). MSNs projecting to the substantia nigra pars reticulata and internal globus pallidus express mostly dynorphin, substance P and D1 dopamine receptors, whereas MSNs projecting to the external globus pallidus preferentially express enkephalin and D2 dopamine receptors (see Chapter 1). These two populations of neurons are thought to participate in distinct, direct and indirect, circuits with opposing functional properties. Cocaine-induced phosphorylation of MSK1 is blocked by the selective D1 receptor antagonist, SCH23390, suggesting that MSK1 is activated in D1 receptor-expressing neurons (Brami-Cherrier et al., 2005). This conclusion is further supported by a study identifying the MSN sub-population in which MSK1 is activated after cocaine injection in mice (Bertran-Gonzalez et al., 2008). Using transgenic mice, in which green fluorescent protein (EGFP) expression is driven by either the D1 receptor promoter (Drd1a-EGFP) or the D2 receptor promoter (Drd2-EGFP), it was shown that acute cocaine treatment induced ERK activation along with MSK1 and H3 phosphorylation exclusively in D1 receptor-expressing neurons. In contrast, phosphorylation of histone H3 in D2 receptor-expressing neurons after haloperidol injection

(a D2 receptor antagonist) is probably independent of MSK1 (Bertran-Gonzalez et al., 2008), suggesting differences in the signalling pathways that mediate histone H3 phosphorylation in the D1 receptor- and D2 receptorexpressing neurons. Furthermore, MSK1 is found at higher levels in D1 receptor-containing MSNs than in D2 receptorcontaining MSNs (Bertran-Gonzalez et al., 2008).

Further studies are now needed to precisely investigate the expression pattern of the other MSK2, and the additional RSK isoforms RSK2, RSK3 and RSK4 in the various brain areas.

## 2. Histone H3 Phosphatases

Although the role of kinases in the regulation of histone H3 phosphorylation has been extensively investigated, phosphatases have also been implicated directly or indirectly in this process. Protein phosphatase-1 (PP1) was first proposed to be implicated directly in dephosphorylation of Ser10-H3 (Chadee et al., 1999) and also in the regulation of histone H3 kinases by inhibiting Aurora B (Murnion et al., 2001). Inhibition of PP1 by cocaine-induced phosphorylation and nuclear translocation of the 32-kDa dopamine-regulated and cyclic-AMP-regulated phosphoprotein (DARPP-32) was shown to be involved in H3 phosphorylation in response to cocaine (Stipanovich et al., 2008). Protein phosphatase-2A (PP2-A) is also known to directly dephosphorylate histone H3 on Ser10 in Drosophila (Nowak et al., 2003). Moreover, PP2A could also inhibit H3 phosphorylation indirectly by dephosphorylating H3 kinase, as demonstrated for Aurora B (Sugiyama et al., 2002).

## C. Histone Methylation

Lysine methyltransferases have enormous specificity when compared to acetyltransferase. They usually modify one single lysine on a single histone and their output can be either activation or repression of transcription (Bannister et al., 2005). Di- or trimethylation of lys4 of H3 usually marks active genes whereas di- or tri-methylation of Lys9 or Lys27 of H3 constitute repressive marks (Delcuve et al., 2009).

# III. CHROMATIN REMODELING AND STRIATAL DYSFUNCTIONS

## A. Chromatin Remodeling in Drug Addiction

Drug addiction is a chronic disorder that can be defined as compulsive drug seeking and taking despite negative consequences (Kalivas and Volkow, 2005; Hyman et al., 2006;

Koob and Kreek, 2007) (see Chapter 33). The neural mechanisms that are responsible for the transition from recreational drug use to a chronically addicted state are supported by long-lasting changes in gene expression in specific brain area (see also Chapter 29). These changes occur in reward areas, including the striatum and nucleus accumbens (NAc), prefrontal cortex (PFC) and ventral tegmental area (VTA). One common characteristic of all drugs of abuse is that they produce increases in extracellular dopamine concentrations within this reward circuitry. By doing so, drugs produce long-term changes and enhance the behavioral aspects of dependence, such as drug seeking (Di Chiara and Bassareo, 2007). Chronic drug exposure produces altered expression of several genes, and results, for example, in accumulation of the transcription factor deltaFosB, which is induced several fold in the NAc and has been implicated in the transition to an addicted state (McClung et al., 2004). Altered expression of specific genes, such as cdk5 and brain-derived neurotrophic factor (BDNF) (Bibb et al., 2001; Grimm et al., 2003) has been reported weeks after the last drug experience, and manipulation of these genes in rodents regulate drug-relapse behavior (Bowers et al., 2004; Graham et al., 2007). Consistent with such stable changes in gene expression, increased histone H3 acetylation was observed on the gene promoters of both cdk5 and BDNF for 1 to 7 days after the final dose of cocaine (Kumar et al., 2005).

The Fos family of IEGs (c-Fos, FosB, Fra1 and Fra2), which are rapidly induced in the striatum after acute exposure to drugs (Hope et al., 1994; Moratalla et al., 1996), has been implicated as important mediators of neuronal plasticity and behavioral alterations induced by drugs of abuse. This induction is transient, lasting only 4-12 hours after drug exposure. In contrast, the splice variant of FosB, delta-FosB, is induced at very low levels after acute administration of drugs, but accumulates after chronic treatment and remains elevated even after weeks of withdrawal, due to the unusually high stability of the protein (Hope et al., 1994; Chen et al., 1997; Kelz et al., 1999; Nestler et al., 2001). These observations led to the proposal that c-Fos is desensitized by chronic administration of cocaine, and deltaFosB is responsible for many of the longer-lived changes in gene expression that underlie addiction (Nestler, 2001). It must be noted, however, that c-Fos can still be detected after chronic administrations when examined at early time points after the last injection, that is, 30 min for mRNA or one hour for protein expression (Steiner and Gerfen, 1993; Rosen et al., 1994; Radwanska et al., 2006). Thus, in both acute and chronic treatments, the induction of *c*-fos is probably one of the prime events of a complex signalling cascade



FIGURE 30.3 Cocaine induces global hyperphosphorylation of H3 and hyperacetylation of H4 in striatal neurons. Mice receiving cocaine were sacrificed 20 minutes later, and striatal sections processed for immunocytochemical detection using (A) phospho-Ser10-H3 (P-H3; left panel) and (B) acetyl-Lys5-H4 (Ac-H4; right panel) specific antibodies. Note the total inhibition of H3 phosphorylation induced by cocaine in MSK1–/– mice and the lack of modification of H4 acetylation levels (From Brami-Cherrier et al., 2005).

leading to cocaine-induced neuronal adaptation and behavioral alterations. c-Fos is associated with Jun-B to form an AP1 complex which will be active for the transcriptional activation of target genes (Radwanska et al., 2006), among these *fosB*, which encodes deltaFosB (Zhang et al., 2002a).

Histone acetylation and phosphorylation have been studied in the context of IEG transcription. In an elegant study using chromatin immunoprecipitation (ChiP) assays, it was shown, that one the prime event at the promoter of c-fos was acetylation of histone H4 and phosphorylation, but not acetylation, of H3 (Kumar et al., 2005). The later conclusion was drawn using a phospho-acetyl H3 antibody, (which recognizes H3 when it is acetylated and phosphorylated at the same tail) along with an anti-acetyl H3 antibody. They found that acute cocaine administration increased levels of phospho-acetylated H3 but not of the solely acetylated form, indicating that only phosphorylation occurred. By contrast, none of these events could be observed when analyzed 1 and 24 hours after a chronic course of cocaine. Histone modifications is quite different at the *fosB* promoter, since ChiP experiments showed hyperacetylation of H4 but no hyperacetylation of H3 by acute cocaine. This increase in histone acetylation is dependent on CBP (Levine et al., 2005). By contrast, after chronic cocaine, no changes in histone H4 acetylation, but hyperacetylation of H3 were found after chronic cocaine (when analyzed one and 24 hours later). No changes for phosphorylation levels of H3 were observed at the *fosB* promoter in either acute or chronic conditions. Using global immunodetection, we also concluded that acute cocaine administration induces rapidly, within 30 minutes,

acetylation of H4, phosphorylation but not acetylation of H3 in striatal neurons (Brami-Cherrier et al., 2005) (Fig. 30.3). Thus post-translational modifications of H3 and H4 seem to be differentially involved in chromatin remodeling. The combinatorial dynamic changes of these histones may depend on the context: the promoter region of the gene, its molecular environment, and signalling pathways activated upon acute or chronic administration.

The induction of c-Fos but not Zif268 protein expression induced by acute cocaine is blocked in MSK1 KO (Brami-Cherrier et al., 2005). Thus, contrasting with *c-fos*, chromatin remodeling at the promoter of *zif268* is not dependent on MSK1 and H3 phosphorylation. Cocaineinduced expression of dynorphin, a peptide which decreases dopamine release by activating kappa opioid receptors on dopamine terminals (Spanagel et al., 1992) (see Chapter 29), is also strongly decreased in MSK1 knock-out mice. However, at this time, no evidence indicates that the promoter of the gene encoding this peptide is under the control of chromatin remodeling via H3 phosphorylation. Instead, we know that dynorphin is regulated by the transcription factor CREB (Carlezon et al., 1998), the phosphorylation of which was decreased in MSK1 KO mice.

Increased locomotor sensitization induced by cocaine is observed after treatment by global inhibitors of HDAC activity, such as sodium butyrate, which increases cocaineinduced H3 phospho-acetylation at the c-*fos* promoter, along with c-*fos* mRNA induction (Kumar et al., 2005). Conversely, viral-mediated over-expression of *Hdac4* or *Hdac5* has the opposite effects. Consistent with these findings, mice deficient in CBP, which possess an intrinsic HAT activity, show reduced cocaine-induced locomotor sensitization, along with altered histone H4 acetylation levels at the *Fos B* promoter (Levine et al., 2005). Thus, hyperacetylation of histones, including H3 and H4, seems to be important for chromatin remodeling and behavioral alterations induced by cocaine.

By contrast, reduced H3 phosphorylation observed in MSK1 KO mice is associated with decreased locomotor sensitization, but an apparent increased sensitivity to cocaine in the place preference paradigm, indicated by a leftward shift of the CPP dose-response curve. ERK is critical for long lasting effects of cocaine since MEK inhibitors totally block locomotor sensitization (Valjent et al., 2005), the induction of conditioned place preference (CPP) (Valjent et al., 2000) and its reconsolidation (Miller and Marshall, 2005; Valjent et al., 2006a), and incubation of craving (Lu et al., 2005). Thus, altogether these data provide clear evidence of an uncoupling of molecular events downstream from ERK for the control of behavioral alterations induced by cocaine, and support recent data from conditional *c-fos* knock-out mice, where locomotor sensitization but not CPP were impaired (Zhang et al., 2006). By contrast, ERK signaling to zif268 appears to be critically involved in cocaine CPP, as demonstrated in homozygous knock-out mice for zif268 (Valjent et al., 2006b).

In addition to the activation of the ERK/MSK1 pathway, recent evidence shows that cocaine and other drugs of abuse inhibit the dephosphorylation of H3 (Stipanovich et al., 2008). Cocaine induces the nuclear accumulation of the active form (phosphorylated on Thr34) of DARPP-32, a potent inhibitor of protein phosphatase-1. This nuclear accumulation results from the dephosphorylation of Ser97, which decreases the export of DARPP-32 from the nucleus to the cytoplasm. Dephosphorylation of Ser97 is achieved through PKA-activation of the B56 $\delta$  subunit of PP2A. Disruption of this mechanism in Ser97Ala DARPP-32 mutant mice altered the behavioral effects of drugs of abuse (Stipanovich et al., 2008), suggesting that regulation of H3 dephosphorylation also contributes to the effects of drug of abuse.

A promising new molecular avenue for the analyses of chromatin remodeling in drug addiction, was recently approached using genome-wide promoter microarrays (ChIP-chip) or high-throughput sequencing (Renthal and Nestler, 2008). These techniques allow the characterization of drug-induced histone modifications across every gene in the genome, and give rise to a wealth of new informations about epigenetic regulation in specific brain areas, as well as novel gene targets for the control of behavioral responses to drugs of abuse.

# **B.** Chromatin Remodeling in Human Neurological Disease

Besides its well documented role in drug addiction, chromatin remodeling in the striatum also plays a role in the pathogenesis of various neurological conditions including HD, dentatorubral-pallidoluysian atrophy and L-DOPA-induced dyskinesias.

## 1. Striatal Chromatin Remodeling in Huntington's Disease

HD is the most frequent neurodegenerative disease caused by an expansion of glutamines repeats (see also Chapter 35). The main clinical manifestations of HD are chorea, cognitive impairment and psychiatric disorders. The transmission of HD is autosomic dominant with a complete penetrance. The mutation responsible for HD, an unstable expansion of CAG repeat sequence, is located at the 5' terminal part of the IT15 gene encoding the Huntingtin (htt). One important characteristic of HD is the vulnerability of a particular brain region, the striatum, despite similar expression of the mutated protein in other brain areas (Roze et al., 2008a). Aggregation of the mutated Htt, transcriptional dysregulation, altered energy metabolism, excitotoxicity, impaired axonal transport, and altered synaptic transmission mediate neuronal dysfunction and death (Roze et al., 2008a). Dysregulation of transcription was first described in HD brain tissues at early neuropathological stages, with altered levels of dopamine receptor and neuropeptide mRNAs. Decreased mRNA levels found in pre-symptomatic HD transgenic mice, indicated that changes in transcription underlie neurodegeneration rather than unspecific degradation of all RNAs in affected neurons (Cha, 2007). Transcriptional dysregulation was further investigated in a series of works, in HD rodent models and human brain tissues, showing deregulation of multiple genes, encoding neurotransmitter receptors, enzymes, proteins involved in the structure of neurons, in their response to stress, or in the axonal transport. This deregulation of transcription (either up- or down-regulation) in large genomic regions occurs in a coordinated fashion and is associated with disease progression (Anderson et al., 2008).

Mutant htt, in its soluble or aggregated form, interacts with transcription factors, interferes with the transcriptional

machinery, and in turn in the transcriptional responses and cell viability (Roze et al., 2008a). In addition to these alterations, chromatin remodeling, particularly in the striatum, is likely to play a key role in the transcriptional deregulation observed in HD. The mutated htt interacts with CBP and blocks its intrinsic HAT activity (Steffan et al., 2001). Administration of HDACis including SAHA, sodium butyrate and phenylbutyrate, have a potential therapeutic effect in several HD mouse models (Steffan et al., 2001; Ferrante et al., 2003; Hockly et al., 2003; Gardian et al., 2005), with improved behavioral performance and neuronal survival. Interestingly, administration of a new benzamide-type HDACi with lower potential toxicity than other HDAC, HDACi 4b, also restores the transcription of critical striatal genes and improves motor and neuropathological phenotype of R6/2 HD mice (Thomas et al., 2008). Finally, it must be emphasized that levels of acetylated histones are not decreased globally in HD mice models, but rather selectively in the promoters of genes that are specifically downregulated in HD (Sadri-Vakili et al., 2007). Since all HDACis act broadly on various classes of HDACs, better benefits to side effects could be obtained with specific HDAC/Sirt inhibitors, as recently proposed in drosophila models of HD (Pallos et al., 2008).

Methylation of histones plays the reverse, inhibiting role on transcription. One of the proteins involved in methyltransferase activity at histone H3 (Lys9) is ESET (ERGassociated protein with SET domain). ESET expression is increased in HD patients and transgenic R6/2 HD mice (Ryu et al., 2006). Sp1 acts as a transcriptional activator of the ESET promoter at guanosine-cytosine (GC)-rich DNA binding sites (Yang et al., 2003). Inhibiting Sp1 binding to these sites, using mitramycin (a clinically approved antitumor antibiotic), suppressed basal ESET promoter activity in a dosedependent manner. The combined pharmacological treatment with mithramycin and cystamine down-regulates ESET gene expression and hypertrimethylation of histone H3. This treatment significantly ameliorates the behavioral and neuropathological phenotype of R6/2 HD mice and improves their survival. In general, the DNA/RNA binding agents, anthracyclins are thought to provide significant therapeutic potential by correcting the pathological nucleosome changes and realigning transcription. Two such agents, chromomycin and mithramycin, were found to improve altered nucleosomal homeostasis, normalizing the shift in the balance between methylation and acetylation in HD mice. This resulted in the regulation of a subset of downregulated genes along with a significant improvement of the

behavioral and neuropathological phenotypes observed in HD mice (Stack et al., 2007).

Although it has been less studied, histone H3 phosphorylation and histone H2A-H2B ubiquitylation are also critical to induce the nucleosomal response and gene transcription at some promoters. The histone H3 kinase MSK1 is deficient in the striatum, specifically, of R6/2 mice and HD patients (Roze et al., 2008b). Restoring MSK1 expression and subsequent striatal H3 phosphorylation in an in vitro model system of HD protects against neuronal alteration induced by the mutated htt including neuritic retraction, aggregate formation and neuronal death (Roze et al., 2008b). Histone H2A ubiquitylation is increased in R6/2 HD mice and associated to DNA regions located in the promoters of down-regulated genes in an in vitro model of HD (Kim et al., 2008a). This transcriptional repression is rescued by restoration of the ubiquitylated H2A level. In addition, histone H2B ubiquitylation is decreased in R6/2 HD mice, and association of ubiquitylated H2B with promoters positively correlates with transcriptional level in R6/2 mice. Reduction in H2A and H2B ubiquitylation produces the opposite effect on transcription via methylation of histone H3 at lysine 9 and lysine 4, respectively. These findings provide the first rationale for targeting histone ubiquitylation for therapy in HD (Kim et al., 2008a).

# 2. Striatal Chromatin Remodeling in Dentatorubral-Pallidoluysian Atrophy

Dentatorubral-pallidoluysian atrophy (DRPLA) is another example of a human autosomal dominant neurodegenerative disorder due to an expanded polyglutamine repeat, located in the exon 5 of the gene coding the atrophin 1 protein. The main clinical manifestations of DRPLA are chorea, cognitive impairment, ataxia, myoclonus, epilepsia and psychiatric disturbances. Similarly to HD, selective brain areas are predominantly affected by the disease, including the striatum, the dentate nucleus, the red nucleus and the subthalamic nucleus. Although far less studied, the mechanisms of neuronal dysfunction are thought to be closed to those involved in HD, including transcriptional dysregulation. This transcriptional dysregulation is an early phenomenon, which increases along with disease progression in a DRPLA mouse model (Sato et al., 2009). Similarly to HD, the polyglutamine stretch interferes with transcription factors and the transcriptional machinery. In particular, dysfunction of CREB-dependent transcription is likely to account, at least in part, for this dysregulation. Thus, most of genes down-regulated in DRPLA models have a CRE site in their promoters, and CRE dependent transcription is altered in vitro, by over-expression of an expanded poly-glutamine stretch (Shimohata et al., 2000; Nucifora et al., 2001; Shimohata et al., 2005). Atrophin 1 itself, is also a transcription regulator inhibiting gene expression through various mechanisms (Wood et al., 2000; Zhang et al., 2002b). These may include recruitment of HDAC1 through an interaction with atrophin 2 (Zoltewicz et al., 2004). The repressive activity of atrophin 1 on transcription is reduced in mutated atrophin 1, in a drosophila model of DRPLA (Zhang et al., 2002b). Treatment of 118Q DRPLA mice with sodium butyrate reverses the global hypoacetylation of histone H3 observed in these mice and improve their motor phenotype (Ying et al., 2006).

### 3. Striatal Chromatin Remodeling in L-DOPA-Induced Dyskinesia

Parkinson's disease is a neurodegenerative disease mainly characterized by the progressive loss of the dopaminergic input to the dorsal striatum (see also Chapter 34), resulting in severe motor dysfunctions with bradykinesia, rigidity and tremor. The dopamine precursor L-DOPA remains the most effective symptomatic pharmacological therapy. Unfortunately, as a result of both disease progression and long duration exposure to L-DOPA, patients develop debilitating involuntary movements referred to as L-DOPAinduced dyskinesias (see Chapter 36). Indeed, severe striatal dopamine depletion alters the response of MSNs to dopaminergic drugs (see Chapter 28). This altered response is mainly due to the sensitization of D1 receptor signaling and its downstream consequences (Berke et al., 1998; Gerfen et al., 2002; Corvol et al., 2004; Kim et al., 2006). Dopamine depletion is associated, in the striatum, with increased level and efficiency of the Golf protein (Corvol et al., 2004; Aubert et al., 2005), activation of the adenylate cyclase (Zhuang et al., 2000; Corvol et al., 2001) and, in turn, enhanced cAMP-dependent PKA-mediated phosphorylation, including DARPP-32 phosphorylation (Picconi et al., 2003; Santini et al., 2007) (see also Chapter 26). In addition, loss of physiological dopamine inputs and subsequent sensitization of D1 receptors enhances the L-DOPAinduced activation of the ERK signalling pathway (Gerfen et al., 2002; Kim et al., 2006; Pavon et al., 2006; Santini et al., 2007; Westin et al., 2007; Nicholas et al., 2008). In an animal model of PD, this activation occurs selectively in the striatonigral (but not striatopallidal) MSNs that express the D1 receptors (see also Chapter 36). The subsequent activation of MSK1 and phosphorylation of histone H3, which are specific to dyskinetic animals, are also restricted to striatonigral MSNs (Santini et al., 2009). Since phosphorylation of DARPP-32 has been involved, at least in part, in the activation of ERK downstream D1 receptors (Valjent et al., 2005), these data suggest a functional coupling between the cAMP/PKA/DARPP-32 cascade and the ERK/MSK1/H3 pathway. Modulation of histone H3 and H4 acetylation is likely to play also a role in the chromatin remodeling involved in L-DOPA-induced dyskinesia, but data on this aspect are scarce and inconsistent (Nicholas et al., 2008). Although it remains to be clearly demonstrated, these histone modifications and the related chromatin remodeling, could underlie, at least in part, the alteration of genes expression pattern observed in the striatal MSNs and the resulting long-term changes in neuronal functions (Andersson et al., 1999; Bordet et al., 2000; Konradi et al., 2004; Carta et al., 2005).

# **IV. CONCLUSIONS**

Thus, a combination of post-translational modifications occurs on histones at specific loci and appears to be critically required for gene transcription to occur. Modifying one of these post-translational events could be sufficient to restore at least in part, molecular and behavioral alterations observed in neurological diseases associated with basal ganglia dysfunctions. Although most attention has been paid on histone acetylation and therapeutic potentials of HDACis, we propose that modulation of the other histone post-translational modifications could be a promising avenue for the treatment of neuropathologies of basal ganglia, including drug and alcohol addiction, HD, DRPLA and L-DOPA-induced dyskinesias.

### REFERENCES

- Alarcon JM, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, Barco A (2004) Chromatin acetylation, memory, and LTP are impaired in CBP+/– mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron 42:947–959.
- Alcorta DA, Crews CM, Sweet LJ, Bankston L, Jones SW, Erikson RL (1989) Sequence and expression of chicken and mouse rsk: homologs of *Xenopus laevis* ribosomal S6 kinase. Mol Cell Biol 9:3850–3859.
- Anderson AN, Roncaroli F, Hodges A, Deprez M, Turkheimer FE (2008) Chromosomal profiles of gene expression in Huntington's disease. Brain 131:381–388.
- Andersson M, Hilbertson A, Cenci MA (1999) Striatal fosB expression is causally linked with I-DOPA-induced abnormal involuntary movements

and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis 6:461–474.

- Arthur JS (2008) MSK activation and physiological roles. Front Biosci 13:5866–5879.
- Arts J, Angibaud P, Marien A, et al. (2007) R306465 is a novel potent inhibitor of class I histone deacetylases with broad-spectrum antitumoral activity against solid and haematological malignancies. Br J Cancer 97:1344–1353.
- Aubert I, Guigoni C, Hakansson K, et al. (2005) Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann Neurol 57:17–26.
- Bannister AJ, Schneider R, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T (2005) Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. J Biol Chem 280:17732–17736.
- Belfield JL, Whittaker C, Cader MZ, Chawla S (2006) Differential effects of Ca<sup>2+</sup> and cAMP on transcription mediated by MEF2D and cAMPresponse element-binding protein in hippocampal neurons. J Biol Chem 281:27724–27732.
- Berger SL (2002) Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12:142–148.
- Berger SL (2007) The complex language of chromatin regulation during transcription. Nature 447:407–412.
- Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR (1998) A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci 18:5301–5310.
- Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Herve D, Valjent E, Girault JA (2008) Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 28:5671–5685.
- Bibb JA, Chen J, Taylor JR, et al. (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410:376–380.
- Bieliauskas AV, Pflum MK (2008) Isoform-selective histone deacetylase inhibitors. Chem Soc Rev 37:1402–1413.
- Bolger TA, Yao TP (2005) Intracellular trafficking of histone deacetylase 4 regulates neuronal cell death. J Neurosci 25:9544–9553.
- Bordet R, Ridray S, Schwartz JC, Sokoloff P (2000) Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. Eur J Neurosci 12:2117–2123.
- Bowers MS, McFarland K, Lake RW, Peterson YK, Lapish CC, Gregory ML, Lanier SM, Kalivas PW (2004) Activator of G protein signaling 3: a gatekeeper of cocaine sensitization and drug seeking. Neuron 42:269–281.
- Brami-Cherrier K, Lavaur J, Pages C, Arthur JS, Caboche J (2007) Glutamate induces histone H3 phosphorylation but not acetylation in striatal neurons: role of mitogen- and stress-activated kinase-1. J Neurochem 101:697–708.
- Brami-Cherrier K, Roze E, Girault JA, Betuing S, Caboche J (2009) Role of the ERK/MSK1 signalling pathway in chromatin remodelling and brain responses to drugs of abuse. J Neurochem 108:1323–1335.
- Brami-Cherrier K, Valjent E, Herve D, et al. (2005) Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci 25:11444–11454.
- Brandl A, Heinzel T, Kramer OH (2009) Histone deacetylases: salesmen and customers in the post-translational modification market. Biol Cell 101:193–205.
- Broide RS, Redwine JM, Aftahi N, Young W, Bloom FE, Winrow CJ (2007) Distribution of histone deacetylases 1-11 in the rat brain. J Mol Neurosci 31:47–58.

- Brownell JE, Allis CD (1996) Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr Opin Genet Dev 6:176–184.
- Bu P, Evrard YA, Lozano G, Dent SY (2007) Loss of Gcn5 acetyltransferase activity leads to neural tube closure defects and exencephaly in mouse embryos. Mol Cell Biol 27:3405–3416.
- Candau R, Zhou JX, Allis CD, Berger SL (1997) Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. EMBO J 16:555–565.
- Carlezon WA Jr., Thome J, Olson VG, et al. (1998) Regulation of cocaine reward by CREB. Science 282:2272–2275.
- Carrozza MJ, Kusch T, Workman JL (2003) Repairing nucleosomes during transcription. Nat Struct Biol 10:879–880.
- Carta AR, Tronci E, Pinna A, Morelli M (2005) Different responsiveness of striatonigral and striatopallidal neurons to L-DOPA after a subchronic intermittent L-DOPA treatment. Eur J Neurosci 21:1196–1204.
- Cha JH (2007) Transcriptional signatures in Huntington's disease. Prog Neurobiol.
- Chadee DN, Hendzel MJ, Tylipski CP, Allis CD, Bazett-Jones DP, Wright JA, Davie JR (1999) Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. J Biol Chem 274:24914–24920.
- Chandramohan Y, Droste SK, Arthur JS, Reul JM (2008) The forced swimming-induced behavioral immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the N-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway. Eur J Neurosci 27:2701–2713.
- Chawla S, Hardingham GE, Quinn DR, Bading H (1998) CBP: a signalregulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science 281:1505–1509.
- Chawla S, Vanhoutte P, Arnold FJ, Huang CL, Bading H (2003) Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. J Neurochem 85:151–159.
- Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. J Neurosci 17:4933–4941.
- Cheung P, Allis CD, Sassone-Corsi P (2000) Signaling to chromatin through histone modifications. Cell 103:263–271.
- Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365:855–859.
- Chwang WB, Arthur JS, Schumacher A, Sweatt JD (2007) The nuclear kinase mitogen- and stress-activated protein kinase 1 regulates hippocampal chromatin remodeling in memory formation. J Neurosci 27:12732–12742.
- Clayton AL, Mahadevan LC (2003) MAP kinase-mediated phosphoacetylation of histone H3 and inducible gene regulation. FEBS Lett 546:51–58.
- Collins BJ, Deak M, Arthur JS, Armit LJ, Alessi DR (2003) In vivo role of the PIF-binding docking site of PDK1 defined by knock-in mutation. Embo J 22:4202–4211.
- Corbit KC, Soh JW, Yoshida K, Eves EM, Weinstein IB, Rosner MR (2000) Different protein kinase C isoforms determine growth factor specificity in neuronal cells. Mol Cell Biol 20:5392–5403.
- Corvol JC, Studler JM, Schonn JS, Girault JA, Herve D (2001) Galpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. J Neurochem 76:1585–1588.

- Corvol JC, Muriel MP, Valjent E, Feger J, Hanoun N, Girault JA, Hirsch EC, Herve D (2004) Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. J Neurosci 24:7007–7014.
- Cress WD, Seto E (2000) Histone deacetylases, transcriptional control, and cancer. J Cell Physiol 184:1–16.
- Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P (2003) Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. J Cell Sci 116:4905–4914.
- Cuthbert GL, Daujat S, Snowden AW, et al. (2004) Histone deimination antagonizes arginine methylation. Cell 118:545–553.
- Deak M, Clifton AD, Lucocq LM, Alessi DR (1998) Mitogen- and stressactivated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. Embo J 17:4426–4441.
- Delcuve GP, Rastegar M, Davie JR (2009) Epigenetic control. J Cell Physiol 219:243–250.
- Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol 7:69–76.
- Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, Benz CC (2005) Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol 45:495–528.
- Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421:448–453.
- Ferrante RJ, Kubilus JK, Lee J, et al. (2003) Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J Neurosci 23:9418–9427.
- Fischle W, Wang Y, Allis CD (2003) Histone and chromatin cross-talk. Curr Opin Cell Biol 15:172–183.
- Frodin M, Jensen CJ, Merienne K, Gammeltoft S (2000) A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1. Embo J 19:2924–2934.
- Frodin M, Antal TL, Dummler BA, Jensen CJ, Deak M, Gammeltoft S, Biondi RM (2002) A phosphoserine/threonine-binding pocket in AGC kinases and PDK1 mediates activation by hydrophobic motif phosphorylation. Embo J 21:5396–5407.
- Garcia-Ramirez M, Rocchini C, Ausio J (1995) Modulation of chromatin folding by histone acetylation. J Biol Chem 270:17923–17928.
- Gardian G, Browne SE, Choi DK, et al. (2005) Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease. J Biol Chem 280:556–563.
- Gerfen CR, Miyachi S, Paletzki R, Brown P (2002) D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 22:5042–5054.
- Giles RH, Peters DJ, Breuning MH (1998) Conjunction dysfunction: CBP/p300 in human disease. Trends Genet 14:178–183.
- Goodman RH, Smolik S (2000) CBP/p300 in cell growth, transformation, and development. Genes Dev 14:1553–1577.
- Graff J, Mansuy IM (2008) Epigenetic codes in cognition and behavior. Behav Brain Res 192:70–87.
- Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW (2007) Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat Neurosci 10:1029–1037.
- Gregoretti IV, Lee YM, Goodson HV (2004) Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. J Mol Biol 338:17–31.
- Grewal SI, Moazed D (2003) Heterochromatin and epigenetic control of gene expression. Science 301:798–802.

- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y (2003) Timedependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742–747.
- Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL (2003) Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc Natl Acad Sci USA 100:4389–4394.
- Hardingham GE, Chawla S, Cruzalegui FH, Bading H (1999) Control of recruitment and transcription-activating function of CBP determines gene regulation by NMDA receptors and L-type calcium channels. Neuron 22:789–798.
- Hassa PO, Haenni SS, Elser M, Hottiger MO (2006) Nuclear ADPribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev 70:789–829.
- Heffron D, Mandell JW (2005) Differential localization of MAPK-activated protein kinases RSK1 and MSK1 in mouse brain. Brain Res Mol Brain Res 136:134–141.
- Hockly E, Richon VM, Woodman B, et al. (2003) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci USA 100:2041–2046.
- Hodawadekar SC, Marmorstein R (2007) Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. Oncogene 26:5528–5540.
- Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 13:1235–1244.
- Hsu JY, Sun ZW, Li X, Reuben M, Tatchell K, Bishop DK, Grushcow JM, Brame CJ, Caldwell JA, Hunt DF, Lin R, Smith MM, Allis CD (2000) Mitotic phosphorylation of histone H3 is governed by Ipl1/ aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. Cell 102:279–291.
- Hu SC, Chrivia J, Ghosh A (1999) Regulation of CBP-mediated transcription by neuronal calcium signaling. Neuron 22:799–808.
- Huang Y, Doherty JJ, Dingledine R (2002) Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. J Neurosci 22:8422–8428.
- Hubbert C, Guardiola A, Shao R, et al. (2002) HDAC6 is a microtubuleassociated deacetylase. Nature 417:455–458.
- Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598.
- Janknecht R, Nordheim A (1996) MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. Biochem Biophys Res Commun 228:831–837.
- Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403–1413.
- Kelz MB, Chen J, Carlezon WA Jr., et al. (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. Nature 401:272–276.
- Kim DS, Palmiter RD, Cummins A, Gerfen CR (2006) Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice. Neuroscience 137:1381–1388.
- Kim MO, Chawla P, Overland RP, Xia E, Sadri-Vakili G, Cha JH (2008a) Altered histone monoubiquitylation mediated by mutant huntingtin induces transcriptional dysregulation. J Neurosci 28:3947–3957.

- Kim WY, Kim S, Kim JH (2008b) Chronic microinjection of valproic acid into the nucleus accumbens attenuates amphetamine-induced locomotor activity. Neurosci Lett 432:54–57.
- Konradi C, Westin JE, Carta M, Eaton ME, Kuter K, Dekundy A, Lundblad M, Cenci MA (2004) Transcriptome analysis in a rat model of L-DOPA-induced dyskinesia. Neurobiol Dis 17:219–236.
- Koob G, Kreek MJ (2007) Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry 164:1149–1159.
- Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972.
- Kouzarides T (2002) Histone methylation in transcriptional control. Curr Opin Genet Dev 12:198–209.
- Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705.
- Kumar A, Choi KH, Renthal W, et al. (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314.
- Kuo MH, Allis CD (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20:615–626.
- Kuo MH, Zhou J, Jambeck P, Churchill ME, Allis CD (1998) Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo. Genes Dev 12:627–639.
- Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD (2004) Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 279:40545–40559.
- Levine AA, Guan Z, Barco A, Xu S, Kandel ER, Schwartz JH (2005) CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. Proc Natl Acad Sci USA 102:19186–19191.
- Li B, Carey M, Workman JL (2007) The role of chromatin during transcription. Cell 128:707–719.
- Liu CL, Kaplan T, Kim M, Buratowski S, Schreiber SL, Friedman N, Rando OJ (2005) Single-nucleosome mapping of histone modifications in *S. cerevisiae*. PLoS Biol 3:e328.
- Lo WS, Duggan L, Emre NC, Belotserkovskya R, Lane WS, Shiekhattar R, Berger SL (2001) Snf1 a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. Science 293:1142–1146.
- Lo WS, Trievel RC, Rojas JR, Duggan L, Hsu JY, Allis CD, Marmorstein R, Berger SL (2000) Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. Mol Cell 5:917–926.
- Lu J, McKinsey TA, Zhang CL, Olson EN (2000) Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. Mol Cell 6:233–244.
- Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y (2005) Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. Nat Neurosci 8:212–219.
- Luger K, Richmond TJ (1998) The histone tails of the nucleosome. Curr Opin Genet Dev 8:140–146.
- Marmorstein R (2001) Structure and function of histone acetyltransferases. Cell Mol Life Sci 58:693–703.
- McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ (2004) DeltaFosB: a molecular switch for long-term adaptation in the brain. Brain Res Mol Brain Res 132:146–154.
- McCoy CE, Campbell DG, Deak M, Bloomberg GB, Arthur JS (2005) MSK1 activity is controlled by multiple phosphorylation sites. Biochem J 387:507–517.

- McCoy CE, Macdonald A, Morrice NA, Campbell DG, Deak M, Toth R, McIlrath J, Arthur JS (2007) Identification of novel phosphorylation sites in MSK1 by precursor ion scanning MS. Biochem J 402:491–501.
- McKinsey TA, Zhang CL, Lu J, Olson EN (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature 408:106–111.
- McManus KJ, Hendzel MJ (2001) CBP, a transcriptional coactivator and acetyltransferase. Biochem Cell Biol 79:253–266.
- Mehler MF (2008) Epigenetic principles and mechanisms underlying nervous system functions in health and disease. Prog Neurobiol 86:305–341.
- Merson TD, Dixon MP, Collin C, Rietze RL, Bartlett PF, Thomas T, Voss AK (2006) The transcriptional coactivator Querkopf controls adult neurogenesis. J Neurosci 26:11359–11370.
- Michishita E, Park JY, Burneskis JM, Barrett JC, Horikawa I (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell 16:4623–4635.
- Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47:873–884.
- Moller DE, Xia CH, Tang W, Zhu AX, Jakubowski M (1994) Human rsk isoforms: cloning and characterization of tissue-specific expression. Am J Physiol 266:C351–C359.
- Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147–156.
- Murnion ME, Adams RR, Callister DM, Allis CD, Earnshaw WC, Swedlow JR (2001) Chromatin-associated protein phosphatase 1 regulates aurora-B and histone H3 phosphorylation. J Biol Chem 276:26656–26665.
- Nakajima T, Fukamizu A, Takahashi J, Gage FH, Fisher T, Blenis J, Montminy MR (1996) The signal-dependent coactivator CBP is a nuclear target for pp90RSK. Cell 86:465–474.
- Nathan D, Sterner DE, Berger SL (2003) Histone modifications: Now summoning sumoylation. Proc Natl Acad Sci USA 100:13118–13120.
- Nelson CJ, Santos-Rosa H, Kouzarides T (2006) Proline isomerization of histone H3 regulates lysine methylation and gene expression. Cell 126:905–916.
- Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128.
- Nestler EJ, Barrot M, Self DW (2001) DeltaFosB: a sustained molecular switch for addiction. Proc Natl Acad Sci USA 98:11042–11046.
- New L, Zhao M, Li Y, Bassett WW, Feng Y, Ludwig S, Padova FD, Gram H, Han J (1999) Cloning and characterization of RLPK, a novel RSK-related protein kinase. J Biol Chem 274:1026–1032.
- Nicholas AP, Lubin FD, Hallett PJ, et al. (2008) Striatal histone modifications in models of levodopa-induced dyskinesia. J Neurochem 106:486–494.
- Nightingale KP, Wellinger RE, Sogo JM, Becker PB (1998) Histone acetylation facilitates RNA polymerase II transcription of the Drosophila hsp26 gene in chromatin. EMBO J 17:2865–2876.
- North BJ, Marshall BL, Borra MT, Denu JM, Verdin E (2003) The human Sir2 ortholog, SIRT2, is an NAD+ -dependent tubulin deacetylase. Mol Cell 11:437–444.
- Nowak SJ, Corces VG (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet 20:214–220.

- Nowak SJ, Pai CY, Corces VG (2003) Protein phosphatase 2A activity affects histone H3 phosphorylation and transcription in *Drosophila melanogaster*. Mol Cell Biol 23:6129–6138.
- Nucifora FC Jr., Sasaki M, Peters MF, et al. (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 291:2423–2428.
- Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953–959.
- Pallos J, Bodai L, Lukacsovich T, Purcell JM, Steffan JS, Thompson LM, Marsh JL (2008) Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a *Drosophila* model of Huntington's disease. Hum Mol Genet 17:3767–3775.
- Pavon N, Martin AB, Mendialdua A, Moratalla R (2006) ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol Psychiatry 59:64–74.
- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci 6:501–506.
- Pierrat B, Correia JS, Mary JL, Tomas-Zuber M, Lesslauer W (1998) RSK-B, a novel ribosomal S6 kinase family member, is a CREB kinase under dominant control of p38alpha mitogen-activated protein kinase (p38alphaMAPK). J Biol Chem 273:29661–29671.
- Putignano E, Lonetti G, Cancedda L, Ratto G, Costa M, Maffei L, Pizzorusso T (2007) Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. Neuron 53:747–759.
- Radwanska K, Valjent E, Trzaskos J, Caboche J, Kaczmarek L (2006) Regulation of cocaine-induced activator protein 1 transcription factors by the extracellular signal-regulated kinase pathway. Neuroscience 137:253–264.
- Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. Trends Mol Med 14:341–350.
- Renthal W, Maze I, Krishnan V, et al. (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56:517–529.
- Robzyk K, Recht J, Osley MA (2000) Rad6-dependent ubiquitination of histone H2B in yeast. Science 287:501–504.
- Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J (2008) Histone deacetylase inhibitors decrease cocaine but not sucrose selfadministration in rats. J Neurosci 28:9342–9348.
- Rosen JB, Chuang E, Iadarola MJ (1994) Differential induction of Fos protein and a Fos-related antigen following acute and repeated cocaine administration. Brain Res Mol Brain Res 25:168–172.
- Roux PP, Blenis J (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68:320–344.
- Roze E, Saudou F, Caboche J (2008a) Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. Curr Opin Neurol 21:497–503.
- Roze E, Betuing S, Deyts C, et al. (2008b) Mitogen- and stress-activated protein kinase-1 deficiency is involved in expanded-huntingtin-induced transcriptional dysregulation and striatal death. FASEB J 22:1083–1093.
- Ryu H, Lee J, Hagerty SW, Soh BY, McAlpin SE, Cormier KA, Smith KM, Ferrante RJ (2006) ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. Proc Natl Acad Sci USA 103:19176–19181.

- Sadri-Vakili G, Bouzou B, Benn CL, et al. (2007) Histones Associated with Downregulated Genes are Hypo-acetylated in Huntington's Disease Models. Hum Mol Genet 16(11):1293–1306.
- Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault JA, Herve D, Greengard P, Fisone G (2009) Critical involvement of cAMP/ DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J Neurosci 27:6995–7005.
- Santini E, Alcacer C, Cacciatore S, Heiman M, Herve D, Greengard P, Girault JA, Valjent E, Fisone G (2009) L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. J Neurochem 108:621–633.
- Santos-Rosa H, Caldas C (2005) Chromatin modifier enzymes, the histone code and cancer. Eur J Cancer 41:2381–2402.
- Sassone-Corsi P, Mizzen CA, Cheung P, Crosio C, Monaco L, Jacquot S, Hanauer A, Allis CD (1999) Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. Science 285:886–891.
- Sato T, Miura M, Yamada M, et al. (2009) Severe neurological phenotypes of Q129 DRPLA transgenic mice serendipitously created by en masse expansion of CAG repeats in Q76 DRPLA mice. Hum Mol Genet 18:723–736.
- Schroeder FA, Lin CL, Crusio WE, Akbarian S (1999) Antidepressantlike effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry Jul 1;62(1):55–64.
- Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861.
- Shiio Y, Eisenman RN (2003) Histone sumoylation is associated with transcriptional repression. Proc Natl Acad Sci USA 100:13225–13230.
- Shilatifard A (2006) Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu Rev Biochem 75:243–269.
- Shimohata M, Shimohata T, Igarashi S, Naruse S, Tsuji S (2005) Interference of CREB-dependent transcriptional activation by expanded polyglutamine stretches – augmentation of transcriptional activation as a potential therapeutic strategy for polyglutamine diseases. J Neurochem 93:654–663.
- Shimohata T, et al. (2000) Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. Nat Genet 26:29–36.
- Sindreu CB, Scheiner ZS, Storm DR (2007) Ca<sup>2+</sup>-stimulated adenylyl cyclases regulate ERK-dependent activation of MSK1 during fear conditioning. Neuron Jan 4;53(1):79–89.
- Smith JA, Poteet-Smith CE, Malarkey K, Sturgill TW (1999) Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK in vivo. J Biol Chem 274:2893–2898.
- Sng JC, Taniura H, Yoneda Y (2006) Histone modifications in kainateinduced status epilepticus. Eur J Neurosci 23:1269–1282.
- Soloaga A, Thomson S, Wiggin GR, Rampersaud N, Dyson MH, Hazzalin CA, Mahadevan LC, Arthur JS (2003) MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. Embo J 22:2788–2797.
- Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 89:2046–2050.
- Stack EC, Del Signore SJ, Luthi-Carter R, et al. (2007) Modulation of Nucleosome Dynamics in Huntington's Disease. Hum Mol Genet 16(10):1164–1175.

- Steffan JS, Bodai L, Pallos J, et al. (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature 413:739–743.
- Steiner H, Gerfen CR (1993) Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci 13:5066–5081.
- Sterner DE, Berger SL (2000) Acetylation of histones and transcriptionrelated factors. Microbiol Mol Biol Rev 64:435–459.
- Stipanovich A, Valjent E, Matamales M, et al. (2008) A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature 453:879–884.
- Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45.
- Sugiyama K, Sugiura K, Hara T, Sugimoto K, Shima H, Honda K, Furukawa K, Yamashita S, Urano T (2002) Aurora-B associated protein phosphatases as negative regulators of kinase activation. Oncogene 21:3103–3111.
- Thomas EA, Coppola G, Desplats PA, et al. (2008) The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. Proc Natl Acad Sci USA 105:15564–15569.
- Thomas T, Voss AK, Chowdhury K, Gruss P (2000) Querkopf, a MYST family histone acetyltransferase, is required for normal cerebral cortex development. Development 127:2537–2548.
- Thomson S, Clayton AL, Mahadevan LC (2001) Independent dynamic regulation of histone phosphorylation and acetylation during immediate-early gene induction. Mol Cell 8:1231–1241.
- Thomson S, Clayton AL, Hazzalin CA, Rose S, Barratt MJ, Mahadevan LC (1999) The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. Embo J 18:4779–4793.
- Tsankova NM, Kumar A, Nestler EJ (2004) Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci 24:5603–5610.
- Tse C, Fletcher TM, Hansen JC (1998a) Enhanced transcription factor access to arrays of histone H3/H4 tetramer. DNA complexes in vitro: implications for replication and transcription. Proc Natl Acad Sci USA 95:12169–12173.
- Tse C, Sera T, Wolffe AP, Hansen JC (1998b) Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. Mol Cell Biol 18:4629–4638.
- Ura K, Kurumizaka H, Dimitrov S, Almouzni G, Wolffe AP (1997) Histone acetylation: influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression. EMBO J 16:2096–2107.
- Valjent E, Corvol JC, Trzaskos JM, Girault JA, Herve D (2006a) Role of the ERK pathway in psychostimulant-induced locomotor sensitization. BMC Neurosci 7:20.
- Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J (2000) Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J Neurosci 20:8701–8709.
- Valjent E, Aubier B, Corbille AG, Brami-Cherrier K, Caboche J, Topilko P, Girault JA, Herve D (2006b) Plasticity-associated gene Krox24/ Zif268 is required for long-lasting behavioral effects of cocaine. J Neurosci 26:4956–4960.
- Valjent E, Pascoli V, Svenningsson P, et al. (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate

signals to activate ERK in the striatum. Proc Natl Acad Sci USA 102:491-496.

- Vecsey CG, Hawk JD, Lattal KM, et al. (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci 27:6128–6140.
- Wang L, Liu L, Berger SL (1998) Critical residues for histone acetylation by Gcn5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. Genes Dev 12:640–653.
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854.
- Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA (2007) Spatiotemporal Pattern of Striatal ERK1/2 Phosphorylation in a Rat Model of L-DOPA-Induced Dyskinesia and the Role of Dopamine D1 Receptors. Biol Psychiatry.
- Wiggin GR, Soloaga A, Foster JM, Murray-Tait V, Cohen P, Arthur JS (2002) MSK1 and MSK2 are required for the mitogen- and stressinduced phosphorylation of CREB and ATF1 in fibroblasts. Mol Cell Biol 22:2871–2881.
- Williams MR, Arthur JS, Balendran A, van der Kaay J, Poli V, Cohen P, Alessi DR (2000) The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells. Curr Biol 10:439–448.
- Wood JD, Nucifora FC Jr., Duan K, Zhang C, Wang J, Kim Y, Schilling G, Sacchi N, Liu JM, Ross CA (2000) Atrophin-1, the dentatorubral and pallido-luysian atrophy gene product, interacts with ETO/ MTG8 in the nuclear matrix and represses transcription. J Cell Biol 150:939–948.
- Yamauchi T, Yamauchi J, Kuwata T, et al. (2000) Distinct but overlapping roles of histone acetylase PCAF and of the closely related PCAF-B/GCN5 in mouse embryogenesis. Proc Natl Acad Sci USA 97:11303–11306.
- Yang L, Mei Q, Zielinska-Kwiatkowska A, et al. (2003) An ERG (etsrelated gene)-associated histone methyltransferase interacts with histone deacetylases 1/2 and transcription co-repressors mSin3A/B. Biochem J 369:651–657.
- Yao TP, Oh SP, Fuchs M, et al. (1998) Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell 93:361–372.
- Ying M, Xu R, Wu X, Zhu H, Zhuang Y, Han M, Xu T (2006) Sodium butyrate ameliorates histone hypoacetylation and neurodegenerative phenotypes in a mouse model for DRPLA. J Biol Chem 281:12580–12586.
- You A, Tong JK, Grozinger CM, Schreiber SL (2001) CoREST is an integral component of the CoREST- human histone deacetylase complex. Proc Natl Acad Sci USA 98:1454–1458.
- Zeniou M, Ding T, Trivier E, Hanauer A (2002) Expression analysis of RSK gene family members: the RSK2 gene, mutated in Coffin-Lowry syndrome, is prominently expressed in brain structures essential for cognitive function and learning. Hum Mol Genet 11:2929–2940.
- Zhang J, Zhang D, McQuade JS, Behbehani M, Tsien JZ, Xu M (2006) c-fos regulates neuronal excitability and survival. Nat Genet 30:416–420.
- Zhang J, Zhang L, Jiao H, Zhang Q, Zhang D, Lou D, Katz JL, Xu M (2006) c-Fos facilitates the acquisition and extinction of cocaineinduced persistent changes. J Neurosci 26:13287–13296.
- Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J, Xu M (2002a). J Neurochem Sep;82(6):1453–64.

- Zhang S, Xu L, Lee J, Xu T (2002b) Drosophila atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. Cell 108:45–56.
- Zhang Y, Reinberg D (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev 15:2343–2360.
- Zhao Y, Bjorbaek C, Moller DE (1996) Regulation and interaction of pp90(rsk) isoforms with mitogen-activated protein kinases. J Biol Chem 271:29773–29779.
- Zhuang X, Belluscio L, Hen R (2000) G(olf)alpha mediates dopamine D1 receptor signaling. J Neurosci 20:RC91.
- Zoltewicz JS, Stewart NJ, Leung R, Peterson AS (2004) Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. Development 131:3–14.

# Phasic Dopamine Signaling and Basal Ganglia Function

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# I. INTRODUCTION

The functional role of individual elements within a complicated system is difficult to ascertain without some appreciation of what the overall system is designed to do. As one of the brain's fundamental processing units, the basal ganglia are an intricate system that contain the ascending dopamine (DA) projections as important integral elements (Lindvall and Bjorklund, 1974) (see Chapter 1). Therefore, in the present chapter we will begin by reviewing briefly some of the influential ideas on basal ganglia function, specifically their involvement in action selection (Mink, 1996; Redgrave et al., 1999b; Grillner et al., 2005; Humphries et al., 2006; Prescott et al., 2006; Hikosaka, 2007; Redgrave, 2007) and reinforcement learning (Wickens, 1993; Schultz, 1998; Salamone and Correa, 2002; Wickens et al., 2003; Wise, 2004; Houk, 2005; Schultz, 2006; Berridge, 2007). Within this context we will then assess the specific contribution of the phasic response of DA neurons to the fundamental processes of reinforcement learning. Although ventral midbrain DA neurons target numerous structures, including intrinsic nuclei of the basal ganglia, frontal cortex, amygdala, hippocampus, septal area, several thalamic nuclei, and the habenula (Lindvall and Bjorklund, 1974), the largest and most thoroughly investigated DA input is to the striatum. We will therefore restrict ourselves to a discussion of how striatal function may be influenced by phasic DA signaling. Perhaps, a clearer understanding of this comparatively well characterized system will then provide important insights about how phasic DA input can modulate function in other parts of the brain.

# II. SELECTION: A FUNDAMENTAL PROBLEM

Despite numerous suggestions implicating the basal ganglia in a wide range of functions including perception, learning, memory, attention, many aspects of motor function, even analgesia and seizure suppression, accumulating evidence points to a fundamental role in basic selection processes (Mink, 1996; Redgrave et al., 1999b; Grillner et al., 2005; Humphries et al., 2006; Prescott et al., 2006; Hikosaka, 2007; Redgrave, 2007). A basic selection problem is common to all multifunctional systems. Briefly, the problem that must be solved is to resolve which functional system, at any point in time, should be permitted to direct "the final common motor path", that is, determine behavioral output. The macro-architecture of the basal ganglia (Fig. 31.1A) may be seen as providing a potential solution to this selection problem (Redgrave et al., 1999b). The critical components involved in selection are the parallel looped components that originate from and return to diverse cortically and subcortically based functional systems (Alexander et al., 1986; McHaffie et al., 2005). Afferent projections to the basal ganglia convey excitatory signals which, according to the "selection" view, are seen as competing bids. In this model (Gurney et al., 2001b, 2001a; Humphries et al., 2006), intrinsic processing, which is responsive to the comparative magnitudes or "saliences" of competing inputs, generates patterns of output in which the external structure(s)/ representation(s) providing the most "salient" input(s) are selectively disinhibited (Chevalier and Deniau, 1990). In the absence of any consideration of biological systems, a conceptually similar control architecture was developed to select the actions of an autonomous mobile robot (Fig. 31.1B) (Snaith and Holland, 1990). Subsequently, it has been confirmed that sequences of actions that allow a mobile robot to forage when "hungry" and hide when "frightened" can be selected appropriately by a biologically constrained model of basal ganglia architecture (Prescott et al., 2006). We will now consider why reinforcement learning should be so closely associated with a generic selection mechanism.

## **III. REINFORCEMENT LEARNING**

The basal ganglia have long been associated with reinforcement learning, in particular, with instrumental conditioning (Wise, 2004; Schultz, 2006; Berridge, 2007). In his famous Law of Effect, Thorndike (1911) declared that "any act which in a given situation produces satisfaction becomes associated with that situation so that when the situation recurs the act is more likely than before to recur also". Using slightly different language, Thorndike is stating that in a given context, an action that is associated with reinforcement is *more likely to be selected* in the future when the same or similar contexts are encountered. Therefore, in essence, reinforcement learning



**FIGURE 31.1** Biological and artificial selection architectures. A. Principle input and output connections of the mammalian basal ganglia. Phasic excitatory inputs (black arrows) are directed to the striatum (STR) from subcortical structures via the thalamus, cerebral cortex and limbic structures (amygdala and hippocampus). The main output nuclei are the substantia nigra pars reticulata (SNr) and the internal globus pallidus (not shown). Tonically active inhibitory connections (medium grey arrows) are indirectly relayed back to the cerebral cortex (via the thalamus) and directly back to midbrain (tectum) and brainstem structures. Light grey arrows represent intrinsic inhibitory connections. B. A schematic illustration of the artificial selection architecture proposed by Snaith and Holland (1990) in which competing behavioral systems (C1–C8) provide phasically active excitatory input to and receive tonically active inhibitory outputs from a central selection mechanism.

can be seen as a process for biasing the operation of a selection machine. The question we will now address is how a reinforcer could bias the operation of a central selection mechanism of the kind represented in the macro-architecture of basal ganglia described above. There are several possibilities but two in particular stand out.

1. Adjust the relative strengths of competing inputs (Fig. 31.2A): Theoretically, selection could be biased simply by increasing the input salience of competing systems associated with a reinforcer. Since the proposed selection process works by assessing the relative magnitudes of input saliences in competing channels (Gurney et al., 2001b, 2001a; Humphries et al., 2006), reinforcement-related



**FIGURE 31.2** Independent reinforcement mechanisms that could bias action selection in the basal ganglia. Reinforcement A: The salience of afferent signals to the striatum in reinforced channels could be potentiated selectively by input from a currently unknown source(s) that is sensitive to reward prediction errors (see text). Reinforcement B: Phasic dopamine signals could reinforce corticostriatal/thalamostriatal neurotransmission selectively at recently activated synapses.

boosting of a particular channel's input would increase the probability of it being selected in any future competition. Much evidence in the biological literature indicates that when a particular stimulus is associated with reward, its representation in afferent structures projecting to the basal ganglia is enhanced (Schultz, 2000; Kobayashi et al., 2002; Watanabe et al., 2002; Ikeda and Hikosaka, 2003; Ding and Hikosaka, 2006; Kobayashi et al., 2006). Although the origin of these reinforcement signals is currently unknown, there are reasons to believe they originate from structures that are sensitive to detailed estimates of outcome value.

2. Change the relative sensitivity of the receiver to reinforced inputs (Fig. 31.2B): A different way to bias selection would be to adjust the sensitivity of basal ganglia intrinsic circuitry by enhancing its responses, specifically to reinforced inputs. Increased activity associated with reinforced inputs could be combined with corresponding reductions in sensitivity to non-reinforced or punished inputs. This version of biasing action selection has received most attention in analyses of the basal ganglia's

role in reinforcement learning (Wickens, 1993; Schultz, 1998; Reynolds and Wickens, 2002; Wickens et al., 2003; Wise, 2004; Everitt and Robbins, 2005; Schultz, 2006; Arbuthnott and Wickens, 2007). Selectivity is achieved by restricting the effects of reinforcement to specific subsets of recently or concurrently active inputs based on comparative signal timing (Arbuthnott and Wickens, 2007). In most contemporary models reward-related actions are thought to be specific inputs from the cerebral cortex that are reinforced by signals from DA neurons in the ventral midbrain (Schultz, 2006). While it is clear that DA neurotransmission operates in a range of different modes (Grace, 1995; Fiorillo et al., 2003; Floresco et al., 2003; Roitman et al., 2004; Heien et al., 2005; Schultz, 2007; Venton and Wightman, 2007), the responses linked most often with reinforcement learning are the short-latency phasic responses which are widely accepted as signaling reward prediction errors (Schultz, 1998).

# IV. ROLE OF DOPAMINE IN REINFORCEMENT LEARNING

# A. Phasic Dopamine Signaling

In most species the unexpected presentation of a primary reward, or a neutral stimulus previously associated with primary rewards, normally evokes a stereotypic phasic DA response (Freeman, 1985; Horvitz et al., 1997; Schultz, 1998; Guarraci and Kapp, 1999). It is characterized by a short latency (70-100 ms), short duration (100-200 ms) burst of activity (Schultz, 1998) (Fig. 31.3B). However, it is the adaptive nature of phasic DA responses when experimental conditions are altered that has attracted most interest (Schultz, 1998; Satoh et al., 2003; Nakahara et al., 2004; Bayer and Glimcher, 2005; Schultz, 2006). The properties of phasic DA signaling on which most contemporary theories are based can be itemized as follows: (i) While neutral sensory events also initially elicit phasic DA responses, they habituate rapidly if not associated with reward (Ljungberg et al., 1992). (ii) If a habituated neutral stimulus is subsequently associated with a primary reward, its capacity to evoke a phasic DA response is re-established (Ljungberg et al., 1992). (iii) As a primary reward becomes increasingly predicted by a prior event, its capacity to provoke a phasic DA response diminishes (Schultz, 1998; Pan et al., 2005). (iv) If a reward is predicted and fails to occur, DA neurons exhibit a brief pause about the time of expected reward delivery (Schultz et al., 1997). These seminal observations



**FIGURE 31.3** A latency constraint associated with visual input to DA neurons. Typical examples show the relative timing of responses evoked by an unexpected visual stimulus in the superior colliculus, substantia nigra pars compacta and substantia nigra pars reticulata. Peri-stimulus histograms from different publications are aligned on stimulus onset. A. Activity in the superior colliculus is characterized by an early sensory response (latency ~40 ms) followed by a later motor response (latency ~200 ms). The latter is responsible for driving the orienting gaze-shift to bring the stimulus onto the fovea. (Modified with permission from Jay and Sparks, 1987.) B. The phasic dopaminergic response (latency ~70 ms) (modified with permission from Schultz 1998) occurs after the collicular sensory response but prior to its pre-saccadic motor response. C. Phasic dopaminergic activity also occurs prior to the output signal from substantia nigra pars reticulata that disinhibits the motor related activity of target neurons in the superior colliculus. (Modified with permission from Hikosaka and Wurtz, 1983).

have been interpreted as indicating that DA neurons signal events that are "better" or "worse" than expected (reward prediction errors) (Montague et al., 1996). Because behavioral experiments have established that unpredicted reward, rather than reward per se is critical for learning (see Schultz, 2006 for review), the phasic response properties of DA neurons have captured the imagination of both the biological (Schultz, 1998; BarGad et al., 2003; O'Doherty et al., 2003; Morris et al., 2004; Ungless, 2004; Bayer and Glimcher, 2005) and computational neuroscience communities (Montague et al., 1996; Sutton and Barto, 1998; Dayan and Balleine, 2002; Montague et al., 2004; Singh et al., 2005). Indeed, the reward prediction error hypothesis of phasic DA signaling has become one of the widely accepted tenets of contemporary neuroscience. However, there is a body of evidence that does not fit easily with this view (Redgrave et al., 1999a; Horvitz, 2000; Redgrave and Gurney, 2006; Redgrave et al., 2008).

## **B.** Inconvenient Observations

- DA neurons exhibit robust phasic responses to unexpected neutral stimuli that are novel and elicit orienting, but have no reinforcement consequences (Horvitz, 2000). Counter claims that such stimuli must therefore be rewarding are circular.
- Phasic DA neuronal responses are remarkably stereotyped (~100 ms latency, ~100 ms duration) across species, numerous experimental paradigms, and are largely independent of sensory modality or perceptual complexity of eliciting events (Schultz, 1998).
- **3.** The latencies of phasic, sensory-evoked DA responses (~100 ms) (Schultz, 1998) are typically shorter than those of the gaze-shift (~150–200 ms) that brings the eliciting event onto the fovea for examination (Jay and Sparks, 1987; Fig. 31.3A, cf. Fig. 31.3B). Therefore, under most natural circumstances, both the identity and conscious appreciation of the event has yet to be established at the time of DA signaling (Stoerig, 2006).
- 4. Pre-saccadic visual input to DA neurons derives largely, if not exclusively from the midbrain superior colliculus (Comoli et al., 2003; Dommett et al., 2005). Note that short latency visual input from the superior colliculus may influence DA neurons either directly via the tectonigral projection or indirectly via other routes including the pedunculopontine tegmental nucleus (Pan et al., 2005) and the subthalamic nucleus (Coizet et al., 2009). However the important point is that while collicular neurons are exquisitely sensitive to the location of luminance changes, they are largely insensitive to color, static contrast and high spatial frequency (Wurtz and Albano, 1980; Sparks, 1986; Grantyn, 1988; Stein and Meredith, 1993). Note also that collicular neurons are responsive at short latency to multisensory stimuli (visual, auditory and somatosensory (Stein and Meredith, 1993), although, the extent to which non-visual activity in the
superior colliculus can influence DA neurons is currently unknown. Thus, in the absence of specific training in highly constrained environments, pre-saccadic visual processing in the superior colliculus is particularly ill equipped to determine the identity, and hence the value of an unexpected event.

5. Despite numerous reports of DA neurons effectively discriminating complex characteristics of visual stimuli used to signal reward magnitude and probability (Schultz, 2006), it must be noted that, with few exceptions (Morris et al., 2004), relevant experiments contain a confound based on numerous (typically 1000s, sometimes 10s of 1000s) prior associations between the location of stimulus presentation (encoded within collicular retinocentric maps; Wurtz and Albano, 1980) and stimulus value. While such procedures may not easily generalize to natural environments (where evolution "designed" relevant neural systems to operate), they may have the ability to differentially sensitize "rewarded" regions of the retinotopic collicular response field (Ikeda and Hikosaka, 2003), thereby giving the impression that the colliculus, and hence DA neurons, can discriminate stimulus value at pre-saccadic latencies.

# C. The Reinforcing Function of Phasic Dopamine: Reward Prediction?

Most versions of the reward prediction error hypothesis assume that phasic DA activity is reward-related and reinforces the selection of behavior that will maximize the future acquisition of reward (Dayan and Balleine, 2002; Schultz, 2006). Here, a reward prediction error represents a difference between the predicted and actual utility value of the current state/event. Unfortunately, when an event is both temporally and spatially unpredictable (i.e., most natural situations), subcortical afferent sensory processing would be in a position to report only preliminary estimates of stimulus valence (Dean et al., 1989). In contrast, after a foveating gaze-shift the event can be analysed by more sophisticated cortical perceptual processing and its identity established (Thorpe and Fabre-Thorpe, 2001). Insofar as an accurate determination of value depends on knowing what the stimulus is, it seems that post-gaze-shift stimulus identification may be a necessary pre-condition for calculating genuine errors of "reward prediction". Accumulating evidence suggests that such calculations can be performed by neurons in pre-frontal cortex (Padoa-Schioppa and Assad, 2006; Potts et al., 2006). However, to date we know of no evidence suggesting that DA neurons can be activated phasically by the appreciation of an unexpected reward determined on the basis of post-gaze-shift analyses of stimulus identity. Consequently, although there is doubt that early, subcortical sensory processing in real-world situations can provide DA neurons with sufficient information to signal reward prediction errors (Redgrave et al., 1999a), evidence that phasic DA is performing an essential reinforcing function is substantially stronger. Thus, biologically significant events (classified by early sensory processing as "not immediately harmful") (Dean et al., 1989), evoke positive DA responses (Schultz, 1998; Horvitz, 2000), while noxious stimuli (Ungless et al., 2004; Coizet et al., 2006) or the failure of predicted salient events (Schultz, 1998), evoke predominantly negative responses. Since DA neurons seem to be made aware that something has happened, rather than what has happened, perhaps it would be safer to regard phasic DA activity as reporting "sensory prediction errors" rather than "reward prediction errors". However, this leaves open the important question of what is being reinforced.

# D. An Alternative Proposal: Reinforcement of Agency Assessment

Relying on what is known of the sensory properties of the superior colliculus (Wurtz and Albano, 1980; Stein and Meredith, 1993; Boehnke and Munoz, 2008), DA neurons are likely to be informed that a biologically significant event has occurred. Typically, if neutral stimuli are not reinforced their capacity to evoke a sensory response in the superior colliculus habituates rapidly (Horn and Hill, 1966; Sprague et al., 1968; Grantyn, 1988). Consequently, in the present context biological significance is established in terms of a non-habituated sensory response in the superior colliculus (Boehnke and Munoz, 2008). In addition, and depending on simple stimulus characteristics such as size, speed and direction of movement (especially loom), the superior colliculus can also assess whether or not a novel event is likely to be immediately harmful (Dean et al., 1989). It is likely, therefore, that short-latency afferent sensory information originating from the superior colliculus contains preliminary estimates of both biological salience (Boehnke and Munoz, 2008) and potential harm (Dean et al., 1989). With this point in mind, we proposed recently (Redgrave and Gurney, 2006; Redgrave et al., 2008) that, rather than reinforcing actions that maximize future reward, afferent information from the superior colliculus may be more suitable for reinforcing assessments of agency (that is, when

some aspect of the agent's behavior is the cause of an initially unpredicted event) and the discovery of novel actions (when the agent identifies which particular aspects of its behavior are the critical cause). Such learning would make notably less stringent demands of early sensory processing than envisaged by the reward prediction error hypothesis, and is more likely to be within the scope of subcortical sensory systems (Comoli et al., 2003; Dommett et al., 2005; Boehnke and Munoz, 2008).

### **V. THE AGENCY HYPOTHESIS**

# A. A Neural Network for Determining Agency

The agency hypothesis (Redgrave and Gurney, 2006; Redgrave et al., 2008), which proposes that the basal ganglia appear ideally configured to determine whether the agent is the likely cause of the unpredicted event, then through DA-related repetition and neural plasticity, discover the causal components of behavioral output (novel actions), was based on an analysis of the functional architecture of the basal ganglia and considerations of signal timing. (Note we use the general term "agent" to accommodate bio-mimetic architectures used to control robot behavior (Prescott et al., 2006) as well as the neural systems in the brains of animals that control their behavior.) For example, the relatively invariant timing of the phasic DA response (Schultz, 1998) highlights the importance of considering the signals that are also likely to be present in targeted structures at the time of phasic DA release, because it is with these signals that DA is most likely to interact. For the purpose of this analysis, we will continue to restrict ourselves primarily to signal processing in the striatum which is the principal target of ascending DA systems (Lindvall and Bjorklund, 1974; Gerfen and Wilson, 1996). A review of relevant literature (Redgrave and Gurney, 2006; Redgrave et al., 2008) suggests there will be at least three additional afferent signals present in the striatum at the time of phasic DA release (Fig. 31.4A):

1. Sensory: Many tectonigral axons that innervate the substantia nigra pars compacta have collateral projections that terminate in regions of the thalamus that provide direct input to the striatum (Coizet et al., 2007) (see Chapter 23). This branched architecture would ensure that a separate short-latency sensory representation of the unexpected event that triggered the phasic DA signal would also elicit a corresponding phasic glutamatergic input to the striatum from the thalamus (Smith et al., 2004; Lacey et al., 2007) (see also Chapter 22). The known timing of these two afferent signals (Dommett et al., 2005; Schulz et al., 2009) suggests they have the potential to converge.



**FIGURE 31.4** Potential interaction between converging inputs to the striatum to determine agency. A. Four classes of anatomically and physiologically identified afferent signals to the striatum: (i) Efference motor copy via branched pathways from motor cortex and subcortical sensorimotor structures (for example, superior colliculus) reach the striatum directly (cortex) or indirectly via the thalamus (subcortical structures); (ii) Striatal neurons are sensitive to experimental context (see text for references); (iii) A sequence of retino-tecto-thalamo-striatal projections convey a short-latency glutamatergic version of unpredicted visual events; (iv) retino-tecto-nigro-striatal projections convey a short-latency dopaminergic version of unpredicted visual events. B. Event caused by agent. Whenever the agent is the cause of the unpredicted event, relevant components of the multidimensional contextual and motor efference copy inputs will directly precede short-latency glutamatergic sensory input from the thalamus and the phasic dopaminergic input from substantia nigra. C. Event caused by external source. When no relevant motor copy inputs precede the phasic sensory inputs (glutamatergic and dopaminergic), the unpredicted event is likely to have been caused by an external source. This figure was modified with permission from the artwork that appeared in Redgrave and Gurney, 2006.

- Contextual: Striatal neurons are influenced by contextual variables related to the general sensory, metabolic and cognitive state of the animal (Hikosaka et al., 1989; Schultz, 2000). Such inputs are likely to originate in cortical, limbic and subcortical (thalamic) structures (Gerfen and Wilson, 1996).
- **3.** *Motor-copy:* Anatomical and physiological data suggest that copies of motor commands from both cortical and subcortical sensorimotor structures to the brainstem are also relayed to the striatum via collaterals of fibres that contact motor and pre-motor neurons in the medulla and spinal cord (Crutcher and DeLong, 1984; Bickford and Hall, 1989; Levesque et al., 1996; Mink, 1996; Reiner et al., 2003; McHaffie et al., 2005) (see also Chapter 18). These efference copy signals of action decisions and motor commands may be seen as providing the striatum with a running record of ongoing behavioral output.

# **B.** Signal Timing and the Determination of Agency

The combination of potentially convergent signals identified in Figure 31.4A could permit the agent to determine whether any aspect of its behavioral output (motor-copy) was related to the onset of a biologically significant sensory event. In cases where the agent is causally responsible, a representation of the critical movement will most often be embedded in the immediately preceding record of motor output (Fig. 31.4B), while if the event was caused by an external source (that is, not the agent), then nothing in the preceding motor record would correlate (Fig. 31.4C). How might such a mechanism operate? A clue to DA's reinforcing role is given by the observation that high levels of DA receptor activation cause segments of behavior to be repeated, for example, pharmacologically induced stereotypies (Robbins and Sahakian, 1981). Consequently, we suppose that sensory-evoked phasic DA release reinforces the re-selection of any immediately preceding behavioral output. In other words, the selection mechanisms within the basal ganglia are biased towards repeating the justperformed action. Perhaps this effect could be considered a special case of DA making the agent "want" to do what it has just done (Berridge, 2007). Thus, if the agent is the cause of the sensory event and prior behavior is repeated then the event will occur again, if not, it won't. At a mechanistic level, we suppose the phasic DA signal causes a form of plasticity that adjusts the "sensitivity of the receiver"

to bias the re-selection of causal behavioral output (i.e., Reinforcement B in Fig. 31.2). However, this comparatively simple story entails a major computational problem - how is the agent/reinforcement system to know, or able to identify, which aspects of its prior behavior are causal? In most circumstances, the contextual and motor record will be multi-dimensional, and at the beginning, possibly with few components causally related to the sensory event. However, if the phasic release of DA promotes repetition of prior behavioral selections with some variability (that is, where not all components are included in each iteration), then by facilitating/potentiating recently active channels when the event occurs, and suppressing active channels when it fails, the system should converge on the causal/relevant components of contextual and behavioral output. This repetitionbias mechanism would ensure that increasingly refined representations of the critical aspects of behavioral output and the consequent sensory outcome will occur more frequently in brain areas external to the basal ganglia which have the capacity to develop and store associative links between action and outcome (Corbit and Balleine, 2003). At the end of this process when critical causal components of behavioral output have been identified and refined, and the sensory consequences of enactment fully predicted, a new action channel would have developed within the basal ganglia and a novel action-outcome pairing stored externally (Singh et al., 2005). At this point, an entirely new action/ response would have been developed which was not previously in the agent's repertoire. However, what happens if the unexpected sensory event caused by the agent is bad? Thus far, we have considered almost exclusively the role of positive DA neuronal responses in promoting action-outcome learning. We will now turn our attention to the issue of phasic suppressions of DA neuronal activity.

# C. Aversive Stimuli and Failures of Predicted Reward

If a caused event was aversive or detrimental, discovering causative aspects of behavior through repetitions promoted by phasic DA responses would be ill-advised, if not dangerous. In such cases, the adaptive option would be to suppress any tendency to repeat immediately prior behavior and avoid the context(s) in which the event occurred. It is significant, therefore, that noxious stimuli can elicit a short latency phasic inhibition of significant numbers of ventral midbrain DA neurons, that generally lasts for the duration of the noxious event (Ungless et al., 2004; Coizet et al., 2006). If positive DA responses are thought to promote behavioral repetitions, it would be natural to suggest that negative DA signals act to reduce the likelihood of re-selecting immediately preceding behavior. To initiate negative DA responses would require afferent sensory processing to discriminate, at short latency, potentially harmful events, and by some means direct inhibitory afferent signals to DA neurons. Recent experimental evidence shows that the parabrachial nucleus, a major target of afferent nociceptive pathways from the spinal cord (Klop et al., 2005), provides direct projections to ventral midbrain regions containing DA neurons (Klop et al., 2005; Overton et al., 2005). The mechanism by which these inputs suppress the activity of DA neurons remains to be determined. Similarly, additional work will be required to determine the precise consequences of a population pause in DA neuronal activity on striatal neurotransmission.

A further detrimental situation where agency could be important is when a well-predicted reward fails to occur. Perhaps it was something the agent did that was responsible for the predicted event failing. If so, the adaptive option would also be to suppress any tendency to repeat immediately prior behavioral selections. Under such circumstances it is well-established that DA neurons typically exhibit a short pause/negative response about the time of the failed reward delivery (Schultz, 1998). Thus, failures of predicted reward may have similar effects in structures targeted by DA, to those elicited by noxious stimuli (Ungless et al., 2004; Coizet et al., 2006). The source(s) of the afferent inhibitory signals that suppress DA activity when a predicted event fails is currently unknown. However, very recent evidence indicates a role of the habenula (Matsumoto and Hikosaka, 2009). A final point is that, as with positive phasic DA signals (Schultz, 1998), negative phasic responses of DA neurons occur at very short latencies following noxious stimulus onset (Coizet et al., 2006) or following the failure of a predicted reward (Schultz, 1998). We will now consider why this might be.

### D. Why are Short-Latency Dopamine Reinforcement Signals so Short?

Anatomical studies indicated that early sensory input to DA neurons in the ventral midbrain comes mainly, if not exclusively, from the comparatively primitive sensory systems of the brainstem (Comoli et al., 2003; Dommett et al., 2005; Overton et al., 2005; Omelchenko and Sesack, 2007). Viewed from the perspective of the reward prediction error hypothesis, it is difficult to understand why the sensory information indicating "current state" that is used to generate a reward prediction error should be based on such limited and preliminary sensory processing. The manifest advantages of waiting just an additional few hundred milliseconds for cortical systems to identify the stimulus and provide incomparably better estimates of utility seem obvious. However, if instead DA neurons are signaling sensory prediction errors that are used to determine agency and discover novel actions, largely independent of immediate outcome value (Redgrave et al., 2006, 2008), pre-saccadic latencies of DA responses are an advantage, not a problem. Indeed, the agency view of phasic DA signaling provides a potential explanation of why the reinforcement signal should occur before any behavior elicited by the onset of an unpredicted biologically significant event, that is, the initial orienting/approach or defensive/withdrawal movements (Wurtz and Albano, 1980; Sparks, 1986; Grantyn, 1988; Dean et al., 1989; Stein and Meredith, 1993). In the proposed basal ganglia network, a critical component is the record of behavioral output relayed to the striatum by collateral projections from sensorimotor regions to motor effector systems in the brainstem (Crutcher and DeLong, 1984; Bickford and Hall, 1989; Levesque et al., 1996; Mink, 1996; Reiner et al., 2003; McHaffie et al., 2005) (Fig. 31.4A). Assuming some aspect of the agent's behavior causes an initially unpredicted event, and reinforcement is delayed until after the event has been properly investigated and identified - which may require combinations of eye, head and body movements associated with orienting gaze-shifts, inspection etc - the record of behavioral output would immediately be contaminated by movements elicited by the event itself, but unrelated to its cause. The overall system would then be faced with a drastically more difficult task of identifying and then reinforcing critical causative components of behavior embedded somewhere further back in the past motor record. This has been recognized as the credit-assignment problem (Izhikevich, 2007). On the other hand, if an agent is the cause of a phasic sensory event, it is its immediately preceding behavior that is most likely to be responsible. Having a DA reinforcement signal (positive or negative) delivered before any event-elicited behavioral reactions would greatly simplify the credit-assignment problem and ensure reinforcement was directed to the most likely cause. This mechanism could explain the profound detrimental effect of delaying reinforcement by just a few seconds on acquisition rates in associative learning (Black et al., 1985; Dickinson, 2001; Elsner and Hommel, 2004; Schultz, 2006). For example, in the case of instrumental conditioning, the longer the delay between causal component of behavior (for example, lever press) and sensory reinforcement (the delivery of reward) the more likely the record of 'motor output' in the striatum will be contaminated with irrelevant behavioral output - which, inadvertently, would be reinforced by the sensory-evoked DA response when reward is delivered. This problem would be exacerbated if the active inputs representing copies of behavioral (motivational, affective, cognitive and sensorimotor) selections, establish a temporary "eligibility trace" (that decays over seconds) that sensitizes the recently active striatal neurons to subsequent DA-related reinforcement [see Artbuthnott and Wickens (2007) for an alternative articulation of the same idea]. The processes by which synaptic efficacies are adjusted following reinforcement-related phasic DA receptor activation (Greengard et al., 1999), and how long they remain in an altered state, will necessarily extend well beyond the duration of the phasic DA response (100-200 ms). Presumably it is this arrangement that permits a discrete "trigger" event (a phasic DA response measured in ms) to produce selective and lasting adjustments (measured in minutes) to an action selection mechanism that biases subsequent selections towards (positive DA) or away from (negative DA) what the agent has just done.

#### VI. SUMMARY AND CONCLUSIONS

This chapter is an elaboration of the recent proposal that short-latency sensory driven DA responses provide the reinforcement signals that enables the brain to discriminate the sensory events for which it is responsible (Redgrave and Gurney, 2006). As part of this process, new responses required in specific circumstances to make events happen are discovered. These basic functions rely on combining the selective and adaptive properties of basal ganglia neural processing. Quick and dirty sensory analysis is sufficient to generate the necessary reinforcing signals, with the system needing to know only if a sensory event is likely to be immediately detrimental, so appropriate (positive or negative) DA reinforcement signals can be generated. We have made a clear distinction between this "sensory prediction error" system, and systems driven by "reward prediction errors" which require outcomes to be identified so useful estimates of utility can be made to guide the choice of future actions. The latter system is thought to be associated with processing in the pre-frontal cortex (Padoa-Schioppa and Assad, 2006; Potts et al., 2006). However, the mechanism(s) by which genuine errors in reward prediction are used to adjust the magnitude of representations of reward-related stimuli in structures with afferent projections to the basal ganglia (Kobayashi et al., 2002; Watanabe et al., 2002; Ikeda and Hikosaka, 2003; Ding and Hikosaka, 2006; Kobayashi et al., 2006; Pleger et al., 2008) (Reinforcement A in Fig. 31.2), remain to be determined.

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#### REFERENCES

- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Ann Rev Neurosci 9:357–381.
- Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30:62–69.
- BarGad I, Morris G, Bergman H (2003) Information processing, dimensionality reduction and reinforcement learning in the basal ganglia. Prog Neurobiol 71:439–473.
- Bayer HM, Glimcher PW (2005) Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47:129–141.
- Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacol (Berl) 191:391–431.
- Bickford ME, Hall WC (1989) Collateral projections of predorsal bundle cells of the superior colliculus in the rat. J Comp Neurol 283:86–106.
- Black J, Belluzzi JD, Stein L (1985) Reinforcement delay of one second severely impairs acquisition of brain self-stimulation. Brain Res 359:113–119.
- Boehnke SE, Munoz DP (2008) On the importance of the transient visual response in the superior colliculus. Curr Opin Neurobiol 18:544–551.
- Chevalier G, Deniau JM (1990) Disinhibition as a basic process in the expression of striatal functions. Trends Neurosci 13:277–281.
- Coizet V, Overton PG, Redgrave P (2007) Collateralization of the tectonigral projection with other major output pathways of superior colliculus in the rat. J Comp Neurol 500:1034–1049.
- Coizet V, Dommett EJ, Redgrave P, Overton PG (2006) Nociceptive responses of midbrain dopaminergic neurones are modulated by the superior colliculus in the rat. Neuroscience 139:1479–1493.
- Coizet V, Graham JH, Moss J, Bolam JP, Savasta M, McHaffie JG, Redgrave P, Overton PG (2009) Short-latency visual input to the subthalamic nucleus is provided by the midbrain superior colliculus. J Neurosci 29:5701–5709.
- Comoli E, Coizet V, Boyes J, Bolam JP, Canteras NS, Quirk RH, Overton PG, Redgrave P (2003) A direct projection from superior colliculus to substantia nigra for detecting salient visual events. Nat Neurosci 6:974–980.
- Corbit LH, Balleine BW (2003) The role of prelimbic cortex in instrumental conditioning. Behav Brain Res 146:145–157.
- Crutcher MD, DeLong MR (1984) Single cell studies of the primate putamen. II. Relations to direction of movement and pattern of muscular activity. Exp Brain Res 53:244–258.

- Dayan P, Balleine BW (2002) Reward, motivation, and reinforcement learning. Neuron 36:285–298.
- Dean P, Redgrave P, Westby GWM (1989) Event or emergency? Two response systems in the mammalian superior colliculus. Trends Neurosci 12:137–147.
- Dickinson A (2001) The 28th Bartlett Memorial Lecture Causal learning: An associative analysis. Quart J Exp Psych B Com Phy P 54:3–25.
- Ding L, Hikosaka O (2006) Comparison of reward modulation in the frontal eye field and caudate of the macaque. J Neurosci 26:6695–6703.
- Dommett E, Coizet V, Blaha CD, et al. (2005) How visual stimuli activate dopaminergic neurons at short latency. Science 307:1476–1479.
- Elsner B, Hommel B (2004) Contiguity and contingency in action-effect learning. Psychol Res 68:138–154.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299:1898–1902.
- Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968–973.
- Freeman AS (1985) Firing properties of substantia nigra dopaminergic neurons in freely moving rats. Life Sci 36:1983–1994.
- Gerfen CR, Wilson CJ (1996). The basal ganglia. *In* Handbook of Chemical Neuroanatomy, Vol 12: Integrated Systems of the CNS, Part III (Swanson LW, Bjorklund A, Hokfelt T, eds), pp. 371–468: Elsevier. Amsterdam.
- Grace AA (1995) The tonic phasic model of dopamine system regulation Its relevance for understanding how stimulant abuse can alter basal ganglia function. Drug Alcohol Depend 37:111–129.
- Grantyn R (1988) Gaze control through superior colliculus: structure and function. In: Neuroanatomy of the Oculomotor System (Buttner-Ennever JA ed), pp. 273–333. Elsevier: Amsterdam.
- Greengard P, Allen PB, Nairn AC (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 23:435–447.
- Grillner S, Helligren J, Menard A, Saitoh K, Wikstrom MA (2005) Mechanisms for selection of basic motor programs - roles for the striatum and pallidum. Trends Neurosci 28:364–370.
- Guarraci FA, Kapp BS (1999) An electrophysiological characterization of ventral tegmental area dopaminergic neurons during differential Pavlovian fear conditioning in the awake rabbit. Behav Brain Res 99:169–179.
- Gurney K, Prescott TJ, Redgrave P (2001a) A computational model of action selection in the basal ganglia. II. Analysis and simulation of behavior. Biol Cybern 84:411–423.
- Gurney K, Prescott TJ, Redgrave P (2001b) A computational model of action selection in the basal ganglia. I. A new functional anatomy. Biol Cybern 84:401–410.
- Heien M, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Wassum KM, Wightman RM (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci USA 102:10023–10028.
- Hikosaka O (2007) GABAergic output of the basal ganglia. Prog Brain Res 160:209–226.
- Hikosaka O, Wurtz RH (1983) Visual and oculomotor function of monkey substantia nigra pars reticulata. I. Relation of visual and auditory responses to saccades. J Neurophysiol 49:1230–1253.
- Hikosaka O, Sakamoto M, Usui S (1989) Functional properties of monkey caudate neurons III. Activities related to expectation of target and reward. J Neurophysiol 61:814–831.

- Horn G, Hill RM (1966) Effect of removing the neocortex on the response to repeated sensory stimulation of neurones in the mid-brain. Nature 211:754–755.
- Horvitz JC (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 96:651–656.
- Horvitz JC, Stewart T, Jacobs BL (1997) Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. Brain Res 759:251–258.
- Houk JC (2005) Agents of the mind. Biol Cybern 92:427-437.
- Humphries MD, Stewart RD, Gurney KN (2006) A physiologically plausible model of action selection and oscillatory activity in the basal ganglia. J Neurosci 26:12921–12942.
- Ikeda T, Hikosaka O (2003) Reward-dependent gain and bias of visual responses in primate superior colliculus. Neuron 39:693–700.
- Izhikevich EM (2007) Solving the distal reward problem through linkage of STDP and dopamine signaling. Cereb Cortex 17: 2443–2452.
- Jay MF, Sparks DL (1987) Sensorimotor integration in the primate superior colliculus. I Motor convergence. J Neurophysiol 57:22–34.
- Klop EM, Mouton LJ, Hulsebosch R, Boers J, Holstege G (2005) In cat four times as many lamina I neurons project to the parabrachial nuclei and twice as many to the periaqueductal gray as to the thalamus. Neuroscience 134:189–197.
- Kobayashi S, Lauwereyns J, Koizumi M, Sakagami M, Hikosaka O (2002) Influence of reward expectation on visuospatial processing in macaque lateral prefrontal cortex. J Neurophysiol 87:1488–1498.
- Kobayashi S, Nomoto K, Watanabe M, Hikosaka O, Schultz W, Sakagami M (2006) Influences of rewarding and aversive outcomes on activity in macaque lateral prefrontal cortex. Neuron 51:861–870.
- Lacey CJ, Bolam JP, Magill PJ (2007) Novel and distinct operational principles of intralaminar thalamic neurons and their striatal projections. J Neurosci 27:4374–4384.
- Levesque M, Charara A, Gagnon S, Parent A, Deschenes M (1996) Corticostriatal projections from layer V cells in rat are collaterals of long-range corticofugal axons. Brain Res 709:311–315.
- Lindvall O, Bjorklund A (1974) The organization of the ascending catcholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol Scand Suppl. 412:1–48.
- Ljungberg T, Apicella P, Schultz W (1992) Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol 67:145–163.
- Matsumoto M, Hikosaka O (2009) Representation of negative motivational value in the primate lateral habenula. Nature Neuroscience 12:77–84.
- McHaffie JG, Stanford TR, Stein BE, Coizet V, Redgrave P (2005) Subcortical loops through the basal ganglia. Trends Neurosci 28:401–407.
- Mink JW (1996) The basal ganglia: Focused selection and inhibition of competing motor programs. Prog Neurobiol 50:381–425.
- Montague PR, Dayan P, Sejnowski TJ (1996) A framework for mesencephalic dopamine systems based on predictive Hebbian learning. J Neurosci 16:1936–1947.
- Montague PR, Hyman SE, Cohen JD (2004) Computational roles for dopamine in behavioral control. Nature 431:760–767.
- Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H (2004) Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron 43:133–143.
- Nakahara H, Itoh H, Kawagoe R, Takikawa Y, Hikosaka O (2004) Dopamine neurons can represent context-dependent prediction error. Neuron 41:269–280.

- O'Doherty JP, Dayan P, Friston K, Critchley H, Dolan RJ (2003) Temporal difference models and reward-related learning in the human brain. Neuron 38:329–337.
- Omelchenko N, Sesack SR (2007) Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. Neuroscience 146:1259–1274.
- Overton PG, Coizet V, Dommett EJ, Redgrave P (2005) The parabrachial nucleus is a source of short latency nociceptive input to midbrain dopaminergic neurones in rat online. Washington DC: Society for Neuroscience.
- Padoa-Schioppa C, Assad JA (2006) Neurons in the orbitofrontal cortex encode economic value. Nature 441:223–226.
- Pan WX, Schmidt R, Wickens JR, Hyland BI (2005) Dopamine cells respond to predicted events during classical conditioning: Evidence for eligibility traces in the reward- learning network. J Neurosci 25:6235–6242.
- Pleger B, Blankenburg F, Ruff CC, Driver J, Dolan RJ (2008) Reward facilitates tactile judgments and modulates hemodynamic responses in human primary somatosensory cortex. J Neurosci 28:8161–8168.
- Potts GF, Martin LE, Burton P, Montague PR (2006) When things are better or worse than expected: The medial frontal cortex and the allocation of processing resources. J Cogn Neurosci 18:1112–1119.
- Prescott TJ, Gonzalez FMM, Gurney K, Humphries MD, Redgrave P (2006) A robot model of the basal ganglia: Behavior and intrinsic processing. Neural Netw 19:31–61.

Redgrave P (2007) Basal ganglia 13246: Scholarpedia.

- Redgrave P, Gurney K (2006) The short-latency dopamine signal: a role in discovering novel actions? Nat Rev Neurosci 7:967–975.
- Redgrave P, Prescott TJ, Gurney K (1999a) Is the short latency dopamine response too short to signal reward error? Trends Neurosci 22:146–151.
- Redgrave P, Prescott T, Gurney KN (1999b) The basal ganglia: A vertebrate solution to the selection problem? Neuroscience 89:1009–1023.
- Redgrave P, Gurney K, Reynolds J (2008) What is reinforced by phasic dopamine signals? Brain Res Rev 58:322–339.
- Reiner A, Jiao Y, DelMar N, Laverghetta AV, Lei WL (2003) Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. J Comp Neurol 457:420–440.
- Reynolds JN, Wickens JR (2002) Dopamine-dependent plasticity of corticostriatal synapses. Neural Netw 15:507–521.
- Robbins TW, Sahakian BJ (1981) Behavioral and neurochemical determinants of drug-induced stereotypy. In: Metabolic Disorder of the Nervous System (Rose FC ed), pp. 244–291. London: Pitman.
- Roitman MF, Stuber GD, Phillips PEM, Wightman RM, Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. J Neurosci 24:1265–1271.
- Salamone JD, Correa M (2002) Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav Brain Res 137:3–25.
- Satoh T, Nakai S, Sato T, Kimura M (2003) Correlated coding of motivation and outcome of decision by dopamine neurons. J Neurosci 23:9913–9923.
- Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27.
- Schultz W (2000) Multiple reward signals in the brain. Nat Rev Neurosci 1:199–207.

- Schultz W (2006) Behavioral theories and the neurophysiology of reward. Annu Rev Psychol 57:87–115.
- Schultz W (2007) Multiple dopamine functions at different time courses. Annu Rev Neurosci 30:259–288.
- Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599.
- Schulz JM, Redgrave P, Mehring C, Aertsen A, Clements KM, Wickens JR, Reynolds JNJ. 2009. Short-latency activation of striatal spiny neurons via subcortical visual pathways. J. Neurosci 29:6336-6347.
- Singh SP, Barto AG, Chentanez N (2005) Intrinsically motivated reinforcement learning. Proc Adv Neural Info Process Syst 17:2181–2188.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Snaith S, Holland O (1990) An investigation of two mediation strategies suitable for behavioral control in animals and animats. In: From Animals to Animats: Proceedings of the First International Conference on the Simulation of Adaptive Behavior (Meyer J-A, Wilson S, eds), pp. 255–262. Cambridge, MA: MIT Press.
- Sparks DL (1986) Translation of sensory signals into commands for control of saccadic eye movements: role of the primate superior colliculus. Physiol Rev 66:118–171.
- Sprague JM, Marchiafava PL, Rixxolatti G (1968) Unit responses to visual stimuli in the superior colliculus of the unanesthetized, mid-pontine cat. Arch Ital Biol 106:169–193.
- Stein BE, Meredith MA (1993) The Merging of the Senses. Cambridge, MA: The MIT Press.
- Stoerig P (2006) Blindsight, conscious vision, and the role of primary visual cortex. In: Visual Perception, Pt 2: Fundamentals Of Awareness: Multi-Sensory Integration And High-Order Perception, pp 217-234.
- Sutton RS, Barto AG (1998) Reinforcement Learning An Introduction. Cambridge, MA: MIT Press.
- Thorndike EL (1911) Animal Intelligence. New York: Macmillan.
- Thorpe SJ, Fabre-Thorpe M (2001) Seeking categories in the brain. Science 291:260–263.
- Ungless MA (2004) Dopamine: the salient issue. Trends Neurosci 27:702–706.
- Ungless MA, Magill PJ, Bolam JP (2004) Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. Science 303:2040–2042.
- Venton BJ, Wightman RM (2007) Pharmacologically induced, subsecond dopamine transients in the caudate-putamen of the anesthetized rat. Synapse 61:37–39.
- Watanabe M, Hikosaka K, Sakagami M, Shirakawa S (2002) Coding and monitoring of motivational context in the primate prefrontal cortex. J Neurosci 22:2391–2400.
- Wickens J (1993) A Theory of the Striatum. Oxford: Pergamon.
- Wickens JR, Reynolds JNJ, Hyland BI (2003) Neural mechanisms of reward-related motor learning. Curr Opin Neurobiol 13:685–690.
- Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483–494.
- Wurtz RH, Albano JE (1980) Visual-motor function of the primate superior colliculus. Ann Rev Neurosci 3:189–226.

# Role of Basal Ganglia in Habit Learning and Memory: Rats, Monkeys, and Humans

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#### I. INTRODUCTION

In a 1950 symposium, Karl Lashley offered the following conclusion on a possible role for the mammalian basal ganglia in learning and memory: "The evidence seems conclusive that in mammals the basal ganglia are not an essential link in the patterning of learned activities" (cited by Iverson, 1979). Five decades later, it is now clear that Lashley's conclusion was premature, and in fact the basal ganglia are critically involved in mammalian learning and memory processes. In particular, extensive evidence indicates that the basal ganglia mediate a form of learning in which "Hullian-like" stimulus-response (S-R) habits (Hull, 1943) are acquired and expressed. This hypothesis has largely been developed within the context of a "multiple systems" approach to memory organization. According to multiple memory systems theory, relatively independent brain systems support the acquisition of different types of memory (for a historical review, see Squire, 2004). Multiple memory systems theory was originally derived from an analysis of the effects of damage to the hippocampal system on behavior across a wide range of learning tasks (e.g., Hirsh, 1974; O'Keefe and Nadel, 1978). This analysis revealed that the effects of hippocampal lesions on learning and memory were selective, producing impairment on tasks involving "cognitive/relational" memory (Tolman, 1932; Hirsh, 1974), and sparing acquisition of tasks that could be acquired using S-R "habit" learning. The hypothesis that the basal ganglia may mediate S-R/ habit learning was subsequently introduced by Mishkin and colleagues in the mid-1980s (Mishkin et al., 1984; Mishkin and Petri, 1984; see also Mahut et al., 1984). The present chapter presents a brief overview of data supporting this hypothesis by focusing on evidence that the role of the basal ganglia in habit learning and memory generalizes across different mammalian species, including rats, monkeys, and humans. The aim of the chapter is to provide converging evidence for this view of the mnemonic function of the basal ganglia by highlighting a few prominent experiments from each of these species. Review of the findings from lower animals (rats and non-human primates) focuses on lesion studies, whereas description of the human research focuses on research involving patients with neuropsychological disorders and neuroimaging studies. These studies predominantly assessed the role of the striatum/caudate nucleus and putamen.

### **II. EVIDENCE FROM RAT STUDIES**

As described above, the idea that the basal ganglia are involved in habit learning and memory was developed within the context of multiple memory systems theory, specifically in an attempt to identify brain regions that may mediate the learning that is spared following damage to a "cognitive" memory system that critically involves hippocampal/medial temporal lobe function. This hypothesis was based in part on anatomical considerations, as the dorsal striatum receives sensory input from all regions of the cortex via topographically arranged corticostriatal projections, and can readily influence motor output via downstream projections to brainstem structures and/or via basal ganglia-thalamo-cortical loops (e.g., Webster, 1961; Van Hoesen et al., 1981; Alexander et al., 1986) (for an overview of the anatomical organization, see Chapter 1). Thus, it was suggested that the dorsal striatum is anatomically well-situated to mediate S-R habit learning across various sensory modalities (Mahut et al., 1984; Mishkin and Petri, 1984).

In rats, early lesion studies conducted prior to the advent of the multiple memory systems approach can be considered consistent with the hypothesis that the basal ganglia play a role in habit learning. Thus, basal ganglia damage impairs acquisition of one-way and two-way active avoidance (e.g., Kirkby and Kimble, 1968; Winocur and Mills, 1969; Neill and Grossman, 1971; Mitcham and Thomas, 1972; Kirkby and Polgar, 1974; Winocur, 1974), and straight-alley runway behavior (Kirkby et al., 1981). However, the first study to directly assess this hypothesis in rats employed dissociation methodology to compare the effects of basal ganglia lesions on acquisition of both cognitive and habit learning tasks in a radial maze (Packard et al., 1989). In the standard "cognitive" memory version of the task, rats obtained food rewards by visiting each arm of the radial maze once within a daily training session, and re-entries into maze arms that were previously visited are scored as errors. This task requires rats to remember which maze arms have been previously visited within a trial, and involves spatial working memory (Olton and Papas, 1979) and/or the use of a cognitive mapping strategy (O'Keefe and Nadel, 1978). In a newly developed "habit" memory version of the task, rats obtained food rewards by visiting four randomly selected and illuminated maze arms twice within a daily training session, and visits to unlit maze arms are scored as errors. Every visit to an illuminated maze arm was reinforced, and thus there was no requirement to use cognitive memory to remember specific arm entries. Instead, this task can be acquired using a habit learning mechanism by which a light cue (i.e., stimulus) evokes approach behavior (i.e., a response). It is important to note that these two tasks were conducted in the same apparatus and were designed to possess the same motivational (food reward), sensory (visual), and motoric (maze running) characteristics. Therefore, any differential effect

of basal ganglia damage in task acquisition could be more readily attributed to a deficit in the type of memory required for task performance. Pre-training electrolytic lesions of the basal ganglia (i.e., dorsal striatum) produced a dissociation in behavior in these two radial maze tasks, impairing acquisition of the habit task and leaving acquisition of the cognitive task unaffected. These findings in these two radial maze tasks were subsequently replicated in a study examining the effects of neurotoxic lesions of the basal ganglia (McDonald and White, 1993). Importantly, the deficit produced by dorsal striatal lesions in the acquisition of this simultaneous visual discrimination task in the radial maze reflects disruption of a S-R (light-approach) association, rather than a stimulus-stimulus (light-food) association. The nature of the association guiding the expression of learned behavior can be assessed in a reinforcer devaluation paradigm (Adams, 1981), in which sensitivity in changes to the "value" of a reinforcer are examined once a learned behavior is acquired. According to S-R learning theory, reinforcer devaluation should not disrupt responding, as S-R associations do not include information concerning the nature of the reinforcer. In the dorsal striatal-dependent radial maze task described above, rats exposed to reinforcer devaluation produced by lithium chloride injections following task acquisition continue to approach illuminated maze arms, consistent with an S-R interpretation of "what" is learned in this task (Sage and Knowlton, 2000; see also Yin et al., 2004; 2005).

An additional early study used two water maze tasks to examine the hypothesis that the basal ganglia play a selective role in habit memory (Packard and McGaugh, 1992). In these tasks two rubber balls differing in visual appearance (vertical versus horizontal black/white stripes) served as visual cues. One ball (correct) was located on top of a platform that could be used to escape the water, and the other ball (incorrect) was located on top of a thin rod that did not provide escape. In a cognitive version of the task the correct platform was located in the same spatial location on every trial, and the visual pattern on the ball associated with the correct platform varied across trials. Therefore, acquisition of this task required animals to use a spatial form of cognitive memory. In a habit version of the task the visual pattern on the ball associated with the correct platform was consistent, but the platform was located in different spatial locations across trials. Therefore, this task can be acquired by a habit learning mechanism that involves performing an approach response to a specific visual cue. Pretraining lesions of the dorsal striatum impair acquisition of the habit task, without affecting acquisition of the cognitive task (Packard and McGaugh, 1992).

A similar dissociation is observed using a single-platform water maze task in which rats are trained to swim to a visible escape platform that is always located in the same spatial location. In this situation, when the visible platform is moved to a new spatial location following training, rats with lesions of the dorsal striatum exhibit a cognitive strategy and swim to the spatial location that the platform was previously located in, whereas control rats exhibit a habit strategy and swim to the visible platform in its new location (McDonald and White, 1994). Taken together with the radial maze experiments described above, these findings indicate that the selective role of the dorsal striatum in habit memory generalizes to aversively motivated learning.

Another example of a lesion study in rats that provides evidence of a selective role for the basal ganglia in habit learning was conducted using a plus-maze task (Packard and McGaugh, 1996). With respect to multiple memory systems theory, the use of this task is of particular interest in part because it was originally introduced as a means of distinguishing between cognitive and S-R habit theories of learning by assessing the relative use of "place" and "response" learning (e.g., Tolman et al., 1946). The plusmaze apparatus is arranged so that a goal box (e.g., east or west) can be approached from one of two start boxes (e.g., north or south). In a "dual-solution" version of the task, rats are trained to obtain food from a consistently baited goal box (e.g., west), from the same start box (e.g., south). According to the cognitive view of learning, rats trained in this task learn the spatial location of the reinforcer, and this information can be used to guide an approach response to the baited goal box. In contrast, according to the S-R habit view of learning, rats learn to approach the baited goal box by acquiring a response tendency (i.e., a specific body turn) at the choice point of the maze. Note that both of these putative learning mechanisms can be used to successfully acquire this dual-solution task. Following acquisition, a probe trial is administered in which rats are started from the opposite start box (e.g., north), and behavior on this trial is used to assess the type of learning acquired. Thus, rats employing cognitive memory for the spatial location of the reinforcer should continue to approach the baited goal box on the probe trial (i.e., place learning). In contrast, rats employing habit memory should display the specific body turn response that was acquired and approach the opposite goal box on the probe trial (i.e., response learning).

In order to examine the role of the basal ganglia in the expression of learned behavior in this task, rats that were cannulated in the dorsolateral striatum were first trained in a daily session using the same start box on each trial (e.g., south) to obtain food from a consistently baited goal box (e.g., west). Following seven days of training rats were performing at asymptotic levels, and on day eight they were given a probe trial to assess the relative use of place and response learning. Prior to the probe trial, a reversible brain lesion was produced via intra-dorsolateral striatal injections of the sodium channel blocker lidocaine. On the day 8 probe trial, rats receiving or saline vehicle or lidocaine injections into the dorsolateral striatum were predominantly place learners. Thus, infusions of lidocaine into the dorsolateral striatum did not impair the expression of cognitive/place learning that was employed by the control rats on the day eight probe trial, consistent with the findings from the radial maze experiment described above. Of course, as the vehicle injected rats displayed a predominant use of place learning on the day eight probe trial, the findings do not allow for any conclusions concerning the potential role of the basal ganglia in response learning. However, an interesting feature of the dual-solution plus-maze task is that with extended training, rats eventually switch from the use of place learning to a response-learning tendency (Ritchie et al., 1950; Hicks, 1964). Therefore, the rats were trained for an additional seven days, and received intra-striatal injections of lidocaine prior to a second probe trial administered on day sixteen. On this second probe trial rats receiving saline vehicle injections were predominantly response learners. In contrast, rats receiving intra-dorsal striatal injections of lidocaine were predominantly place learners, indicating a blockade of the expression of response learning. The results suggest that the shift or transition from the use of place learning to response learning in this task involves the gradual recruitment of a dorsal-striatal based habit learning system to guide behavior. Interestingly, the "switch" to response learning can be accelerated by post-training intradorsolateral striatal injections of glutamate (Packard, 1999).

The dorsolateral striatum also mediates the acquisition of a single-solution plus-maze task that requires rats to use habit/response learning by varying the start point on each trial and reinforcing the same body turn at the choice point (Chang and Gold, 2004; for an extended review of the role of the basal ganglia in plus-maze behavior, see Packard, 2009). The selective impairment in response learning in the plusmaze following lesions of the basal ganglia is consistent with other studies implicating this brain region in "egocentric" habit learning (e.g., Potegal, 1972; Cook and Kesner, 1988).

It is important to note that lesions of the hippocampal system produce the opposite effect of dorsal striatal lesions in both the plus-maze (Packard and McGaugh, 1996), and radial maze (Packard et al., 1989; McDonald and White, 1993), tasks described above. Thus, consistent with multiple memory systems theory the double dissociations observed following manipulations of these two brain regions provide compelling evidence of the functional independence of cognitive and habit memory. In sum, the findings of the two experiments described above indicate that the basal ganglia play a selective role in habit learning and memory in rats. Numerous other studies in rats employing brain lesion, pharmacological, electrophysiological, and molecular approaches are also consistent with this hypothesis (for extended reviews see White and McDonald, 2002; Packard and Knowlton, 2002; Graybiel, 2008; Packard, 2009).

#### III. EVIDENCE FROM MONKEY STUDIES

As previously described, the idea that the basal ganglia are involved in habit learning was originally introduced based in large part on anatomical consideration of brain regions that might suitably mediate the spared learning that is observed following hippocampal/medial temporal lobe damage in monkeys (Mishkin and Petri, 1984; Mishkin et al., 1984; Mahut et al., 1984). However, in contrast to the large number of studies supporting this idea that have been conducted using rats, relatively few lesion experiments have directly assessed this hypothesis in non-human primates.

Two early brain lesion experiments provided some evidence (albeit inconclusive) of a role for the basal ganglia in habit learning in non-human primates. The first of these studies was designed to further examine the hypothesis that interconnected regions of the mammalian cortex and basal ganglia are "equipotential" in function (Divac et al., 1967). Ablation of the inferotemporal cortex had been previously shown to impair acquisition of a visual discrimination habit (Mishkin and Pribram, 1954), and one of the subcortical projection sites of this cortical region is the tail of the caudate nucleus (Whitlock and Nauta, 1956). Therefore, monkeys with pre-training lesions of different regions of the caudate nucleus, including a group that received lesions of a rostral part of the caudate tail, were trained in a visual pattern discrimination task that can be acquired using an S-R habit learning mechanism. Monkeys with lesions of the caudate tail required twice as many trials to reach criterion in this task compared to other groups of monkeys with lesions in the head of the caudate nucleus, although they were eventually able to acquire the task (Divac et al., 1967).

A second early study of the effects of basal ganglia lesions on habit learning in monkeys examined the effects of lesions of the caudoventral putamen on retention of visual and auditory discrimination habit tasks (Buerger et al., 1974). Like the tail of the caudate nucleus, the monkey caudoventral putamen also receives projections from the inferotemporal cortex (Reitz and Pibram, 1969). In this study, lesions of the putamen impaired post-operative retention of the visual discrimination task, without affecting behavior in the auditory discrimination task. Subjects with the largest amount of ventral putamen damage displayed the greatest retention deficit. Although the findings were consistent with a possible role for the basal ganglia in habit learning and memory, the findings were ultimately inconclusive due to inadvertent lesion damage to the adjacent white matter.

Several years subsequent to these two early primate experiments, and following the numerous studies conducted in rats, the hypothesis that the monkey basal ganglia plays a selective role in habit memory was examined (Fernandez-Ruiz et al., 2001). This study compared the effects of neurotoxic-induced lesions of the rostral tail of the caudate nucleus and overlying ventrocaudal putamen on behavior in a visual "habit" task and a "cognitive" delayed non-matching-tosample (DNMS) task. In the visual habit task, monkeys were presented concurrently with pairs of objects (20 different pairs), and one of the objects in each pair was consistently baited with a food reward located in an underlying well. The repetition of each pair occurred using a 24 hour inter-trial interval. Monkeys were first trained to criterion using one set of 20 objects, and following surgery were trained on three new sets of 20 objects each. In the DNMS task, monkeys were trained to displace a baited sample object, followed by a choice using various delay intervals between the previously baited object (i.e., which was now unbaited), and a novel baited object. The findings indicated that monkeys with lesions of the ventrocaudal neostriatum were impaired on the concurrent object visual discrimination task, with animals with the largest lesion extent displaying the greatest impairment (Fernandez-Ruiz et al., 2001). Importantly, the basal ganglia lesions did not impair behavior in the DNMS task, indicating a dissociation of the effects of the lesions on habit and cognitive memory. Taken together with previous evidence that performance on the DNMS task, but not the visual habit task is impaired by lesions of the medial temporal lobe (e.g., perirhinal/entorhinal cortices; Buffalo et al., 1999), the findings suggest a double dissociation between the mnemonic functions of these brain regions. An additional finding from a lesion study in non-human primates also implicates

basal ganglia function in habit learning and memory (Teng et al., 2000). In this study, monkeys with lesions restricted to the hippocampal system (hippocampus proper, dentate gyrus, and subiculum) were unimpaired on an 8-object concurrent visual discrimination task. However, a subset of animals that had also sustained damage to the tail of the caudate nucleus were impaired in concurrent discrimination learning, as well as on a pattern discrimination task.

Finally, although outside of the scope of the present brief review, evidence from several studies employing electrophysiological approaches also provide evidence consistent with a role for the monkey basal ganglia in habit learning and memory (for reviews see Hikosaka, 2007; Graybiel, 2008).

### **IV. EVIDENCE FROM HUMAN STUDIES**

The hypothesis that regions of the basal ganglia are involved in habit learning and memory is also supported by studies in humans (for review see Packard and Knowlton, 2002). This line of research includes experiments in patients with neuropsychological disorders that involve basal ganglia dysfunction, including Parkinson's and Huntington's disease (e.g., Paulsen et al., 1993; Butters et al., 1994; Knowlton et al., 1996), as well as studies employing noninvasive neuroimaging (e.g., Jenkins et al., 1994; Doyon et al., 1996; Rauch et al., 1997; Dong et al., 2000; Poldrack et al., 2001; Hartley et. al., 2003; Iaria et al., 2003).

An early study designed to directly assess a possible selective mnemonic role for the basal ganglia involved a comparison of cognitive and habit memory in temporal lobe amnesics, and patients with Parkinson's disease (Knowlton et al., 1996). This experiment employed a classification learning task in which each of four individual visual cues were probabilistically related either 25, 43, 57, or 75% of the time to one outcome (sunshine) and 75, 57, 43, or 25% of the time with a second outcome (rain). On each trial one, two, or three of these cues were on a computer screen and subjects pressed a key to predict the "weather" outcome, and feedback (i.e., correct or incorrect prediction) was provided. Previous research had demonstrated that probabilistic learning is spared in temporal lobe amnesic patients, suggesting that the use of cognitive/declarative memory is not necessary for acquisition (Knowlton et al., 1994). Rather, the task is learned gradually over several trials, and subjects can perform the task without awareness of the specific information they have acquired. Nondemented patients with Parkinson's disease failed to acquire the probabilistic classification task, performing at approximately 50-55% correct following 50 trials, whereas age and education-matched control subjects performed at approximately 70% correct (Knowlton et al., 1996). The hypothesis that this impairment involves a selective deficit in habit learning was supported by the finding that the same Parkinson's patients displayed normal cognitive/declarative memory for the training episode as assessed by a post-session questionnaire. Equally important, amnesic patients with bilateral damage to the hippocampus or diencephalic midline displayed the opposite behavioral pattern; acquiring the probabilistic classification task to control levels, but presenting a severe impairment in cognitive/declarative memory for the training episode. Therefore, this human study provides a double dissociation between the roles of the basal ganglia and medial temporal lobe in habit and cognitive memory, respectively, analogous to that observed in rats and monkeys. Whereas the study by Knowlton and colleagues notably involved a direct comparison of basal ganglia function in cognitive and habit memory, acquisition of the "weather prediction" task (Marsh et al., 2004; 2005) is also impaired in patients with Tourette's syndrome, a neuropsychiatric disorder known to involve basal ganglia dysfunction.

Basal ganglia function in habit learning and memory has also been investigated in human research using "virtual reality" maze tasks designed to be analogous in part to those used in earlier animal research. For example, Iaria and colleagues used functional magnetic resonance imaging (fMRI) and trained subjects in a virtual environment radial maze task that could be acquired using a cognitive/spatial learning or a habit/egocentric learning strategy (Iaria et al., 2003). Following acquisition, subjects reported in a debriefing session on how they had solved the task. Based on their response, subjects were categorized as using a cognitive strategy (i.e., use of extra-maze landmarks to guide spatial choice behavior), or a habit strategy (i.e., use of an egocentric turning response from a single maze starting point to guide choice behavior). The fMRI results revealed subjects using a spatial strategy showed increased activation in the hippocampus. In contrast, subjects that used a nonspatial strategy showed increases in activity within the basal ganglia (caudate nucleus). Moreover, consistent with the previously described lower animal research, in which rats "switched" to the use of caudate-dependent habit learning with extended training (Packard and McGaugh, 1996), human subjects who switched from a spatial to nonspatial strategy in the virtual maze task also showed increases in caudate nucleus activation over training (Iaria et al., 2003). Activation of the dorsal striatum/caudate nucleus has also been shown to correlate with accurate "route-following" (repeatedly following a fixed route), whereas hippocampal activation is correlated with successful "wayfinding" (the use of novel short routes) in a virtual reality "town" environment (Hartley et al., 2003).

Consistent with the research employing virtual reality mazes, a role for the human dorsal striatum in habit learning has also been demonstrated using the reinforcer devaluation procedure described earlier (Tricomi et al., 2009). In this fMRI study, subjects were shown visual cues that were associated with two different food rewards, and were trained on a variable interval reinforcement schedule to make a key press response press on one of two different keys. Following task acquisition, reinforcer devaluation was instigated by allowing subjects to satiate to one of the two food rewards. Relative to subjects who had received limited (i.e., one day) of training, those who had been "overtrained" for three days did not display any effect of the reinforcer devaluation procedure, responding to both cues despite the satiation experience. Moreover, consistent with a role for the basal ganglia in habit formation, the fMRI data indicated greater activation in the dorsolateral striatum in subjects receiving the more extensive training.

Finally, numerous neuropsychological and neuroimaging human studies implicate the basal ganglia in both the acquisition and expression of various motor skills that may putatively be acquired as habits (for reviews, see Salmon and Butters, 1995; Doyon et al., 2009).

# V. CONCLUSIONS, MODIFICATIONS, AND IMPLICATIONS

As described above, findings of studies in rats, monkeys, and humans provide converging evidence supporting the hypothesis that the basal ganglia are involved in habit learning and memory. With regards to the research conducted in rats, the examples provided in the present brief chapter focused on a set of brain lesion experiments (for an extended review on the effects of basal ganglia lesions on habit learning in rats see, White, 2009). However, it should be noted that this hypothesis is also supported by numerous studies in rats employing pharmacological (e.g., Packard and White, 1991; Packard and Teather, 1997; 1999), molecular (e.g., Colombo et al., 2003; Teather et. al., 2005), and electrophysiological (e.g., Jog et al., 1999; for review see Graybiel, 2008) approaches. In addition, mechanisms of synaptic plasticity (e.g., long-term potentiation, long-term depression, and spike-dependent timing) that may mediate habit learning in the rat basal ganglia have also been extensively investigated (for reviews, see Di Filippo et al., 2009; Wickens, 2009).

The present discussion referred to the "dorsal striatum" (i.e., caudate-putamen), in general. However, the hypothesis that functional heterogeneity may exist in the learning and memory processes mediated by the dorsal striatum is suggested by anatomical evidence that this brain structure receives input from all areas of the neocortex (e.g., Webster, 1961; 1965; Kemp and Powell, 1970; Veening et al., 1980; McGeorge and Faull, 1989). In recent years increasing evidence indicates that the role of the dorsal striatum in habit learning and memory may be particularly associated with lateral regions of this brain structure (for reviews, see Yin and Knowlton, 2006; White, 2009). In contrast, medial regions of the dorsal striatum that receive input from the frontal cortex may subserve more "cognitive-like" mnemonic functions, although it should be noted that there is evidence from lesion studies in rats that appears inconsistent with this idea (e.g., DeCoteau and Kesner, 2000; Adams, Kesner and Ragozzino, 2001; Sakamoto and Okaichi, 2001). Nonetheless, one useful modification of the general hypothesis that the dorsal striatum plays a selective role in habit learning and memory is that this mnemonic function may be more closely tied to the dorsolateral striatum. For example, a dissociation between the role of the dorsomedial and dorsolateral striatum in action-outcome learning and S-R habit learning has been observed in the rat (Yin et al., 2004; 2005). In this context it might also be noted that whereas particular disease states have clearly been associated with basal ganglia dysfunction, the extent of accompanying damage to various brain regions interconnected with the basal ganglia (e.g., areas of prefrontal cortex) can vary depending on disease stage. Thus, mnemonic deficits have also been observed in Parkinson's and Huntington's disease that likely reflect an interaction with executive functions of the frontal cortex (for review, see Grahn et al., 2009). In addition, it should be noted that deficits in habit learning in Parkinson's disease may in part reflect an interaction with dopaminergic medication (e.g., Frank, 2005; Shohamy et al., 2006).

Finally, the implications of the general hypothesis that the basal ganglia play a critical role in habit learning and memory can be appreciated when one considers that theoretical approaches to a wide range of human behavior have begun to incorporate this idea. Examples include theories of social interaction and self-regulation (e.g., Lieberman, 2000; Wood et al., 2002; Wood and Neal, 2007), culturally-based rituals (Graybiel, 2008), and various psychopathologies (e.g., White, 1996; Marsh et al., 2004; Everitt and Robbins, 2005; Graybiel, 2008) (see Chapter 33 for discussion of a role for the basal ganglia and habit learning in drug addiction).

### REFERENCES

- Adams S, Kesner RP, Ragozzino ME (2001) Role of the medial and lateral caudate-putamen in mediating an auditory conditional response association. Neurobiol Learning Memory 76:106–116.
- Adams CD (1981) Variations in the sensitivity of instrumental responding to reinforcer devaluation. Q J Exp Psychol 34B:77–98.
- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381.
- Buerger AA, Gross CG, Rocha-Miranda CE (1974) Effects of ventral putamen lesions on discrimination learning by monkeys. J Comp Physiol Psychol 86:440–446.
- Buffalo EA, Ramus SJ, Clark RE, Teng E, Squire LR, Zola ZM (1999) Dissociation between the effects of damage to the perirhinal cortex and area TE. Learning and Memory 6:572–599.
- Butters N, Salmon DP, Heindel WC (1994) Specificity of the memory deficits associated with basal ganglia dysfunction. Rev Neurol (Paris) 150:580–587.
- Colombo PJ, Brightwell JJ, Countryman RA (2003) Cognitive strategyspecific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. J Neurosci 23:3547–3554.
- Chang Q, Gold PE (2004) Inactivation of dorsolateral striatum impairs acquisition of response learning in cue-deficient, but not cue-available, conditions. Behav Neurosci 118:383–388.
- Cook D, Kesner RP (1988) Caudate nucleus and memory for egocentric localization. Behav Neural Biol 49:332–343.
- DeCoteau WE, Kesner RP (2000) A double dissociation between the rat hippocampus and medial caudoputamen in processing two forms of knowledge. Behav Neurosci 114:1096–1108.
- Di Filippo M, Picconi B, Tantucci M, et al. (2009) Short-term and longterm plasticity at corticostriatal synapses: implications for learning and memory. Behav Brain Res 199:108–118.
- Divac I, Rosvold HE, Szwarcbart MK (1967) Behavioral effects of selective ablation of the caudate nucleus. J Comp Physiol Psychol 63:183–190.
- Dong Y, Fukuyama H, Honda M, Okada T, Hanakawa T, et al. (2000) Essential role of the right superior parietal cortex in Japanese kana mirror reading: an fMRI study. Brain 123:790–799.
- Doyon J, Owen AM, Petrides M, Sziklas V, Evans AC (1996) Functional anatomy of visuomotor skill learning in human subjects examined with positron emission tomography. Eur J Neurosci 8:637–648.
- Doyon J, Bellec P, Amsel R, Penhune V, Monchi O, Carrier J, Lehericy S, Benali B (2009) Contributions of the basal ganglia and functionally related brain structures to motor learning. Behav Brain Res 199:61–75.
- Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Fernandez-Ruiz J, Wang J, Aigner TG, Mishkin M (2001) Visual habit formation in monkeys with neurotoxic lesions of the ventrocaudal neostriatum.. Proc Natl Acad Sci USA 98:4196–4201.
- Frank MJ (2005) Dynamic dopamine modulation in the basal ganglia: A neurocomputational account of cognitive deficits in medicated and non-medicated Parkinsonism. J Cog Neurosci 17:51–72.

- Grahn JA, Parkinson JA, Owen AM (2009) The role of the basal ganglia in learning and memory: neuropsychological studies. Behav Brain Res 199:53–60.
- Graybiel AM (2008) Habits, rituals, and the evaluative brain. Ann Rev Neurosci 31:359–387.
- Hartley T, Maguire EA, Spiers HJ, Burgess N (2003) The well-worn route and the path less traveled: distinct neural bases of route following and wayfinding in humans. Neuron 37:877–888.
- Hicks LH (1964) Effects of overtraining on acquisition and reversal of place and response learning. Psychol Report 15:459–462.
- Hikosaka O (2007) Basal ganglia mechanisms of reward-oriented eye movement. Ann NY Acad Sci 1104:229–249.
- Hirsh R (1974) The hippocampus and contextual retrieval of information from memory: a theory. Behav Biol 12:421–444.
- Hull CL (1943) Principles of Behavior. New York: Appleton-Century Crofts.
- Iaria G, Petrides M, Dagher A, Pike B, Bohbot V (2003) Cognitive strategies dependent on the hippocampus and caudate nucleus in human navigation. J Neurosci 23:5945–5952.
- Iversen SD (1979) Behaviour after neostriatal lesions in animals. In: The Neostriatum (Divac I, Oberg RGE, eds), pp. 291–313. Oxford, UK: Permagon Press.
- Jenkins IH, Brooks DJ, Nixon PD, Frackowiak RSJ, Passingham RE (1994) Motor sequence learning: a study with positron emission tomography. J Neurosci 14:3775–3790.
- Jog MS, Kubota Y, Connolly CI, Hillegaart V, Graybiel AM (1999) Building neural representations of habits. Sci 286:1745–1749.
- Kemp JM, Powell TP (1970) The cortico-striate projection in the monkey. Brain 93:525–546.
- Kesner RP, Bolland BL, Dakis M (1993) Memory for spatial locations, motor responses, and objects: triple dissociation among the hippocampus, caudate nucleus, and extrastriate visual cortex. Exp Brain Res 93:462–470.
- Kesner RP, Wilburn MW (1974) A review of electrical stimulation of the brain in context of learning and retention. Behav Biol 10:259–293.
- Kirkby RJ, Kimble DP (1968) Avoidance and escape behavior following striatal lesions in the rat. Exp Neurol 20:215–227.
- Kirkby RJ, Polgar S, Coyle IR (1981) Caudate nucleus lesions impair the ability of rats to learn a simple straight-alley task.. Percep Mot Skills 52:499–502.
- Kirkby RJ, Polgar S (1974) Active avoidance in the laboratory rats following lesions of the dorsal or ventral caudate nucleus. Physiol Psychol 2:301–306.
- Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Sci 273:1399–1402.
- Knowlton BJ, Squire LR, Gluck MA (1994) Probabilistic category learning in amnesia. Learn Mem 1:106–120.
- Lieberman MD (2000) Intuition: a social cognitive neuroscience approach. Psychol Bull 126:109–137.
- Mahut HS, Zola-Morgan S, Moss M (1984) Consolidation of memory: The hippocampus revisited. In: The Neuropsychology of Memory (Butters N, Squire LR, eds), pp. 297–315. New York: Guilford.
- Marsh R, Alexander GM, Packard MG, Zhu H, Wingard JC, Quackenbush G, Peterson BS (2004) Habit learning in Tourette syndrome: a translational neuroscience approach to a developmental psychopathology. Arch Gen Psychiat 61:1259–1268.
- Marsh R, Alexander GM, Packard MG, Zhu H, Peterson BS (2005) Perceptual-motor skill learning in Tourette syndrome: Evidence for multiple procedural learning and memory systems. Neuropsychologia 43:1456–1465.

- McDonald RJ, White NM (1993) A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 107:3–22.
- McDonald RJ, White NM (1994) Parallel information processing in the water maze: evidence for independent memory systems involving the dorsal striatum and hippocampus. Behav Neural Biol 61:260–270.
- McGeorge AJ, Faull RLM (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neurosci 29:503–537.
- Mishkin M, Malamut B, Bachevalier J (1984) Memories and habits: two neural systems. In: Neurobiology of Learning and Memory (Lynch G, McGaugh JI, Weinberger NM, eds), pp. 65–77. New York: Guilford.
- Mishkin M, Petri HL (1984) Memories and habits: some implications for the analysis of learning and retention. In: Neuropsychology of Memory (Butters N, Squire LR, eds), pp. 287–296. New York: Guilford.
- Mishkin M, Pribram KH (1954) Visual discrimination performance following partial ablations of the temporal lobe. I. Ventral vs. lateral. J Comp Physiol Psych 47:14–20.
- Mitcham JC, Thomas RK (1972) Effects of substantia nigra and caudate nucleus lesions on avoidance learning in rats. J Comp Physiol Psychol 81:101–107.
- Neill DB, Grossman SP (1971) Behavioral effects of lesions or cholinergic blockade of the dorsal and ventral caudate of rats. J Comp Physiol Psychol 71:311–317.
- O'Keefe J, Nadel L (1978) The Hippocampus as a Cognitive Map. Oxford, UK: Oxford University Press.
- Olton DS, Papas BC (1979) Spatial memory and hippocampal function. Neuropsychologia 17:669–682.
- Packard MG, Hirsh R, White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. J Neurosci 9:1465–1472.
- Packard MG, McGaugh JL (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks: further evidence for multiple memory systems. Behav Neurosci 106:439–446.
- Packard MG, McGaugh JL (1996) Inactivation of the hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. Neurobiol Learn Mem 65:65–72.
- Packard MG (2009) Exhumed from thought: basal ganglia and response learning in the plus-maze. Behav Brain Res 199:24–31.
- Packard MG (1999) Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning.. Proc Natl Acad Sci USA 96:12881–12886.
- Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Ann Rev Neurosci 25:563–593.
- Packard MG, Teather LA (1997) Double dissociation of hippocampal and dorsal striatal memory systems by post-training intracerebral injections of 2-aminophosphonopentanoic acid. Behav Neurosci 111:543–551.
- Packard MG, Teather LA (1999) Dissociation of multiple memory systems by post-training intracerebral injections of glutamate. Psychobiol 27:40–50.
- Packard MG, White NM (1991) Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. Behav Neurosci 105:295–306.
- Paulsen JS, Butters N, Salmon DP, Heindel WC, Swenson MR (1993) Prism adaptation in Alzheimer's and Huntington's disease. Neuropsychol 7:73–81.
- Poldrack RA, Clark J, Pare-Blagoev J, et al. (2001) Interactive memory systems in the human brain. Nature 29:546–550.
- Potegal M (1972) The caudate nucleus egocentric localization system. Acta Neurobiol Exp (Warsz) 32:479–494.

- Rauch SL, Whalen PJ, Savage CR, Curran T, Kendrick A, et al. (1997) Striatal recruitment during an implicit sequence learning task as measured by functional magnetic resonance imaging. Hum Brain Mapp 5:124–132.
- Reitz SL, Pribram KH (1969) Some subcortical projections of the inferotemporal gyrus of the monkey. Exp Neurol 25:632–645.
- Ritchie BF, Aeschliman B, Pierce P (1950) Studies in spatial learning: VIII. Place performance and acquisition of place dispositions. J Comp Physiol Psychol 43:73–85.
- Salmon DP, Butters N (1995) Neurobiology of skill and habit learning. Curr Opin Neurobiol 5:184–190.
- Sakamoto T, Okaichi H (2001) Use of win-stay and win-shift strategies in place and cue tasks by medial caudate putamen (MCPu) lesioned rats. Neurobiol Learn Mem 76:192–208.
- Sage JR, Knowlton BJ (2000) Effects of US devaluation on win-stay and win-shift radial arm maze performance in rats. Behav Neurosci 114:295–306.
- Shohamy D, Myers CE, Geghman KD, Sage J, Gluck MA (2006) L-Dopa impairs learning, but spares generalization, in Parkinson's disease. Neuropsychologia 44:774–784.
- Squire LR (2004) Memory systems of the brain: a brief history and current perspective. Neurobiol Learn Mem 82:171–177.
- Teather LA, Packard MG, Smith DE, Ellis-Behnke RG, Bazan NG (2005) Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks. Neurobiol Learn Mem 84:75–84.
- Teng E, Stefanacci L, Squire LR, Zola SM (2000) Contrasting effects on discrimination learning after hippocampal lesions and conjoint hippocampal-caudate lesions in monkeys. J Neurosci 20:3853–3863.
- Thorndike EL (1933) A proof of the law of effect. Sci 77:173-175.
- Tolman EC (1932) Purposive Behavior in Animals and Men. New York: Appleton-Century Crofts.
- Tolman EC, Ritchie BF, Kalish D (1946) Studies in spatial learning II. Place versus response learning. J Exp Psychol 36:221–229.
- Tricomi E, Balleine B, O'Doherty J (2009) A specific role for posterior dorsolateral striatum in human habit learning. Eur J Neurosci 29:2225–2232.
- Van Hoesen GW, Yeterian EH, Lavizzo-Mourey R (1981) Widespread corticostriate projections from temporal cortex of the rhesus monkey. J Comp Neurol 199:205–219.
- Veening JG, Cornelissen FM, Lieven JM (1980) The topical organization of the afferents to the caudatoputamen of the rat. A horseradish peroxidase study. Neurosci 5:1253–1268.
- Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95:532–544.
- Webster KE (1965) The Corticostriatal Projection in the cat. J Anat 99: 329–337.
- Wickens JR (2009) Synaptic plasticity in the basal ganglia. Behav Brain Res 199:119–128.
- White NM (1996) Addictive drugs as reinforcers: multiple partial actions on memory systems. Addiction 91:921–949.
- White NM, McDonald RJ (2002) Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem 77:125–184.
- White NM (2009) Some highlights of research on the effects of caudate nucleus lesions over the past 200 years. Behav Brain Res 199:3–23.
- Winocur G, Mills JA (1969) Effects of caudate lesions on avoidance behavior in rats. J Comp Physiol Psychol 65:552–557.
- Winocur G (1974) Functional dissociation within the caudate nucleus of rats. J Comp Physiol Psychol 86:432–439.

- Whitlock DC, Nauta WJH (1956) Sobcortical projections form the temporal neocortex in *Macca mulatta*. J Comp Neurol 106:183–212.
- Wood W, Quinn JM, Kashy DA (2002) Habits in everyday life: thought, emotion, and action. J Personality Soc Psychol 83:1281–1297.
- Wood W, Neal DT (2007) A new look at habits and the habit-goal interface. Psychol Rev 114:843–863.
- Yin HH, Knowlton BJ (2004) Contributions of striatal subregions to place and response learning. Learn Mem 11:459–463.
- Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci 7:464–476.
- Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.
- Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513–523.

# Drug Addiction: the Neural and Psychological Basis of a Compulsive Incentive Habit

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# I. INTRODUCTION

In this chapter we discuss the central role of the basal ganglia in the pathophysiology of stimulant addiction. More precisely we will emphasize that drug addiction, which we describe as a **compulsive incentive habit**, results from the progressive subversion by addictive drugs of striatum-dependent instrumental and Pavlovian learning mechanisms that are normally involved in the control over behavior by natural reinforcers. The basal ganglia are predominantly organized in functionally segregated parallel cortico-striato-pallido-thalamo-cortical loops involved in cognitive, motor and motivational processes (see Chapter 1 for an overview of the anatomical organization of these circuits). However, increasing evidence suggests that the neural organization of the basal ganglia is more complex, and that serial integrative projections may link motivational, emotional and motor functions in the establishment of instrumental behavior. Circuitry linking ventral and dorsal areas of the striatum via so-called 'spiraling' connections with midbrain dopamine (DA) neurons (see Chapter 24) may thus be one aspect of this circuitry mediating the modulation by drug-associated Pavlovian incentive influences of drug seeking and taking instrumental responses that are themselves under the control of both action-outcome and stimulus-response (S-R) mechanisms. Here we suggest that protracted exposure to psychostimulants abnormally recruits these serial and dopamine-dependent striato-nigro-striatal ascending spirals linking the nucleus accumbens to more dorsal regions of the striatum, thereby underlying a shift from action-outcome to S-R mechanisms in the control over drug seeking. When this shift in the associative structure governing instrumental control over drug-seeking is combined with drug-associated Pavlovian influences from limbic cortical structures, such as the amygdala and the orbitofrontal cortex, drug seeking behavior becomes established as an incentive habit. Though not sufficient to explain the compulsive nature of the drug seeking and taking behavior that characterizes drug addiction, the instantiation of striatal processing of drug-associated stimuli and their influence on instrumental responding might be a key mechanism underlying the development of drug addiction.

## II. DRUG ADDICTION: A NEUROPSYCHIATRIC DISORDER DEPENDENT UPON THE BASAL GANGLIA AND THEIR CORTICAL INPUTS

Drug addiction is a brain disease (Leshner, 1997) currently defined as a chronic relapsing disorder characterized by loss of control over drug intake and compulsive drug use that persists despite adverse consequences (DSM IV, APA, 2000), such that drug addicts eventually jeopardize their social and professional lives as well as their health in their pursuit of drug use (Gawin and Ellinwood Jr, 1989; Gawin, 1989, 1991). Over the course of the development of the pathology, drug seeking becomes progressively controlled by drug-associated stimuli in the environment (O'Brien et al., 1992a, 1992b, 1998), acting as both conditioned reinforcers and as Pavlovian elicitors of craving and relapse. Although there is not yet a generally agreed account of the neurobiological basis of addiction, within and between systems

adaptations in the brain have been identified that may contribute to the establishment of compulsive drug taking. Thus psychostimulants engage positive reinforcement mechanisms, perhaps sensitizing the incentive properties of the cues in the environment that are repeatedly associated with them, a process termed "incentive sensitization" (Robinson and Berridge, 1993, 2000, 2001, 2003). But psychostimulants also trigger between-systems adaptations that lead to increased drug seeking through negative reinforcement: withdrawal from psychostimulants triggers progressively increasing negative affective states, a process known as "hedonic allostasis" (Koob and Le Moal, 2001, 2005, 2008), which may contribute to compulsive drug use. Finally, not only do psychostimulants act upon Pavlovian associations, but they also influence the mechanisms underlying instrumental learning and performance. Psychostimulants accelerate the establishment of habitual responding for the drug, such that drug seeking becomes to some extent divorced from the hedonic value of the drug itself and more controlled by drugassociated stimuli in the environment (Everitt and Robbins, 2005). Cocaine and amphetamine exposure may also impair prefrontal cortex-dependent executive functions such as behavioral control (Bornovalova et al., 2005; Verdejo-García et al., 2007) and planning (Bechara et al., 2001; Bolla et al., 2003; Franken et al., 2007).

Thus, drug addiction may be viewed as a multifactorial pathology involving dysfunctional prefrontal cortical executive control over abnormally strong Pavlovian and instrumental learning mechanisms, which in turn depend upon the striatum and its limbic-cortical afferents (Everitt et al., 2001; Everitt and Robbins, 2005). The neural basis of these Pavlovian and instrumental learning mechanisms has been extensively investigated for behavior directed towards natural rewards. Much evidence suggests that addictive drugs aberrantly engage these mechanisms via their effects upon monoaminergic, glutamatergic and other neurotransmitter systems (Berke and Hyman, 2000; Hyman and Malenka, 2001; Nestler, 2001a; Nestler, 2002; Robbins and Everitt, 2002; Frenois et al., 2005; Hyman, 2005; Hyman et al., 2006; Dalley and Everitt, 2009), upon neuronal morphology (Kolb et al., 2003; Jedynak et al., 2007) and upon functional interactions within and between cortical, striatal and brainstem structures (Takahashi et al., 2007; Belin and Everitt, 2008). In this chapter we will discuss how the basal ganglia and their cortical inputs are involved in the pathophysiology of psychostimulant addiction.

## III. NEUROPHYSIOLOGICAL MAPPING OF THE CONSEQUENCES OF PSYCHOSTIMULANT EXPOSURE IN THE BASAL GANGLIA

Psychostimulants exert their primary neurobiological effects by altering the function of proteins involved in the regulation of DA transmission. Cocaine binds to the DA transporter (DAT) and blocks the reuptake of DA, as well as serotonin and noradrenaline, whereas amphetamine also acts at the vesicular monoamine transporter (VMAT) to promote the release of DA and other monoamines (see also Chapter 17). By increasing DA transmission at dopaminergic synapses in the ventral and dorsal striatum, pallidum, subthalamic nucleus (STN) and also limbic cortical structures including the amygdala and the prefrontal cortex (Brown et al., 1979; Meibach and Katzman, 1979; Campbell et al., 1985; Lavoie et al., 1989), psychostimulants mimic DA increases secondary to phasic DA neuron firing involved in coding salience and reward (Schultz, 2000), but greatly potentiate these mechanisms through both the magnitude and duration of drug-induced increases in DA transmission.

Functional imaging studies in rats have revealed that exposure to cocaine not only influences the activity of many cortical areas, including medial prefrontal (mPFC), anterior cingulate (ACC), somatosensory and motor cortices (Febo et al., 2005), but also the striatum, STN and pallidum (Pontieri et al., 1995; Febo et al., 2005). These findings have been corroborated by cellular imaging studies, using c-fos mRNA as a marker of cellular activation after exposure to psychostimulants, which have revealed that the dorsomedial and dorsolateral striatum, the core (NAcC) and shell (NAcS) of the nucleus accumbens (NAc), the STN and the ventral pallidum (VP) are activated after single, or repeated exposure to amphetamine or cocaine (e.g., Bachand et al., 2009) (see Chapter 29). Repeated exposure to psychostimulants has also been reported to alter gene expression in all the structures of the basal ganglia (Frankel et al., 2008).

Thus, not only the striatum, but other structures grouped within the basal ganglia, are directly affected by exposure to psychostimulants and may be implicated in the pathophysiology of drug addiction. Thus, the STN has been suggested differentially to mediate the motivational properties of food versus drug rewards, since lesions of this structure decrease motivation for, and conditioned approach to stimuli associated with, cocaine, but increase these behavioral measures for food in a progressive ratio schedule of reinforcement and conditioned place preference tests, respectively (Baunez et al., 2005). The pallidum is also involved in the reinforcing effects of psychostimulants since dopaminergic or excitotoxic lesions of this structure respectively block the acquisition of conditioned place preference for cocaine (Gong et al., 1997) or impair the acquisition of cocaine self-administration (Hubner and Koob, 1990). Moreover, reinstatement of cocaine seeking behavior is dependent upon  $\mu$ -opioid receptors in the ventral pallidum (Tang et al., 2005), suggesting that this structure is also involved in the circuitry that mediates relapse to drug seeking behavior (McFarland and Kalivas, 2001). Increasing evidence suggests that both parallel and serial striatal mechanisms underlying Pavlovian and instrumental learning play a major role in the establishment and the expression of drug addictive behavior.

### IV. DRUG REINFORCEMENT: A MECHANISM DEPENDENT UPON VENTRAL CORTICO-STRIATO-PALLIDAL LOOPS

Since all drugs of abuse share the commonality of increasing DA transmission within the NAc (Di Chiara and Imperato, 1988), this system has been a major neurobiological target for addiction research for almost 50 years. Thus, following the initial studies on intracranial self-stimulation of the brain (Olds and Milner, 1954), the mesolimbic pathway, comprising projections from the dopaminergic neurons of the ventral tegmental area innervating the NAc as well as the olfactory tubercle, has been shown to be of importance in mediating the reinforcing effects of addictive drugs (Ikemoto, 2007). Imaging studies in humans have revealed that the subjective effects of self-administered psychostimulants, e.g., euphoria, high and rush, are correlated with DA transmission in the ventral striatum (Breiter et al., 1997; Drevets et al., 2001). However, it is difficult to infer any causal relationship between NAc DA levels and euphoria since DA in the NAc is involved in several processes mediating, for example, stimulus salience and novelty, which may play a role in the subjective interpretation of the effects of stimulants.

# A. From Reward to Reinforcement, the Dopamine Hypothesis

The mesolimbic dopamine hypothesis of reward (Wise, 2004) has been a major focus of research because of the functional position of the ventral striatum in corticostriatal circuitry. The NAc was suggested to be the interface between emotion, motivation and action (Mogenson et al., 1980) on the basis of its major inputs from the limbic cortices

such as the amygdala, OFC and hippocampus (Floresco et al., 2001; Ambroggi et al., 2008; Belujon and Grace, 2008) (see also Chapter 21). Thus, the hypothesis that drugs, especially psychostimulants such as cocaine and amphetamine, exert their rewarding effects via an increase in NAc DA transmission has greatly influenced drug addiction research, generating abundant experimental tests of a very difficult hypothesis to refute, not least because subjective states of "pleasure" or "liking" (as it is now so often referred to) cannot easily be measured in animals. Although NAc DA is not apparently involved in the presumed hedonic reactions to the taste of food reinforcers, whereas opiate mechanisms in the NAc and globus pallidus are (Berridge and Robinson, 1998; Kelley and Berridge, 2002), there are no related data in animals concerning hedonic or 'liking' responses to addictive drugs.

However, it is clear that hedonic responses to addictive drugs must inevitably reflect the subjective perception of their neurochemical effects, including DA release in the NAc and elsewhere (Volkow et al., 1999), and these may be correlated with activity in other systems that are involved more directly with hedonic responses to natural and drug reinforcers (Everitt and Robbins, 2005). It also seems unlikely that 'pleasure' can be mediated by neurochemical mechanisms occurring solely in subcortical structures such as the NAc or globus pallidus and that activity in striatopallidal circuitry and in other sites following drug self-administration, or consumption of natural rewards, is subject to further processing in cortical, perhaps especially insular (Critchley et al., 2004) and other prefrontal cortical areas, before attribution and accompanying subjective commentary - or "feelings" - can occur (Altman et al., 1996; Everitt and Robbins, 2005).

If the relationship between DA and the hedonic response to psychostimulants remains a matter of debate there is, however, wide agreement that DA transmission, especially in the ventral cortico-striato-pallidal loop of the basal ganglia, provides a neurochemical mechanism of drug reinforcement in the brain. Several lines of evidence suggest that the dopaminergic innervation of the NAcS and olfactory tubercle underlies the primary reinforcing effects of cocaine and amphetamine (Di Chiara et al., 2004; Wise, 2004; Ikemoto et al., 2005) as measured in drug self-administration procedures. Thus, not only is extracellular DA in the NAc increased in response to administration of addictive drugs (Di Chiara and Imperato, 1988; Carboni et al., 1989), but intra-NAc infusions of direct and indirect DA receptor agonists are reinforcing

(Phillips et al., 1994; Ikemoto et al., 1997) and natural- and drug-reinforced responding depends on NAc DA (Pettit et al., 1984; Salamone et al., 2003).

## **B.** From Positive to Negative Reinforcement: Reduced Striatal Dopamine Transmission and Beyond

Repeated exposure to psychostimulants also triggers neurophysiological adaptations within the basal ganglia that are associated with negative reinforcement and hedonic allostasis (Koob and Le Moal, 2008; Koob, 2009). Withdrawal from chronic exposure to psychostimulants is associated with negative affective and psychological states such as chronic irritability, emotional pain, dysphoria, anxiety, and loss of interest in natural sources of reward (Koob and Le Moal, 2005). These negative states may contribute to the establishment of compulsive drug use in that they maintain drug seeking by addicts through attempts to selfmedicate (Koob and Le Moal, 2008; Koob, 2009).

At the neurobiological level, acute withdrawal from extended access to cocaine in rats is associated with decreased levels of DA in the NAc (Weiss et al., 1992), that have been suggested to contribute to boredom and lack of interest in natural rewards and which, together with increased expression of dynorphin in the NAc, contribute to dysphoria (Koob and Le Moal, 2008; Koob, 2009) (see Chapter 29 for a review of the suggested role of dynorphin in addiction). Interestingly, low striatal D2 DA receptor levels have been observed in cocaine addicts and to be associated with reduced frontal metabolism, suggesting a general alteration of limbic corticostriatal circuitry function in psychostimulant addicts (Volkow et al., 1993). However, recent studies in drug naive monkeys have revealed that low D2-DA receptor levels in the ventral striatum also predict the subsequent selfadministration of cocaine (Nader et al., 2008). Moreover, a reduced NAc binding potential in vivo of the DA D2/3 receptor ligand [18F]fallypride, has been shown to predict escalation of cocaine self-administration and high levels of impulsivity in rats (Dalley et al., 2007), a behavioral marker of vulnerability for the transition from controlled to compulsive cocaine self-administration (Belin et al., 2008b). Thus, whereas cocaine exposure is associated with decreased striatal DA levels that may be involved in mediating hedonic allostasis, pre-existing low DA D2 receptor levels in the NAc may predispose to psychostimulant addiction.

One of the most striking neurobiological adaptations to repeated exposure to psychostimulants that plays a role in negative states is the progressive recruitment of stress systems, including CRF and noradrenaline, in the "extended amygdala", which includes the BNST and the CeA, as well as perhaps the NAcS. These adaptations provide additional drive for drug taking as a self-medication strategy (Koob and Moal, 1997, 2001, 2005, 2008). The recruitment of CRF and NA systems has been suggested to contribute to the establishment of compulsive drug use (Koob and Le Moal, 2005, 2008), and may be important modulatory factors involved in the control of incentive habits.

### C. Neurochemical Sensitization of Striatal Dopamine Transmission by Repeated Exposure to Psychostimulants

Repeated exposure to psychostimulants induces a neurochemical sensitization of the dopaminergic system, characterized by a progressive enhancement of drug-induced DA release to the same dose of cocaine or amphetamine (Robinson and Becker, 1982; Robinson et al., 1982; Robinson et al., 1988; Pierce and Kalivas, 1997; Belin et al., 2007). This long lasting neuroadaptation has been reported to be measurable in cocaine addicts (Schlaepfer et al., 1997) and is associated with an increased behavioral response to a challenge with the drug that is in turn correlated with cellular and molecular adaptations in both dopaminergic and NAc neurons (Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000; Belin et al., 2007).

The sensitized DA response to psychostimulant administration has been suggested to contribute to the narrowing of the behavioral repertoire of drug addicts who focus on drug seeking and drug taking behaviors at the expense of other sources of reward. Thus, superimposed on the decreased basal DA levels in the striatum that accompanies withdrawal from stimulants, a sensitized DA response to the drug may ultimately become an important mechanism enabling an optimal level of reinforcement in the brain, thereby providing stimulants, and perhaps other, addictive drugs with the ability to increase DA transmission sufficiently to maintain drug seeking behavior (Goldstein and Volkow, 2002).

At the psychological level, behavioral sensitization has been suggested to reflect maladaptive learning mechanisms, whereby repeated exposure to psychostimulants impacts upon Pavlovian incentive mechanisms dependent upon the ventral striatum, thereby abnormally increasing the incentive properties of drug-associated stimuli which induce a pathological "wanting", as opposed to "liking" for the drug (Robinson and Berridge, 1993, 2000, 2001, 2003).

Sensitization of the dopaminergic system has strongly been argued to play a role in the pathophysiology of psychostimulant addiction (Robinson and Berridge, 1993, 2000), but the nature of the psychological processes affected by this neuroadaptation is still a matter of debate. The "incentive sensitization" theory emphasizes Pavlovian mechanisms that influence motivational states through, for example, Pavlovian-instrumental transfer (PIT), suggested to be one manifestation of drug "wanting" (Wyvell and Berridge, 2001). However, amphetamine or cocaine sensitization also facilitate the development of habitual responding for food (Nelson and Killcross, 2006; Nordquist et al., 2007), suggesting that behavioral sensitization may also reflect adaptations within the corticodorsal striatal circuitry that controls instrumental performance (Everitt and Wolf, 2002; Belin et al., 2008a). Additionally, the demonstration that repeated cocaine exposure fails to induce behavioral sensitization in Pitx3-Deficient mice lacking a nigrostriatal pathway (Beeler et al., 2008) suggests that the dorsal striatum, the locus of S-R (habit) learning mechanisms (see Chapter 32), may be an important substrate for the establishment of a neurobiological process that has been considered previously mainly to involve the ventral striatum.

The dichotomy illustrated above is far-reaching since drug addiction is a pathology encompassing both Pavlovian and instrumental learning mechanisms (Everitt et al., 2001; Everitt and Robbins, 2005; Belin et al., 2008a; Everitt et al., 2008) in which both dorsal and ventral basal ganglia circuits are implicated. Although not having been the major focus of drug addiction research until recently, the dorsal striatum, which is involved in cognitive and motor processes, clearly provides a core neurobiological substrate of both goal-directed and habitual control of instrumental responding (Yin et al., 2004; Everitt and Robbins, 2005; Faure, 2005; Yin et al., 2005; Yin and Knowlton, 2006) and thus is viewed as being of increasing importance within the neurobiological framework of addictive processes (Garavan et al., 2000; Everitt et al., 2001; Everitt and Robbins, 2005; Volkow, 2006; Belin and Everitt, 2008; Belin et al., 2008a). Addiction cannot simply be viewed as involving neurobiological adaptations within an "incentive motivational" mesolimbic DA pathway innervating the nucleus accumbens; it also appears to involve recruitment and even reorganization of the inter- and intra-striatal mechanisms normally involved in controlling instrumental behavior (Everitt and Robbins, 2005).

## V. STRIATAL-DEPENDENT PAVLOVIAN AND INSTRUMENTAL LEARNING MECHANISMS IN THE DEVELOPMENT OF DRUG ADDICTION

# A. Instrumental Learning Processes: the Acquisition of Drug Taking Behavior

Identification of the neurobiological basis of the acquisition of instrumental responding for addictive drugs has proven problematic because of the difficulty in disentangling the neural control of instrumental conditioning from that of the effects of the self-administered drugs themselves, which include "rewarding", reinforcing and motor effects. Thus, gross DA depletion from the striatum, especially the ventral striatum, greatly impairs the acquisition of cocaine self-administration (Roberts et al., 1977), in large part by decreasing the reinforcing effects of the drug. However, pretraining lesions of either the NAcS or NAcC do not impair the acquisition of cocaine self-administration (Ito et al., 2004), while lesion or inactivation of limbic cortical structures, including the basolateral amygdala (BLA), subiculum or medial prefrontal cortices, have no or only minor effects on the acquisition of this behavior (Whitelaw et al., 1996; Weissenborn et al., 1997; Caine et al., 2001). Nevertheless, it is widely held that the dopaminergic innervation of the striatum, particularly the NAc, is central to the acquisition of instrumental responding for drugs, as it is for natural rewards (Cousins et al., 1993; Cousins and Salamone, 1996a, 1996b). It has been hypothesized that drugs of abuse induce abnormally strong consolidation of instrumental learning mechanisms, thereby greatly enhancing the propensity to engage in a drug seeking response the next time that the context and cues signal that responding is opportune. The neuronal networks within the NAc that fire during lever pressing for natural or cocaine rewards differ within the same animals (Carelli et al., 2000; Carelli and Wondolowski, 2003; Carelli, 2004), suggesting that addictive drugs not only subvert instrumental conditioning mechanisms but also recruit non-overlapping and distinct ventral striatal networks.

# **B.** Goal-Directed and Habitual Drug Seeking Behavior

The same instrumental response can be mediated by different associative structures, i.e., goal-directed or S-R (habit). We have hypothesized that the mechanism controlling drug seeking behavior shifts from the former to the latter during the development of drug addiction (Everitt and Robbins, 2005; Belin et al., 2008a). When instrumental responding is goal-directed, the initiation of the response is under the direct control of the current value of the outcome and, at least for ingestive reinforcers, appears to be controlled by the dorsomedial striatum (Yin et al., 2005; Yin et al., 2006). Habitual responding<sup>1</sup>, by contrast, is dependent upon the dorsolateral striatum (Yin et al., 2004; Yin and Knowlton, 2006) (see Chapter 32), and is characterized by direct initiation of responding by a conditioned stimulus and/or context (Balleine and Dickinson, 1998). During early stages of instrumental conditioning, responding is assumed to be goal-directed, while prolonged training under unchanged circumstances eventually facilitates behavioral autonomy. In addition, the development of habitual control of behavior is facilitated by schedules of reinforcement in which the contingency between responding and reward delivery is weak, such as in interval schedules of reinforcement (Dickinson et al., 1983; Hilário and Costa, 2008), including second-order schedules of cocaine reinforcement that we have employed (Everitt and Robbins, 2000). These two modes of control over instrumental behavior can be differentiated experimentally through reinforcer devaluation procedures (Mackintosh, 1974): whereas goal-directed behavior is sensitive to devaluation of the outcome, habitual responding is not (Balleine et al., 1995; Balleine and Dickinson, 1998; Yin et al., 2004; Faure, 2005; Yin et al., 2005; Yin et al., 2006).

It has not proven possible to date using the test of devaluation of non-ingestive drug rewards (e.g., intravenous cocaine) to probe the associative structure underlying performance of drug self-administration. However, when drugs of abuse were offered in liquid form, the presence of cocaine or alcohol in a sucrose solution led to accelerated emergence of habitual control of behavior (Dickinson

<sup>&</sup>lt;sup>1</sup>Automatic behavioral responses that depend upon the dorsal striatum reflect the operation of two different processes (i) the psychological mechanisms that trigger the response and (ii) the psychological mechanisms that control the performance of this response. Here we discuss a habit in terms of only the first of these two mechanisms. It has already been well described that automatization of behavior (Balleine and Dickinson, 1998) is associated with increased performance and faster onset of response in a subjectively less demanding task, therefore reflecting a cognitive-motor network that is shaped by extended training. Such skills, or procedural memory, may involve the second mechanism referred to above and, in the context of addiction, in the repetitive motor sequences associated with drug seeking and drug taking. Habitual responding, as operationally defined by Logan (Logan, 1998a; Logan, 1998b) and Dickinson (Dickinson, 1985) is triggered by the presentation of a stimulus without any explicit relationship with the outcome. Such stimulus-response (S-R) processes can encompass other behavioral subroutines either of the same S-R nature or be goal-directed.

et al., 2002; Miles et al., 2003). Since the dorsal, particularly dorsolateral, striatum has been implicated in S-R (habit) learning, it is of note that chronic exposure to cocaine progressively affects the physiology of the dorsal striatum, modifying the matrix/striosome activation ratio of this structure in favor of the striosomes (Canales, 2005), that may receive more limbic cortical projections than the matrix neurons of the same area, and that metamphetamine sensitization is associated with increased spine density on medium spiny neurons of the dorsolateral but not dorsomedial striatum (Jedynak et al., 2007).

At the behavioral level, inactivation of the dorsolateral striatum in rats impairs relapse to drug seeking after long-term withdrawal or "abstinence" (Fuchs, 2006; See et al., 2007), and bilateral infusion of the D1 DA receptor antagonist SCH23390 into the dorsolateral striatum blocks context-induced reinstatement of heroin seeking behavior, which may reflect contextual control of an S-R habit (Bossert et al., 2009). These results are consistent with activation of the dorsal striatum following the presentation to drug addicts of cocaine-associated cues that elicit craving (Garavan et al., 2000; Volkow, 2006; Wong et al., 2006).

The body of evidence reported above, in combination with the sensitivity of the development of habitual control of behavior to DA depletion in the dorsal striatum (Faure, 2005), strongly suggest that drug seeking may rapidly become habitual. This might depend upon the ability of addictive drugs to enhance a naturally occurring DA sensitization process that has been shown to develop after protracted instrumental training (Ahn and Phillips, 2007), as well as to usurp DA-dependent striatal memory consolidation mechanisms (Tiffany, 1990; Everitt et al., 2001; Wise, 2004; Everitt and Robbins, 2005). In principle, the more direct motivational coupling between a taking response and its affective outcome would be expected to preserve goaldirected control of drug taking for a longer period of time than drug seeking (Adams and Dickinson, 1981), although the large number of drug taking events in which addicts engage, combined with the repetitive dopaminergic activations associated with each drug taking event, could eventually surmount that motivational control to produce consummatory habits (Cardinal et al., 2002). The implications of such a mechanism are far reaching, because whereas habitual drug seeking could, in the event of reward devaluation, become inflexible, persistent - or compulsive - seeking, the actual consumption of procured drug reward might be expected never to fail to align with drug reward value. However, if both drug seeking and taking become habitual, devalued drug rewards may be procured and consumed repeatedly, even compulsively, especially when compounded by a failure of prefrontal control mechanisms that evaluate the changed affective consequences of actions (Schoenbaum, 2004).

# C. Pavlovian Conditioning: the Establishment of Drug "Cues"

Pavlovian conditioning refers to the behavioral and physiological changes brought about by experiencing a predictive relationship between a neutral stimulus and a consequent biologically significant event (Pavlov, 1927). In a Pavlovian conditioning experiment, a contingency is arranged between the presentation of the neutral stimulus, and the delivery of a biologically significant outcome, so that the animal learns that a specific stimulus predicts the impending delivery of the unconditioned stimulus. Both stimuli are presented independently of the animal's behavior. The acquisition of Pavlovian conditioning has been well established for both appetitive and aversive reinforcers.

Pavlovian conditioning may manifest itself behaviorally in procedures that measure "Pavlovian approach" (or "autoshaping"), Pavlovian-to-instrumental transfer (or PIT) and conditioned reinforcement. Thus, Pavlovian conditioned stimuli not only become attractants that grab attention and subserve approach behavior, they can energize or motivate instrumental behavior (as in the PIT paradigm), and they can also act as conditioned reinforcers of instrumental behavior. All three processes may contribute significantly to the development of compulsive responding. We will discuss them separately in the following sections.

#### D. Conditioned Approach

Conditioned approach, sometimes referred to as signtracking, is one of the automatic behavioral responses elicited by Pavlovian conditioned stimuli (Brown and Jenkins, 1968; Tomie et al., 1989) that may serve to bring animals closer to sources of natural reinforcement, or in the context of addiction, to addictive drugs (Tiffany, 1990; Altman et al., 1996). Drug-paired conditioned stimuli have now been shown to support approach responses in rats. It appears that Pavlovian approach can be observed with relatively long, but not shorter drug inter-infusion intervals (Kearns and Weiss, 2004; Uslaner and Robinson, 2006), suggesting that relatively predictive, as opposed to coincident, CS-US pairings do produce approach to drug-paired stimuli.

Little is known about the neural basis of approach to drug-paired stimuli. However, it might be predicted that similar structures to those underlying approach to foodassociated stimuli, such as the central amygdala (CeA) and nucleus accumbens core (Parkinson et al., 1999; Parkinson et al., 2000; Cardinal et al., 2002; Parkinson et al., 2002) also underlie approach to psychostimulant-associated cues, although perhaps with abnormal strength due to the intrinsic DA activating effects of stimulant drugs which might amplify associative mechanisms in the CeA. Indeed, increased DA transmission in the CeA enhances Pavlovian conditioned approach to a natural reward CS (Harmer and Phillips, 1999). Drug-induced potentiation of learning is also suggested by the finding that exposure to psychostimulants, through sensitization, renders conditioned approach insensitive to lithiuminduced devaluation of a natural reward (Schoenbaum, 2004). This finding suggests that during the development of addiction, drug-associated Pavlovian predictors may elicit approach responses regardless of the current motivational value of the drug. At the clinical level, such Pavlovian mechanisms might be relevant for automatic or reflexive approach to places where the drug is available (Tiffany, 1990), such as approaching a bar or other sources of drug supply. Individual differences in sign-tracking to food-associated cues also predict subsequent drug self-administration, suggesting that the propensity for this form of learning might indicate a vulnerability to subsequent drug abuse and addiction (Flagel et al., 2007; Flagel et al., 2008a,b).

### E. Pavlovian-to-Instrumental Transfer

PIT has been hypothesized to play a significant role in drug addictive behavior (Berridge et al., 2009; Yin and Knowlton, 2006) since it describes the enhancement of instrumental performance by non-contingently presented Pavlovian CSs. Behaviorally, Pavlovian CSs may enhance instrumental responding when that instrumental response has been trained extensively under lean reinforcement conditions, whereas minimal training under rich reinforcement schedules renders responding less susceptible to Pavlovian influences (Lovibond, 1981; Holland and Gallagher, 2003). Accordingly, it is not surprising that the PIT effect is stronger when instrumental responding is controlled by habitual (S-R) rather than goal-directed mechanisms (Holland, 2004).

Although PIT has been shown in smokers working for cigarettes and money (Hogarth et al., 2007), in rodents non-contingent presentation of a cocaine-paired CS, that is known to facilitate DA transmission in the NAc (Gratton and Wise, 1994; Neisewander et al., 1996; Di Ciano et al., 1998; Ito et al., 2000; Weiss et al., 2000), does not appear to potentiate responding for the drug, but instead significantly

depresses instrumental performance (Di Ciano and Everitt, 2003). It has also been reported that non-contingent presentation of a drug-paired CS after extinction does not reinstate cocaine seeking, whereas response-contingent CS presentation does (Grimm et al., 2000).

There are at least two different potential explanations for this somewhat surprising set of results. Firstly, responding for non-ingestive drug rewards may simply not be potentiated by non-contingent presentation of drug-associated CSs (Everitt and Robbins, 2005) because the sensory and reward-processing substrates upon which they act are fundamentally different from those engaged by natural rewards. For example, there are no obvious counterparts to consummatory ingestive behavior in animals receiving intravenous drug infusion. Secondly, and perhaps most importantly, CSs in psychostimulant selfadministration studies may themselves be processed as highly salient, rewarding stimuli (Robinson and Berridge, 1993), which could explain the observed depression in responding, since CS presentation might even distract an animal from performing its seeking responses (Flagel et al., 2008a,b). Thus, the frequent coincident presentation of CS and US in drug self-administration studies and the stimulusenhancing effects of psychostimulants may lead rats to process a drug-paired CS both as highly salient and as rewarding in its own right, consistent with its persistent conditioned reinforcing properties (Di Ciano and Everitt, 2004a).

#### F. Conditioned Reinforcement

As noted above, stimuli that are associated with unconditioned rewards may also themselves gain rewarding properties, thereby acting as conditioned reinforcers. Conditioned reinforcers can support responding for long periods of time, thereby bridging delays between seeking and obtaining the drug (Everitt and Robbins, 2000). Such properties can be demonstrated in procedures where animals work to obtain presentation of a CS, often in the absence of the unconditioned reward. Conditioned stimuli will therefore support the acquisition of a new instrumental response provided they are presented response-contingently (Robbins, 1976; Williams and Dunn, 1991; Williams, 1992). Under a second-order schedule of reinforcement, the seeking of drugs (as well as food or a sexual partner) can be maintained over long periods if a drug CS is presented response-contingently (usually under a fixed ratio schedule), but with cocaine (or other reward) delivered under an overall fixed interval or fixed ratio schedule (Goldberg et al., 1975; Everitt and Robbins, 2000).

Conditioned reinforcement plays an important role in the foraging for drugs over long periods of time both in humans (Panlilio et al., 2005) and animals (Arroyo et al., 1998; Goldberg et al. 1975; 1981). Cocaine and amphetamine-associated CSs acting as conditioned reinforcers markedly enhance drug seeking (Goldberg et al., 1975; Arroyo et al., 1998) and can support the acquisition of a new instrumental response (Di Ciano and Everitt, 2004a; Lee et al., 2006). In addition, it has been shown that omission of CS presentation under a second order schedule of reinforcement results in a more disruptive effect on cocaine seeking than on food seeking, suggesting that prolonged psychostimulant seeking is particularly dependent upon conditioned reinforcers (Goldberg et al., 1981).

The neural basis of conditioned reinforcement for drug-paired CSs is well characterized. The acquisition of cue-controlled cocaine seeking depends upon the BLA (Whitelaw et al., 1996), the NAcC, but not the NAcS (Di Ciano and Everitt, 2001; Ito et al., 2004) and the interaction between the BLA and the NAcC (Di Ciano, 2004). Lesions of the orbitofrontal cortex also affect the acquisition of cocaine seeking (Hutcheson and Everitt, 2003; Everitt et al., 2007). Performance of cue-controlled cocaine seeking is also influenced by the VTA (Di Ciano and Everitt, 2004b) while the potentiation by cocaine of drug seeking under a second order schedule depends upon the NAcS (Ito et al., 2004).

Although the acquisition of cue-controlled cocaine seeking depends upon the NAcC and the BLA, it is the dopaminergic innervation of the dorsal striatum that is involved in mediating well established cue-controlled cocaine seeking. A first indication that this is the case was the observation that during well-established cocaine seeking under a secondorder schedule, contingent presentations of a cocaine-associated conditioned reinforcer were associated with increased extracellular DA in the dorsolateral striatum but not in the NAcC, nor in the NAcS (Ito et al., 2002). Moreover, bilateral intra-dorsolateral striatum infusions of the DA receptor antagonist,  $\alpha$ -flupenthixol, selectively reduced cue-controlled cocaine seeking habits (Vanderschuren, 2005; Belin and Everitt, 2008).

Therefore, between the acquisition and the subsequent performance, or maintenance, of cue-controlled cocaine seeking there is an apparent shift in the locus of control from the NAc to the dorsolateral striatum, which, we have hypothesized, reflects the development of habitual drug seeking (Everitt and Robbins, 2005). This devolution of control over behavior to DA-influenced mechanisms in the dorsolateral striatum is strongly supported by the observation in monkeys that the alterations of striatal metabolic activity, DA transporter binding and DA D2 receptors following cocaine self-administration are initially restricted to the NAc, but spread dorsally throughout the entire striatum after protracted exposure to the drug, eventually to encompass the dorsolateral striatum (Letchworth et al., 2001; Porrino, 2004). Additionally, cue-selective evoked neuronal firing in a discrimination learning/reversal task is altered in the ventral striatum but increased in the dorsolateral striatum by protracted exposure to cocaine (Takahashi et al., 2007), while relapse to cocaine-seeking behavior after imposed abstinence depends upon the dorsolateral striatum (See et al., 2007). These preclinical data are consistent with evidence obtained from imaging studies that have revealed activation of the dorsal striatum by cocaine (Garavan et al., 2000), and a relationship between dorsal striatal DA transmission and cue-induced cocaine craving (Volkow, 2006). Thus, both in humans and animals, chronic cocaine exposure appears to facilitate a shift from ventral to dorsal striatal control over behavior that we have shown to be associated with the development of habitual, cue-controlled cocaine seeking.

We have established in rats that this progressive devolution of the control over cue-controlled cocaine seeking depends upon the serial, DA-dependent connectivity linking the NAcC to the dorsolateral striatum initially described in non human primates (Haber et al., 2000) (see Chapter 24) and also in rats (Ikemoto, 2007). This corticostriatal circuitry has been proposed to be a substrate for integrative mechanisms linking incentive motivation to cognitive processes (Everitt and Wolf, 2002; Everitt and Robbins, 2005; Yin and Knowlton, 2006; Belin et al., 2008a; Everitt et al., 2008; Haber, 2008). Disconnection of the NAcC and its regulation of DA transmission in the dorsolateral striatum impairs habitual cue-controlled cocaine seeking to the same extent as bilateral DA receptor blockade in the dorsolateral striatum alone (Belin and Everitt, 2008). This asymmetric manipulation does not impair general operant responding. Thus combining a unilateral excitotoxic lesion of the NAcC with an infusion of  $\alpha$ flupenthixol into the contralateral dorsolateral striatum does not impair a newly acquired chain pulling task for sucrose (Belin and Everitt, 2008) or cocaine seeking assessed after eleven days of continuous reinforcement for cocaine at a point, therefore, when responding is under action-outcome control (Belin D, Besson M and Everitt BJ, unpublished observations).

Therefore, based on these observations we have hypothesized that the Pavlovian incentive influences exerted by the BLA over the NAcC in turn recruit an increasingly dominant habit system that is dependent upon dorsolateral striatal mechanisms engaged by re-entrant dopaminergic circuitry, thereby establishing an incentive habit. Drug seeking in addicted individuals might then be seen as a result of pathological drug-influenced Pavlovian mechanisms that engage a drug seeking habit that is therefore difficult to relinquish. Such a mechanism may account for the observation in humans that presentation of cocaine-associated stimuli activates the dorsolateral striatum (Garavan et al., 2000; Volkow, 2006) together with limbic cortical areas (Childress et al., 1999). It is further supported by studies in animals showing that repeated exposure to psychostimulants, whether selfadministered or experimenter-delivered, increases the incentive properties of cocaine (Deroche et al., 1999; Ahmed and Cador, 2006), recruits dorsolateral striatum-dependent adaptations (Letchworth et al., 2001; Porrino, 2004) and facilitates the formation of an S-R habit (Nelson and Killcross, 2006; Nordquist et al., 2007). These observations might reflect the powerfully strong recruitment by drugs of abuse of the DA-dependent ascending, or spiraling, striatal circuitry that provides a neuroanatomical and functional substrate for linking incentive influences from the amygdala to S-R processes in the dorsolateral striatum via the NAcC.

Although we argue that incentive habits play an important role in the pathophysiology of drug addiction, they cannot account for different behavioral aspects of the pathology, especially compulsive drug use, i.e., maintained drug use despite adverse consequences, which is a hallmark of drug addiction. Indeed, incentive habits may provide the neurobiological and psychological substrate for drugassociated impulses to be behaviorally mediated without explicit control over the action, helping to explain the persistence in doing so despite knowledge of the negative consequences of taking the drug. Compulsive drug use may thus result from a failure in top–down executive control over incentive habits, not only involving striatal mechanisms but also disrupted prefontal cortical functions (Everitt and Robbins, 2005; Belin et al., 2008a; Everitt et al., 2008).

### VI. CELLULAR AND MOLECULAR SUBSTRATES OF DRUG ADDICTION: ROLE OF CORTICOSTRIATAL MECHANISMS

## A. Psychostimulant-Induced Plasticity in the Corticostriatal Circuitry: When Addictive Drugs Usurp Basal Ganglia-Dependent Learning Mechanisms, such as Striatal LTP and LTD

At the cellular level, emphasis has been placed on the view that addiction develops as a consequence of adaptive neuroplasticity such as long-term potentiation (LTP) and long-term depression (LTD) in midbrain DA neurons and the targets of their projections, particularly the striatum, but increasingly in limbic and cortical areas as well (e.g., the hippocampus, amygdala and prefrontal cortex). At the core of these studies is the view that the synaptic plasticity measured as LTP and LTD (see also Chapters 9 and 12) underpins alterations in neural circuitry induced by acute or chronic exposure to addictive drugs and thereby altered reward, Pavlovian and instrumental learning processes (Hyman and Malenka, 2001; Jones and Bonci, 2005; Saal and Malenka, 2005; Kauer and Malenka, 2007).

There are abundant data showing that exposure to psychostimulants elicits LTP at excitatory synapses on VTA neurons. Measured 24h after a single in vivo exposure to cocaine, LTP was shown no longer to be induced at synapses onto DA neurons suggesting that they were already potentiated (Ungless et al., 2001). In addition, the AMPA/ NMDA ratio (the ratio between AMPA receptor-mediated and NMDA receptor-mediated EPSCs) was increased twofold in VTA slices. This effect was blocked by an NMDA receptor antagonist, indicating that NMDA receptor activation was necessary for cocaine to instantiate LTP (Ungless et al., 2001). The effect appears to be selective to DA neurons in the VTA as there was no such effect of cocaine on LTP or AMPA/NMDA ratios on GABAergic neurons (Ungless et al., 2001). Amphetamine too has been shown to increase AMPA/NMDA ratios 24h after treatment in vivo (Saal et al., 2003). The LTP induced by cocaine is now known to involve the insertion of Glu-R2-lacking AMPA receptors into the neuronal membrane (Bellone and Luscher, 2006). The cocaine-evoked plasticity persists for about 5 days, but is not evident after 10 days (Ungless et al., 2001).

Psychostimulant-induced LTP in the VTA has been shown to correlate with behavioral sensitization (Kauer and Malenka, 2007) which is known to be blocked by glutamate receptor antagonists infused into the VTA (Vanderschuren and Kalivas, 2000). Since over-expression of the GluR1 AMPA receptor subunit in the VTA has been reported to enhance sensitization and some motivational effects of drugs of abuse (Carlezon and Nestler, 2002), it has been hypothesized that LTP induced in VTA DA neurons by addictive drugs may have an important, albeit transient effect to increase their "rewarding effects" (Hyman et al., 2006). Nevertheless, it is not clear how such acute and transient effects of addictive drugs on neuronal plasticity contribute to the development of some of the behavioral characteristics of addiction, such as escalation of drug intake, compulsive drug seeking and an enhanced propensity to relapse during withdrawal (but see the review by Thomas et al., 2008).

However, it has been shown that whilst the passive administration of cocaine results in the transient appearance of LTP in VTA neurons, cocaine self-administration results in a much more persistent potentiation of VTA excitatory synapses that is still detectable 3 months following the last cocaine exposure (Thomas et al., 2001). Moreover, food or sucrose ingestion resulted only in the transient form of LTP in VTA neurons. These studies are important both because they take into account that, in addiction, drugs are self-administered and not passively received and also because they suggest that plasticity induced by self-administered cocaine is different, being more persistent, than that following exposure to voluntarily ingested natural rewards. These findings may indeed be more readily related to phenomena such as cued reinstatement or relapse to drug seeking after abstinence (Chen et al., 2008; Thomas et al., 2008).

Plasticity in the primary striatal target of mesolimbic DA neurons has also been demonstrated. But unlike VTA synaptic potentiation, the predominant response in NAc neurons is LTD-like, rather than LTP-like, since AMPA/NMDA ratios are depressed. This LTD is only seen after repeated (5 days) cocaine treatment, not after a single injection (Thomas et al., 2001; Kourrich et al., 2007). The picture is, though, somewhat more complicated, since after 1-2 weeks of withdrawal, there is an increase in AMPA/NMDA ratio and synaptic potentiation, not depression (Boudreau and Wolf, 2005). Furthermore, if cocaine is self-administered rather than given non-contingently, there is in addition a marked increase in the AMPA receptor subunit GluR1 in NAc neurons and a reduction in GluR2 subunits (Conrad et al., 2008), so that these neurons become calcium permeable and more excitable (Luscher and Bellone, 2008). In animals that had self-administered cocaine and studied after 1 day of withdrawal, LTD is actually depressed in the NAcC and NAcS, but after 3 weeks of withdrawal from cocaine, LTD was abolished specifically in the NAcC (Martin et al., 2006). Whilst cocaine dosing procedures and other methodological differences do not allow a simple functional interpretation of these data, they do show that cocaine is able to induce long-lasting changes in the NAcC that may be related to drug seeking behavior and relapse. As Martin et al. (2006) speculate, a failure to elicit LTD in the NAcC of rats having self-administered cocaine might be related to the consolidation of the instrumental drug taking response, or to the readiness with which drug-associated CSs induce relapse, or other behavioral processes that depend upon the NAcC (Martin et al., 2006). Given the role of the dorsal striatum in instrumental habit learning (Yin et al., 2004; Yin et al., 2006) and the enhancement of habit learning by stimulant drugs (Nelson and Killcross, 2006; Nordquist et al., 2007), investigation of synaptic LTD in the dorsal striatum following the self-administration of cocaine and other drugs under conditions that are associated with the development of drug seeking habits (Vanderschuren, 2005; Belin and Everitt, 2008) would also provide a valuable means for linking neuronal plasticity and addictive behavior (Luscher and Bellone, 2008). Interestingly, synaptic plasticity is altered by cocaine exposure at corticostriatal synapses in the dorsolateral striatum (Centonze et al., 2006) and amphetamine sensitization increases the density of dendritic spines on medium spiny neurons in the dorsolateral striatum (Li et al., 2003), thereby suggesting that structural and synaptic plasticity mechanisms in the dorsal striatum are influenced by psychostimulants.

These striatal synaptic plasticity mechanisms are accompanied by structural changes in the dopaminergic neurons of the ventral midbrain (Nestler, 2005b), the medium spiny neurons of the striatum and also cortical neurons (Robinson and Kolb, 1997; Robinson and Kolb, 1999; Robinson et al., 2001; Li et al., 2003; Robinson and Kolb, 2004; Crombag et al., 2005). Interestingly, striatal neuronal morphological changes induced by repeated exposure to psychostimulants are not restricted to the ventral striatum where, specifically in the NAcC, they are associated with the induction of behavioral sensitization (Li et al., 2004). They also occur in the dorsolateral striatum, where prior exposure to methamphetamine produced a significant increase in mushroom and thin spines on medium spiny neurons, but not in the dorsomedial striatum where a significant decrease in mushroom spines is observed (Jedynak et al., 2007). Thus, not only is striatal synaptic function altered by exposure to psychostimulants but striatal networks appear to be completely reorganized by these drugs, thereby altering the integrative mechanisms of the striatal complex. Whether these progressive morphological adaptations play a role in the recruitment of the intrastriatal mechanisms involved in the establishment of incentive habits remains to be established, but the dorsomedial/dorsolateral striatum spine density dissociation suggests that morphological alterations may parallel the progressive recruitment of the dorsolateral striatum by chronic exposure to psychostimulants (Porrino, 2004).

### B. Psychostimulant-Induced Molecular Adaptations within the Basal Ganglia: Implications for Drug Addiction

The behavioral, neural and cellular adaptations involved in the development of psychostimulant addiction are associated with profound alterations of gene expression in the basal ganglia and their limbic cortical and dopaminergic inputs (Nestler, 1993; Nestler and Aghajanian, 1997; Nestler, 2000; Gonzalez-Nicolini and McGinty, 2002; Bannon et al., 2005; Goldman et al., 2005; Yuferov et al., 2005; Albertson et al., 2006) (see Chapter 29), although a clear relationship between each level remains to be established. While repeated exposure to psychostimulants induces forms of cellular adaptation other than gene expression, including activation of transduction pathways by phosphorylation of their components such as DARPP-32, CREB and ERK (Carrasquillo and Sweatt, 2005; Lu et al., 2005; Mattson et al., 2005; Zachariou et al., 2006b) (see also Chapter 26), addressing receptors at the membrane, or reorganization of post-synaptic density scaffolding, we will focus here on modification of gene expression which might provide the molecular substrate for the long-lasting nature of drug addiction (Nestler and Aghajanian, 1997; Nestler, 2000) (see also Chapter 30). Thus, the transient activation of CREB in the NAc by repeated exposure to cocaine or amphetamine is associated with a decrease in the rewarding properties of these drugs (Carlezon et al., 1998), very likely through its inducting of the expression of the peptide dynorphin. This peptide binds to kappa opioid receptors on the soma of DA neurons in the VTA as well as on their terminals, thereby decreasing DA release in the NAc (Spanagel et al., 1992), and is also associated with dysphoria (Hyman and Malenka, 2001) (see Chapter 29).

Drug-induced gene expression has been shown progressively to be recruited in corticostriatal circuitry as successive "waves", the first induced by the acute effects of exposure to cocaine or amphetamine and the subsequent waves emerging during protracted exposure to the drug and withdrawal episodes (Kalivas and Volkow, 2005). Moreover, a shift in the locus of these gene expression waves may occur from ventral to more dorsal loops of the cortico-striato-pallidal circuitry, as described at the metabolic and functional level in monkeys chronically administering cocaine (Letchworth et al., 2001; Porrino, 2004). Thus the picture obtained from post-mortem analyses of brain transcriptomes of cocaine addicts (Albertson et al., 2006; Kristiansen et al., 2009) represents the end-point of a series of transitions that may be crucial in the etiology of drug addiction, but remain fully to be elucidated.

Considerable interest has been generated in the induction of the Fos family of genes in the ventral and the dorsal striatum after acute exposure to addictive drugs (Hope et al., 1992; Hope et al., 1994). These immediate early genes are transiently activated by exposure to cocaine or amphetamine and are involved in mediating subsequent drug-induced changes in gene expression (Nestler, 2005a; Nestler, 2005b; Nestler, 2008). However, they cannot account for the protracted nature of behavioral changes observed in drug addicts. It is thus important to focus on the long-term effects of chronic drug exposure which have greater heuristic value with regard to the human condition (Nestler, 2009). Thus, after protracted exposure to psychostimulants most Fos-like transcription factors are desensitized, in that their inducibility is blunted (Hope et al., 1992) (see Chapter 29). In contrast, a truncated form of FosB, namely  $\Delta$ FosB, that mediates long-term neural and behavioral plasticity (Nestler et al., 1999), accumulates in substance P and dynorphin-containing striatal neurons and contributes to long-lasting drug-induced modifications in gene expression (Nestler, 2001b).

Cocaine-induced  $\Delta$ FosB in the NAcS and NAcC, but also in the BLA, is correlated with preference for cocaine compared to novelty, as measured in a CPP procedure (Harris et al., 2007). Expression of  $\Delta$ FosB in striatal dynorphin-containing neurons of transgenic mice increases the sensitivity to the locomotor activating effects of cocaine and the rewarding properties of cocaine, as measured in a CPP procedure (Kelz et al., 1999) or self-administration of low doses of the drug (Colby et al., 2003). Conversely, the expression of  $\Delta$ c-Jun, a dominant negative antagonist of  $\Delta$ FosB, in the NAc and dorsal striatum decreases cocaine CPP, suggesting reduced sensitivity to the rewarding effects of the drug (Peakman et al., 2003).

 $\Delta$ FosB controls the expression of several genes which have been suggested to be involved in the development of addiction-like behavior in animal models. Thus, it regulates the expression of dynorphin, although unlike CREB it decreases it (Zachariou et al., 2006a), as well as cdk5, a protein involved in regulating dopaminergic and glutamatergic transmission in the striatum (Chergui et al., 2004). Cdk5 has been shown to modulate the rewarding properties of cocaine (Benavides et al., 2007) and, more importantly, to be involved in cocaine-induced morphological changes of striatal neurons (Meyer et al., 2008).

Another mechanism by which  $\Delta$ FosB may exert control over behavioral and molecular responses to addictive drugs is its potential to control epigenetic mechanisms, i.e., regulation by chromatin compaction of the availability of genes to control the transcription machinery (Nestler, 2009) (see Chapter 30). For example,  $\Delta$ FosB is implicated in the desensitization of Fos-like genes after repeated exposure to amphetamine through an epigenetic mechanism (Renthal et al., 2008).

### VII. TOWARDS AN UNDERSTANDING OF PSYCHOSTIMULANT ADDICTION: DYSREGULATION OF CORTICOSTRIATAL CIRCUITRY AND INCENTIVE HABITS

Drug addiction develops as the result of within and between systems adaptations (Everitt and Robbins, 2005; Koob and Moal, 2005), modulating positive and negative reinforcement processes, but hypothesized ultimately to be expressed as pathologically instantiated instrumental and Pavlovian mechanisms over which top down executive control is impaired (Everitt and Robbins, 2005). Positive and negative reinforcement, as well as Pavlovian reflexive responses, instrumental learning and the control by Pavlovian incentives over instrumental performance are dependent upon parallel and serial striatal, integrative mechanisms thereby affording the basal ganglia a central role in the neurobiology of drug addiction.

### A. Parallel Mechanisms

Studies of natural reinforcement have proven to be very valuable for the understanding of the learning mechanisms dependent upon the basal ganglia that are involved both in voluntary behavior and psychostimulant addiction. Three parallel systems can be identified as playing different or complementary roles in instrumental learning and instrumental performance.

- A ventral system, including the ventral striatum, and particularly the NAc, and its inputs especially from the amygdala, hippocampus and anterior cingulate cortex as well as "teaching signal" DA inputs from the VTA. It is important for the acquisition of instrumental drug seeking behavior and for mediating the impact of Pavlovian stimuli on instrumental performance, including conditioned reinforcement and Pavlovianinstrumental transfer (Cardinal et al., 2002; Everitt and Robbins, 2005; Belin et al., 2008a).
- 2. A dorsomedial system, including the DMS and its afferents from the cerebral cortex, most notably the prefrontal and prelimbic cortices (Reep et al., 2003), is involved in executive processes, including the control of goal-directed instrumental behavior. Within this dorsomedial system, the pDMS receiving projections from the BLA (Kelley et al., 1982), might play a predominant role in action-outcome learning mechanisms and perhaps the modulation of instrumental incentive learning (Balleine, 2005) when behavior is goal-directed.

**3.** A dorsolateral system, including the dorsolateral striatum, its inputs from the sensorimotor and associative cortex (Alexander et al., 1986; Alexander and Crutcher, 1990) and its DA innervation (Faure, 2005), which mediates habitual, or S-R, learning and performance mechanisms (Balleine, 2005).

Therefore, it appears that the striatum can be segregated into different, though interacting, functional domains in which the ventral part, or NAc, is involved in the acquisition, performance and modulation of instrumental behavior by Pavlovian CSs (Cardinal et al., 2002), whereas the dorsal territories of the striatum are involved in learning action-outcome and S-R associations. Because of the topographic organization of basal ganglia circuitry (Alexander et al., 1986; Alexander and Crutcher, 1990) these domains convey parallel and somewhat independent information required for instrumental behavior, given that lesions of one domain apparently do not impair the behavioral output of the others (Yin et al., 2004; Yin et al., 2005; Yin and Knowlton, 2006; Yin et al., 2006). Such parallel mechanisms can also be seen during the acquisition and expression of Pavlovian reflexive responses. Thus specific ventral circuitry is involved in the acquisition and expression of autoshaping (or sign-tracking). This ventral circuit involves the CeA, the anterior cingulate cortex, the NAcC and its inputs from the VTA (Cardinal et al. 2002). It is dissociable from a more dorsal circuit that is involved in conditioned orienting (Han et al., 1997), involving direct projections from the CeA to influence the nigrostriatal pathway. Dopamine plays an important role in detecting unpredicted salient stimuli as well as the actions that result in the presentation of these stimuli (Redgrave and Gurney, 2006) (see Chapter 31), thereby gating incentive teaching signals in the basal ganglia and monitoring their accuracy (prediction/error detection) (Schultz et al., 1997; Schultz, 1998; Schultz and Dickinson, 2000; Schultz, 2007). Because Pavlovian learning mechanisms gain access to instrumental performance through the NAc, parallel processing alone cannot account for these interactions when instrumental performance depends upon different, seemingly separate, neural systems, such as that involving the dorsolateral striatum (Belin and Everitt, 2008).

### **B.** Integrative Mechanisms

Pavlovian influences over instrumental performance require integrative processes within the basal ganglia that we have suggested (Belin and Everitt, 2008) depend to a considerable extent upon the DA-dependent ascending spiralling circuitry linking the NAc to the dorsal striatum (Haber et al., 2000; Haber, 2003; Ikemoto, 2007; Haber, 2008) (Fig. 33.1). The neural mechanisms of conditioned reinforcement provide a very clear behavioral illustration of integrative processes within the basal ganglia. Responding with conditioned reinforcement, as measured at early stages of training under a second order schedule of reinforcement, depends upon DA transmission in the BLA, glutamatergic transmission in the NAcC, interactions between these



FIGURE 33.1 Drug addiction as a failure in top-down executive control over drug-oriented incentive habits. Basal ganglia circuitry is fundamentally involved in the mechanisms underlying the development and persistence of drug addiction. The reinforcing, and possibly the hedonic (H), effects of psychostimulants depend upon the shell of the nucleus accumbens (NAcS), the olfactory tubercle and the ventral pallidum (GPe-GPi), whereas the motivational balance between natural and drug rewards (NR/DR) may depend upon the subthalamic nucleus (STN). More importantly, exposure to addictive drugs triggers neurobiological, and hence, functional modifications, in neural networks involved in implicit subcortical, and declarative cortical, mechanisms. At the subcortical level, addictive drugs alter both Pavlovian and instrumental learning mechanisms. (1) Addictive drugs enhance the Pavlovian incentive influences from the basolateral nucleus of the amygdala (BLA) to the core of the nucleus accumbens (NAcC) and alter the Pavlovian incentive processing between the BLA and the orbitofrontal cortex (OFc) thereby leading to increased incentive salience of drugs and environmental stimuli associated with them. Alterations of hippocampal (hipp) function may also contribute to an enhanced incentive control of contextual cues over drug seeking and drug taking behavior. (2) Addictive drugs facilitate the instantiation of habitual responding, whereby drug seeking behavior is no longer under the direct control of the motivational properties of the drug itself, but instead, governed by stimuli in the environment. The development of habitual drug seeking and drug taking behavior may be related to a ventral to dorsal striatal shift in the locus of control over behavior, which depends at least in part upon the ascending dopamine-dependent circuitry linking the ventral to the dorsolateral striatum (DLS) via recurrent connections with the dopaminergic neurons in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) in the ventral midbrain. Thus, maladaptive, drug focused, Pavlovian incentive processes that control "drug-oriented incentive impulses" in the NAcC eventually influence the dorsal striatum-dependent stimulusresponse (S-R), or "habit" system, thereby giving rise to incentive habits. Additionally, exposure to psychostimulants shifts the striatal matrix/striosome functional output towards the striosomes which receive limbic inputs and hence may represent another way to gate limbic influence over dorsolateral striatal (DLS)-dependent processes. However, incentive habits cannot alone account for the development of compulsive drug seeking and taking behavior which, instead, may arise from the interaction between subcortical mechanisms that tend to drive the addict towards drugs and drug-associated stimuli and declarative cortical mechanisms. Indeed, exposure to addictive drugs triggers a change in the balance of cortical neuroadaptations from ventromedial to dorsolateral prefrontal cortex, which might be associated with drug-induced deficits in top-down executive control over instrumental behavior. Drug addicts and drug-exposed animals display cognitive inflexibility, impaired decision making processes and high rates of impulsivity, suggesting impairment of prefrontal cortical function. Thus, once incentive habits develop and are progressively less under the control of prefrontal executive function, drug use is no longer under the control of the individual and can be described as compulsive.

structures and also the integrity of the orbitofrontal cortex (Everitt et al., 2008). However, after protracted training to seek cocaine it is the dorsal striatal dopaminergic system that is important in instrumental performance (Ito et al., 2002; Vanderschuren, 2005) and the recruitment of the dorsolateral striatum has been demonstrated to depend upon the dopaminergic interactions linking NAcC to the dorsolateral striatum (Belin and Everitt, 2008). These data emphasize intra-striatal integrative mechanisms in the interactions between Pavlovian and instrumental learning. However it is still unclear whether this transition from ventral to dorsal striatum demonstrated for cocaine-seeking under a second order schedule of reinforcement is specific to addictive drugs or if it reflects a greater capacity of stimulants such as cocaine to hasten the integrative process that is usually recruited by natural rewards.

# C. Addiction: Towards the View of an Incentive Habit

Although not definitively demonstrated, addictive drugs may through their potent effects on DA transmission underlie the abnormally strong consolidation of Pavlovian and instrumental learning mechanisms. This greatly enhances the propensity to engage in drug seeking in response to contexts and cues associated with the drug, particularly when these cues act as sub-goals for instrumental behavior by acting as conditioned reinforcers (Everitt and Robbins, 2005). Moreover, treatment with stimulant drugs that sensitize DA transmission also facilitates the development of habits over goal-directed instrumental responses for natural rewards (Nelson and Killcross, 2006; Nordquist et al., 2007), while orally ingested drug rewards such as alcohol and cocaine engage S-R habits more rapidly than do natural reinforcers (Dickinson et al., 2002; Miles et al., 2003).

We have summarized here experimental support for the hypothesis that drug seeking habits progressively dominate goal-directed drug-seeking behavior and that they are highly influenced by Pavlovian incentive mechanisms. Hence we have introduced the term **incentive habit** (Belin et al., 2008a) to describe this phenomenon. We argue that incentive habits depend at least in part upon serial processing between the BLA, the NAcC and the ascending, DA-mediated circuitry that links this system to the dorsolateral striatum. Incentive habits can be triggered by drugassociated stimuli, withdrawal-associated stimuli or internal states that influence the motivational value of these stimuli. Therefore, incentive sensitization (Robinson and Berridge, 1993; Robinson and Berridge, 2000; Robinson and Berridge, 2001; Robinson and Berridge, 2003) and negative affective states (Koob and Moal, 1997, 2001, 2005, 2008) play an important role in the establishment and persistent performance of incentive habits by generating an increased incentive value of drug-associated stimuli and/or a with-drawal (including conditioned withdrawal) induced drive towards drug taking, respectively.

### D. Top-down Inhibitory or "Executive" Control

There is no reason to think that incentive habits are uniquely established in individuals responding for drugs. Such habits are also adaptively established in animals responding for natural rewards. However, in the context of drug addiction, it is the development of a **pathological incentive habit** that is of major interest and importance and the mechanisms we have so far described are perhaps not sufficient by themselves to explain the development of compulsive drug use, i.e. that which is maintained despite negative consequences, the hallmark of addiction in DSM-IV (APA, 2000).

We and others have hypothesized that the development of compulsive drug use may reflect a loss of prefrontal executive control over incentive habits that underlie drug seeking and taking (Jentsch and Taylor, 1999; Robbins and Everitt, 1999; Everitt and Robbins, 2005; Kalivas and Volkow, 2005). Repeated exposure to drugs of abuse is associated with many cognitive and behavioral deficits including those in visual attention, delay discounting, reversal learning, impulsivity or decision making in both humans (Moeller et al., 2002; Hester and Garavan, 2004; Kirby and Petry, 2004) and in animal models of addiction (Paine et al., 2003; Paine and Olmstead, 2004; Schoenbaum et al., 2004; Dalley et al., 2005a; Dalley et al., 2005b; Black, 2006; Calu et al., 2007; George et al., 2008). These processes require the functional integrity of areas of the PFC. Therefore, protracted exposure to addictive drugs may diminish the influence of top-down executive control by the PFC (Fig. 33.1), thereby facilitating the impact of Pavlovian motivational influences on instrumental drug seeking responses (Schoenbaum et al., 2004; Schoenbaum, 2004; Schoenbaum et al., 2006; Schoenbaum and Shaham, 2008). Additionally, by subverting orbitofrontal-dependent decision-making processes (Jentsch and Taylor, 1999; Bolla et al., 2003), drugs of abuse may bias individual choices towards drugs and diminish sensitivity to negative feedback, thereby promoting compulsive drug seeking (Everitt and Robbins, 2005).

An interesting avenue for future research will be to determine whether the drug-induced development of incentive habits and failure of top-down executive control are parallel or interactive processes. Studies in monkeys have reported that the progressive engagement of the dorsal striatum by cocaine self-administration parallels a progressive functional engagement of dorsolateral parts of the PFC after extended exposure to cocaine (Porrino et al., 2007). This observation suggests that both cortical and ventral to dorsal striatal shifts co-occur in the course of addiction and is in agreement with studies in humans showing that low DA D2-receptor binding in the dorsal striatum of cocaine addicts is associated with decreased metabolic activity in the PFC, especially in the orbitofrontal cortex (Volkow et al., 1993). However, whether it is intra-striatal adaptations that trigger or contribute to cortical adaptations in response to protracted cocaine selfadministration, or vice-versa, remains to be established. Nor is it clear whether reduced cortical metabolism (Volkow et al., 2001; Volkow et al., 2002) or reductions in, for example, striatal DA D2 receptors (Volkow et al., 2001) seen in drug addicted individuals are purely the result of chronic drug taking, or are causal factors leading to drug abuse and addiction, or result from an interaction between the two (Dalley et al., 2007). Nevertheless, we suggest that the development of incentive habits that parallels the progressively greater control of the dorsal striatum over drug seeking may be facilitated by drug-induced prefrontal cortical impairments. Indeed, S-R habits require less executive control than goal-directed actions (Muller et al., 2007; Tanji and Hoshi, 2008) and may therefore be strengthened by weaker prefrontal control over behavior resulting from toxic effects of chronically and even acutely (Schoenbaum et al., 2006) self-administered addictive drugs.

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#### REFERENCES

Adams C, Dickinson A (1981) Instrumental responding following reinforcer devaluation. Q J Exp Psychol Comp Physiol Psychol 33:109–121.

- Ahmed S, Cador M (2006) Dissociation of psychomotor sensitization from compulsive cocaine consumption. Neuropsychopharmacology 31:563–571.
- Ahn S, Phillips A (2007) Dopamine efflux in the nucleus accumbens during within-session extinction, outcome-dependent, and habit-based instrumental responding for food reward. Psychopharmacology 191:641–651.
- Albertson DN, Schmidt CJ, Kapatos G, Bannon MJ (2006) Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. Neuropsychopharmacology 31:2304–2312.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Ann Rev Neurosci 9:357–381.
- Altman J, Everitt B, Robbins T, Glautier S (1996) The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology 125:285–345.
- Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648–661.
- APA (2000) Diagnostic and Statistical Manual of Mental Disorders: American Psychiatric Association.
- Arroyo M, Markou A, Robbins T, Everitt B (1998) Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology (Berl) 140:331–344.
- Bachand KD, Guthrie KM, Wolgin DL (2009) Expression of c-fos mRNA in the basal ganglia associated with contingent tolerance to amphetamine-induced hypophagia. Behav Brain Res 198:388–396.
- Balleine B (2005) Neural bases of food-seeking: Affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav 86:717–730.
- Balleine B, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37:407–419.
- Balleine B, Gerner C, Dickinson A (1995) Instrumental outcome devaluation is attenuated by the anti-emetic ondansetron. Q J Exp Psychol B 48:235–251.
- Bannon M, Kapatos G, Albertson D (2005) Gene expression profiling in the brains of human cocaine abusers. Addict Biol 10:119–126.
- Baunez C, Dias C, Cador M, Amalric M (2005) The subthalamic nucleus exerts opposite control on cocaine and "natural" rewards. Nat Neurosci 8:484–489.
- Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE (2001) Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 39:376–389.
- Beeler J, Cao Z, Kheirbek M, Zhuang X (2009) Loss of cocaine locomotor response in pitx3-deficient mice lacking a nigrostriatal pathway. Neuropsychopharmacology 34:1149–1161.
- Belin D, Deroche-Gamonet V, Jaber M (2007) Cocaine-induced sensitization is associated with altered dynamics of transcriptional responses of the dopamine transporter, tyrosine hydroxylase, and dopamine D2 receptors in C57Bl/6J mice. Psychopharmacology (Berl) 193:567–578.
- Belin D, Everitt B (2008) Cocaine-seeking habits depend upon dopaminedependent serial connectivity linking the ventral with the dorsal striatum. Neuron 57:432–441.

- Belin D, Jonkman S, Dickinson A, Robbins T, Everitt B (2008a) Parallel and interactive learning processes within the basal ganglia: Relevance for the understanding of addiction. Behav Brain Res 199:89–102.
- Belin D, Mar A, Dalley J, Robbins T, Everitt B (2008b) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320:1352–1355.
- Bellone C, Luscher C (2006) Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. Nat Neurosci 9:636–641.
- Belujon P, Grace AA (2008) Critical role of the prefrontal cortex in the regulation of hippocampus-accumbens information flow. J Neurosci 28:9797–9805.
- Benavides D, Quinn J, Zhong P, et al. (2007) Cdk5 modulates cocaine reward, motivation, and striatal neuron excitability. J Neurosci 27:12967–12976.
- Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25:515–532.
- Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?. Brain Res Brain Res Rev 28:309–369.
- Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: "liking", "wanting", and learning. Curr Opin Pharmacol 9(1):65–73.
- Black Y (2006) Altered attention and prefrontal cortex gene expression in rats after binge-like exposure to cocaine during adolescence. J Neurosci 26:9656–9665.
- Bolla KI, Eldreth DA, London ED, et al. (2003) Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. NeuroImage 19:1085–1094.
- Bornovalova M, Daughters S, Hernandez G, Richards J, Lejuez C (2005) Differences in impulsivity and risk-taking propensity between primary users of crack cocaine and primary users of heroin in a residential substance-use program. Exp Clin Psychopharmacol 13:311–318.
- Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25:9144–9151.
- Bossert JM, Wihbey KA, Pickens CL, Nair SG, Shaham Y (2009) Role of dopamine D1-family receptors in dorsolateral striatum in contextinduced reinstatement of heroin seeking in rats. Psychopharmacology 206(1): 51–60.
- Breiter HC, Gollub RL, Weisskoff RM, Kennedy D (1997) Acute effects of cocaine on human brain activity and emotion. Neuron 19:591–611.
- Brown LL, Markman MH, Wolfson LI, Dvorkin B, Warner C, Katzman R (1979) A direct role of dopamine in the rat subthalamic nucleus and an adjacent intrapeduncular area. Science 206:1416–1418.
- Brown PL, Jenkins HM (1968) Auto-shaping of the pigeon's key-peck. J Exp Anal Behav 11:1–8.
- Caine SB, Humby T, Robbins TW, Everitt BJ (2001) Behavioral effects of psychomotor stimulants in rats with dorsal or ventral subiculum lesions: locomotion, cocaine self-administration, and prepulse inhibition of startle. Behav Neurosci 115:880–894.
- Calu D, Stalnaker T, Franz T, Singh T, Shaham Y, Schoenbaum G (2007) Withdrawal from cocaine self-administration produces long-lasting deficits in orbitofrontal-dependent reversal learning in rats. Learning and Memory 14:325–328.
- Campbell GA, Eckardt MJ, Weight FF (1985) Dopaminergic mechanisms in subthalamic nucleus of rat: analysis using horseradish peroxidase and microiontophoresis. Brain Res 333:261–270.

- Canales J (2005) Stimulant-induced adaptations in neostriatal matrix and striosome systems: Transiting from instrumental responding to habitual behavior in drug addiction. Neurobiol Learning and Memory 83:93–103.
- Carboni E, Imperato A, Perezzani L, Di Chiara G (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661.
- Cardinal R, Parkinson JA, Hall J, Everitt B (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321–352.
- Carelli R, Ijames SG, Crumling AJ (2000) Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus "natural" (water and food) reward. J Neurosci 20:4255–4266.
- Carelli R, Wondolowski J (2003) Selective encoding of cocaine versus natural rewards by nucleus accumbens neurons is not related to chronic drug exposure. J Neurosci 23:11214–11223.
- Carelli RM (2004) Nucleus accumbens cell firing and rapid dopamine signaling during goal-directed behaviors in rats. Neuropharmacology 47(Suppl 1):180–189.
- Carlezon WAJ, Nestler EJ (2002) Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? Trends Neurosci 25:610–615.
- Carlezon WAJ, Thome J, Olson VG, et al. (1998) Regulation of cocaine reward by CREB. Science 282:2272–2275.
- Carrasquillo Y, Sweatt JD (2005) Craving cocaine pERKs up the amygdala. Nat Neurosci 8:129–130.
- Centonze D, Costa C, Rossi S, et al. (2006) chronic cocaine prevents depotentiation at corticostriatal synapses. Biol Psychiatr 60:436–443.
- Chen BT, Bowers MS, Martin M, et al. (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron 59:288–297.
- Chergui K, Svenningsson P, Greengard P (2004) Cyclin-dependent kinase 5 regulates dopaminergic and glutamatergic transmission in the striatum. Proc Natl Acad Sci USA 101:2191–2196.
- Childress AR, Mozley PD, McElgin W, et al. (1999) Limbic activation during cue-induced cocaine craving.. Am J Psychiatr 156:11–18.
- Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW (2003) Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. J Neurosci 23:2488–2493.
- Conrad K, Tseng K, Uejima J, Reimers J, Heng L, Shaham Y, Marinelli M, Wolf M (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118–121.
- Cousins MS, Salamone JD (1996a) Involvement of ventrolateral striatal dopamine in movement initiation and execution: a microdialysis and behavioral investigation. Neuroscience 70:849–859.
- Cousins MS, Salamone JD (1996b) Skilled motor deficits in rats induced by ventrolateral striatal dopamine depletions: behavioral and pharmacological characterization. Brain Res 732:186–194.
- Cousins MS, Sokolowski JD, Salamone JD (1993) Different effects of nucleus accumbens and ventrolateral striatal dopamine depletions on instrumental response selection in the rat. Pharmacol Biochem Behav 46:943–951.
- Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ (2004) Neural systems supporting interoceptive awareness. Nat Neurosci 7:189–195.
- Crombag H, Gorny G, Li Y, Kolb B, Robinson T (2005) Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. Cereb Cortex 15:341–348.

- Dalley J, Fryer T, Brichard L, et al. (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 315:1267–1270.
- Dalley J, Lääne K, Pena Y, Theobald D, Everitt B, Robbins T (2005a) Attentional and motivational deficits in rats withdrawn from intravenous self-administration of cocaine or heroin. Psychopharmacology 182:579–587.
- Dalley J, Theobald D, Berry D, Milstein J, Lääne K, Everitt B, Robbins T (2005b) Cognitive sequelae of intravenous amphetamine selfadministration in rats: evidence for selective effects on attentional performance. Neuropsychopharmacology 30:525–537.
- Dalley JW, Everitt BJ (2009) Dopamine receptors in the learning, memory and drug reward circuitry. Semin Cell Develop Biol 20:403–410.
- Deroche V, Le Moal M, Piazza PV (1999) Cocaine self-administration increases the incentive motivational properties of the drug in rats. Eur J Neurosci 11:2731–2736.
- Di Chiara G, Bassareo V, Fenu S, et al. (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47(Suppl 1):227–241.
- Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85:5274–5278.
- Di Ciano P (2004) direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J Neurosci 24:7167–7173.
- Di Ciano P, Blaha CD, Phillips AG (1998) Conditioned changes in dopamine oxidation currents in the nucleus accumbens of rats by stimuli paired with self-administration or yoked-administration of damphetamine. Eur J Neurosci 10:1121–1127.
- Di Ciano P, Everitt B (2001) Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology 25:341–360.
- Di Ciano P, Everitt B (2003) Differential control over drug-seeking behavior by drug-associated conditioned reinforcers and discriminative stimuli predictive of drug availability. Behav Neurosci 117:952–960.
- Di Ciano P, Everitt B (2004a) Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behavior. Neuropharmacology 47:202–213.
- Di Ciano P, Everitt B (2004b) Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. Eur J Neurosci 19:1661–1667.
- Di Ciano P, Everitt B (2005) Neuropsychopharmacology of drug seeking: Insights from studies with second-order schedules of drug reinforcement. Eur J Pharmacol 526(1-3):186–198.
- Dickinson A (1985) Actions and habits: the development of behavioral autonomy. Phil Trans Roy Soc Lond B 308:67–78.
- Dickinson A, Nicholas DJ, Adams CD (1983) The effect of the instrumental training contingency on susceptibility to reinforcer devaluation. Q J Exp Psychol 35:35–51.
- Dickinson A, Wood N, Smith J (2002) Alcohol seeking by rats: Action or habit? Quart J Exp Psychol Sect B 55:331–348.
- Drevets WC, Gautier C, Price JL, Kupfer DJ (2001) Amphetamineinduced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatr 19:81–96.
- Everitt B, Belin D, Economidou D, Pelloux Y, Dalley J, Robbins T (2008) Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos Trans R Soc Lond B Biol Sci 363:3125–3135.
- Everitt B, Dickinson A, Robbins T (2001) The neuropsychological basis of addictive behavior. Brain Res Rev 36:129–138.

- Everitt B, Hutcheson D, Ersche K, Pelloux Y, Dalley J, Robbins T (2007) The orbital prefrontal cortex and drug addiction in laboratory animals and humans. Ann NY Acad Sci 1121(1):576–597.
- Everitt B, Robbins T (2000) Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behavior. Psychopharmacology 153:17–30.
- Everitt B, Robbins T (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.
- Everitt B, Wolf ME (2002) Psychomotor stimulant addiction: a neural systems perspective. J Neurosci 22:3312–3320.
- Everitt BJ, Belin D, Dalley JW, Robbins TW Dopaminergic Mechanisms in Drug-Seeking Habits and the Vulnerability to Drug Addiction in Dopamine Handbook, Iversen L, Iversen SD, Dunnett SB, Bjorklund A (eds.), Oxford University Press, New York. In Press.
- Faure A (2005) Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation. J Neurosci 25:2771–2780.
- Febo M, Segarra A, Nair G, Schmidt K, Duong T, Ferris C (2005) The neural consequences of repeated cocaine exposure revealed by functional MRI in awake rats. Neuropsychopharmacology 30:936–943.
- Flagel S, Watson S, Akil H, Robinson T (2008a) Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization. Behav Brain Res 186:48–56.
- Flagel S, Watson S, Robinson T, Akil H (2007) Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. Psychopharmacology 191:599–607.
- Flagel SB, Akil H, Robinson TE (2008b) Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology 56:139–148.
- Floresco SB, Todd CL, Grace AA (2001) Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. J Neurosci 21:4915–4922.
- Frankel PS, Alburges ME, Bush L, Hanson GR, Kish SJ (2008) Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. Neuropharmacology 55:41–46.
- Franken I, van Strien JW, Franzek EJ, van de Wetering BJ (2007) Errorprocessing deficits in patients with cocaine dependence. Biol Psychol 75:45–51.
- Frenois F, Stinus L, Di Blasi F, Cador M, Le Moine C (2005) A specific limbic circuit underlies opiate withdrawal memories. J Neurosci 25:1366–1374.
- Fuchs R (2006) Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. J Neurosci 26:3584–3588.
- Garavan H, Pankiewicz J, Bloom A, et al. (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. Am J Psychiatr 157:1789–1798.
- Gawin FH (1989) Cocaine abuse and addiction. J Fam Pract 29:193-197.
- Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251:1580–1586.
- Gawin FH, Ellinwood EH Jr (1989) Cocaine dependence. Ann Rev Med 40:149–161.
- George O, Mandyam C, Wee S, Koob G (2008) Extended access to cocaine self-administration produces long-lasting prefrontal cortexdependent working memory impairments. Neuropsychopharmacology 33:2474–2482.
- Goldberg SR, Kelleher RT, Goldberg DM (1981) Fixed-ratio responding under second-order schedules of food presentation or cocaine injection. J Pharmacol Exp Ther 218:271–281.

- Goldberg SR, Kelleher RT, Morse WH (1975) Second-order schedules of drug injection. Fed Proc 34:1771–1776.
- Goldman D, Oroszi G, Ducci F (2005) The genetics of addictions: uncovering the genes. Nat Rev Genet 6:521–532.
- Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642–1652.
- Gong W, Neill D, Justice JBJ (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res 754:103–112.
- Gonzalez-Nicolini V, McGinty JF (2002) Gene expression profile from the striatum of amphetamine-treated rats: a cDNA array and in situ hybridization histochemical study. Brain Res Gene Exp Patterns 1:193–198.
- Gratton A, Wise RA (1994) Drug-and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. J Neurosci 14:4130–4146.
- Grimm J, Kruzich PJ, See R (2000) Contingent access to stimuli associated with cocaine self-administration is required for reinstatement of drug-seeking behavior. Psychobiology 28:383–386.
- Haber S (2003) The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.
- Haber S (2008) Parallel and integrative processing through the basal ganglia reward circuit: lessons from addiction. Biol Psychiatr 64:173–174.
- Haber S, Fudge JL, McFarland NR (2000) striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Han JS, McMahan RW, Holland PC, Gallagher M (1997) The role of an amygdalo-nigrostriatal pathway in associative learning. J Neurosci 17:3913–3919.
- Harmer CJ, Phillips GD (1999) Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated D-amphetamine. Neuroscience 90:119–130.
- Harris GC, Hummel M, Wimmer M, Mague SD, Aston-Jones G (2007) Elevations of FosB in the nucleus accumbens during forced cocaine abstinence correlate with divergent changes in reward function. Neuroscience 147:583–591.
- Hester R, Garavan H (2004) Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 24:11017–11022.
- Hilário MR, Costa RM (2008) High on habits. Front Neurosci 2:208-217.
- Hogarth L, Dickinson A, Wright A, Kouvaraki M, Duka T (2007) The role of drug expectancy in the control of human drug seeking. J Exp Psychol Anim Behav Process 33:484–496.
- Holland P (2004) relations between Pavlovian-instrumental transfer and reinforcer devaluation. J Exp Psychol Anim Behav Process 30:104–117.
- Holland P, Gallagher M (2003) Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimuluspotentiated feeding and Pavlovian-instrumental transfer. Eur J Neurosci 17:1680–1694.
- Hope B, Kosofsky B, Hyman SE, Nestler EJ (1992) Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci USA 89:5764–5768.
- Hope BT, Nye HE, Kelz MB, et al. (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 13:1235–1244.
- Hubner CB, Koob GF (1990) The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. Brain Res 508:20–29.

- Hutcheson DM, Everitt B (2003) The effects of selective orbitofrontal cortex lesions on the acquisition and performance of cue-controlled cocaine seeking in rats. Ann NY Acad Sci 1003:410–411.
- Hyman S (2005) Addiction: A disease of learning and memory. Am J Psychiatr 162:1414–1422.
- Hyman S, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2:695–703.
- Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598.
- Ikemoto S, Glazier BS, Murphy JM, McBride WJ (1997) Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. J Neurosci 17:8580–8587.
- Ikemoto S, Qin M, Liu ZH (2005) The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid? J Neurosci 25:5061–5065.
- Ikemoto S (2007) Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27–78.
- Ito R, Dalley J, Howes SR, Robbins T, Everitt B (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 20:7489–7495.
- Ito R, Dalley J, Robbins T, Everitt B (2002) Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J Neurosci 22:6247–6253.
- Ito R, Robbins T, Everitt B (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci 7:389–397.
- Jedynak J, Uslaner J, Esteban J, Robinson T (2007) Methamphetamineinduced structural plasticity in the dorsal striatum. Eur J Neurosci 25:847–853.
- Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology 146:373–390.
- Jones S, Bonci A (2005) Synaptic plasticity and drug addiction. Curr Opin Pharmacol 5:20–25.
- Kalivas P, Volkow N (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatr 162:1403–1413.
- Kauer J, Malenka R (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844–858.
- Kearns DN, Weiss SJ (2004) Sign-tracking (autoshaping) in rats: a comparison of cocaine and food as unconditioned stimuli. Learn Behav 32:463–476.
- Kelley A, Berridge K (2002) The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 22:3306–3311.
- Kelley AE, Domesick VB, Nauta WJ (1982) The amygdalostriatal projection in the rat – an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7:615–630.
- Kelz MB, Chen J, Carlezon WA Jr, Whisler K (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. Nature 401:272–276.
- Kirby KN, Petry NM (2004) Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. Addiction 99:461–471.
- Kolb B, Gorny G, Li Y, Samaha AN, Robinson T (2003) Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. Proc Natl Acad Sci USA 100:10523–10528.

- Koob G, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97–129.
- Koob G, Le Moal M (2005) Plasticity of reward neurocircuitry and the "dark side" of drug addiction. Nat Neurosci 8:1442–1444.
- Koob G, Le Moal M (2008) Addiction and the brain antireward system. Annu Rev Psychol 59:29–53.
- Koob G, Moal ML (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52–58.
- Koob GF, Moal ML (2005) Neurobiology of Addiction. New York: Academic Press.
- Koob GF (2009) Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology 56(Suppl 1):18–31.
- Kourrich S, Rothwell PE, Klug JR, Thomas MJ (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci 27:7921–7928.
- Kristiansen LV, Bannon MJ, Meador-Woodruff J (2009) Expression of transcripts for myelin related genes in postmortem brain from cocaine abusers. Neurochem Res 34:46–54.
- Lavoie B, Smith Y, Parent A (1989) Dopaminergic innervation of the basal ganglia in the squirrel monkey as revealed by tyrosine hydroxylase immunohistochemistry. J Comp Neurol 289:36–52.
- Lee J, Milton AL, Everitt B (2006) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. J Neurosci 26:5881–5887.
- Leshner AI (1997) Addiction is a brain disease, and it matters. Science 278:45–47.
- Letchworth SR, Nader MA, Smith HR, Friedman DP, Porrino L (2001) Progression of changes in dopamine transporter binding site density as a result of cocaine self-administration in rhesus monkeys. J Neurosci 21:2799–2807.
- Li Y, Acerbo M, Robinson T (2004) The induction of behavioral sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 20:1647–1654.
- Li Y, Kolb B, Robinson T (2003) The location of persistent amphetamineinduced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. Neuropsychopharmacology 28:1082–1085.
- Logan GD (1998a) Toward an instance theory of automatization. Psychol Rev 95:492–527.
- Logan GD (1998b) What is learned during automatization? II. Obligatory encoding of spatial location. J Exp Psychol Hum Percept Perform 24:1720–1736.
- Lovibond PF (1981) Appetitive Pavlovian-instrumental interactions: effects of inter-stimulus interval and baseline reinforcement conditions. Q J Exp Psychol B 33:257–269.
- Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y (2005) Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. Nat Neurosci 8:212–219.
- Luscher C, Bellone C (2008) Cocaine-evoked synaptic plasticity: a key to addiction?. Nat Neurosci 11:737–738.
- Mackintosh NJ (1974) The Psychology of Animal Learning. Oxford: Academic Press.
- Martin M, Chen BT, Hopf FW, Bowers MS, Bonci A (2006) Cocaine selfadministration selectively abolishes LTD in the core of the nucleus accumbens. Nat Neurosci 9:868–869.
- Mattson BJ, Bossert JM, Simmons DE, Nozaki N, Nagarkar D, Kreuter JD, Hope BT (2005) Cocaine-induced CREB phosphorylation in nucleus accumbens of cocaine-sensitized rats is enabled by enhanced activation of extracellular signal-related kinase, but not protein kinase A. J Neurochem 95:1481–1494.

- Mcfarland K, Kalivas P (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.
- Meibach RC, Katzman R (1979) Catecholaminergic innervation of the subthalamic nucleus: evidence for a rostral continuation of the A9 (substantia nigra) dopaminergic cell group. Brain Res 173:364–368.
- Meyer DA, Richer E, Benkovic SA, et al. (2008) Striatal dysregulation of Cdk5 alters locomotor responses to cocaine, motor learning, and dendritic morphology. Proc Natl Acad Sci USA 105:18561–18566.
- Miles F, Everitt B, Dickinson A (2003) Oral cocaine seeking by rats: Action or habit? Behav Neurosci 117:927–938.
- Moeller FG, Dougherty DM, Barratt ES, Oderinde V, Mathias CW, Harper RA, Swann AC (2002) Increased impulsivity in cocaine dependent subjects independent of antisocial personality disorder and aggression. Drug Alcohol Depend 68:105–111.
- Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97.
- Muller J, Dreisbach G, Goschke T, Hensch T, Lesch KP, Brocke B (2007) Dopamine and cognitive control: the prospect of monetary gains influences the balance between flexibility and stability in a set-shifting paradigm. Eur J Neurosci 26:3661–3668.
- Nader MA, Czoty PW, Gould RW, Riddick NV (2008) Review. Positron emission tomography imaging studies of dopamine receptors in primate models of addiction. Philos Trans R Soc Lond B Biol Sci 363:3223–3232.
- Neisewander JL, O'Dell LE, Tran-Nguyen LT, Castaneda E, Fuchs R (1996) Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. Neuropsychopharmacology 15:506–514.
- Nelson A, Killcross S (2006) Amphetamine exposure enhances habit formation. J Neurosci 26:3805–3812.
- Nestler EJ (1993) Cellular responses to chronic treatment with drugs of abuse. Crit Rev Neurobiol 7:23–39.
- Nestler EJ (2000) Genes and addiction. Nat Genet 26:277–281.
- Nestler EJ (2001a) Neurobiology. Total recall-the memory of addiction. Science 292:2266–2267.
- Nestler EJ (2001b) Molecular neurobiology of addiction. Am J Addict 10:201–217.
- Nestler EJ (2002) Common molecular and cellular substrates of addiction and memory. Neurobiol Learn Mem 78:637–647.
- Nestler EJ (2005a) The neurobiology of cocaine addiction. Sci Pract Perspect 3:4–10.
- Nestler EJ (2005b) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445–1449.
- Nestler EJ (2008) Review. Transcriptional mechanisms of addiction: role of DeltaFosB. Philos Trans R Soc Lond B Biol Sci 363:3245–3255.
- Nestler EJ (2009) Epigenetic mechanisms in psychiatry. Biol Psychiatry 65:189–190.
- Nestler EJ, Aghajanian GK (1997) Molecular and cellular basis of addiction. Science 278:58–63.
- Nestler EJ, Kelz MB, Chen J (1999) DeltaFosB: a molecular mediator of long-term neural and behavioral plasticity. Brain Res 835:10–17.
- Nordquist RE, Voorn P, de Mooij-van Malsen JG, et al. (2007) Augmented reinforcer value and accelerated habit formation after repeated amphetamine treatment. Eur Neuropsychopharmacol 17:532–540.
- O'Brien CP, Childress AR, Ehrman R, Robbins SJ (1998) Conditioning factors in drug abuse: can they explain compulsion? J Psychopharmacol 12:15–22.
- O'Brien CP, Childress AR, Mclellan A, Ehrman R (1992a) A learning model of addiction. Res Publ Assoc Res Nerv Ment Dis 70:157–177.
- O'Brien CP, Childress AR, Mclellan A, Ehrman R (1992b) Classical conditioning in drug-dependent humans. Ann NY Acad Sci 654:400–415.
- Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419–427.
- Paine T, Olmstead M (2004) Cocaine disrupts both behavioral inhibition and conditional discrimination in rats. Psychopharmacology (Berl) 175:443–450.
- Paine TA, Dringenberg HC, Olmstead MC (2003) Effects of chronic cocaine on impulsivity: relation to cortical serotonin mechanisms. Behav Brain Res 147:135–147.
- Panlilio LV, Yasar S, Nemeth-Coslett R, et al. (2005) Human cocaineseeking behavior and its control by drug-associated stimuli in the laboratory. Neuropsychopharmacology 30:433–443.
- Parkinson JA, Dalley J, Cardinal R, Bamford A (2002) Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behavior: implications for mesoaccumbens dopamine function. Behav Brain Res 137:149–163.
- Parkinson JA, Olmstead M, Burns LH, Robbins T (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 19:2401–2411.
- Parkinson JA, Robbins T, Everitt B (2000) Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. Eur J Neurosci 12:405–413.
- Pavlov IP (1927) Conditioned reflexes. London: Oxford University Press.
- Peakman MC, Colby C, Perrotti LI, et al. (2003) Inducible, brain regionspecific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. Brain Res 970:73–86.
- Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84:167–173.
- Phillips GD, Robbins TW, Everitt BJ (1994) Bilateral intraaccumbens self-administration of d-amphetamine: antagonism with intra-accumbens SCH-23390 and sulpiride. Psychopharmacology (Berl) 114:477–485.
- Pierce R, Kalivas P (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Rev 25:192–216.
- Pontieri FE, Mainero C, La Riccia M, Passarelli F, Orzi F (1995) Functional correlates of repeated administration of cocaine and apomorphine in the rat. Eur J Pharmacol 284:205–209.
- Porrino L (2004) Cocaine self-administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci 24:3554–3562.
- Porrino L, Smith HR, Nader MA, Beveridge TJ (2007) The effects of cocaine: a shifting target over the course of addiction. Prog Neuropsychopharmacol Biol Psychiatr 31:1593–1600.
- Reep RL, Cheatwood JL, Corwin JV (2003) The associative striatum: organization of cortical projections to the dorsocentral striatum in rats. J Comp Neurol 467:271–292.
- Renthal W, Carle TL, Maze I, et al. (2008) Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J Neurosci 28:7344–7349.
- Robbins T (1976) Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. Nature 264:57–59.
- Robbins T, Everitt B (1999) Drug addiction: bad habits add up. Nature 398:567–570.

- Robbins T, Everitt BJ (2002) Limbic-striatal memory systems and drug addiction. Neurobiol Learn Mem 78:625–636.
- Roberts DC, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615–620.
- Robinson T, Becker JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur J Pharmacol 85:253–254.
- Robinson T, Becker JB, Presty SK (1982) Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. Brain Res 253:231–241.
- Robinson T, Berridge K (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291.
- Robinson T, Berridge K (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95:S91–S117.
- Robinson T, Berridge K (2001) Incentive-sensitization and addiction. Addiction 96:103–114.
- Robinson T, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257–266.
- Robinson T, Jurson PA, Bennett JA, Bentgen KM (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain Res 462:211–222.
- Robinson T, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491–8497.
- Robinson T, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598–1604.
- Robinson T, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 47(Suppl 1):33–46.
- Robinson TE, Berridge KC (2003) Addiction. Annu Rev Psychol 54:25–53.
- Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577–582.
- Saal D, Malenka RC (2005) The role of synaptic plasticity in addiction. Clin Neurosci Res 5:141–146.
- Salamone JD, Correa M, Mingote S, Weber SM (2003) Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry, and drug abuse. J Pharmacol Exp Ther 305:1–8.
- Schlaepfer TE, Pearlson GD, Wong DF, Marenco S, Dannals RF (1997) PET study of competition between intravenous cocaine and [11C]raclopride at dopamine receptors in human subjects. Am J Psychiatr 154:1209–1213.
- Schoenbaum G (2004) Cocaine makes actions insensitive to outcomes but not extinction: implications for altered orbitofrontal-amygdalar function. Cerebral Cortex 15:1162–1169.
- Schoenbaum G, Roesch M, Stalnaker T (2006) Orbitofrontal cortex, decision-making and drug addiction. Trends Neurosci 29:116–124.
- Schoenbaum G, Saddoris MP, Ramus SJ, Shaham Y, Setlow B (2004) Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. Eur J Neurosci 19:1997–2002.
- Schoenbaum G, Shaham Y (2008) The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. Biol Psychiatr 63: 256–262.

- Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80(1):1–27.
- Schultz W (2000) Multiple reward signals in the brain. Nat Rev Neurosci 1:199–207.
- Schultz W (2007) Behavioral dopamine signals. Trends Neurosci 30:203–210.
- Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599.
- Schultz W, Dickinson A (2000) Neuronal coding of prediction errors. Ann Rev Neurosci 23:473–500.
- See R, Elliott J, Feltenstein M (2007) The role of dorsal vs ventral striatal pathways in cocaine-seeking behavior after prolonged abstinence in rats. Psychopharmacology 194:321–331.
- Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci USA 89:2046–2050.
- Takahashi Y, Roesch M, Stalnaker T, Schoenbaum G (2007) Cocaine exposure shifts the balance of associative encoding from ventral to dorsolateral striatum. Front Integ Neurosci:1–11.
- Tang XC, McFarland K, Cagle S, Kalivas PW (2005) Cocaine-induced reinstatement requires endogenous stimulation of mu-opioid receptors in the ventral pallidum. J Neurosci 25:4512–4520.
- Tanji J, Hoshi E (2008) Role of the lateral prefrontal cortex in executive behavioral control. Physiol Rev 88:37–57.
- Thomas MJ, Kalivas P, Shaham Y (2008) Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. Br J Pharmacol 154:327–342.
- Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nat Neurosci 4:1217–1223.
- Tiffany ST (1990) A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. Psychol Rev 97:147–168.
- Tomie A, Brooks W, Zito B (1989) Sign-tracking: The search for reward. In Klein SB and Mowrer RR (eds.), Contemporary learning theory: Pavlovian conditioning and the status of traditional learning theory (pp. 191–223). Hillsdale, NJ: Lawrence Erlbaum.
- Tomie A, Brooks W, Zito B (1989) Sign-tracking: the search for reward. Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory. 191-223.
- Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411:583–587.
- Uslaner JM, Robinson TE (2006) Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice – mediation by enhanced incentive motivation? Eur J Neurosci 24:2345–2354.
- Vanderschuren L (2005) Involvement of the dorsal striatum in cue-controlled cocaine seeking. J Neurosci 25:8665–8670.
- Vanderschuren L, Kalivas P (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151:99–120.
- Verdejo-García AJ, Perales JC, Pérez-García M (2007) Cognitive impulsivity in cocaine and heroin polysubstance abusers. Addictive Behav 32:950–966.
- Volkow N (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26:6583–6588.
- Volkow N, Fowler J, Wang G, Hitzemann R (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 14:169–177.
- Volkow ND, Chang L, Wang GJ, et al. (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatr 158:2015–2021.

- Volkow ND, Fowler JS, Wang GJ (1999) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. J Psychopharmacol 13:337–345.
- Volkow ND, Fowler JS, Wang GJ, Goldstein RZ (2002) Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. Neurobiol Learn Mem 78:610–624.
- Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O (2000) Control of cocaine-seeking behavior by drugassociated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci USA 97:4321–4326.
- Weiss F, Markou A, Lorang MT, Koob G (1992) Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Res 593:314–318.
- Weissenborn R, Robbins TW, Everitt BJ (1997) Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. Psychopharmacology (Berl) 134:242–257.
- Whitelaw RB, Markou A, Robbins TW, Everitt BJ (1996) Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaineseeking behavior under a second-order schedule of reinforcement. Psychopharmacology (Berl) 127:213–224.
- Williams BA (1992) Inverse relations between preference and contrast. J Exp Anal Behav 58:303–312.
- Williams BA, Dunn R (1991) Substitutability between conditioned and primary reinforcers in discrimination acquisition. J Exp Anal Behav 55:21–35.
- Wise R (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483–494.
- Wong D, Kuwabara H, Schretlen D, et al. (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacology 31:2716–2727.
- Wyvell CL, Berridge K (2001) Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. J Neurosci 21:7831–7840.
- Yin H, Knowlton B (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci 7:464–476.
- Yin H, Knowlton B, Balleine B (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.
- Yin H, Knowlton B, Balleine B (2005) Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. Eur J Neurosci 22:505–512.
- Yin H, Knowlton B, Balleine B (2006) Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. Behavioral Brain Res 166:189–196.
- Yin H, Ostlund SB, Knowlton B, Balleine B (2005) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513–523.
- Yuferov V, Nielsen D, Butelman E, Kreek M (2005) Microarray studies of psychostimulant-induced changes in gene expression. Addict Biol 10:101–118.
- Zachariou V, Bolanos CA, Selley DE, et al. (2006a) An essential role for DeltaFosB in the nucleus accumbens in morphine action. Nat Neurosci 9:205–211.
- Zachariou V, Sgambato-Faure V, Sasaki T, et al. (2006b) Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action. Neuropsychopharmacology 31:555–562.

# Parkinson's Disease: Cross-Talk Between Environmental Factors and Gene Defects

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## I. INTRODUCTION

The etiology of Parkinson's disease (PD) is poorly understood. However, numerous studies point to environmental poisons, such as synthetic toxins, pesticides and heavy metals, as potential risk factors. Indeed, the environmental hypothesis of PD was born in the early to mid 1980s, when Langston and colleagues discovered that the toxin, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) could cause Parkinsonism in humans (Langston et al., 1983; Langston et al., 1984). Nevertheless, humans are exposed to numerous pesticides and toxins in nature, yet not everyone develops PD, suggesting that the factors required for disease development are more complex. Many studies, using animal models or patients, point to interactions between an individual's genetic background and exposure to environmental toxins, which has led to the multiple hit hypothesis of PD, i.e., more than one risk factor trigger disease development and progression (for review see Carvey et al., 2006). In addition, inflammation is emerging

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II. ENVIRONMENTAL HYPOTHESIS OF PARKINSON'S DISEASE
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## A. Toxin Exposure and Animal Models

Epidemiological studies provide strong support for the environmental basis of PD, for they have consistently found a high incidence of PD among people who live in rural areas or are involved in farming (Priyadarshi et al., 2001; Kamel et al., 2007). This increased risk has led scientists to concentrate on exposure to toxins as precipitating factors in disease development. In Table 34.1, we provide a summary of the most common toxins and their effects.

as an important feature of the disease, one that may contribute to the neurodegenerative process (Boka et al., 1994;

Hunot et al., 1999; McGeer et al., 2001). This chapter will

examine the contributions of environmental factors, genetic

TABLE 34.1 Summary of environmental toxins and their effects					
Environmental toxin	Penetration of the blood-brain barrier (BBB)	Mechanism of action	Associated with Parkinsonism or risk of developing PD in humans		
мртр	Lipophilic and readily crosses BBB	Inhibits complex I	Yes		
Paraquat	Carrier-mediated transport across the BBB	Generates oxygen radicals and inhibits complex I with a low potency	Yes		
Maneb	Unknown but may cross with a carrier- mediated process as paraquat does	Inhibits complex III and increases lipid peroxidation	Yes		
Dieldrin	Lipophilic and readily crosses the BBB	Decreases dopamine transport and stimulates glial production of oxygen radicals	Yes		
Rotenone	Lipophilic and readily crosses BBB	Inhibits complex I	Not known		
Heavy metals	Readily cross the BBB	Reduces dopamine production and alters dopamine turnover	Yes		

### 1. 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine

The discovery that the toxic metabolite of MPTP, 1methyl-4-phenylpyridiumn ion (MPP<sup>+</sup>), was a potent complex I inhibitor in dopamine neurons opened a new era in PD research (see also Chapter 38). When this toxin is injected in humans, it replicates most features of sporadic PD (Davis et al., 1979; Langston et al., 1983). In primates other than humans, the effects of MPTP also mimic those in man, and if given acutely or chronically to mice, 40-80% of dopamine neurons in the substantia nigra die (for reviews, see Meredith and Kang, 2006; Meredith et al., 2008). MPTP's actions in rodents are limited, for it has no effect on rats (Zuddas et al., 1994), and despite killing dopamine neurons in mice, frequent injections and large doses are required to reach significant dopamine depletion (Sonsalla and Heikkila, 1986) and, behaviorally, MPTP-treated mice show few of the typical motor symptoms (Meredith and Kang, 2006).

MPTP is highly lipophilic and readily crosses the blood-brain barrier (Markey et al., 1984). Once in the brain, astrocytes, and certain types of neurons, take it up and oxidize the molecule to a protoxin via monoamine oxidase B (Fig. 34.1). This protoxin is then converted to MPP<sup>+</sup>, which is somehow released into the extracellular space, where it is actively transported into dopamine neurons via the dopamine transporter (Fig. 34.1). Once inside the cell, MPP<sup>+</sup> either binds the vesicular monoamine transporter-2 and moves into vesicles, or it penetrates mitochondrial

membranes and disrupts mitochondrial respiration at the level of complex I, or it remains in the cytosol, where it interacts with enzymes carrying negative charges (Ramsay and Singer, 1986; Lee et al., 1992; Klaidman et al., 1993). It is the movement into the mitochondria that damages respiration, reduces ATP production, and augments oxidative stress (Meredith et al., 2004; Przedborski et al., 2004). Other pesticides/fungicides that are similar in their chemical structures to MPTP, are therefore thought to induce Parkinsonism through their ability to inhibit mitochondrial proteins (Fig. 34.1). There are also naturally occurring compounds in nature with a substituted pyridinium structure similar to synthetic pesticides, which can kill dopamine neurons in vitro (Michel et al., 1989).

### 2. Paraquat (PQ)

A quarternary ammonium herbicide, N-N'-dimethyl-4,4'bipyridium dichloride (PQ) structurally resembles MPTP and its toxic metabolite, MPP<sup>+</sup>. Paraquat does not easily cross the blood-brain-barrier (BBB; Shimizu et al., 2001), but once it does, this toxin is transported into the mitochondria by a carrier-mediated process (Shimizu et al., 2001; Dick et al., 2007), where it becomes a potent redox cycler (Thiruchelvam et al., 2005). Neurotoxicity arises with the formation of reactive oxygen and nitrogen species due to inhibition of mitochondrial and cytoplasmic malate dehydrogenase activities (Fukushima et al., 1993). Earlier reports speculated that PQ acts by inhibiting complex I, due to its

**FIGURE 34.1** Schematic diagram illustrating how environmental toxicants reach the mitochondria of a dopamine neuron in the substantia nigra. MPTP crosses the blood–brain barrier and is taken up by astrocytes, where it is oxidized by monoamine oxidase B (MAO-B) to form two metabolites, MPDP+ and MPP+ (Chiba et al., 1984). The metabolite MPP+ is generally considered to be the toxic metabolite, although MPDP+ also has some toxicity but is thought to be the major peripheral metabolite. MPP+ is transported into the dopamine neurons via the dopamine transporter (DAT) and is either sequestered in organelles (see text) but especially in mitochondria, where it targets complex I of the electron transport chain. Many agricultural toxins are lipophilic and penetrate the plasma and mitochondrial membranes targeting either complex I or III in the transport chain. All these toxins augment the production of reactive oxygen and nitrogen species and reduce the synthesis of ATP.

structural similarity to MPTP (Fukushima et al., 1994; Tawara et al., 1996; Dawson and Dawson, 2003). Richardson and colleagues (2005) demonstrated a low in vitro potency of PQ to inhibit complex I, as compared to MPP<sup>+</sup> in isolated mitochondria. Nevertheless, other more recent studies have shown that PQ can become reduced to a cation  $(PQ^+)$ by NAD(P)H dehydrogenase, a process that generates oxygen radicals within the mitochondria (Cocheme and Murphy, 2008). Castello and colleagues (Castello et al., 2007) also show inhibition of the electron transport chain but suggest that superoxide production involves inhibiting complex III. Thus, PQ appears to damage mitochondria, through the enhanced production of oxygen radicals, and its mechanism of action may occur through inhibition of complex I or, possibly, III (Fig. 34.1). Clearly, further work on the structuretoxicity relationship is required to understand how the pyridium ion damages dopamine neurons but, more importantly, why structurally similar ions, such as those generated by MPTP and PQ, could have different mechanisms of action at the level of the mitochondrion.

#### 3. Maneb

Manganese ethylene-bis-dithiocarbamate (Maneb) is a heavy metal fungicide that inhibits glutamate transport and dose-dependently reduces high affinity dopamine uptake and release (Soleo et al., 1996; Vaccari et al., 1996; Vaccari et al., 1998). Maneb induces Parkinsonism in agricultural workers, who are chronically exposed to the fungicide (Ferraz et al., 1988; Meco et al., 1994), and its effects are enhanced when individuals have also been exposed to the pesticide, paraquat. Maneb is used to model PD in mice and is thought to inhibit complex III and increase lipid peroxidation (Fig. 34.1; Thiruchelvam et al., 2005). In in vitro studies, Maneb catalyzes catechol oxidation, which may underlie its selectivity in targeting dopamine neurons (Fitsanakis et al., 2002). It is generally co-administered with paraquat to enhance toxicity in order to destroy a significant number of dopamine neurons (Thiruchelvam et al., 2000a; Thiruchelvam et al., 2000b; Thiruchelvam et al., 2000c).

### 4. Dieldrin

A diorthosubstituted polychlorinated biphenyl, dieldrin, like DDT, is a chlorinated insecticide. These compounds are cyclodiene insecticides. They are stable and are highly lipophilic, thus they cross the BBB readily. They tend to accumulate and persist in nature. They were commonly used from the 1950s to the 1970s and have been detected, postmortem, in the brains of PD patients (Corrigan et al., 1998). Recent studies have demonstrated that developmental exposure of mice to dieldrin leads to persistent changes in the nigrostriatal system and an increased susceptibility to other dopamine toxins (Richardson et al., 2006). Dieldrin decreases striatal expression of the dopamine transporter, thus altering dopamine uptake, but in the absence of dopamine cell loss (Hatcher et al., 2007). This insecticide also stimulates microglia to generate reactive oxygen species (Mao et al., 2007).

#### 5. Rotenone

This is a common pesticide and insecticide, and a natural mitochondrial poison extracted from tropical legumes. It is lipophilic, readily crosses cell membranes, and easily penetrates the BBB (Betarbet et al., 2000). It is a potent mitochondrial inhibitor and targets complex I in a manner



similar to MPTP (Fig. 34.1). Greenamyre and colleagues have shown that rotenone delivered chronically by minipumps kills dopamine neurons and induces a bradykinetic behavioral profile (Betarbet et al., 2000; Sherer et al., 2003b). They have also shown that rotenone-treated rats display other features of PD, including accumulation and aggregation of alpha-synuclein, microgliosis and iron accumulation in the SN, as well as loss of enteric neurons and cardiac sympathetic denervation. Rotenone was originally thought to specifically target the nigrostriatal dopamine system (Hoglinger et al., 2003), but the data support a more general toxicity including damage to peripheral organs such as the stomach and liver as well as induce non-specific CNS damage (Lapointe et al., 2004). In addition, the ability to reproduce this model has proven difficult. More recent work by Greenamyre and colleagues have shown less variability in this model through the use of i.p. injections of the toxin (reviewed in Meredith et al., 2008).

### 6. Other Environmental Toxicants

Substances that may increase the risk of PD include heavy metals, such as manganese and zinc, found in the carbamate herbicides, mancozeb and zineb, respectively. Exposure to these metals over time can lead to detrimental changes in dopamine turnover (Soleo et al., 1996). Lead exposure can also lead to similar changes in dopamine turnover. Lead can replace iron in brain tissue and since iron is a co-factor for L-dopa, it can reduce dopamine production and increase oxidative radicals (Jenner, 1998). Other industrial chemicals such as toluene, trichloroethylene, carbon monoxide, and carbon disulfide have also been linked to PD (Tanner, 1992; Hageman et al., 1999; Gash et al., 2008).

## **B.** Toxin Exposure and Human Susceptibility to PD

Epidemiological studies support the environmental basis for PD (Priyadarshi et al., 2001; Kamel et al., 2007) and experimental animals exposed to environmental toxins mimic many pathological hallmarks of PD thereby strongly implicating toxin exposure in the pathology (Betarbet et al., 2000; Thiruchelvam et al., 2000a; Petroske et al., 2001; Meredith et al., 2002; Przedborski and Vila, 2003; Thiruchelvam et al., 2006; Zeevalk et al., 2007). Nevertheless, there is little evidence to state that toxin exposure leads to the development of PD. Certainly, human exposure to certain industrial chemicals or chronic exposure to toxins such

as dieldrin or maneb, reduce dopamine production in the human brain and increase certain Parkinsonian symptoms, as described above. One important factor to consider is that humans could accumulate these toxins over time and once they reach physiologically relevant quantities, dopamine neuron loss accelerates.

One clear difference between PQ and MPTP is the dicationic nature of PQ and the requirement that PQ is actively transported across the BBB (Shimizu et al., 2001; Dick et al., 2007). Paraquat exposure has been linked with an increase in the risk of contracting PD (Hertzman et al., 1990; Hertzman et al., 1994). Even though PQ does not readily cross the BBB, repeated subchronic exposure could lead to PQ accumulation and damage to the mitochondria (LoPachin and Gavin, 2008). Rotenone also damages mitochondria but has yet to be linked directly to Parkinson's disease in humans. Heavy metal exposure, i.e. iron or manganese, is associated with Parkinsonism in humans. One study identified a small group of professional welders who developed Parkinsonism, but the onset and pathology differed somewhat from those features in other PD patients (Racette et al., 2001).

Exposure to toxins during development may also enhance susceptibility to PD later in life. One study exposed juvenile mice to zineb or the mixture of endosulfan and zineb through i.p. injections over a week and found that this exposure was enough to reduce dopamine levels and increase protein aggregations in the cortex following re-exposure at 8 months of age (Jia and Misra, 2007). Even though this study was conducted in experimental animals, scientists agree that there are significant risks to infants and children, if they are exposed to pesticides (Kimmel and Makris, 2001; Dietert et al., 2002). Finally, the environmental susceptibility to toxins by humans could be related to genetic damage or genetic predisposition (Potashkin and Meredith, 2006; Meredith et al., 2008).

## C. Toxins and Oxidative Stress

Features of oxidative stress, such as ATP loss, neuroinflammation, large accumulations of lysosomes and lipofuscins, and protein aggregation, appear selectively in dopamine neurons following treatment with a variety of toxins. Even though many of the same features appear in cells during the aging process, such characteristics are accelerated in PD and can be mimicked in toxin models of the disease (Dauer and Przedborski, 2003; Meredith et al., 2004; Carvey et al., 2006). Normal mitochondrial respiration is critical to the maintenance of adequate ATP for basic cell processes. Any loss of ATP can disrupt functional ion pumps and reduce the plasma membrane potential, which in turn decreases the activation threshold for N-methyl-D-aspartate (NMDA) receptors (Novelli et al., 1988; Smith and Grace, 1992; Meredith et al., 2009), thus increasing a neuron's vulnerability to damage by glutamate (Blandini et al., 1996; Przedborski and Jackson-Lewis, 1998). Particularly damaging is the production of peroxynitrite and oxygen radicals.

There are data supporting reduced mitochondrial respiration as a causative factor in PD. Post-mortem, the substantia nigra shows a reduction of about 30% in mitochondrial respiration, possibly through damage to complex I (Parker et al., 1989; Schapira et al., 1990a; Schapira et al., 1990b). In addition, humans administered mitochondrial poisons, such as MPTP, develop Parkinsonism (Langston et al., 1983). It is not only dopamine neurons that have a reduced capacity for many normal physiological functions in PD. Recent in vivo imaging (high resolution 31 phosphorous magnetic resonance spectroscopy) of the brain of PD patients found a mitochondrial dysfunction in parts of the cortex not associated the nigrostriatal system. Rango and colleagues (2006) found an energy imbalance in cortical neurons under increased oxidative metabolism requirements. Although these data provide strong evidence for mitochondrial dysfunction in PD, it remains unclear whether mitochondrial defects actually induce the disorder. Certainly individuals with typical mitochondrial diseases do not show the same symptoms as PD patients (Przedborski, 2009). Moreover, since post-mortem studies are conducted on individuals in an advanced disease state with very few remaining dopamine cells, the mitochondrial disturbance may be concentrated in non-dopamine cells.

Oxidative stress remains an important factor for PD progression. Bioenergetic restraints increase cellular stress and greatly augment the formation of radicals (Fariello, 1988; Jenner, 1998), particularly peroxynitrite and oxygen radicals. These harm physiological functions, such as proteolysis, and damage cytosolic proteins. Protein accumulations further contribute to cellular stress and presumably form Lewy bodies, which are large, "wagon-wheel" accumulations of insoluble proteins.

### **D.** Inclusion Formation

Misfolded, unassembled or damaged proteins, and in particular alpha-synuclein, are potentially toxic to the cell, and must therefore be rapidly cleared from the cytoplasm, a process generally attributed to the ubiquitin-proteasomal system (UPS). However, the UPS is energy expensive and reserved for proteins with short half-lives (Glickman and Ciechanover, 2002; Cuervo, 2004). Disruptions in ATP production could also saturate the UPS. Such saturation would help explain why ubiquitinated complexes accumulate in the dopamine cells of PD midbrains (Gai et al., 2000; McNaught and Jenner, 2001). These proteins accumulate in circular bodies called Lewy bodies (see Box 34.1) in the cytoplasm of dopamine neurons.

Animal models do not adequately replicate Lewy body formation. Eosinophilic inclusions have been reported in old primates treated with MPTP (Forno et al., 1986; Forno et al., 1993), but Lewy bodies are seemingly absent from nigral dopamine cells in humans, who became Parkinsonian by injecting MPTP (Langston et al., 1999). There are limited reports of alpha-synuclein-positive aggregates in the chronic MPTP mouse model, but only in mice that had lost more than 70 percent of their dopamine neurons after treatment (Meredith et al., 2002; Meredith et al., 2004). However, the aggregates are granular and do not resemble Lewy bodies (Fig. 34.2). Other groups using the same MPTP regimen failed to find alpha-synucleinpositive inclusions (Shimoji et al., 2005; Alvarez-Fischer et al., 2008), but dopamine cell loss was considerably less (40%; Alvarez-Fischer et al., 2008). In a rotenone rat model, where cell death in the substantia nigra is progressive, alpha-synuclein-immunoreactive inclusions are found in the substantia nigra neurons (Betarbet et al., 2000). No inclusions were seen in rotenone-treated rats that had no dopamine lesions. In addition, the inclusions in the rotenone model were small, but had a solid core with a fibrillar halo as seen in Lewy bodies. Data from these models suggest that this protein pathology appears once oxidative damage and cell loss have become extensive. Certainly, rotenone-induced inhibition of complex I in cell cultures progressively damages the cells oxidatively (Sherer et al., 2002). Also, inhibition of complex I, and augmented oxidative stress promote alpha-synclein aggregation (Masliah et al., 2000; Parihar et al., 2008).

The current concept of protein aggregation in PD points to protein stress and proteolytic failure as underlying causes, but how alpha-synuclein contributes to proteinaceous aggregations is not clear. Initially, reports described alpha-synuclein as being degraded by the UPS (Bennett et al., 1999; Tofaris et al., 2001), suggestions that led to the theory that malfunction in the UPS degradation of

#### Box 34.1 Lewy Bodies

Distinctive neuronal inclusions considered a diagnostic hallmark of idiopathic Parkinson's disease are called Lewy bodies (Beal, 2001). Lewy bodies are eosinophilic structures in the neuronal cytoplasm. Immunohistochemically, they stain with a variety of antibodies, including those raised against neurofilament, ubiquitin, and alpha-synuclein (Spillantini et al., 1997; Spillantini et al., 1998). In the brainstem, they are usually circular with a dense protein/ lipid core surrounded by a peripheral halo (Roy and Wolman, 1969). In the cerebral cortex, they are often much less eosinophilic, more elongate and diffuse than the brainstem bodies. In the cortex, they also may lack the peripheral halo (Meredith et al., 2002; Katsuse et al., 2003). Lewy bodies may be single or multiple within a neuron and their size can vary. Ultrastructurally, they have a dense central core, which is lipid-rich and surrounded by radiating filaments (7 to 20 nm in diameter; Meredith et al., 2004). In the cortex, Lewy bodies have a random arrangement of intermediate filaments but which lack a radial orientation. Lipids are a significant proportion of cortical Lewy bodies and tend to be concentrated in the core if the bodies are of a concentric formation. Alpha-synuclein and ubiquitin in the cortical bodies appear in overlapping layers or are partial segregated into layers (Gai et al., 2000).

Lewy bodies may result from disrupted neurofilament transport or metabolism leading to an accumulation of altered cytoskeletal elements including a variety of insoluble proteins (Fig. 34.3). Lewy bodies are also present in other neurodegenerative conditions such as Lewy body dementia. They are also found in older people with no neurological or psychiatric disorders, but at lower densities than in neurodegenerative conditions (Del Tredici et al., 2002).

The fibrillization and aggregation of proteins such as alpha-synuclein may represent the pathological state of the neuron or simply be a hallmark of the ongoing degeneration (Goldberg and Lansbury, 2000). Regardless, these inclusions disrupt the cytoskeleton of the cell (Braak and Braak, 2000), and could even trigger the demise of the neuron. Complex I dysfunction and the ensuing oxidative damage to cells seems to promote protein aggregation (for reviews see Dawson and Dawson, 2003; Meredith et al., 2004). This is because oxidative radicals can damage proteins and promote formation of intermediate fibrillization stages. Thus, oxidative stress and dysfunction of complex I may be central to the spiral that leads to protein aggregation and cell death (Fig. 34.4).

alpha-synuclein is a cause of PD. However, subsequent studies that inhibited the UPS were unable to confirm changes in cellular quantities of alpha-synuclein (Ancolio et al., 2000; Rideout et al., 2001; Rideout and Stefanis, 2002). Proteins with long half-lives, such as alpha-synuclein, appear instead to be degraded by autophagic pathways in lysosomes. Lysosomal inhibitors can increase the aggregation of alpha-synuclein (Webb et al., 2003; Lee et al., 2004) and mutated alpha-synuclein impairs the trafficking of this protein to lysosomes by chaperone-mediated autophagy (Cuervo, 2004). Autophagy plays a neuroprotective role by rapidly trafficking proteins and organelles to lysosomes for degradation. Such continuous turnover of molecules is important for it prevents the accretion of toxic residues and can even stave off apoptosis (Kiffin et al., 2004; Komatsu et al., 2007). However, this recycling is energetically expensive and under conditions of prolonged stress, proteins can become misfolded and need assistance from chaperones for degradation (Cuervo, 2004). Recent work has shown that when wild-type alpha-synuclein interacts with oxidized dopamine, the end product is able to block chaperonemediated autophagy, an interference that could lead to detrimental accumulations of alpha-synuclein in the cytoplasm of dopamine neurons (Martinez-Vicente et al., 2008).

Proteins and organelles can form lipofuscin granules inside lysosomes, by an iron-catalyzed oxidation of the protein and lipid residues (Meredith et al., 2004). These granules sensitize lysosomes to oxidative stress (Terman and Brunk, 2004) and oxidative radicals, such as hydrogen peroxide, diffuse easily into the granules where they reduce ferrous iron and form peroxidation byproducts, such as protein carbonyls (Dauer and Przedborski, 2003; Meredith et al., 2004). The low pH in the lysosomal compartments is an environment compatible with these activities. Lipofuscin granules accumulate with age, but grow rapidly in number and augment their load of indigestible residues in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Mayer et al., 1992; Nixon et al., 1992; Braak et al., 2001; Meredith et al., 2004; Terman and Brunk, 2004). Lipofuscins can interfere with autophagy, which suggests that these granules represent lysosomal dysfunction and a decline in proteolysis (Terman and Brunk, 2004). It seems reasonable therefore to assume that over time in neurodegenerative disease, abnormal residues and their chaperones increase. Lysosomes overburdened with lipofuscin granules and lipid lyse, releasing their toxic, low pH, contents into the cytosol, an action that could lead to the seeding of Lewy bodies (Fig. 34.3), or the protein load leads to cellular exhaustion and a second type of programmed cell death, i.e. autophagic death (Alirezaei et al., 2008). Protein aggregation may also be associated with the failure to scavenge oxidative radicals (Sherer et al., 2002; Testa et al., 2005).



**FIGURE 34.2** Ultrastructural micrographs of cytoplasmic inclusions in dopamine neurons of the substantia nigra of mice treated chronically with MPTP. (A) A tyrosine hydroxylase-immunoreactive neuron. Arrows and inset point to proteinaceous accumulations in the cytoplasm. Scale bar =  $5 \mu m$  (B) Higher power EM micrograph illustrating membrane-bound lysosomal bodies filled with lipids and immunoreactive protein (arrowhead) in an alpha-synuclein-immunopositive (asterisks mark the immunoreaction product) neuron. Scale bar =  $1 \mu m$ . (C) lipofuscin granules and protein accumulation (arrowhead) alongside parallel filaments (asterisk). Scale bar =  $1 \mu m$ . (D) Large lysosomes with protein accumulations and parallel membranes resembling debris from mitochondria and lipid (asterisk). Scale bar =  $1 \mu m$ . Figure adapted from Meredith and colleagues (2002) and reprinted by permission from Brain Research.



FIGURE 34.3 Schematic diagram that proposes a mechanism for proteolysis of alpha-synuclein in dopamine neurons compromised by toxins. (A) Insoluble alpha-synuclein in beta sheet formation, i.e. protofibrils (red), is tagged by ubiquitin for degradation. (B) Alpha-synuclein is transported via chaperone-mediated autophagy for degradation in lysosomes (Cuervo, 2004). (C) Lysosomes gather proteins and membranes for break down in the low pH compartment. Lipid droplets are illustrated in gray and represent byproducts of membrane recycling. (D) Lysosomes erupt when overloaded and energy supplies are insufficient to support their proteolytic activities. Cells undergo protein stress and enter death pathways (programmed either via apoptosis or autophagy) when cytochrome c from mitochondria leaks into the cytoplasm and ruptured lysosomes release acidic contents. If cell death pathways are not activated, (E) the molecules released from lysosomes could "seed" the Lewy body formation. Each Lewy body has a dense lipid core and radiating filaments of alpha-synuclein and ubiquitin (adapted from Meredith et al., 2004). To view a color version of this image please visit http:// www.elsevierdirect.com/companion/9780123747679

## III. ENVIRONMENTAL TOXINS AND INFLAMMATION

In normal adult brain, microglia display ramified morphology and, together with astrocytes, play a key role in the immune response. Microglia are the resident lymphocytes of the central nervous system, comprising nearly a fifth of all brain cells. When an infection, a toxic insult or trauma occurs, these glia proliferate and move from the resting to the activated state. They morph from their small, spider-like state into an amoeboid form with enlarged cytoplasmic processes capable of phagocytosis (Giulian, 1987). Activated microglia upregulate cell surface molecules such as complement receptors and secrete a range of neurotrophic molecules (Nakajima et al., 2001). *In vitro* studies point to a role in neuronal survival, because microglia produce neurotrophins, including brain derived neurotrophic factor and neurotrophin 4/5 (Miwa et al., 1997). However, when the environment signals a need to clear damaged neurons or debris, microglia secrete pro-inflammatory molecules. The phagocytic activity is beneficial, as it effectively removes dead and dying cells, and microglia may be directed to the site of the lesion by nitric oxide, one of these factors (Duan et al., 2009). However, dysregulation or excessive activation increase cytokine signaling by microglia, a process that causes further damage to struggling cells and has been reported in the substantia nigra of PD patients postmortem (Boka et al., 1994; Hunot et al., 1999; Liu et al., 2000).

Nigral dopamine neurons may be selectively at risk due to the high levels of microglia in this brain region (Lawson et al., 1990; Kim et al., 2000). The chronic inflammatory response seen in the chronic MPTP and rotenone rodent models of PD is consistent with that described for human PD (McGeer et al., 1988; Meredith et al., 2003; Sherer et al., 2003a). Moreover, the observation that microgliosis persists for years in humans and non-human primates following acute exposure to MPTP (Langston et al., 1999; McGeer et al., 2003), indicates that the inflammatory response continues in the absence of more exposures to the neurotoxicant. Recent studies by Benner and colleagues (2004) and Brochard et al. (2009) suggest an important role for the CD4<sup>+</sup> T cells in MPTP-treated mice. Infiltration of T cells leads to accelerated dopamine cell death in the substantia nigra in this model. Therefore, the current view that microglial activation and T cell production in PD are part of an ongoing, self-reinforcing cycle of inflammation is compelling, since such activities arise initially from toxin exposure or infectious agents (Liu and Hong, 2003), and could contribute to disease progression.

## IV. ENVIRONMENTAL TOXINS AND GENETIC VULNERABILITY

Most cases of PD are sporadic and a strong environmental influence is likely. Nevertheless, the argument for a genetic component is strong (see Box 34.2), even though recent epidemiological studies suggest that the risk for offspring of patients with PD is the same as that for nieces and nephews. There is significant familial clustering beyond the nuclear family (Sveinbjornsdottir et al., 2000). Certain genes that regulate mitochondrial function and proteolysis are mutated in familial PD (Box 34.2), and several genes have been identified in these cases but these cases are rare (Dawson and Dawson, 2003). Animal models created from these mutations share important defects with toxin-induced models giving rise to the expectation that a certain genetic profile could increase susceptibility for disease (see Meredith et al., 2008 for review). Moreover, oxidative radicals augmented by mitochondrial defects from environmental toxins can have devastating effects on

genes and permanently alter gene products, changes that enhance oxidative stress, inflammation and lead to cell death (Potashkin and Meredith, 2006). The identification of susceptibility gene(s) in PD and determining how those genes are altered, could help identify the environmental "triggers" for gene expression.

The current thinking that environmental and genetic factors interact means that disease development could involve molecular changes that contribute to altered gene or protein expression. The importance of protein ubiquitination for degradation is well understood (see above) and mutations in certain genes, such as Parkin, produce a "loss-offunction" in ubiquitination and lead to familial forms of PD (Marin and Ferrus, 2002). The fact that dysregulation of some of the same genes in sporadic PD highlights the importance of oxidative stress and protein misfolding and is fundamental to disease development and progression (Dauer, 2003). Furthermore, free radical-mediated cellular damage may be caused by endogenous processes, such as inflammation (see above) or physiological stressors from the environment (toxins). During mitochondria respiration, most oxygen atoms are reduced to water but a small percentage escapes as reactive oxygen species. Energy released from heat or radiation increases the release of these radicals. Free radicals pair with other hydrogen atoms and thereby exert oxidant stress on cellular substrates (Sacheck and Blumberg, 2001). Nuclear DNA can be affected by these radicals, through nicking, even though the nucleus is poorly oxygenated and the DNA is bound by histones that quench radicals (Wei, 1998). Mitochondrial DNA is more sensitive than nuclear DNA to oxidative damage because of its proximity to the respiratory chain, the absence of protective histones, and limited DNA repair capabilities (Richter et al., 1988; Wei, 1998). Nucleic acid damage by oxidation produces 8-hydroxyguanosine (80HG) immunoreactivity, which can be used as a marker for evaluating the effect of oxidative stress on nucleic acids (Potashkin and Meredith, 2006). This molecule, which can be induced by environmental toxins, permanently damages cytoplasmic RNA and mitochondrial DNA, and there is evidence that 80HG immunoreactivity increases in neurons of the substantia nigra in PD (Zhang et al., 1999).

Free radicals induce apoptosis by increasing mitochondrial membrane permeability and the release of cytochrome c into the cytosol (Lee and Wei, 2005). Offspring of maternal victims of PD show deficits in complex I that would increase production of these radicals (Swerdlow et al., 1998). Below critical levels, oxidative radicals presumably induce stress responses by altering the expression

#### Box 34.2 Parkinson's Disease Mutations

Most PD cases arise without a family history of the disease. There are however several mutations linked to familial PD. These include mutations in PARK1, LRRK2, PARK2, PARK6, and PARK7 genes (Gasser, 2001a). The genes GBA, SNCAIP and UCH-L1 are associated with familial PD (Gasser, 2001a, b). Polymorphisms at the PARK1, PARK2 and PARK8 loci have been implicated in idiopathic PD (Farrer et al., 2001). In addition, genes that regulate dopamine transmission and xenobiotic metabolism have been associated with sporadic PD (Gasser, 2001a,b).

#### Loci and mode of inheritance for known mutations involved in familial PD

Locus	Chromosome location	Gene	Mode of inheritance	Possible role
PARK1 (also known as SNCA)	4q21	Alpha-synuclein	Autosomal dominant	Sustaining neurotransmission; "gain- of-function" detrimental effect
PARK2	6q25-27	Parkin	Autosomal recessive	Protein degradation
PARK3	2p13	?SPR (enzyme in tetrahydrobiopterin biosynthesis)	Autosomal dominant	
PARK4	4p15-16	Unknown (now known to be a form of PARK1)	Autosomal dominant	
PARK5	4p14	UCH-L1	Autosomal dominant	Protein degradation
PARK6	1p35-37	PINK1	Autosomal recessive	Mitochondrial protection
PARK7	1p36	DJ-1	Autosomal recessive	Protect from oxidative stress
PARK8	12p11.2-q13.1	LRRK2	Autosomal dominant	Protein-protein interactions
PARK9	1p36	ATP13A2	Autosomal recessive	P5 subfamily of ATPases
PARK10	1p32	?USP24	Late-onset susceptibility gene	Ubiquitin specific peptidase
	1q21	GBA		Active in lysosomes
SNCAIP	5q23.1-23.3	Synphilin 1		Synaptic function

of nuclear genes in order to try to rescue the cell. However, persistent upregulation of oxidative stress damages DNA and its protein products ultimately leading to cell death. The interaction of the environment with genetic factors is poorly understood in PD, but the implications of cross-talk between them are strong. It appears therefore that disease development in PD could lie in the toxin-induced oxidative damage to DNA and/or the toxic products of the mutated genes.

There are many levels at which the exposure to toxins in the environment might interact with genetic mutations to produce PD. Although some mutations are sufficient to produce the rare cases of familial PD presumably without toxin exposure, we consider that certain genetic mutations will increase the susceptibility to damage. A mutation that reduces the individual's ability to degrade abnormally folded proteins is an obvious example, but it is also likely that differences in toxin metabolism might influence vulnerability. Mutations in one particular gene, LRRK2, were first identified in 2004 in familial cases of PD (Paisan-Ruiz et al., 2004), and soon after, the G2019S mutation in the LRRK2 gene was found in 1–2% of sporadic PD cases (Gilks et al., 2005). Although there is a lack of a Mendelian pattern of inheritance for this mutation, there is digenic or polygenic inheritance. There is also a large variability in onset age and other clinical features, even among members of the same G2019S family (Bonifati, 2006). As yet, we have no data on whether environmental factors play a role in the sporadic PD cases with the G2019S mutation, but it is not unreasonable to assume that such factors could modify the expression and progression of the disease in these individuals.

### V. SUMMARY AND CONCLUSIONS

Environmental contributions to PD indicate that common features of damage caused by environmental toxins include the disruption of mitochondrial respiration and ensuing energy deficits. This energy deficiency can lead either directly, or indirectly via the production of damaging free radicals, to abnormal regulation of protein production and destruction, which may underlie the characteristic cell death in PD (Fig. 34.4). Inflammation resulting directly from toxin exposure can contribute to the ultimate dopamine denervation and that such degeneration might trigger further inflammation and damage DNA through the release of dangerous radicals, in agreement with the multiple hit hypothesis that is widely accepted by PD researchers (Carvey et al., 2006). We try to distinguish between changes in the brain that might be causative and those, which might underpin disease progression. What is interesting is that both protein degradation pathways and inflammation are usually regarded as neuroprotective and it is the abnormalities in these processes that are destructive.

Another attractive proposition for environmentalgenetic interactions in PD is that the toxic process itself can induce alterations in an individual's DNA, particularly since mitochondrial DNA would seem to be particularly at risk to this form of damage. The question of whether there is a difference between age-related changes in neuroprotective processes and toxin-induced degeneration may be explained by these observations. It is likely that deterioration in mitochondrial DNA occurs with age, and changes will be cumulative. Perhaps exposure to toxins speeds up this normal process in idiopathic PD. Certain DNA sequences might be particularly at risk to this sort of oxidative stress-induced damage, which would explain why some of the degenerative processes seen in PD resemble accelerated age-related alterations.

In summary, it seems likely that PD is multifactorial and does not reflect the same causes in all sufferers. In some, a single gene mutation results in onset of the (familial) condition, in others accidental exposure to a toxin in the environment is sufficient to trigger the (sporadic) illness. In



**FIGURE 34.4** Illustration of the multifactorial basis for PD. Exposure to environmental factors such as toxins, gives rise to mitochondrial defects, which can damage genes and lead to protein accumulations through impaired transcription, translation and/or proteolysis. Inherited, mutated genes may also lead to the same chronic protein stress. Autophagy, a neuroprotective mechanism that can even rescue neurons from apoptosis, shifts the cell towards degeneration and death pathways if the build-up of abnormal protein residues and their chaperones lead to cellular exhaustion. Inflammation, generally regarded as neuroprotective, can also shift the cell towards death once pro-inflammatory cytokines are released from activated glial cells.

both cases, the patients show a similar pathology, which includes abnormal metabolism and dysfunctional handling of proteins. Added to these, there is a large population of PD patients who have been exposed to toxins. In some of these, one or more mutations will predispose them to damage by the toxin. In the remaining patients, the effects of toxin exposure probably interact with the mitochondrial or nuclear DNA damage to enhance toxic effects. Finally, similarities exist between the pathology of normal aging and PD because both processes involve the more vulnerable parts of the mitochondrial genome and mitochondrial function.

### REFERENCES

- Alirezaei M, Kiosses WB, Flynn CT, Brady NR, Fox HS (2008) Disruption of neuronal autophagy by infected microglia results in neurodegeneration. PLoS ONE 3:e2906.
- Alvarez-Fischer D, Guerreiro S, Hunot S, et al. (2008) Modelling Parkinson-like neurodegeneration via osmotic minipump delivery of MPTP and probenecid. J Neurochem 107:701–711.
- Ancolio K, Alves da Costa C, Ueda K, Checler F (2000) Alpha-synuclein and the Parkinson's disease-related mutant Ala53Thr-alpha-synuclein do not undergo proteasomal degradation in HEK293 and neuronal cells. Neurosci Lett 285:79–82.

- Beal MF (2001) Experimental models of Parkinson's disease. Nat Rev Neurosci 2:325–334.
- Benner EJ, Mosley RL, Destache CJ, et al. (2004) Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. Proc Natl Acad Sci USA 101:9435–9440.
- Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM (1999) Degradation of alpha-synuclein by proteasome. J Biol Chem 274:33855–33858.
- Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3:1301–1306.
- Blandini F, Porter RH, Greenamyre JT (1996) Glutamate and Parkinson's disease. Mol Neurobiol 12:73–94.
- Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC (1994) Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett 172:151–154.
- Bonifati V (2006) Parkinson's disease: the LRRK2-G2019S mutation: opening a novel era in Parkinson's disease genetics. Eur J Hum Genet 14:1061–1062.
- Braak E, Sandmann-Keil D, Rub U, Gai WP, de Vos RAI, Steur ENHJ, Arai K, Braak H (2001) Alpha-synuclein immunopositive Parkinson's disease-related inclusion bodies in lower brain stem nuclei. Acta Neuropathol (Berl) 101:195–201.
- Braak H, Braak E (2000) Pathoanatomy of Parkinson's disease. J Neurol 247(Suppl 2):II3–10.
- Brochard V, Combadiere B, Prigent A, et al. (2009) Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 119:182–192.
- Carvey PM, Punati A, Newman MB (2006) Progressive dopamine neuron loss in Parkinson's disease: the multiple hit hypothesis. Cell Transplant 15:239–250.
- Castello PR, Drechsel DA, Patel M (2007) Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. J Biol Chem 282:14186–14193.
- Chiba K, Trevor A, Castagnoli N, Jr. (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Commun 120:574–578.
- Cocheme HM, Murphy MP (2008) Complex I is the major site of mitochondrial superoxide production by paraquat. J Biol Chem 283:1786–1798.
- Corrigan FM, Murray L, Wyatt CL, Shore RF (1998) Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. Exp Neurol 150:339–342.
- Cuervo AM (2004) Autophagy: many paths to the same end. Mol Cell Biochem 263:55–72.
- Dauer W, Przedborski S (2003) Parkinson's disease: Mechanisms and models. Neuron 39:889–909.
- Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, Kopin IJ (1979) Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1:249–254.
- Dawson TM, Dawson VL (2003) Molecular pathways of neurodegeneration in Parkinson's disease. Science 302:819–822.
- Del Tredici K, Rub U, De Vos RA, Bohl JR, Braak H (2002) Where does parkinson disease pathology begin in the brain? J Neuropathol Exp Neurol 61:413–426.
- Dick FD, De Palma G, Ahmadi A, et al. (2007) Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study. Occup Environ Med 64:666–672.
- Dietert RR, Lee JE, Bunn TL (2002) Developmental immunotoxicology: emerging issues. Hum Exp Toxicol 21:479–485.

- Duan Y, Sahley CL, Muller KJ (2009) ATP and NO dually control migration of microglia to nerve lesions. Dev Neurobiol 69:60–72.
- Fariello RG (1988) Experimental support for the implication of oxidative stress in the genesis of parkinsonian syndromes. Funct Neurol 3:407–412.
- Farrer M, Maraganore DM, Lockhart P, et al. (2001) alpha-Synuclein gene haplotypes are associated with Parkinson's disease. Hum Mol Genet 10:1847–1851.
- Ferraz HB, Bertolucci PH, Pereira JS, Lima JG, Andrade LA (1988) Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology 38:550–553.
- Fitsanakis VA, Amarnath V, Moore JT, Montine KS, Zhang J, Montine TJ (2002) Catalysis of catechol oxidation by metal-dithiocarbamate complexes in pesticides. Free Radic Biol Med 33:1714–1723.
- Forno LS, DeLanney LE, Irwin I, Langston JW (1993) Similarities and differences between MPTP-induced parkinsonsim and Parkinson's disease. Neuropathologic considerations. Adv Neurol 60:600–608.
- Forno LS, Langston JW, DeLanney LE, Irwin I, Ricaurte GA (1986) Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. Ann Neurol 20:449–455.
- Fukushima T, Yamada K, Isobe A, Shiwaku K, Yamane Y (1993) Mechanism of cytotoxicity of paraquat. I. NADH oxidation and paraquat radical formation via complex I. Exp Toxicol Pathol 45:345–349.
- Fukushima T, Yamada K, Hojo N, Isobe A, Shiwaku K, Yamane Y (1994) Mechanism of cytotoxicity of paraquat. III. The effects of acute paraquat exposure on the electron transport system in rat mitochondria. Exp Toxicol Pathol 46:437–441.
- Gai WP, Yuan HX, Li XQ, Power JT, Blumbergs PC, Jensen PH (2000) In situ and in vitro study of colocalization and segregation of alpha-synuclein, ubiquitin, and lipids in Lewy bodies. Exp Neurol 166:324–333.
- Gash DM, Rutland K, Hudson NL, et al. (2008) Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity. Ann Neurol 63:184–192.
- Gasser T (2001a) Genetics of Parkinson's disease. J Neurol 248:833-840.
- Gasser T (2001b) Molecular genetics of Parkinson's disease. Adv Neurol 86:23–32.
- Gilks WP, Abou-Sleiman PM, Gandhi S, et al. (2005) A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 365:415–416.
- Giulian D (1987) Ameboid microglia as effectors of inflammation in the central nervous system. J Neurosci Res 18:155–171.
- Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428.
- Goldberg MS, Lansbury PT, Jr. (2000) Is there a cause-and-effect relationship between alpha-synuclein fibrillization and Parkinson's disease? Nat Cell Biol 2:E115–119.
- Hageman G, van der Hoek J, van Hout M, van der Laan G, Steur EJ, de Bruin W, Herholz K (1999) Parkinsonism, pyramidal signs, polyneuropathy, and cognitive decline after long-term occupational solvent exposure. J Neurol 246:198–206.
- Hatcher JM, Richardson JR, Guillot TS, et al. (2007) Dieldrin exposure induces oxidative damage in the mouse nigrostriatal dopamine system. Exp Neurol 204:619–630.
- Hertzman C, Wiens M, Bowering D, Snow B, Calne D (1990) Parkinson's disease: a case-control study of occupational and environmental risk factors. Am J Ind Med 17:349–355.
- Hertzman C, Wiens M, Snow B, Kelly S, Calne D (1994) A casecontrol study of Parkinson's disease in a horticultural region of British Columbia. Mov Disord 9:69–75.

- Hoglinger GU, Feger J, Prigent A, et al. (2003) Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. J Neurochem 84:491–502.
- Hunot S, Dugas N, Faucheux B, et al. (1999) FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. J Neurosci 19:3440–3447.
- Jenner P (1998) Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov Disord 1:24–34.
- Jia Z, Misra HP (2007) Developmental exposure to pesticides zineb and/ or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals later in life. Neurotoxicology 28:727–735.
- Kamel F, Tanner C, Umbach D, et al. (2007) Pesticide exposure and selfreported Parkinson's disease in the agricultural health study. Am J Epidemiol 165:364–374.
- Katsuse O, Iseki E, Marui W, Kosaka K (2003) Developmental stages of cortical Lewy bodies and their relation to axonal transport blockage in brains of patients with dementia with Lewy bodies. J Neurol Sci 211:29–35.
- Kiffin R, Christian C, Knecht E, Cuervo AM (2004) Activation of chaperone-mediated autophagy during oxidative stress. Mol Biol Cell 15:4829–4840.
- Kim WG, Mohney RP, Wilson B, Jeohn GH, Liu B, Hong JS (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. J Neurosci 20:6309–6316.
- Kimmel CA, Makris SL (2001) Recent developments in regulatory requirements for developmental toxicology. Toxicol Lett 120:73–82.
- Klaidman LK, Adams JD, Jr., Leung AC, Kim SS, Cadenas E (1993) Redox cycling of MPP+: evidence for a new mechanism involving hydride transfer with xanthine oxidase, aldehyde dehydrogenase, and lipoamide dehydrogenase. Free Radic Biol Med 15:169–179.
- Komatsu M, Ueno T, Waguri S, Uchiyama Y, Kominami E, Tanaka K (2007) Constitutive autophagy: vital role in clearance of unfavorable proteins in neurons. Cell Death Differ 14:887–894.
- Langston JW, Langston EB, Irwin I (1984) MPTP-induced parkinsonism in human and non-human primates – clinical and experimental aspects. Acta Neurol Scand Suppl 100:49–54.
- Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979–980.
- Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 46:598–605.
- Lapointe N, St-Hilaire M, Martinoli MG, Blanchet J, Gould P, Rouillard C, Cicchetti F (2004) Rotenone induces non-specific central nervous system and systemic toxicity. Faseb J 18:717–719.
- Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151–170.
- Lee EH, Liu SP, Lu KT, Lin WR (1992) Comparative studies of the neurotoxicity of MPTP in rats of different ages. Chin J Physiol 35:317–336.
- Lee HC, Wei YH (2005) Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. Int J Biochem Cell Biol 37:822–834.
- Lee HJ, Khoshaghideh F, Patel S, Lee SJ (2004) Clearance of alphasynuclein oligomeric intermediates via the lysosomal degradation pathway. J Neurosci 24:1888–1896.

- Liu B, Hong JS (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther 304:1–7.
- Liu B, Du L, Hong JS (2000) Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation. J Pharmacol Exp Ther 293:607–617.
- LoPachin RM, Gavin T (2008) Response to "Paraquat: the red herring of Parkinson's disease research". Toxicol Sci 103:219–22; author reply 222–213.
- Mao H, Fang X, Floyd KM, Polcz JE, Zhang P, Liu B (2007) Induction of microglial reactive oxygen species production by the organochlorinated pesticide dieldrin. Brain Res 1186:267–274.
- Marin I, Ferrus A (2002) Comparative genomics of the RBR family, including the Parkinson's disease-related gene parkin and the genes of the ariadne subfamily. Mol Biol Evol 19:2039–2050.
- Markey SP, Johannessen JN, Chiueh CC, Burns RS, Herkenham MA (1984) Intraneuronal generation of a pyridinium metabolite may cause drug- induced parkinsonism. Nature 311:464–467.
- Martinez-Vicente M, Talloczy Z, Kaushik S, et al. (2008) Dopaminemodified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest 118:777–788.
- Masliah E, Rockenstein E, Veinbergs I, et al. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287:1265–1269.
- Mayer RJ, Landon M, Laszlo L, Lennox G, Lowe J (1992) Protein processing in lysosomes: the new therapeutic target in neurodegenerative disease. Lancet 340:156–159.
- McGeer PL, Yasojima K, McGeer EG (2001) Inflammation in Parkinson's disease. Adv Neurol 86:83–89.
- McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38:1285–1291.
- McGeer PL, Schwab C, Parent A, Doudet D (2003) Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. Ann Neurol 54:599–604.
- McNaught KS, Jenner P (2001) Proteasomal function is impaired in substantia nigra in Parkinson's disease. Neurosci Lett 297:191–194.
- Meco G, Bonifati V, Vanacore N, Fabrizio E (1994) Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bisdithiocarbamate). Scand J Work Environ Health 20:301–305.
- Meredith GE, Kang UJ (2006) Behavioral models of Parkinson's disease in rodents: a new look at an old problem. Mov Disord 21:1595–1606.
- Meredith GE, Dervan AG, Totterdell S (2003) Microglial-neuronal interactions in the substantia nigra pars compacta in the chronic MPTP/ probenecid-treated mouse: An ultrastructural study. Intl PD Symp Abs.
- Meredith GE, Sonsalla PK, Chesselet MF (2008) Animal models of Parkinson's disease progression. Acta Neuropathol 115:385–398.
- Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC, Jr., Lau YS (2002) Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. Brain Res 956:156–165.
- Meredith GE, Halliday GM, Totterdell S (2004) A critical review of the development and importance of proteinaceous aggregates in animal models of Parkinson's disease: New insights into Lewy body formation. Parkinsonism Relat Disord 10:191–202.
- Meredith GE, Totterdell S, Beales M, Meshul CK (2009) Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. Exp Neurol 219:334–340.

- Michel PP, Dandapani BK, Sanchez-Ramos J, Efange S, Pressman BC, Hefti F (1989) Toxic effects of potential environmental neurotoxins related to 1-methyl-4-phenylpyridinium on cultured rat dopaminergic neurons. J Pharmacol Exp Ther 248:842–850.
- Miwa T, Furukawa S, Nakajima K, Furukawa Y, Kohsaka S (1997) Lipopolysaccharide enhances synthesis of brain-derived neurotrophic factor in cultured rat microglia. J Neurosci Res 50:1023–1029.
- Nakajima K, Honda S, Tohyama Y, Imai Y, Kohsaka S, Kurihara T (2001) Neurotrophin secretion from cultured microglia. J Neurosci Res 65:322–331.
- Nixon RA, Cataldo AM, Paskevich PA, Hamilton DJ, Wheelock TR, Kanaley-Andrews L (1992) The lysosomal system in neurons. Involvement at multiple stages of Alzheimer's disease pathogenesis. Ann NY Acad Sci 674:65–88.
- Novelli A, Reilly JA, Lysko PG, Henneberry RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res 451:205–212.
- Paisan-Ruiz C, et al. (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44:595–600.
- Parihar MS, Parihar A, Fujita M, Hashimoto M, Ghafourifar P (2008) Mitochondrial association of alpha-synuclein causes oxidative stress. Cell Mol Life Sci 65:1272–1284.
- Parker WD, Jr., Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 26:719–723.
- Petroske E, Meredith GE, Callen S, Totterdell S, Lau YS (2001) Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. Neuroscience 106:589–601.
- Potashkin JA, Meredith GE (2006) The role of oxidative stress in the dysregulation of gene expression and protein metabolism in neurodegenerative disease. Antioxid Redox Signal 8:144–151.
- Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS (2001) Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res 86:122–127.
- Przedborski S (2009) Mitochondria are a primary problem in the cause of Parkinson's disease no. Moving Along 13:1–10.
- Przedborski S, Jackson-Lewis V (1998) Experimental developments in movement disorders: update on proposed free radical mechanisms. Curr Opin Neurol 11:335–339.
- Przedborski S, Vila M (2003) The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease. Ann NY Acad Sci 991:189–198.
- Przedborski S, Tieu K, Perier C, Vila M (2004) MPTP as a mitochondrial neurotoxic model of Parkinson's disease. J Bioenerg Biomembr 36:375–379.
- Racette BA, McGee-Minnich L, Moerlein SM, Mink JW, Videen TO, Perlmutter JS (2001) Welding-related parkinsonism: clinical features, treatment, and pathophysiology. Neurology 56:8–13.
- Ramsay RR, Singer TP (1986) Energy-dependent uptake of N-methyl-4phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine, by mitochondria. J Biol Chem 261:7585–7587.
- Rango M, Bonifati C, Bresolin N (2006) Parkinson's disease and brain mitochondrial dysfunction: a functional phosphorus magnetic resonance spectroscopy study. J Cereb Blood Flow Metab 26:283–290.
- Richardson JR, Quan Y, Sherer TB, Greenamyre JT, Miller GW (2005) Paraquat neurotoxicity is distinct from that of MPTP and rotenone. Toxicol Sci 88:193–201.
- Richardson JR, Caudle WM, Wang M, Dean ED, Pennell KD, Miller GW (2006) Developmental exposure to the pesticide dieldrin alters the

dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. Faseb J 20:1695–1697.

- Richter C, Park JW, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA 85:6465–6467.
- Rideout HJ, Stefanis L (2002) Proteasomal inhibition-induced inclusion formation and death in cortical neurons require transcription and ubiquitination. Mol Cell Neurosci 21:223–238.
- Rideout HJ, Larsen KE, Sulzer D, Stefanis L (2001) Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. J Neurochem 78:899–908.
- Roy S, Wolman L (1969) Ultrastructural observations in Parkinsonism. J Pathol 99:39–44.
- Sacheck JM, Blumberg JB (2001) Role of vitamin E and oxidative stress in exercise. Nutrition 17:809–814.
- Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990a) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827.
- Schapira AH, Holt IJ, Sweeney M, Harding AE, Jenner P, Marsden CD (1990b) Mitochondrial DNA analysis in Parkinson's disease. Mov Disord 5:294–297.
- Sherer TB, Betarbet R, Kim JH, Greenamyre JT (2003a) Selective microglial activation in the rat rotenone model of Parkinson's disease. Neurosci Lett 341:87–90.
- Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR, Greenamyre JT (2002) An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 22:7006–7015.
- Sherer TB, Betarbet R, Testa CM, et al. (2003b) Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 23:10756–10764.
- Shimizu K, Ohtaki K, Matsubara K, et al. (2001) Carrier-mediated processes in blood--brain barrier penetration and neural uptake of paraquat. Brain Res 906:135–142.
- Shimoji M, Zhang L, Mandir AS, Dawson VL, Dawson TM (2005) Absence of inclusion body formation in the MPTP mouse model of Parkinson's disease. Brain Res Mol Brain Res 134:103–108.
- Smith ID, Grace AA (1992) Role of the subthalamic nucleus in the regulation of nigral dopamine neuron activity. Synapse 12:287–303.
- Soleo L, Defazio G, Scarselli R, Zefferino R, Livrea P, Foa V (1996) Toxicity of fungicides containing ethylene-bis-dithiocarbamate in serumless dissociated mesencephalic-striatal primary coculture. Arch Toxicol 70:678–682.
- Sonsalla PK, Heikkila RE (1986) The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. Eur J Pharmacol 129:339–345.
- Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95:6469–6473.
- Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840.
- Sveinbjornsdottir S, Hicks AA, Jonsson T, et al. (2000) Familial aggregation of Parkinson's disease in Iceland. N Engl J Med 343:1765–1770.
- Swerdlow RH, Parks JK, Davis JN 2nd, et al. (1998) Matrilineal inheritance of complex I dysfunction in a multigenerational Parkinson's disease family. Ann Neurol 44:873–881.
- Tanner CM (1992) Occupational and environmental causes of parkinsonism. Occup Med 7:503–513.

- Tawara T, Fukushima T, Hojo N, Isobe A, Shiwaku K, Setogawa T, Yamane Y (1996) Effects of paraquat on mitochondrial electron transport system and catecholamine contents in rat brain. Arch Toxicol 70:585–589.
- Terman A, Brunk UT (2004) Lipofuscin. Int J Biochem Cell Biol 36:1400–1404.
- Testa CM, Sherer TB, Greenamyre JT (2005) Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. Brain Res Mol Brain Res 134:109–118.
- Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA (2000a) Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? Brain Res 873:225–234.
- Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA (2000b) The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. J Neurosci 20:9207–9214.
- Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA (2000c) Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? Brain Res 873:225–234.
- Thiruchelvam M, Prokopenko O, Cory-Slechta DA, Buckley B, Mirochnitchenko O (2005) Overexpression of superoxide dismutase or glutathione peroxidase protects against the paraquat + maneb-induced Parkinson disease phenotype. J Biol Chem 280:22530–22539.
- Thiruchelvam M, McCormack A, Richfield EK, et al. (2003) Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. Eur J Neurosci 18:589–600.
- Tofaris GK, Layfield R, Spillantini MG (2001) alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. FEBS Lett 509:22–26.

- Vaccari A, Saba PL, Ruiu S, Collu M, Devoto P (1996) Disulfiram and diethyldithiocarbamate intoxication affects the storage and release of striatal dopamine. Toxicol Appl Pharmacol 139:102–108.
- Vaccari A, Ferraro L, Saba P, Ruiu S, Mocci I, Antonelli T, Tanganelli S (1998) Differential mechanisms in the effects of disulfiram and diethyldithiocarbamate intoxication on striatal release and vesicular transport of glutamate. J Pharmacol Exp Ther 285:961–967.
- Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC (2003) Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 278:25009–25013.
- Wei YH (1998) Oxidative stress and mitochondrial DNA mutations in human aging. Proc Soc Exp Biol Med 217:53–63.
- Yazdani U, German DC, Liang CL, Manzino L, Sonsalla PK, Zeevalk GD (2006) Rat model of Parkinson's disease: Chronic central delivery of 1-methyl-4-phenylpyridinium (MPP(+)). Exp Neurol 200:172–183.
- Zeevalk GD, Manzino L, Sonsalla PK, Bernard LP (2007) Characterization of intracellular elevation of glutathione (GSH) with glutathione monoethyl ester and GSH in brain and neuronal cultures: relevance to Parkinson's disease. Exp Neurol 203:512–520.
- Zhang J, Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ (1999) Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 154:1423–1429.
- Zuddas A, Fascetti F, Corsini GU, Piccardi MP (1994) In brown Norway rats, MPP+ is accumulated in the nigrostriatal dopaminergic terminals but it is not neurotoxic: a model of natural resistance to MPTP toxicity. Exp Neurol 127:54–61.

# Alterations in Corticostriatal Synaptic Function in Huntington's and Parkinson's Diseases

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## I. INTRODUCTION

The striatum is centrally located to receive a variety of synaptic inputs that are integrated and distributed to appropriate output regions producing adaptive behaviors, through cortico-basal ganglia-thalamocortical loops (Alexander and Crutcher, 1990) (for an overview, see Chapter 1). These functions are precisely regulated by the coordinated pre- and postsynaptic actions of dopamine (DA) modulating voltage- and ligand-gated conductances in mediumsized spiny neurons (MSNs), the principal cell type in striatum (see Chapter 5), as well as a variety of interneurons. Huntington's disease (HD) involves dysfunction and

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. eventual degeneration of striatal and cortical neurons, whereas dysfunction and subsequent degeneration of DA neurons lead to Parkinson's disease (PD). With the introduction of animal models of HD and PD, it has become possible to study electrophysiological changes in basal ganglia neurons and, more specifically, alterations along the corticostriatal pathway in each of these disorders. The role of changes in this pathway that contribute to cognitive and motor disturbances in HD and PD models is the primary topic of this chapter. As the literature on neurotoxic models of these diseases is already very extensive, we will concentrate on alterations of the corticostriatal pathway in genetic mouse models.

### **II. STRIATAL ORGANIZATION**

MSNs constitute about 95% of all striatal neurons and utilize  $\gamma$ -aminobutyric acid (GABA) as their principal neurotransmitter (Kita and Kitai, 1988). The dorsal striatum receives parallel sets of diffuse glutamatergic inputs from almost all cortical areas (Wilson, 1990) (see Chapters 18, 19 and 20). These inputs synapse onto spines of MSNs (Kemp and Powell, 1970). The striatum also contains a number of modulatory components including DA projections from the substantia nigra pars compacta (Graybiel, 1990), and cholinergic (see Chapter 7) and GABAergic interneurons (see Chapter 8), including tonically active interneurons (Pisani et al., 2007) and fast-spiking interneurons (Tepper et al., 2008), respectively. These elements constitute a basic striatal microcircuit (Tepper et al., 2004).

Although some overlap exists (Shuen et al., 2008), the striatal output is largely segregated into two populations of GABAergic MSNs with distinct projections, DA receptor and neuropeptide expression (Kawaguchi et al., 1990) (see Chapter 1). The direct pathway consists of MSNs that predominantly express D1 DA receptors (Gerfen et al., 1990), substance P (Haber and Nauta, 1983) and dynorphin (Vincent et al., 1982), and project to the substantia nigra pars reticulata and the internal segment of the globus pallidus (Albin et al., 1989; Gerfen et al., 1990). The indirect pathway is comprised of MSNs that express predominantly D2 receptors (Gerfen et al., 1990), met-enkephalin and neurotensin (Haber and Nauta, 1983; Steiner and Gerfen, 1999), and project to the external segment of the globus pallidus (Albin et al., 1989; Kawaguchi et al., 1990).

Anatomical evidence has demonstrated differential excitatory inputs onto D1 and D2 receptor-containing MSNs (Reiner et al., 2003) (see Chapter 18). Two types of pyramidal corticostriatal projections have been identified; one is ipsilateral and arises from collaterals of the pyramidal tract (PT-type), and the other is bilateral and projects only intratelencephalically to the cortex and striatum (IT-type). Terminals making asymmetric axospinous contact with striatonigral (direct pathway) or D1 receptor-containing neurons are significantly smaller than those making contact with striatopallidal (indirect pathway) or D2 receptor-containing MSNs (Lei et al., 2004). The direct pathway neurons preferentially receive inputs from IT-type cortical neurons, whereas indirect pathway neurons receive greater numbers of inputs from PT-type cortical neurons, suggesting that D2 cells are subject to increased glutamate release from corticostriatal terminals (Reiner et al., 2003). Although this

scheme has recently been challenged (Ballion et al., 2008), our electrophysiological data support differential excitatory inputs to MSNs of the direct and indirect pathways.

## A. Electrophysiological Properties of Striatal D1 and D2 Dopamine Receptor-Containing MSNs

It was generally believed that MSNs giving rise to the direct and indirect pathways were morphologically and electrophysiologically identical. This view has changed primarily due to the recent generation of mice expressing enhanced green fluorescent protein (EGFP) driven by promoters of D1 or D2 receptors, permitting visualization of specific subpopulations of MSNs. Studies in vitro demonstrate that cell input resistance, capacitance, time constant and resting membrane potentials are similar in D1 and D2 receptor-containing MSNs (Kreitzer and Malenka, 2007; Cepeda et al., 2008), although one recent study found that D2 cells have higher input resistances, due to smaller dendritic surface areas, and are slightly but significantly more depolarized than D1 cells (Gertler et al., 2008). D2 cells are more excitable than D1 cells as, at similar current intensities, D2 cells fire more action potentials (Kreitzer and Malenka, 2007), have a lower threshold for action potential generation (Cepeda et al., 2008), and exhibit a lower rheobase (Gertler et al., 2008).

There are also differences in synaptic inputs between subpopulations of MSNs. The frequency of spontaneous excitatory postsynaptic currents (EPSCs) is higher in D2 compared to D1 cells and large-amplitude events (>100 pA) only occur in D2 cells (Kreitzer and Malenka, 2007; Cepeda et al., 2008). After addition of GABA<sub>A</sub> receptor blockers, which induce epileptiform activity in cortical pyramidal neurons, D2 cells display large membrane depolarizations rarely seen in D1 cells (Cepeda et al., 2008), supporting data demonstrating that enkephalin-positive neurons are selectively activated by cortical stimulation (Uhl et al., 1988; Berretta et al., 1997).

These results imply that D2 receptor-containing MSNs reflect on-going cortical activity, particularly the activity generated by PT-type neurons, more faithfully than D1 cells, i.e., inputs from PT-type neurons may provide D2 MSNs with a copy of the cortical motor signal (Reiner et al., 2003). This signal could be crucial for motor coordination. Another implication of increased intrinsic excitability and tighter corticostriatal synaptic coupling of D2 receptorexpressing MSNs is that cells of the indirect pathway are more readily available for activation than cells of the direct pathway. In contrast, the smaller size and diffuse nature of the projections of the IT-type terminals will produce less activation of D1 neurons.

MSNs also receive excitatory inputs from the thalamus (see Chapter 22). The nature of these inputs is beginning to be unraveled in slice preparations that preserve both cortico- and thalamostriatal inputs (Smeal et al., 2007; Ding et al., 2008). In rats, at similar stimulation intensities, responses evoked by thalamic stimulation are smaller and display significant paired-pulse facilitation relative to cortical stimulation (Smeal et al., 2007). They also have a greater NMDA/AMPA ratio than do the synapses mediating cortical input to the same MSN (Smeal et al., 2008). In contrast, in mice some of these findings are almost exactly opposite (Ding et al., 2008). The basis for these differences remains unclear, but it could be related to procedural or species differences, as well as the age of the animals (Smeal et al., 2008).

### **B.** Dopamine Functions in Striatum

DA modulation of responses mediated by activation of glutamate receptor is critical for striatal function (see Chapter 6). Our studies demonstrated that the outcome of DA modulation of glutamatergic inputs depends not only on the type of DA receptor preferentially activated but also on the glutamate receptor subtype activated. Thus, DA via D1 receptors enhances NMDA receptor-mediated responses, whereas via D2 receptors it reduces AMPA receptor-mediated responses (Cepeda et al., 1993; Levine et al., 1996; Hernandez-Echeagaray et al., 2004). Although the other two interactions are less predictable, activation of D1 receptors generally enhances AMPA responses (Yan et al., 1999) and activation of D2 receptors decreases NMDA responses (Levine et al., 1996). The mechanisms by which DA produces differential effects on glutamatergic transmission are complex and involve modulation of voltage-gated currents as well as a number of intracellular signaling pathways (Cepeda and Levine, 2006) (see also Chapter 6).

# C. Presynaptic Modulation of Striatal Glutamatergic Inputs by Dopamine

While most studies of DA function in striatum have focused on its postsynaptic effects on striatal output, DA also alters glutamate and/or GABAergic inputs onto MSNs by presynaptic mechanisms. DA receptors are localized on presynaptic terminals where they can modulate neurotransmitter release (Wang and Pickel, 2002). In the dorsal striatum, D2 receptors have been found on corticostriatal terminal endings and function to decrease glutamate release by presynaptic mechanisms (Flores-Hernandez et al., 1997; Cepeda et al., 2001). Studies in mice lacking D2 receptors provide evidence of increased glutamate release indicating that such receptors function as gatekeepers, i.e., primarily preventing excessive excitation in the striatum (Cepeda et al., 2001). Further, optical techniques visualizing neurotransmitter release via destaining of terminals after incorporation of FM1-43, a styryl dye, have provided definite confirmation that D2 receptor activation alters glutamate release at corticostriatal synapses (Bamford et al., 2004b). Inhibition of glutamate release is frequency-dependent and it can act as a low-pass filter selective for terminals with low probability of release (Dani and Zhou, 2004). In this way, DA released by salient stimuli can directly regulate striatal neurotransmission by selecting specific sets of corticostriatal projections (Bamford et al., 2004b).

## D. Other Receptors Regulating Glutamate Release in the Corticostriatal Pathway

As continuous exposure to glutamate inputs could make MSNs vulnerable to excitotoxic damage, a number of receptors strategically placed on corticostriatal terminals regulate glutamate release. Group II metabotropic glutamate (mGluR<sub>2</sub> and mGluR<sub>3</sub>) (Lovinger and McCool, 1995), GABA<sub>B</sub> (Nisenbaum et al., 1992), cannabinoid (CB<sub>1</sub>) (Huang et al., 2001), and adenosine (A<sub>1</sub>) receptors (Lovinger and Choi, 1995) all have been involved. In dorsal striatum DA also modulates excitatory transmission by means of retrograde endocannabinoid signaling, which reduces glutamate release by activation of CB<sub>1</sub> receptors on presynaptic terminals (Yin and Lovinger, 2006) (see Chapter 9).

Regardless of the mechanisms of presynaptic modulation of glutamate release, the relevance is that functional alterations in cortical pyramidal neurons or in receptor expression on presynaptic endings of the corticostriatal pathway, the gatekeepers of striatal excitability, have an important role in HD and PD neuropathology. The major issue then becomes what are the potential consequences of dysregulation of glutamate release along the corticostriatal pathway in HD and PD?

# III. THE CORTICOSTRIATAL PATHWAY IN HUNTINGTON'S DISEASE

HD is a genetic, progressive neurological disorder that is inherited in an autosomal dominant fashion. The symptoms include abnormal dance-like movements (chorea), cognitive disturbances, and disorders of mood (Harper, 1996). The HD gene (IT15) is located on the short arm of chromosome 4 and contains an expansion in the normal number of CAG (glutamine) repeats, generally >40 (The Huntington's Disease Collaborative Research Group, 1993). HD is typically a late onset disease although juvenile variants occur, usually when more CAG repeats are present. In young children with HD, the symptoms almost invariably include epileptic seizures (Rasmussen et al., 2000). Neuropathologically, HD is primarily characterized by neuronal loss in striatum and cortex (Vonsattel and DiFiglia, 1998). In the striatum, MSNs are most affected (Vonsattel et al., 1985). Although it has been generally believed that the progression of symptoms in the disorder is due to neurodegeneration of MSNs, it has become apparent that severe neuronal dysfunction precedes degeneration and is probably the major cause of many symptoms (Levine et al., 2004). Of equal importance, the understanding of mechanisms causing neuronal dysfunction will provide new targets for therapeutics that can be useful before degeneration has occurred.

The protein coded by the HD gene (huntingtin) is a large protein that is highly conserved and expressed ubiquitously throughout the body (Strong et al., 1993). Huntingtin is a cytoplasmic protein closely associated with vesicle membranes and microtubules, suggesting it may have a role in vesicle trafficking, exocytosis and endocytosis (DiFiglia et al., 1995). In addition, its distribution is very similar to that of synaptophysin (Wood et al., 1996) and it has been shown to associate with various proteins involved in synaptic function. Thus, there is considerable evidence that mutant huntingtin can cause abnormal synaptic transmission in HD (Smith et al., 2005).

There are numerous in depth reviews of genetic mouse models of HD (Bates and Murphy, 2002; Menalled and Chesselet, 2002; Levine et al., 2004; Cepeda et al., 2007; Fan and Raymond, 2007; Gil and Rego, 2008). In the next section we will provide a succinct description of some of the mouse models used to examine electrophysiology of the corticostriatal pathway.

### A. Genetic Mouse Models of HD

Genetic models of HD permit examination of the progression of the disease and elucidation of cause–effect relationships. There are many models of HD currently in use, mostly in mice but at least one in rats (von Horsten et al., 2003) and one in primates (Yang et al., 2008). Mouse models include transgenic (with a fragment or full-length human mutant gene), knock-in, and conditional models. None of the rodent models recapitulates the human disorder in its entirety, nor displays the degree of neurodegeneration



FIGURE 35.1 Proposed changes in corticostriatal synaptic transmission in Huntington's disease. (A) The simplified striatal circuit is composed of medium-sized spiny neurons that receive excitatory glutamatergic (GLU) corticostriatal projections and modulatory dopaminergic (DA) nigrostriatal terminals (SN). Glutamate release is modulated by presynaptic D2 DA receptors. In this diagram only a D2 receptor-expressing neuron is illustrated as they are the more vulnerable. (B) The HD mutation produces age-dependent changes in corticostriatal activity. Glutamate release from cortical terminals is increased in pre-symptomatic HD mice. In R6/2 mice there is also a slight decrease in DA release. (C) In late stages of the disease glutamate release is markedly reduced in symptomatic animals and, because there is a significant loss of spines and synaptic sites, glutamate release activates extrasynaptic NMDA receptors that mediate a pro-apoptotic pathway. At this stage DA release is greatly reduced in R6/2 transgenic mice and D2 filtering is diminished (illustrated here by a thinner D2 receptor).

seen in human HD, but each has relevance to some of the symptoms (Levine et al., 2004).

The R6 line of transgenic mice (Mangiarini et al., 1996) is one of the most widely used models, not only because it was the first model generated but also because it offers many advantages. In particular, R6/2 mice (~150 CAG repeats) manifest a very aggressive, rapidly progressing form of HD. Transgenic R6/2 animals display overt behavioral symptoms as early as 4-5 weeks of age and die of unknown causes at about 15 weeks. Alterations include the formation of neuronal intranuclear inclusions (Davies et al., 1997), changes in neurotransmitter receptor expression (Cha et al., 1998; Ariano et al., 2002), and altered signaling mechanisms (Bibb et al., 2000; Luthi-Carter et al., 2000; Menalled et al., 2000). Many of these alterations are correlated with motor (Carter et al., 1999) and learning deficits (Lione et al., 1999; Murphy et al., 2000). The early occurrence of phenotypic changes has allowed careful and systematic examination of the development of electrophysiological alterations using methods such as infrared videomicroscopy and whole-cell patch clamp recordings that are difficult to use in the more slowly progressing models.

Questions have been raised about the validity of the R6/2 model and whether or not it recapitulates adult-onset HD. It has been considered a better model of juvenile HD. However, there are numerous commonalities between juvenile and adult-onset HD that make the R6/2 a very reasonable model for both types. In a careful and systematic comparison of fragment versus full-length (knock-in) models of HD it was concluded that, when strain back-ground and CAG repeat length are controlled for, fragment and knock-in models develop comparable phenotypes (Woodman et al., 2007). Furthermore, the R6/2 model is extremely useful for *in vivo* drug screening and has become the gold standard for drug testing (Gil and Rego, 2008).

R6/1 mice ( $\sim$ 110 CAG repeats) display a phenotype similar to that of the R6/2, but in a more protracted form (Mangiarini et al., 1996). Significant weight loss and clasping can be observed at 19–23 weeks of age and become more pronounced with age. In addition, aberrant hippocampal synaptic plasticity, which occurs prior to the formation of nuclear aggregates, has been described in these mice (Milnerwood et al., 2006).

The most widely used full-length models use yeast artificial chromosomes (YAC) expressing normal (YAC18) and mutant (YAC46, YAC72 and YAC128) huntingtin (Hodgson et al., 1999; Slow et al., 2003). YAC72 mice display behavioral changes around 7 months, as well as selective degeneration of MSNs in the lateral striatum by 12 months. Neurodegeneration can be present in the absence of aggregates in YAC mice, indicating that aggregates may not be essential to initiate neuronal death (Hodgson et al., 1999). YAC128 mice display similar but more severe alterations which occur earlier than in YAC72 mice (Slow et al., 2003), exhibiting increased open field activity at about 3 months, followed by rotarod abnormalities at 6 months. By 12 months, open field activity is diminished significantly compared to controls. In addition, modest (~10%) striatal atrophy and neuronal loss occur in the striatum and cortex of YAC128 mice (Van Raamsdonk et al., 2005).

Knock-in models also have emerged as major contributors to our understanding of HD. Several models that differ mainly in the number of CAG repeats (from 48 to 150) have been generated (White et al., 1997; Levine et al., 1999; Shelbourne et al., 1999; Wheeler et al., 2000; Lin et al., 2001). Although in knock-in mice overt behavioral changes are subtle, more sensitive and careful testing demonstrates behavioral abnormalities as early as 1–2 months of age (Menalled and Chesselet, 2002; Menalled et al., 2003). Further, a consistent feature in knock-in mice is the presence of nuclear staining and microaggregates at 2–6 months, which is relatively early in the course of the disease. By contrast, nuclear inclusions only are observed in older mice (10–18 months) (Menalled and Chesselet, 2002), and loss of striatal neurons occurs at about 2 years (Hickey et al., 2008).

Cre/LoxP conditional HD mice expressing mutant huntingtin with 103 glutamine repeats, either in all neurons of the brain or restricted to the vulnerable cortical pyramidal neurons, have been generated also (Gu et al., 2005). Interestingly, in these mice huntingtin aggregation was shown to be a cell-autonomous process, whereas motor deficits and cortical neuropathology were observed only when mutant huntingtin expression occurred in multiple neuronal types, including cortical interneurons, but not when it was restricted to cortical pyramidal neurons (Gu et al., 2005).

The most recently developed mouse model of HD uses a bacterial artificial chromosome (BAC) expressing full-length human mutant huntingtin (fl-mhtt) with 97 glutamine repeats. BACHD mice exhibit progressive motor deficits, neuronal synaptic dysfunction, and late-onset selective neuropathology. Importantly, in this model the progressive and selective pathogenic process in HD can occur without diffuse nuclear accumulation of mhtt. Instead, a relatively steady-state level of fl-mhtt and a small amount of N-terminal fragments are sufficient to elicit the disease process (Gray et al., 2008b).

# **B.** Biphasic Alterations in Glutamatergic Neurotransmission

In R6/2 and YAC128 HD mice, alterations in glutamatergic function along the corticostriatal pathway are not uniform, but change dynamically in a biphasic manner. Although a progressive reduction in spontaneous and evoked glutamatergic synaptic activity, coinciding with the appearance of overt behavioral alterations is the most noticeable change in R6/2 mice (Klapstein et al., 2001; Cepeda et al., 2003), dysregulation of glutamatergic input occurs early and is manifested by the presence of large-amplitude and complex synaptic events that peak around 5–7 weeks of age (Cepeda et al., 2003). We attributed these events to increased cortical excitability and possibly a reduction in presynaptic receptor function, including D2, mGluR<sub>2–3</sub> and CB<sub>1</sub> receptors (Cha et al., 1998; Luthi-Carter et al., 2000; Ariano et al., 2002).

Predicted hyperexcitability in cortical networks has been confirmed in the R6/2 and other mouse models. Examination of somatosensory cortical pyramidal neurons in layers II/III in slices from R6/2 mice revealed that spontaneous EPSCs occurred at a higher frequency in behaviorally phenotypic mice while IPSCs were initially increased in frequency and subsequently decreased at 80–90 days. In addition, compared to controls, R6/2 mice demonstrated increased epileptiform activity in slices and seizure susceptibility in vivo after blockade of GABA<sub>A</sub> receptors (Cummings et al., 2009). Decreased inhibition in cortical pyramidal neurons, manifested by a reduction in spontaneous IPSCs, was also observed in the BACHD model at 6 months, when motor dysfunction occurs (Spampanato et al., 2008).

As striatal neuronal action potential generation is highly dependent on cortical inputs, the presence of large-amplitude synaptic events in the striatum of R6/2 mice at 5–7 weeks predicts transiently increased activity along the corticostriatal pathway in a subset of MSNs. Consistent with this idea, in vivo recordings of striatal neurons demonstrated that cell firing is elevated in transgenic relative to WT mice at 6–9 weeks of age (Rebec et al., 2006). Furthermore, restoring extracellular ascorbate to the WT levels reversed this effect suggesting a role for ascorbate in normalizing neuronal function in HD (Rebec et al., 2006). Increased cell firing could be explained by depolarized resting membrane potentials and increased input resistances (Levine et al., 1999; Klapstein et al., 2001), caused by reduced inward rectification of MSNs in this mouse model (Ariano et al., 2005). Not only the frequency but also the burst activity and correlated firing patterns were altered in HD mice (Miller et al., 2008). Thus, correlated firing and coincident bursts between pairs of MSNs were prominent in cells from WTs but reduced in R6/2 and knock-in models suggesting that information processing at both the single-neuron and population level is compromised in the striatum and cortex of symptomatic HD mice (Miller et al., 2008).

Recently we examined alterations in glutamate release in the corticostriatal pathway of YAC128 mice at different stages of disease progression (1, 7 and 12 months), using combined optical and electrophysiological methods. Similar to results from R6/2 mice, the results in YAC128 mice demonstrated biphasic age-dependent changes in corticostriatal function. At 1 month, before the behavioral phenotype develops, AMPA receptor-mediated synaptic currents and glutamate release evoked by cortical stimulation were increased. At 7 and 12 months, after the development of the behavioral phenotype, glutamate release and AMPA synaptic currents were significantly reduced (Joshi et al., 2009). These effects were due to combined pre- and postsynaptic alterations. The susceptibility to excitotoxic stress in YAC128 mice also changes in a biphasic manner (Graham et al., 2009). At 1 month, before phenotypic changes occur, mice display increased sensitivity to NMDA and quinolinic acid. In contrast, at 7-10 months symptomatic mice are resistant to quinolinic acid neurotoxicity. These changes are paralleled by increased NMDA receptor-mediated synaptic currents in slices and increased postsynaptic currents in dissociated MSNs from presymptomatic followed by reduced currents in symptomatic YAC128 mice (Graham et al., 2009). Increased NMDA currents due to reduced Mg<sup>2+</sup> sensitivity are also observed in a subset of MSNs in young R6/2 mice (Starling et al., 2005).

Multiple alterations in corticostriatal synaptic function also occur depending on the pathogenicity of mutant huntingtin (Milnerwood and Raymond, 2007). Presynaptic dysfunction and a propensity towards synaptic depression in YAC72 and YAC128 compared to YAC18 mice occur at 1 month. In YAC128 mice (line 53), reduced AMPA responses evoked by intrastriatal stimulation also were observed. In contrast, when normalized to evoked AMPA currents, postsynaptic NMDA currents were enhanced in all three pathologic HD YAC variants (Milnerwood and Raymond, 2007).

This series of electrophysiological studies points to important alterations in corticostriatal synaptic function. Whether gains or deficits in glutamate release and/or receptor function occur, the HD mutation has deleterious consequences on corticostriatal communication. Although the release of glutamate is under strict control by a multitude of presynaptic receptors, we will concentrate on DA receptors since they have been most frequently studied.

## C. Dopamine Receptor Modulation of Corticostriatal Transmission in HD

In clinical HD and in animal models, changes in the DA system occur early in the disease and may contribute to cognitive and motor abnormalities. In humans, bradykinesia is an underlying manifestation of HD and becomes more apparent in late stages of the disease when choreic movements begin to subside (Thompson et al., 1988). Most animal models also undergo changes in motor activity from hyper- to hypokinesia (Levine et al., 2004). In R6/2 mice, DA levels are decreased and the animals display reduced responses to cocaine (Hickey et al., 2002). DA release also is reduced at 6 weeks and this reduction becomes more pronounced with age (Johnson et al., 2006). Interestingly, in CAG 140 knock-in mice, regions with early pathology receive dense DA inputs, supporting a role of DA in HD pathology (Menalled et al., 2003). Lesions of the DA nigrostriatal pathway are protective in HD models (Stack et al., 2007), again suggesting a deleterious role for DA.

In our optical measurements of glutamate release in the YAC128 model, we also detected alterations in DA modulation of glutamate release at corticostriatal synapses. In WT mice the inhibitory effect of the D2 receptor agonist quinpirole on glutamate release remained stable over age, while in YAC128 mice quinpirole produced an increase in corticostriatal inhibition at 1 month but it became much less effective at 12 months (Joshi et al., 2009). Reductions in DA receptor function in R6/1 mice could also be responsible for deficits in cortical LTD as these can be reversed by quinpirole (Cummings et al., 2006).

## D. Consequences of Corticostriatal Pathway Dysfunction in HD

In HD, D2 receptor-containing MSNs projecting to the external globus pallidus are lost before D1 receptorcontaining neurons. This fact has aroused major interest and speculation about how and why this occurs. The major question concerns whether or not alterations in glutamatergic inputs from the cerebral cortex are responsible for MSN dysfunction and eventual loss in HD. In other words, is striatal neuronal degeneration a cell autonomous process or does it depend on cell–cell interactions? Based on our own and other studies demonstrating a tight synaptic coupling between cortical inputs onto D2 receptor-containing MSNs, we speculated that early dysregulation of cortical inputs induces adaptive changes in MSNs in an attempt to cope with glutamate surges. This unfortunately leads to a progressive disconnection between cortex and striatum that ultimately changes the topography of glutamate receptor activation to an extrasynaptic location that promotes an apoptotic pathway (Papadia and Hardingham, 2007; Milnerwood et al., 2008) and deprives MSNs of important trophic factors released during corticostriatal activation such as BDNF (Zuccato and Cattaneo, 2007).

To address the role of pathological cell-cell interactions in HD, in a recent study (in collaboration with Dr. William Yang, UCLA) a conditional mouse (RosaHD with 103 CAG repeats in exon 1) selectively expressing mutant huntingtin in striatal neurons and a subset of cortical interneurons, was generated and compared with mice expressing mutant huntingtin in the whole brain. In the striatal model of HD, a progressive and cell-autonomous nuclear accumulation of mutant huntingtin aggregates in MSNs occurred but, in contrast to the mouse model expressing mutant huntingtin in all the neurons, the striatal model lacked significant locomotor deficits and striatal neuropathology. Electrophysiological analysis also revealed a cellautonomous deficit in NMDA receptor sensitivity to Mg<sup>2+</sup>, suggesting that both cell-autonomous toxicity and pathological cell-cell interactions are critical for HD pathogenesis (Gu et al., 2007).

To further examine the role of the cortex, BACHD mice were crossed with Emx1-Cre to selectively inactivate full-length mutant huntingtin expression in cortical pyramidal neurons while maintaining expression elsewhere in the brain. Compared to BACHD mice at 12 months of age, BACHD/Emx1-Cre double transgenic mice exhibited significant rescue of synaptic deficits at corticostriatal synapses as well as significant reduction of striatal pathology, suggesting that cell-cell interactions between cortical and striatal neurons are critical for HD pathology (Gray et al., 2008a). In a different HD model (N171-98Q), striatal expression of mutant huntingtin was sufficient to produce intranuclear inclusion bodies and motor impairment that the authors attributed to cell-autonomous transcriptional dysregulation (Brown et al., 2008). The reasons for differences between these models remain unknown.

### E. Some Unresolved Questions

The cortico-basal ganglia-thalamocortical loop is above all an interconnected circuit and, as such, any alteration in a discrete part will alter transmission throughout the system. Two regions appear mainly involved in the onset of HD symptoms, the striatum and the cerebral cortex. However, we still do not know whether pathological and functional changes start earlier in one of these regions, or whether they occur simultaneously throughout the brain. Changes in striatal and cortical local circuits also occur in HD models. An increase in spontaneous GABAergic synaptic activity occurs in conjunction with the earliest alterations in glutamate transmission (Cepeda et al., 2004). Is this a compensatory mechanism to prevent excessive glutamate release? Regardless, increases in local GABA activity will reduce striatal output to other basal ganglia regions and in consequence will affect the circuit.

Another unresolved question concerns the contribution of glutamatergic thalamic inputs to striatal dysfunction and pathology. Are D1 MSNs less sensitive to the HD mutation because they are selectively innervated by IT-type pyramidal and thalamic neurons thus overall receiving less glutamatergic inputs than D2 cells? In a related note, is it possible that the initial hyperkinesia results from alterations in indirect pathway neurons whereas the late hypokinesia reflects involvement of direct pathway neurons?

One may think that the levels of huntingtin expression could account for selective vulnerability. Thus, large cholinergic interneurons do not appear to express huntingtin and they do not degenerate, although these findings have been questioned (Fusco et al., 1999). Interestingly, huntingtin is expressed in a greater proportion in substance P-positive neurons forming the direct striatonigral pathway than in the enkephalin-positive neurons forming the indirect striatopallidal output (Fusco et al., 2003), indicating that huntingtin expression levels may not be a reliable predictor of cell vulnerability. In contrast, what is consistent is that corticostriatal neurons are enriched in huntingtin, suggesting that the HD mutation may render corticostriatal neurons dysfunctional first and potentially destructive upon some MSNs, rather than render all striatal neurons vulnerable (Fusco et al., 1999).

Even though many questions still remain, the corticostriatal pathway is becoming a prime target for treatment of HD symptoms (Li et al., 2003). If cortical inputs are necessary to observe significant pathology in HD (Gu et al., 2007), reducing glutamatergic transmission and cortical hyperexcitability could be beneficial. In agreement, drugs that reduce cortical excitability like riluzole and benzodiazepines (alprazolam) ameliorate symptoms in animal models of HD (Cepeda et al., 2003; Pallier et al., 2007). Large, complex synaptic events are reduced by removing cortical inputs in slices (Cepeda et al., 2003) and decortication also ameliorates the phenotype in vivo (Stack et al., 2007). However, at later stages of disease progression, when the striatum becomes disconnected from the cortex, reducing cortical inputs may be deleterious and attempts should be made to restore normal synaptic activity.

## IV. THE CORTICOSTRIATAL PATHWAY IN PARKINSON'S DISEASE

PD is a degenerative disorder of the central nervous system, primarily affecting DA neurons in the substantia nigra, that produces impairments in motor function, skills, language and cognition (Jankovic, 2008) (see Chapter 34). In humans, PD is characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia) and, in extreme cases, a loss of physical movement (akinesia). The primary symptoms are the result of decreased activation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of DA in the striatum.

PD, as well as other neurodegenerative disorders that affect motor function, also is associated with abnormal neurotransmission along the corticostriatal pathway. In this section we will examine how the reduction in DA availability in PD produces changes in striatal synaptic function. Striatal adaptations after DA dysfunction, by altering glutamatergic neurotransmission along this pathway, could be sufficient to produce some of the cardinal symptoms of PD (bradykinesia, in the DA-deficient state and motor dyskinesias following treatment).

## A. Mouse Models of Parkinsonism

A variety of mouse models have been used to study the effects of DA deficiency on corticostriatal activity. DA depletion has been achieved using different methods including acute catecholamine-depleting agents such as reserpine (Fischer and Heller, 1967), neurotoxins that target catecholamine-producing cells, e.g., 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968; Blandini et al., 2008), MPTP (Meredith et al., 2008), or environmental toxins (Greenamyre et al., 2003) (see Chapter 34). Although these models have been invaluable in elucidating the consequences of the loss of DA



**FIGURE 35.2** Proposed changes in corticostriatal synaptic transmission in Parkinson's disease. (A) The simplified striatal circuit is composed of medium-sized spiny neurons that receive excitatory glutamatergic (GLU) corticostriatal projections and modulatory dopaminergic (DA) nigrostriatal terminals (SN). Glutamate release is modulated by presynaptic D2 DA receptors. (B) In DA-depleted states, DA receptors become sensitized (indicated here by a thicker D2 receptor). Glutamate release may increase as a consequence of reduced DA availability, and corticostriatal filtering is obliterated. Increased release can be excitotoxic, leading to the collapse of dendritic spines in D2 receptor-containing medium-sized spiny neurons. (C) In genetic models of PD (Parkin or DJ-1 KO, and  $\alpha$ -synuclein over-expressing mice), glutamate release is reduced possibly because of increased DA tone at the corticostriatal synapse, which leads to hyposensitive D2 receptors (illustrated here by a thinner D2 receptor).

neurons, their usefulness to study the development of pathophysiological alterations in PD remains limited because they are mostly based on neurotoxic mechanisms and acute administration. PD is a slowly progressing disorder involving significantly more than simply DA depletion (Chesselet et al., 2008).

A genetic mouse model of DA-deficiency has been used to replicate the effects of chronic DA depletion. DA-deficient mice were generated by a targeted deletion of the tyrosine hydroxylase (TH) gene in DA neurons while restoring TH function in noradrenergic and adrenergic cells (Zhou and Palmiter, 1995). DA-deficient mice manifest normal DA neurons, neuronal connections (Zhou and Palmiter, 1995) and D2 autoreceptors (Paladini et al., 2003). However, DAdeficient mice require daily injections of L-3, 4-dihydroxyphenylalanine (I-DOPA) for survival (Zhou and Palmiter, 1995). I-DOPA partially restores brain DA to  $\sim 10\%$  of normal in these mice when measured 1h after treatment but declines to <1% of control levels after 24 h (Zhou and Palmiter, 1995; Bamford et al., 2004a). Without treatment, DA-deficient mice become severely hypophagic and die at  $\sim$ 3 weeks of age. Systemic treatment with 1-DOPA rescues the mouse but produces a transient hyperactive state and induces robust immediate early gene expression in the striatum (Kim et al., 2000; Chartoff et al., 2001), suggesting that DA-deficiency results in hypersensitive D1 (Kim et al., 2000) and D2 receptors (Bamford et al., 2004a).

Genetic mouse models of PD based on the expression of mutations known to cause the disease in humans offer a way to study the full extent of the PD pathology and to perform mechanistic studies by allowing the examination of early pathogenic steps in neurodegeneration (Levine et al., 2004). Several rodent models of PD have been created. A rare mutation in  $\alpha$ -synuclein was the first genetic anomaly shown to cause familial PD (Polymeropoulos et al., 1997). This discovery led to the identification of  $\alpha$ -synuclein, a vesicular protein that is a major component of Lewy bodies (Spillantini et al., 1997) (see Chapter 34). Mice overexpressing the normal or mutated forms of  $\alpha$ -synuclein (ASO) have been generated but their phenotype is highly variable, probably as a consequence of the different promoters used for the transgene (Hashimoto et al., 2003). In one of these models mice overexpressing  $\alpha$ -synuclein under the Thy-1 promoter were examined (Masliah et al., 2000). This model confers widespread, high levels of WT human  $\alpha$ -synuclein overexpression in cortical and subcortical neurons, including the substantia nigra pars compacta (Rockenstein et al., 2002). ASO mice show progressive sensorimotor alterations that are similar to deficits observed in mouse models displaying nigrostriatal degeneration (Fleming et al., 2005), as well as early non-motor olfactory deficits and autonomic changes characteristic of preclinical PD (Fleming et al., 2008; Wang et al., 2008). ASO mice have a full complement of striatal DA terminals at the time when they show deficits in these tests, raising the question of which mechanisms lead to behavioral abnormalities (Chesselet et al., 2008). However, the same mice exhibit altered responses to amphetamine. In particular, they do not show amphetamine-induced stereotypies (Fleming et al., 2006).

The second type of mutation shown to cause familial Parkinsonism occurs in the gene encoding parkin, an E3 ligase (Shimura et al., 2000). Because parkin mutations are loss of function mutations, models have focused on parkin knock-outs (KO). In general, these mice show mild abnormalities (Goldberg et al., 2003; Itier et al., 2003). Mice defective in exon 3 display progressive motor anomalies as well as deficits in sensorimotor integration, starting as early as 2-4 months of age. Surprisingly, these mice have increased basal release of DA and reduced synaptic excitability in the striatum (Goldberg et al., 2003). Overall, these defects are consistent with those observed in another line of mice with a similar mutation (Itier et al., 2003). Neither mouse line, however, displays clear loss of DA or noradrenergic neurons. Therefore, similar to ASO mice, parkin KO mice fall short of reproducing the full spectrum of anomalies observed in PD patients, in particular the loss of nigrostriatal DA neurons. However, parkin KO mice display reduced numbers of proteins involved in mitochondrial function or oxidative stress, providing evidence of mitochondrial dysfunction and oxidative damage in the absence of nigral degeneration in a genetic mouse model of PD (Palacino et al., 2004).

Mice with DJ-1 deletion have normal numbers of DA neurons in the substantia nigra, but they are less sensitive to the inhibitory effects of D2 autoreceptor stimulation and display hypoactivity in an open field test. Furthermore, while corticostriatal LTP is normal in MSNs of DJ-1 KO mice, LTD is absent but this deficit can be reversed by treatment with D2 but not D1 receptor agonists (Goldberg et al., 2005).

## **B.** Alterations in Glutamatergic Neurotransmission and Dopamine Modulation

With the introduction of genetic mouse models of PD and electrophysiological characterization of alterations in the corticostriatal pathway, it has become evident that the mechanisms of motor dysfunction are different in genetic compared to neurotoxic models. Initial data from DA-depleted rats and cats indicated that the firing rate of striatal neurons increased in the ipsilateral side of the lesion (Hull et al., 1974; Schultz and Ungerstedt, 1978). Furthermore, after DA depletion there was an increase in the frequency of spontaneous synaptic membrane depolarizations in MSNs (Galarraga et al., 1987; Cepeda et al., 1989; Calabresi et al., 1993), along with increased gap junctional communication (Cepeda et al., 1989), suggesting that presynaptic filtering by D2 receptors on corticostriatal terminals was reduced in PD. DA depletion also increased cell firing in striatopallidal neurons (Mallet et al., 2006), decreased the threshold to evoke cortical responses (Florio et al., 1993) and facilitated the occurrence of cortically-generated membrane oscillations in a subpopulation of striatal neurons (Tseng et al., 2001). It is thus likely that the facilitation in corticostriatal input is selective to MSNs of the indirect pathway. In fact, the cells of the direct pathway appear to have reduced firing frequency probably as a consequence of decreased cortical activity (Mallet et al., 2006).

DA-deficient states sensitize presynaptic D2 receptor responses (Schultz, 1982; Calabresi et al., 1993; Kim et al., 2000) and likely influence cortical function by modifying cortical-basal ganglia circuits. Optical recordings have determined the effect of DA deficiency and replenishment at single cortical synaptic terminals in mouse models of acute and chronic DA depletion (Bamford et al., 2004a). Using reserpine treated (Bamford et al., 2004a) and DAdeficient (Zhou and Palmiter, 1995) mice, these investigations demonstrated that DA depletion produced sensitized presynaptic D2 receptor responses and altered DA-mediated responses from subsets of corticostriatal terminals (Bamford et al., 2004a). Thus, in control mice, DA was found to inhibit exocytosis from the majority ( $\sim 85\%$ ) of cortical terminals. This presynaptic inhibition is produced through D2 receptors that depress exocytosis from terminals with a low probability of release, while fast-releasing terminals remain unperturbed. In contrast, for both reserpine-treated and DA-deficient mice, D2 receptor stimulation more broadly depressed exocytosis as the reduction was observed in both fast and slow-releasing terminals. Since steady-state expression of the total population of D2 receptors remains unchanged in DA-deficient mice (Kim et al., 2000), it is likely that alterations in selected subpopulations of corticostriatal terminals following DA depletion reflect adaptations in D2 receptor sensitivity (Bamford et al., 2004a). Alterations in D2 receptor sensitivity due to DA deficiency in humans would impair presynaptic filtering and likely lead to bradykinesia under DA-depleted conditions and dyskinesias following DA replenishment (Bamford et al., 2004a).

After a unilateral 6-OHDA injection into the medial forebrain bundle, MSNs ipsilateral to the injection site have a lower density of dendritic spines than those on the contralateral side (Ingham et al., 1993). It was later demonstrated that loss of spines in models of PD selectively affects MSNs of the indirect pathway (Day et al., 2006) (see also Chapter

6). Although strong evidence was presented that this effect could be attributed to dysregulation of postsynaptic L-type  $Ca^{2+}$  channels, a presynaptic contribution also is possible. As indicated above, DA-depleting lesions increase spontaneous glutamate-mediated synaptic events. As this increase selectively affects D2 receptor-containing MSNs, it is possible that excessive glutamate release can become neurotoxic and induce spine elimination, similar to effects that may occur in HD. Membrane loss can potentially induce increases in input resistance (Galarraga et al., 1987) making these cells even more electrotonically compact and excitable, thereby increasing susceptibility to glutamate. Supporting experimental evidence for this idea was obtained recently by the demonstration that dendritic remodeling of MSNs seen in models of PD occurs secondary only to increases in corticostriatal glutamatergic drive (Neely et al., 2007).

## C. Comparison Between Dopamine-Depletion and Genetic Models of PD

Based on data from genetic models, it is becoming increasingly clear that changes in the corticostriatal pathway are different and sometimes opposite to those found in acute or chronic toxic models of DA depletion. Thus, the initial perturbation in genetic models appears to be increased DA tissue concentration that leads to reduced D2 receptor function. For example, ASO mice exhibit a significantly lower frequency of spontaneous EPSCs in MSNs compared to age-matched WT littermates (Wu et al., 2005). In addition, whereas application of amphetamine reduces spontaneous EPSC frequency in control mice, it had little or no effect in ASO mice and DA D2 receptor agonists or antagonists produced contrasting effects in cells from ASO compared to WT mice. Together, these observations suggest that abnormal accumulation of  $\alpha$ -synuclein alters corticostriatal synaptic function and DA modulation and contribute to some of the behavioral abnormalities in ASO mice (Wu et al., 2005). In addition, the altered electrophysiology of the DA system could explain why these mice do not show stereotypies when challenged with amphetamine (Fleming et al., 2006).

Some of these findings resemble those found in DA transporter (DAT) knock-down mice (Wu et al., 2007). These mice display increased DA in the extracellular space due to reduced clearance. Whereas in control mice amphetamine reduces the frequency of spontaneous EPSCs by activation of D2 receptors, in DAT knock-down mice either no changes or small increases in frequency occur suggesting altered sensitivity of D2 receptors (Wu et al., 2007).

Furthermore, in DAT knock-down mice, amphetamine also elicited abnormal responses (Zhuang et al., 2001).

Alterations in corticostriatal synaptic plasticity in the corticostriatal pathway of transgenic ASO mice also are observed (Watson et al., 2009). Whereas striatal LTD occurred in ASO striatum, it did not occur in WTs. Interestingly paired-pulse facilitation increased after induction of LTD, suggesting that  $\alpha$ -synuclein may impact longterm forms of synaptic plasticity by preferentially reducing presynaptic glutamate release from corticostriatal terminals (Watson et al., 2009).

### D. Some Unresolved Questions

Similar to HD studies, adaptations in striatal microcircuits play an important role in motor and cognitive manifestations of PD. Changes in these microcircuits are beginning to be addressed and will be important in understanding mechanisms of alterations in striatal physiology. Studies in EGFP mice using dual patch recordings in D1 and D2 cells demonstrated that the strength of recurrent connections is dramatically reduced in PD models potentially contributing to pathological alterations in MSN activity patterns and psychomotor symptoms (Taverna et al., 2008). The role of cholinergic interneurons, particularly in PD, has aroused renewed interest thanks to the possibility of visualizing these rare interneurons for electrophysiological recordings in slices. In PD diminished striatal DA signaling leads to increased release of acetylcholine, distorting network function. In contrast, in HD there is a reduction in striatal cholinergic markers (Picconi et al., 2006). Electrophysiological studies in striatal TH-positive interneurons that increase dramatically after DA depletion (Porritt et al., 2000) (see Chapter 8), as well as in fast-spiking GABAergic interneurons (Mallet et al., 2006), should be conducted to determine how they influence MSNs in PD models.

The observation that synaptic pruning in DA-depletion models is selective for indirect pathway neurons (Day et al., 2006) is particularly interesting as it is reminiscent of the selective vulnerability of the same cells in HD. Interestingly, in the reserpine model of acute DA depletion spontaneous miniature EPSCs were reduced in frequency (Day et al., 2006), a result at odds with chronic depletion models (6-OHDA) that show increased frequency of spontaneous and miniature EPSCs (Galarraga et al., 1987; Cepeda et al., 1989; Gubellini et al., 2002), suggesting the existence of presynaptic alterations. However, this difference appeared related not to the model used but, surprisingly, to the recording solution in the patch electrode. Thus, using a K<sup>+</sup> based solution which more closely replicates physiological conditions, a significant increase in mEPSC frequency in DA-depleted compared to controls was observed, in line with previous investigations using the 6-OHDA model. This indicates either that spine loss does not necessarily correlate with reduced synapses, or that postsynaptic changes in excitability, e.g., increased input resistance, occur after DA-depletion. Evidence for the second alternative has been documented (Galarraga et al., 1987), although this is not a universal finding (Calabresi et al., 1993) probably because these latter studies could not differentiate D1 and D2 MSNs. Increased postsynaptic excitability can be compounded by the normally higher membrane input resistance and intrinsic excitability of D2 cells (Kreitzer and Malenka, 2007; Cepeda et al., 2008; Gertler et al., 2008). However, presynaptic changes can not be ruled out (Neely et al., 2007). In fact, after 6-OHDA lesions cortical drive into striatopallidal neurons is facilitated while the opposite is true for striatonigral neurons (Mallet et al., 2006), which could explain increased firing of this type of neurons after DA-depleting lesions. In addition, increased sensitivity of D2 receptors at the presynaptic terminal could be a mechanism to cope with increased glutamate release at the corticostriatal terminal (Bamford et al., 2004a), as these inputs appear to target D2 cells preferentially (Cepeda et al., 2008). Obviously, more studies are required to determine if spine loss in PD is due to purely pre- and/or postsynaptic mechanisms.

### **V. CONCLUSIONS**

In both HD and PD, adaptive functional and structural changes in MSNs occur and are manifested by alterations in synaptic activity along the corticostriatal pathway and by dendritic spine loss with preference for MSNs of the indirect pathway. It is of paramount importance to remember that these changes are dynamic and can manifest in opposite directions throughout the progression of the disease, as in early versus late HD, or in genetic versus toxin models of PD.

Why are D2 cells more vulnerable to the deleterious effects of excess glutamate? Tight synaptic coupling with cortical inputs and increased intrinsic excitability are good possibilities. In HD, preferential loss of MSNs giving rise to the indirect pathway points to the potentially deleterious dysregulated glutamate release from cortical neurons. However, the mechanisms leading to glutamate dysregulation are different in HD and PD. In the former, dysregulation seems to be caused by increased cortical excitability, reduced inhibition, and early loss of D2 and other presynaptic receptors controlling glutamate release. In contrast, in PD lack of DA produces D2 receptor supersensitivity, at least in DA-depletion models, but also dysregulated glutamate release that appears more selective to MSNs of the indirect pathway. Increases in input resistance occur in MSNs in both HD and PD, but it is not known if this change is the cause or the consequence of spine loss.

Development of symptoms in human idiopathic PD is protracted, allowing for important adaptations before DA deficiency reaches a critical level (see also Chapter 37). These changes are clearly seen in genetic mouse models compared to acute or chronic DA depletion. Thus, before any DA cell loss, changes in corticostriatal activity and D2 receptor sensitivity are observed. Interestingly, electrophysiological studies in these models are beginning to unravel an unexpected mechanism of striatal neuron dysfunction that involves early and paradoxical increases in DA content. Increased DA in striatum appears to be a common finding in several genetic animal models of PD, including parkin KO (Goldberg et al., 2003), ASO mice (Maidment et al., 2006), and DJ-1 null mice (Chen et al., 2005), suggesting that increased DA may contribute to substantia nigra DA neuron stress and eventual degeneration, either by increased local, dendritic release or, alternatively, by a possible retrograde mechanism. One may wonder if reduced DA input in both PD and HD is another adaptation, similar to reduced glutamatergic input in the late stages of HD, to cope with the potential neurotoxicity of DA (Cyr et al., 2003; Chen et al., 2008).

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### REFERENCES

- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Ariano MA, Aronin N, Difiglia M, Tagle DA, Sibley DR, Leavitt BR, Hayden MR, Levine MS (2002) Striatal neurochemical changes in transgenic models of Huntington's disease. J Neurosci Res 68:716–729.

- Ariano MA, Cepeda C, Calvert CR, et al. (2005) Striatal potassium channel dysfunction in Huntington's disease transgenic mice. J Neurophysiol 93:2565–2574.
- Ballion B, Mallet N, Bezard E, Lanciego JL, Gonon F (2008) Intratelencephalic corticostriatal neurons equally excite striatonigral and striatopallidal neurons and their discharge activity is selectively reduced in experimental parkinsonism. Eur J Neurosci 27:2313–2321.
- Bamford NS, Robinson S, Palmiter RD, Joyce JA, Moore C, Meshul CK (2004a) Dopamine modulates release from corticostriatal terminals. J Neurosci 24:9541–9552.
- Bamford NS, Zhang H, Schmitz Y, et al. (2004b) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 42:653–663.
- Bates GP, Murphy KP (2002) Mouse models of Huntington's disease. In: Huntington's Disease (GP Bates, AL Harper, AL Jones, Eds.), pp. 387–426. Oxford, UK: Oxford University Press.
- Berretta S, Parthasarathy HB, Graybiel AM (1997) Local release of GABAergic inhibition in the motor cortex induces immediate-early gene expression in indirect pathway neurons of the striatum. J Neurosci 17:4752–4763.
- Bibb JA, Yan Z, Svenningsson P, et al. (2000) Severe deficiences in dopamine signaling in presymptomatic Huntington's disease mice. Proc Natl Acad Sci USA 97:6809–6814.
- Blandini F, Armentero MT, Martignoni E (2008) The 6-hydroxydopamine model: news from the past. Parkinsonism Relat Disord 14(Suppl 2): S124–S129.
- Brown TB, Bogush AI, Ehrlich ME (2008) Neocortical expression of mutant huntingtin is not required for alterations in striatal gene expression or motor dysfunction in a transgenic mouse. Hum Mol Genet 17:3095–3104.
- Calabresi P, Mercuri NB, Sancesario G, Bernardi G (1993) Electrophysiology of dopamine-denervated striatal neurons. Implications for Parkinson's disease. Brain 116(Pt 2):433–452.
- Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 19:3248–3257.
- Cepeda C, Levine MS (2006) Where do you think you are going? The NMDA-D1 receptor trap. Sci STKE 2006:pe20.
- Cepeda C, Buchwald NA, Levine MS (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc Natl Acad Sci USA 90:9576–9580.
- Cepeda C, Wu N, Andre VM, Cummings DM, Levine MS (2007) The corticostriatal pathway in Huntington's disease. Prog Neurobiol 81:253–271.
- Cepeda C, Walsh JP, Hull CD, Howard SG, Buchwald NA, Levine MS (1989) Dye-coupling in the neostriatum of the rat: I. Modulation by dopamine-depleting lesions. Synapse 4:229–237.
- Cepeda C, Andre VM, Yamazaki I, Wu N, Kleiman-Weiner M, Levine MS (2008) Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. Eur J Neurosci 27:671–682.
- Cepeda C, Hurst RS, Calvert CR, et al. (2003) Transient and progressive electrophysiological alterations in the corticostriatal pathway in a mouse model of Huntington's disease. J Neurosci 23:961–969.
- Cepeda C, Starling AJ, Wu N, Nguyen OK, Uzgil B, Soda T, Andre VM, Ariano MA, Levine MS (2004) Increased GABAergic function in mouse models of Huntington's disease: reversal by BDNF. J Neurosci Res 78:855–867.

- Cepeda C, Hurst RS, Altemus KL, et al. (2001) Facilitated glutamatergic transmission in the striatum of D2 dopamine receptor-deficient mice. J Neurophysiol 85:659–670.
- Cha JH, Kosinski CM, Kerner JA, et al. (1998) Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. Proc Natl Acad Sci USA 95:6480–6485.
- Chartoff EH, Marck BT, Matsumoto AM, Dorsa DM, Palmiter RD (2001) Induction of stereotypy in dopamine-deficient mice requires striatal D1 receptor activation. Proc Natl Acad Sci USA 98:10451–10456.
- Chen L, Ding Y, Cagniard B, et al. (2008) Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. J Neurosci 28:425–433.
- Chen L, Cagniard B, Mathews T, et al. (2005) Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. J Biol Chem 280:21418–21426.
- Chesselet MF, Fleming S, Mortazavi F, Meurers B (2008) Strengths and limitations of genetic mouse models of Parkinson's disease. Parkinsonism Relat Disord 14(Suppl 2):S84–S87.
- Cummings DM, Milnerwood AJ, Dallerac GM, et al. (2006) Aberrant cortical synaptic plasticity and dopaminergic dysfunction in a mouse model of Huntington's disease. Hum Mol Genet 15:2856–2868.
- Cummings DM, André VM, Uzgil BO, Gee SM, Fisher YE, Cepeda C, Levine MS (2009) Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. J Neurosci 29:10371–10386.
- Cyr M, Beaulieu JM, Laakso A, et al. (2003) Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. Proc Natl Acad Sci USA 100:11035–11040.
- Dani JA, Zhou FM (2004) Selective dopamine filter of glutamate striatal afferents. Neuron 42:522–524.
- Davies SW, Turmaine M, Cozens BA, et al. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548.
- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- DiFiglia M, Sapp E, Chase K, et al. (1995) Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 14:1075–1081.
- Ding J, Peterson JD, Surmeier DJ (2008) Corticostriatal and thalamostriatal synapses have distinctive properties. J Neurosci 28:6483–6492.
- Fan MM, Raymond LA (2007) N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. Prog Neurobiol 81:272–293.
- Fischer E, Heller B (1967) Pharmacology of the mechanism of certain effects of reserpine in the rat. Nature 216:1221–1222.
- Fleming SM, Fernagut PO, Chesselet MF (2005) Genetic mouse models of parkinsonism: strengths and limitations. NeuroRx 2:495–503.
- Fleming SM, Tetreault NA, Mulligan CK, Hutson CB, Masliah E, Chesselet MF (2008) Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. Eur J Neurosci 28:247–256.
- Fleming SM, Salcedo J, Hutson CB, Rockenstein E, Masliah E, Levine MS, Chesselet MF (2006) Behavioral effects of dopaminergic agonists in transgenic mice overexpressing human wildtype alphasynuclein. Neuroscience 142:1245–1253.
- Flores-Hernandez J, Galarraga E, Bargas J (1997) Dopamine selects glutamatergic inputs to neostriatal neurons. Synapse 25:185–195.

- Florio T, Di Loreto S, Cerrito F, Scarnati E (1993) Influence of prelimbic and sensorimotor cortices on striatal neurons in the rat: electrophysiological evidence for converging inputs and the effects of 6-OHDAinduced degeneration of the substantia nigra. Brain Res 619:180–188.
- Fusco FR, Martorana A, De March Z, Viscomi MT, Sancesario G, Bernardi G (2003) Huntingtin distribution among striatal output neurons of normal rat brain. Neurosci Lett 339:53–56.
- Fusco FR, Chen Q, Lamoreaux WJ, et al. (1999) Cellular localization of huntingtin in striatal and cortical neurons in rats: lack of correlation with neuronal vulnerability in Huntington's disease. J Neurosci 19:1189–1202.
- Galarraga E, Bargas J, Martinez-Fong D, Aceves J (1987) Spontaneous synaptic potentials in dopamine-denervated neostriatal neurons. Neurosci Lett 81:351–355.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.
- Gertler TS, Chan CS, Surmeier DJ (2008) Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci 28:10814–10824.
- Gil JM, Rego AC (2008) Mechanisms of neurodegeneration in Huntington's disease. Eur J Neurosci 27:2803–2820.
- Goldberg MS, Pisani A, Haburcak M, et al. (2005) Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45:489–496.
- Goldberg MS, Fleming SM, Palacino JJ, et al. (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278:43628–43635.
- Graham RK, Pouladi MA, Joshi P, et al. (2009) Differential susceptibility to excitotoxic stress in YAC128 mouse models of Huntington disease between initiation and progression of disease. J Neurosci 29:2193–2204.
- Gray M, Gu X, Shiraski D, Cepeda C, Yamazaki I, Levine MS, Yang X (2008a) Cortical control of striatal pathogenesis in the Cre/LoxP conditional BAC transgenic mouse model of Huntington's Disease (BACHD) Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience Program No. 114.6.
- Gray M, Shirasaki DI, Cepeda C, et al. (2008b) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195.
- Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254.
- Greenamyre JT, Betarbet R, Sherer TB (2003) The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Relat Disord 9(Suppl 2):S59–S64.
- Gu X, Andre VM, Cepeda C, Li SH, Li XJ, Levine MS, Yang XW (2007) Pathological cell–cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. Mol Neurodegen 2:8.
- Gu X, Li C, Wei W, et al. (2005) Pathological cell–cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron 46:433–444.
- Gubellini P, Picconi B, Bari M, et al. (2002) Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. J Neurosci 22:6900–6907.
- Haber SN, Nauta WJ (1983) Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry. Neuroscience 9:245–260.

- Harper PS (1996) New genes for old diseases: the molecular basis of myotonic dystrophy and Huntington's disease. The Lumleian Lecture 1995. J R Coll Physicians Lond 30:221–231.
- Hashimoto M, Rockenstein E, Masliah E (2003) Transgenic models of alpha-synuclein pathology: past, present, and future. Ann NY Acad Sci 991:171–188.
- Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS (2004) Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? Eur J Neurosci 19:2455–2463.
- Hickey MA, Reynolds GP, Morton AJ (2002) The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. J Neurochem 81:46–59.
- Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, Chesselet MF (2008) Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. Neuroscience 157:280–295.
- Hodgson JG, Agopyan N, Gutekunst CA, et al. (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 23:181–192.
- Huang CC, Lo SW, Hsu KS (2001) Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. J Physiol 532:731–748.
- Hull CD, Levine MS, Buchwald NA, Heller A, Browning RA (1974) The spontaneous firing pattern of forebrain neurons. I. The effects of dopamine and non-dopamine depleting lesions on caudate unit firing patterns. Brain Res 73:241–262.
- Ingham CA, Hood SH, van Maldegem B, Weenink A, Arbuthnott GW (1993) Morphological changes in the rat neostriatum after unilateral 6-hydroxydopamine injections into the nigrostriatal pathway. Exp Brain Res 93:17–27.
- Itier JM, Ibanez P, Mena MA, et al. (2003) Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet 12:2277–2291.
- Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–376.
- Johnson MA, Rajan V, Miller CE, Wightman RM (2006) Dopamine release is severely compromised in the R6/2 mouse model of Huntington's disease. J Neurochem 97:737–746.
- Joshi PR, Wu NP, Andre VM, et al. (2009) Age-dependent alterations of corticostriatal activity in the YAC128 mouse model of Huntington disease. J Neurosci 29:2414–2427.
- Kawaguchi Y, Wilson CJ, Emson PC (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 10:3421–3438.
- Kemp JM, Powell TP (1970) The cortico-striate projection in the monkey. Brain 93:525–546.
- Kim DS, Szczypka MS, Palmiter RD (2000) Dopamine-deficient mice are hypersensitive to dopamine receptor agonists. J Neurosci 20:4405–4413.
- Kita H, Kitai ST (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res 447:346–352.
- Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet MF, Levine MS (2001) Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice. J Neurophysiol 86:2667–2677.

- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Lei W, Jiao Y, Del Mar N, Reiner A (2004) Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 24:8289–8299.
- Levine MS, Li Z, Cepeda C, Cromwell HC, Altemus KL (1996) Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. Synapse 24:65–78.
- Levine MS, Cepeda C, Hickey MA, Fleming SM, Chesselet MF (2004) Genetic mouse models of Huntington's and Parkinson's diseases: illuminating but imperfect. Trends Neurosci 27:691–697.
- Levine MS, Klapstein GJ, Koppel A, et al. (1999) Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. J Neurosci Res 58:515–532.
- Li JY, Plomann M, Brundin P (2003) Huntington's disease: a synaptopathy? Trends Mol Med 9:414–420.
- Lin CH, Tallaksen-Greene S, Chien WM, et al. (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10:137–144.
- Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB (1999) Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. J Neurosci 19:10428–10437.
- Lovinger DM, Choi S (1995) Activation of adenosine A1 receptors initiates short-term synaptic depression in rat striatum. Neurosci Lett 199:9–12.
- Lovinger DM, McCool BA (1995) Metabotropic glutamate receptormediated presynaptic depression at corticostriatal synapses involves mGLuR2 or 3. J Neurophysiol 73:1076–1083.
- Luthi-Carter R, Strand A, Peters NL, et al. (2000) Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. Hum Mol Genet 9:1259–1271.
- Maidment NT, Lam HA, Ackerson LC, Rockenstein E, Masliah E (2006) Dysregulation of dopamine transmission in mice overexpressing human wildtype alpha-synuclein. Abstract Viewer/Itinerary Planner. Atlanta, GA: Society for Neuroscience Program No. 378.4.
- Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 26:3875–3884.
- Mangiarini L, Sathasivam K, Seller M, et al. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506.
- Masliah E, Rockenstein E, Veinbergs I, et al. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287:1265–1269.
- Menalled L, Zanjani H, MacKenzie L, Koppel A, Carpenter E, Zeitlin S, Chesselet MF (2000) Decrease in striatal enkephalin mRNA in mouse models of Huntington's disease. Exp Neurol 162:328–342.
- Menalled LB, Chesselet MF (2002) Mouse models of Huntington's disease. Trends Pharmacol Sci 23:32–39.
- Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF (2003) Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J Comp Neurol 465:11–26.
- Meredith GE, Totterdell S, Potashkin JA, Surmeier DJ (2008) Modeling PD pathogenesis in mice: advantages of a chronic MPTP protocol. Parkinsonism Relat Disord 14(2):S112–S115.
- Miller BR, Walker AG, Shah AS, Barton SJ, Rebec GV (2008) Dysregulated information processing by medium spiny neurons in striatum of freely behaving mouse models of Huntington's disease. J Neurophysiol 100:2205–2216.

- Milnerwood AJ, Raymond LA (2007) Corticostriatal synaptic function in mouse models of Huntington's disease: early effects of huntingtin repeat length and protein load. J Physiol 585:817–831.
- Milnerwood AJ, Gladding CM, Vasuta OC, Graham RK, Hayden MR, Murphy TH, Raymond LA (2008) Increased extrasynaptic NMDA receptor signalling in a transgenic mouse model of Huntington's disease. Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience Program No. 547.14.
- Milnerwood AJ, Cummings DM, Dallerac GM, et al. (2006) Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. Hum Mol Genet 15:1690–1703.
- Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (2000) Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. J Neurosci 20:5115–5123.
- Neely MD, Schmidt DE, Deutch AY (2007) Cortical regulation of dopamine depletion-induced dendritic spine loss in striatal medium spiny neurons. Neuroscience 149:457–464.
- Nisenbaum ES, Berger TW, Grace AA (1992) Presynaptic modulation by GABAB receptors of glutamatergic excitation and GABAergic inhibition of neostriatal neurons. J Neurophysiol 67:477–481.
- Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J (2004) Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J Biol Chem 279:18614–18622.
- Paladini CA, Robinson S, Morikawa H, Williams JT, Palmiter RD (2003) Dopamine controls the firing pattern of dopamine neurons via a network feedback mechanism. Proc Natl Acad Sci USA 100:2866–2871.
- Pallier PN, Maywood ES, Zheng Z, et al. (2007) Pharmacological imposition of sleep slows cognitive decline and reverses dysregulation of circadian gene expression in a transgenic mouse model of Huntington's disease. J Neurosci 27:7869–7878.
- Papadia S, Hardingham GE (2007) The dichotomy of NMDA receptor signaling. Neuroscientist 13:572–579.
- Picconi B, Passino E, Sgobio C, et al. (2006) Plastic and behavioral abnormalities in experimental Huntington's disease: a crucial role for cholinergic interneurons. Neurobiol Dis 22:143–152.
- Pisani A, Bernardi G, Ding J, Surmeier DJ (2007) Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30:545–553.
- Polymeropoulos MH, Lavedan C, Leroy E, et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047.
- Porritt MJ, Batchelor PE, Hughes AJ, Kalnins R, Donnan GA, Howells DW (2000) New dopaminergic neurons in Parkinson's disease striatum. Lancet 356:44–45.
- Rasmussen A, Macias R, Yescas P, Ochoa A, Davila G, Alonso E (2000) Huntington disease in children: genotype–phenotype correlation. Neuropediatrics 31:190–194.
- Rebec GV, Conroy SK, Barton SJ (2006) Hyperactive striatal neurons in symptomatic Huntington R6/2 mice: variations with behavioral state and repeated ascorbate treatment. Neuroscience 137:327–336.
- Reiner A, Jiao Y, Del Mar N, Laverghetta AV, Lei WL (2003) Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. J Comp Neurol 457:420–440.
- Rockenstein E, Mallory M, Hashimoto M, Song D, Shults CW, Lang I, Masliah E (2002) Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. J Neurosci Res 68:568–578.

- Schultz W (1982) Depletion of dopamine in the striatum as an experimental model of Parkinsonism: direct effects and adaptive mechanisms. Prog Neurobiol 18:121–166.
- Schultz W, Ungerstedt U (1978) Short-term increase and long-term reversion of striatal cell activity after degeneration of the nigrostriatal dopamine system. Exp Brain Res 33:159–171.
- Shelbourne PF, Killeen N, Hevner RF, et al. (1999) A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. Hum Mol Genet 8:763–774.
- Shimura H, Hattori N, Kubo S, et al. (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 25:302–305.
- Shuen JA, Chen M, Gloss B, Calakos N (2008) Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. J Neurosci 28:2681–2685.
- Slow EJ, van Raamsdonk J, Rogers D, et al. (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567.
- Smeal RM, Keefe KA, Wilcox KS (2008) Differences in excitatory transmission between thalamic and cortical afferents to single spiny efferent neurons of rat dorsal striatum. Eur J Neurosci 28:2041–2052.
- Smeal RM, Gaspar RC, Keefe KA, Wilcox KS (2007) A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents. J Neurosci Methods 159:224–235.
- Smith R, Brundin P, Li JY (2005) Synaptic dysfunction in Huntington's disease: a new perspective. Cell Mol Life Sci 62:1901–1912.
- Spampanato J, Gu X, Yang XW, Mody I (2008) Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. Neuroscience 157:606–620.
- Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840.
- Stack EC, Dedeoglu A, Smith KM, et al. (2007) Neuroprotective effects of synaptic modulation in Huntington's disease R6/2 mice. J Neurosci 27:12908–12915.
- Starling AJ, Andre VM, Cepeda C, de Lima M, Chandler SH, Levine MS (2005) Alterations in N-methyl-D-aspartate receptor sensitivity and magnesium blockade occur early in development in the R6/2 mouse model of Huntington's disease. J Neurosci Res 82:377–386.
- Steiner H, Gerfen CR (1999) Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. Neuroscience 88:795–810.
- Strong TV, Tagle DA, Valdes JM, et al. (1993) Widespread expression of the human and rat Huntington's disease gene in brain and non-neural tissues. Nat Genet 5:259–265.
- Taverna S, Ilijic E, Surmeier DJ (2008) Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's disease. J Neurosci 28:5504–5512.
- Tepper JM, Koos T, Wilson CJ (2004) GABAergic microcircuits in the neostriatum. Trends Neurosci 27:662–669.
- Tepper JM, Wilson CJ, Koos T (2008) Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons. Brain Res Rev 58:272–281.
- The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983.
- Thompson PD, Berardelli A, Rothwell JC, Day BL, Dick JP, Benecke R, Marsden CD (1988) The coexistence of bradykinesia and chorea in Huntington's disease and its implications for theories of basal ganglia control of movement. Brain 111(Pt 2):223–244.

- Tseng KY, Kasanetz F, Kargieman L, Riquelme LA, Murer MG (2001) Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions. J Neurosci 21:6430–6439.
- Uhl GR, Navia B, Douglas J (1988) Differential expression of preproenkephalin and preprodynorphin mRNAs in striatal neurons: high levels of preproenkephalin expression depend on cerebral cortical afferents. J Neurosci 8:4755–4764.
- Ungerstedt U (1968) 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110.
- Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR (2005) Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci 25:4169–4180.
- Vincent S, Hokfelt T, Christensson I, Terenius L (1982) Immunohistochemical evidence for a dynorphin immunoreactive striato-nigral pathway. Eur J Pharmacol 85:251–252.
- von Horsten S, Schmitt I, Nguyen HP, et al. (2003) Transgenic rat model of Huntington's disease. Hum Mol Genet 12:617–624.
- Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384.
- Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr. (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44:559–577.
- Wang H, Pickel VM (2002) Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. J Comp Neurol 442:392–404.
- Wang L, Fleming SM, Chesselet MF, Tache Y (2008) Abnormal colonic motility in mice overexpressing human wild-type alpha-synuclein. Neuroreport 19:873–876.
- Watson JB, Hatami A, David H, Masliah E, Roberts K, Evans CE, Levine MS (2009) Alterations in corticostriatal synaptic plasticity in mice overexpressing human alpha-synuclein. Neuroscience 159:501–513.
- Wheeler VC, White JK, Gutekunst CA, et al. (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. Hum Mol Genet 9:503–513.
- White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL, MacDonald ME (1997) Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17:404–410.
- Wilson CJ (1990) Basal ganglia. In: The Synaptic Organization of the Brain (GW Shepherd Ed.), pp. 279–316. Oxford: Oxford University Press.
- Wood JD, MacMillan JC, Harper PS, Lowenstein PR, Jones AL (1996) Partial characterisation of murine huntingtin and apparent variations in the subcellular localisation of huntingtin in human, mouse and rat brain. Hum Mol Genet 5:481–487.
- Woodman B, Butler R, Landles C, et al. (2007) The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain Res Bull 72:83–97.
- Wu N, Cepeda C, Masliah E, Levine MS (2005) Abnormal glutamate and dopamine receptor function in the striatum of α-synuclein-overexpressing mice. Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience Program No. 85.12.
- Wu N, Cepeda C, Zhuang X, Levine MS (2007) Altered corticostriatal neurotransmission and modulation in dopamine transporter knockdown mice. J Neurophysiol 98:423–432.

- Yan Z, Hsieh-Wilson L, Feng J, Tomizawa K, Allen PB, Fienberg AA, Nairn AC, Greengard P (1999) Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin. Nat Neurosci 2:13–17.
- Yang SH, Cheng PH, Banta H, et al. (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453:921–924.
- Yin HH, Lovinger DM (2006) Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 103:8251–8256.
- Zhou QY, Palmiter RD (1995) Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 83:1197–1209.
- Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, Hen R (2001) Hyperactivity and impaired response habituation in hyperdopaminergic mice.. Proc Natl Acad Sci USA 98:1982–1987.
- Zuccato C, Cattaneo E (2007) Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol 81:294–330.

# Molecular Mechanisms of L-DOPA-Induced Dyskinesia

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### I. INTRODUCTION

The dopamine (DA) precursor, 3,4-dihydroxyphenyl-Lalanine (L-DOPA), is a very cost-effective treatment for the signs and symptoms of Parkinson's disease (PD) (Rascol et al., 2003). Unfortunately, however, the response to L-DOPA changes during the progression of PD. As the disease becomes more severe, the need for symptomatic medications becomes larger, while the response to the treatment becomes complicated by motor fluctuations and abnormal involuntary movements (dyskinesia). Meta-analyses of published studies indicate that these motor complications affect approximately 40% of PD patients after 4-6 years of L-DOPA therapy, and up to 90% of the patients by 10 years of treatment (Ahlskog and Muenter, 2001; Manson and Schrag, 2006). Using longacting DA receptor agonists instead of L-DOPA reduces the incidence of motor complications, but these agents achieve a poorer symptomatic control and have important side effects (Stowe et al., 2008). The vast majority of PD patients will

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. therefore continue to require treatment with L-DOPA at some point during the course of the disease. The phenomenological features of dyskinesia and motor fluctuations in PD have been described in some excellent clinical reviews (see, e.g., Luquin et al., 1992; Quinn, 1998; Fabbrini et al., 2007), and will not be discussed in this chapter. The risk factors for L-DOPA-induced dyskinesia (LID), as identified by clinical epidemiological studies, will be mentioned because they give us clues of neurobiological significance. The main factors predisposing to dyskinesia in PD are disease severity and duration, high initial and cumulative doses of L-DOPA, and young age at PD onset (reviewed in Manson and Schrag, 2006). The impact of PD severity and duration on the risk of LID suggests that a large degree of striatal DA denervation predisposes to this movement disorder (discussed in Cenci and Lundblad, 2006). The clear relationship between dyskinesia and L-DOPA dosage indicates, however, that the treatment has a prime causative role. The high risk for LID in young PD patients has been attributed

to the high propensity for neuroplasticity in the young brain (Linazasoro, 2005). This interpretation is in keeping with a commonly held tenet, equating dyskinesia to a disorder of brain plasticity (reviewed in Linazasoro, 2005). Indeed, dyskinesia develops gradually during the course of L-DOPA pharmacotherapy, and once developed, it can be evoked by stress or drugs other than L-DOPA (reviewed in Cenci and Lundblad, 2006; Jenner, 2008). While there is ample evidence of maladaptive neuroplasticity in LID, it remains to be proven that young age predisposes to LID through a general enhancement of brain plasticity mechanisms, and an involvement of etiopathological factors characteristic of young-onset PD cannot be ruled out. By contrast, the causal roles of striatal DA denervation, on one hand, and L-DOPA dosage, on the other hand, have now been demonstrated in basic experimental studies. In both non-human primate and rodent models of LID, the incidence and severity of LID are conditioned by the degree of nigrostriatal DA denervation and by the L-DOPA dose/treatment regimen (Winkler et al., 2002; Jenner, 2003; Lindgren et al., 2007). While DA denervation dramatically lowers the dyskinesia threshold dose, the movement disorder is caused by L-DOPA in a time-and dose-dependent manner (reviewed in Cenci and Lundblad, 2006). In both non-human primate and rodent models of PD, L-DOPA-induced abnormal involuntary movements (AIMs) occur during two-three hours following drug administration, exhibiting the same time course as peak-of-dose dyskinesia in PD. Moreover, the AIMs show an increasing incidence and severity upon repeated administration of the same L-DOPA dose (reviewed in Cenci and Lundblad, 2006).

For the above reasons, this review on the molecular basis of LID will elaborate on two main questions: (i) through what mechanisms does DA denervation predispose to dyskinesia? (ii) what are the molecular pathways through which L-DOPA triggers and aggravates this movement disorder? Our understanding of these basic questions has been considerably advanced by recent studies in animal models of LID, although much more research is required to clarify essential issues, which will exemplified in the following. The bulk of most recent data were produced in rodents, which provide cost-effective models accessible to most laboratories. Rodent models of L-DOPA-induced AIMs utilize rats or mice with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal DA pathway. The animals are treated daily with L-DOPA for a couple of weeks in order to induce movements with hyperkinetic and dystonic features, affecting the forelimb contralateral to the lesion, the trunk and the orofacial musculature (full descriptions are provided in (Cenci and Lundblad, 2007). In most, but not all cases (see Winkler et al., 2002), the AIMs are accompanied by turning behavior in the direction contralateral to the lesion, which is regarded as a model of LID by some investigators (Henry et al., 1998; Carta et al., 2006, but see Cenci et al., 2002; Lundblad et al., 2002; Marin et al., 2006).

## II. MOLECULAR AND CELLULAR CHANGES FOLLOWING DOPAMINE DENERVATION

### A. Presynaptic Alterations

In an intact brain, nigrostriatal DA neurons convert L-DOPA to DA, store exogenously derived DA in synaptic vesicles, and release it when and where needed. Moreover, DA neurons are endowed with presynaptic DA autoreceptors and high-affinity DA transporters at the plasma membrane, thus maintaining extracellular DA levels within a narrow physiological range. A lesion of the nigrostriatal DA projection disrupts the presynaptic control of DA release and clearance. This results in large increases in extracellular DA levels concomitant with the dosing of L-DOPA (reviewed in Cenci and Lundblad, 2006; Cenci, 2007; Cenci and Lindgren, 2007). This concept was developed already in the nineties by seminal microdialysis studies performed in 6-OHDA-lesioned rats. The lesioned animals exhibited a dramatic reduction in baseline extracellular DA concentrations in the striatum, but an abnormally large increase following treatment with L-DOPA (Abercrombie et al., 1990). These findings have been widely replicated in recent literature (Meissner et al., 2006; Lee et al., 2008; Lindgren et al., 2009b). The observed large increases in extracellular DA levels reflect the uptake and conversion of exogenous L-DOPA by cells other than nigrostriatal DA neurons. Most cell types in the brain have indeed the ability to transport L-DOPA across the plasma membrane, and several cell types contain the enzyme converting L-DOPA to DA (aromatic amino acid decarboxylase) (reviewed in Cenci and Lundblad, 2006). In addition, monoaminergic cells, such as serotonin neurons (Peter et al., 1995), also contain a specific transport protein that packages DA into vesicles (vesicular monoamine transporter 2, VMAT2), thus protecting it from cytosolic degradation. However, the uptake and conversion of exogenous L-DOPA by non-dopaminergic cells results in non-regulated DA efflux and defective clearance of extracellular DA. In 6-OHDA-lesioned rats,

treatment with the serotonergic neurotoxin 5,7-dihydroxy tryptamine (5,7-DHT) attenuates the L-DOPA-induced increase in striatal extracellular DA levels by 80% (Tanaka et al., 1999). Interestingly, the same type of neurotoxic lesion completely abolishes L-DOPA-induced AIMs (Carta et al., 2007). Agonists of serotonin 5-HT1A and 5-HT1B receptors, which reduce transmitter release from serotonergic neurons, exert pronounced antidyskinetic effects in animal models of LID (Bishop et al., 2006; Carta et al., 2007; Dekundy et al., 2007; Munoz et al., 2008). The same agents blunt the peak of brain extracellular DA concentrations following a peripheral injection of L-DOPA (Kannari et al., 2001; Lindgren et al., 2009b).

Notwithstanding the considerable progress made in this area, it would be misleading to propose that presynaptic abnormalities in DA release and clearance are sufficient to explain LID. Indeed, dyskinesias indistinguishable from those induced by L-DOPA are evoked by some DA receptor agonists in both animal models (Delfino et al., 2007; Lindgren et al., 2009a) and PD patients (Rascol et al., 2001). These drugs obviate the need for uptake, conversion and release by specific cell categories in the brain. Even if induced by the DA agonists, dyskinesia does, however, occur only when the nigrostriatal DA pathway is severely damaged. This argues for a critical role of postsynaptic mechanisms in the predisposition to LID that is established by DA denervation.

## **B.** Postsynaptic Signal-Transduction Mechanisms

Physiological DA transmission maintains a certain level of sensitivity in postsynaptic neurons, and the absence of DA causes postsynaptic receptors to become supersensitive. Denervation-induced supersensitivity of striatal DA receptors has long been regarded as an important determinant of LID (Klawans et al., 1977; Nutt, 1990; Marconi et al., 1994) (see also Chapter 28). Such a supersensitivity does not appear to stem from an increased receptor number, because no consistent changes in ligand-binding activities at D1, D2, or D3 receptors have been found in dyskinetic PD patients, and findings from animal models have been contradictory in this regard (Rinne et al., 1991; Hurley et al., 1996b; Hurley et al., 1996a; Turjanski et al., 1997; Quik et al., 2000; Bezard et al., 2003; Aubert et al., 2005). Recent studies have reported an increased surface expression of D1 receptors in striatal neurons in both DA-denervated animals and chronically L-DOPA treated, dyskinetic ones (Guigoni et al., 2007; Berthet et al., 2009). Other studies, however, have reported increased internalization of D1 receptors following DA denervation and/or L-DOPA treatment (Muriel et al., 2002; Fiorentini et al., 2006).

Overall, the available data indicate that the denervation-induced supersensitivity of DA receptors reflects postsynaptic signal-transduction mechanisms, consisting in (or resulting from): (i) aberrant activation of signaling cascades that would not be recruited by DA receptors under normal conditions; (ii) exuberant activation of the "physiological" signaling pathways downstream of DA receptors; (iii) reduced expression of negative signaling modulators. A particularly relevant example of aberrant signalingpathway activation has been provided by Gerfen and collaborators, showing that severe DA denervation confers inducibility of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in the striatum upon treatment with D1 DA receptor agonists (Gerfen et al., 2002) (see Chapter 28). Because of its proven role in LID, this molecular response will be extensively commented upon in Section IIIA. As to the "exaggeration" of physiological signaling, a welldocumented and important example consists in the pronounced overactivity of the D1 receptor-adenylate cyclase signal transduction pathways following DA denervation (Mishra et al., 1974; Pifl et al., 1992) (see also Chapter 26). This exuberant response does not appear to be normalized by standard antiparkinsonian pharmacotherapies. Indeed, the ability of DA to stimulate adenylyl cyclase via activation of D1 receptors was found to be enhanced in postmortem striatal tissue from parkinsonian patients (Tong et al., 2004), all of whom received treatment with L-DOPA. Among the possible mechanisms underlying this hyperactive response, an increased coupling of DA receptors to their G proteins seems to play a crucial role. The G-protein coupling efficiency of striatal DA receptors has been studied in both rats and monkey models of PD by measuring DA agonist-induced guanosine 5'-O-(gamma[35S]thio) triphosphate ([35S]GTP-gammaS) binding. These studies have shown that agonist-induced G protein-binding activity is increased at both D2- and D1-type receptors following a DA denervating lesion (Geurts et al., 1999; Aubert et al., 2005), and that enhanced G protein-coupling activity of D1 receptors is particularly important to LID (Aubert et al., 2005). Indeed, a comprehensive study performed in MPTP-intoxicated and L-DOPA-treated monkeys has revealed a positive linear relationship between D1 agonistinduced GTP binding activity in the striatum and dyskinesia severity scores (Aubert et al., 2005). The increased GTP
binding activity at D1 receptors is likely to depend, at least in part, on an upregulated expression of G $\alpha$ -olf protein, to which this type of receptor is coupled (Herve et al., 1993; Corvol et al., 2004). Indeed, DA-denervating lesions upregulate G $\alpha$ -olf levels in striatal neurons, and L-DOPA pharmacotherapy in PD does not seem to normalize this change (Corvol et al., 2004).

Finally, a diminished expression and/or efficiency of negative signaling modulators appears to contribute to the denervation-induced DA receptor supersensitivity. Two classes of negative signaling modulators that are altered by DA denervation have been reported in the literature. The Ras Homolog Enriched in Striatum (Rhes) encodes a GTPbinding protein that inhibits ligand-mediated signaling through G protein-complexes (Vargiu et al., 2004), and is believed to preferentially affect Galpha s/olf-dependent signaling (Harrison and LaHoste, 2006). Removal of DA input to striatal neurons by either surgical denervation or reserpine treatment causes a long-lasting downregulation of Rhes, which may contribute to increased D1 receptor-dependent signaling (Harrison and LaHoste, 2006; Harrison et al., 2008). In support of this hypothesis, Rhes knockout mice exhibit enhanced D1-dependent stimulation of motor activity and cAMP/PKA signaling, and a modest upregulation of Golf protein levels in the striatum (Errico et al., 2008)

Another important class of negative signaling modulators are a family of proteins named, regulators of G protein signaling (RGS), which promote GTP hydrolysis by the alpha subunit of heterotrimeric G proteins, thereby inactivating the G protein and rapidly switching off G proteincoupled receptor signaling pathways (De Vries et al., 2000). A selective increase in the mRNA levels of RGS2, 5 and 8, and a decrease in RGS4 and 9 mRNA, have been described in the rat striatum following DA denervation (Geurts et al., 2003). The downregulation of RGS9, in particular, may play a role in the supersensitivity of DA receptor-signaling associated with LID. Indeed, viral vector-mediated overexpression of RGS9-2 in the striatum reduces the severity of LID in both rat and monkey models of PD (Gold et al., 2007).

# C. Structural and Synaptic Alterations in Striatal Microcircuits

In addition to receptor supersensitivity, the nigrostriatal DA lesion causes changes in dendritic and synaptic morphology in striatal neurons, which have been studied at both the light and the electron microscopic levels. These structural alterations are likely to contribute to the profound deficits in activity-dependent synaptic plasticity that have been detected in the DA-denervated striatum (see Chapters 6, 12 and 35). Studies applying the Golgi silver-impregnation technique to 6-OHDA-lesioned rats have shown that the loss of DA input is rapidly followed by a decrease in the number of dendritic spines in striatal medium-sized spiny (MSN) neurons, and that this structural modification is virtually permanent (Ingham et al., 1989; Ingham et al., 1993). Using mice engineered to express green fluorescent protein (GFP) under the control of the D1- or D2 receptor promoter, Day et al. (2006) showed that denervationinduced spine pruning (accompanied by a loss of corticostriatal synapses) selectively affects D2 receptor-rich, striatopallidal neurons. This study also revealed that the loss of spines is caused by the sustained activation of L-type calcium channels occurring in striatopallidal neurons after DA depletion. Interestingly, post-mortem studies in human PD patients have revealed structural changes in striatal morphology similar to those observed in the animal models (Zaja-Milatovic et al., 2005). Because the patients had been treated with L-DOPA for many years, these results suggest that pharmacological dopaminergic therapies do not normalize the dendritic structure of striatal neurons. The treatment's inability to restore essential structural features of the nigrostriatal microcircuitry is attracting growing attention as a potential determinant of LID (Jenner, 2008). It has been proposed that changes in dendritic morphology and spine loss may cause abnormalities in corticostriatal transmission that are pivotal to both parkinsonian motor symptoms and LID (Day et al., 2006; Deutch, 2006; Jenner, 2008). A recent study would, however, suggest that denervation-induced, striatopallidal spine pruning does not play a critical role in the development of LID. In this study, 6-OHDA-lesioned rats were chronically treated with the L-type calcium channel antagonist, isradipine, starting on the same day of the toxin injection, and through a period of chronic L-DOPA treatment (Schuster et al., 2008b). While totally preventing the loss of corticostriatal synapses in striatopallidal neurons, isradipine achieved just a partial attenuation of LID (Schuster et al., 2008b). These findings do not, however, rule out a major implication of neuronal and synaptic remodeling in the pathophysiology of LID. Conceivably, both DA denervation and the subsequent treatment with L-DOPA produce complex structural and synaptic changes in neurons of the basal ganglia, which are unlikely to be prevented by a single calcium-channel antagonist. Thus, the role of structural dendritic alterations in the pathophysiology of LID remains an open question

for future investigation. This sort of investigation will be aided by modern cell-imaging technologies combined with the use of transgenic mice in which specific populations of neurons are labeled with GFP (see Chapter 6).

## III. MOLECULAR AND CELLULAR CHANGES CAUSED BY L-DOPA TREATMENT

## A. Signaling-Pathway Activation in Striatal Neurons

In the advanced stages of PD, treatment with L-DOPA engages the perturbed signaling machinery of striatal neurons (cf. Section IIB), bringing about molecular responses that favor the expression and consolidation of LID. Such responses are the subject of this paragraph.

Overall, the available data indicate that denervationinduced supersensitivity of DA receptors is not normalized by standard dopaminergic pharmacotherapies. Thus, every dose of L-DOPA is bound to produce an exuberant stimulation of both D1- and D2 receptors, resulting in a large activation and inhibition, respectively, of cAMP-dependent signaling pathways in D1-rich and D2-rich striatal neurons (see also Chapters 1 and 26). While the contribution of D2rich, "indirect pathway" neurons to LID has not yet been elucidated, the overactivity of D1-mediated signaling in "direct pathway" neurons plays a major pathophysiological role in LID, as established in mouse (Santini et al., 2007), rat (Westin et al., 2007) and macaque models (Aubert et al., 2005) of this movement disorder. Several independent studies have provided evidence of altered D1-dependent activation and/or expression of intracellular signaling proteins in L-DOPA-treated dyskinetic rodents. In particular, chronically L-DOPA treated, dyskinetic rats and mice show increased striatal phosphorylation of dopamineand-cAMP-regulated phosphoprotein of 32 KDa (DARPP-32) at the threonine-34 residue (Picconi et al., 2003; Santini et al., 2007). Phosphorylation of DARPP-32 at threonine 34 is induced by L-DOPA through the stimulation of D1 receptors and protein kinase A (Svenningsson et al., 2004). Because phospho-Thr34-DARPP32 is a potent inhibitor of protein phosphatase-1 (PP-1) (Svenningsson et al., 2004), it is not surprising that the striatal levels of several phosphorylated substrates are elevated in the "dyskinetic" striatum. An imbalanced activation of protein tyrosine kinases and phosphatases contributes to the accumulation of phosphorylated substrated in DA-denervated striatal neurons upon treatment with DA agonists (Zhen et al., 2002; Dunah et al., 2004). Particularly important substrates include subunits of NMDA (Chase and Oh, 2000; Dunah et al., 2000) and AMPA (Santini et al., 2007) receptors, and extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Pavon et al., 2006; Santini et al., 2007; Westin et al., 2007). The implications of altered glutamate receptor-phosphorylation are discussed in Section IIIB. The following paragraph will focus on evidence linking the ERK1/2 signaling cascade to LID.

Extracellular signal-regulated kinases 1 and 2 (ERK1/2) belong to the mitogen-activated protein kinases (MAPK) family of signaling cascades, which share the motif of three serially linked kinases regulating each other by sequential phosphorylation (Seger and Krebs, 1995) (see Chapter 26). The ERKs are abundantly expressed in neurons, and play a pivotal role in synaptic plasticity, learning and memory (reviewed in Sweatt, 2001). Pronounced striatal activation of ERK1/2 is attributed a particularly important role in the supersensitive molecular and behavioral responses induced by DA agonists in DA denervated rats (Cai et al., 2000; Gerfen et al., 2002) (see Chapter 28). In rodents with 6-OHDA lesions, both acute and chronic treatments with L-DOPA induce high levels of active (Thr202/Tyr204phosphorylated) ERK1/2 in striatal neurons. However, while acute L-DOPA treatment induces phospho-ERK1/2 in all DA-denervated animals, the response to chronic L-DOPA treatment is significantly different in dyskinetic vs. non-dyskinetic animals. Rats that develop dyskinesia during the treatment continue to respond to L-DOPA with a sustained phosphorylation of ERK1/2 (which is even larger in magnitude than in the acutely treated cases), whereas rats that remain free from dyskinesia show a much attenuated response, which does not differ significantly from that seen in saline-injected control animals (Westin et al., 2007). In both rat (Westin et al., 2007) and mouse models of PD (Santini et al., 2007), striatal levels of phosphorylated ERK1/2 are positively correlated with the L-DOPA-induced AIMs scores. These findings have prompted the suggestion that the core signaling alteration associated with dyskinesia consists in an inability to desensitize the phospho-ERK1/2 response upon repeated exposure to L-DOPA (Westin et al., 2007). The importance of this molecular alteration to LID is highlighted by the marked antidyskinetic effect of treatments that block ERK1/2 phosphorylation, such as an inhibitor of the ERK1/2 upstream kinase, MEK1/2 (Santini et al., 2007; Lindgren et al., 2009a) or the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, lovastatin (Schuster et al., 2008a). The striatal activation of ERK1/2 by L-DOPA is dose-dependently suppressed by antagonists of D1-like receptors, which also produce a similar, dose-dependent suppression of L-DOPA-induced AIMs (Westin et al., 2007). Moreover, genetic inactivation of DARPP-32 achieves a parallel attenuation of L-DOPAinduced AIMs and phospho-ERK1/2 levels in the striatum (Santini et al., 2007). These findings demonstrate the critical role of D1 receptors and cAMP-dependent signaling pathways in the striatal ERK1/2 response to L-DOPA, as well as the importance of this response to dyskinesia.

Through what mechanisms does a pronounced striatal activation of ERK1/2 lead to dyskinesia? We can envisage two types of mechanisms. The short-term consequences of ERK1/2 activation, which shape the response of striatal neurons to each dose of L-DOPA, rely on the phosphorylation of membrane-bound receptors, ion channels, and synaptic proteins (Reszka et al., 1995; Sweatt, 2001). In addition, ERK1/2 bring about long-term cellular adaptations that involve some nuclear substrates of ERK1/2, such as histone kinases (Hauge and Frodin, 2006) and transcription factors (Sgambato et al., 1998) (Fig. 36.1). In rodent models of LID, the histone H3 kinase, mitogen-and-stress activated protein kinase-1 (MSK-1) is co-activated and co-regulated with ERK1/2 in striatal neurons (Santini et al., 2007; Westin et al., 2007), resulting in striatal histone modifications (Santini et al., 2007; Nicholas et al., 2008) that are known to play an important role in the induction of gene transcription (Nowak and Corces, 2000) (see also Chapter 30).

# **B.** Altered Glutamate Receptor Function and Corticostriatal Synaptic Plasticity

Subunit phosphorylation is a major regulatory mechanism for NMDA and AMPA receptors, governing subunit trafficking and aggregation at the synapse, and the rate of receptor desensitization (Swope et al., 1992). Several lines of evidence point to an altered function of striatal ionotropic glutamate receptors in LID (reviewed in Cenci and Lundblad, 2006) (see also Chapter 35). There is a growing consensus that the most critical alteration consists in an abnormal intracellular trafficking and synaptic abundance of particular receptor subunits. This phenomenon has been studied extensively in both monkey and rat models of LID with respect to the NMDA receptor. In parkinsonian macaques, NR1 and NR2B subunits were found to be significantly decreased in synaptosomal membranes, while the abundance of NR2A was unaltered (Hallett et al., 2005). Dyskinesiogenic L-DOPA treatment normalized NR1 and NR2B and increased NR2A subunits to 150% of unlesioned levels. These results led to the suggestion that a relative enhancement in the synaptic abundance of NR2A plays a particularly important role in LID (Hallett et al., 2005). By contrast, two independent studies in 6-OHDAlesioned rats have emphasized that the receptor-trafficking alteration most critically associated with LID consists in a re-distribution of NR2B subunits between synaptic and extrasynaptic membranes. In these studies, the levels of NR2B proteins were reduced in a striatal postsynaptic density-fraction, but not in total striatal homogenates, from chronically L-DOPA-treated dyskinetic rats (Fiorentini et al., 2006; Gardoni et al., 2006). These animals showed, in addition, reduced synaptic levels of NMDA/D1 receptor complexes (Fiorentini et al., 2006). Intrastriatal delivery of a cell-permeable peptide, which disrupted the interaction between NR2B and its anchoring proteins, reduced the synaptic abundance of NR2B and conferred susceptibility to L-DOPA-induced AIMs to rats previously classified as non-dyskinetic (Gardoni et al., 2006).

Further studies are required to clarify the extent and mechanisms by which an abnormal trafficking of ionotropic glutamate receptor subunits depends on their altered state of phosphorylation. Very few studies thus far have addressed this important issue, and the experimental protocol used in these studies did not include chronic treatment with L-DOPA to elicit dyskinesia (Dunah et al., 2004). Another crucial, open question is the impact of altered subunit distribution on synaptic integration and plasticity in striatal neurons.

Altered activity-dependent plasticity of corticostriatal synapses has been documented in the rat LID model (see also Chapter 12). In corticostriatal slices from dyskinetic rats, high-frequency stimulation (HFS) of cortical afferents was found to induce a normal long-term potentiation (LTP) response, but LTP could not be reversed by subsequent low-frequency stimulation of the same afferent pathway (Picconi et al., 2003; Picconi et al., 2008). This loss of bidirectional synaptic plasticity is of great potential significance to LID, as it would disrupt the ability of striatal neurons to "erase" irrelevant information when processing cortically driven motor commands. The mechanisms behind the loss of synaptic depotentiation in dyskinetic animals have not been completely resolved. The alteration was originally attributed to an overactive signaling downstream of D1 receptors, leading to persistent blockade of intracellular phosphatases by DARPP-32 (Picconi et al., 2003). Further investigations are, however, required to clarify which phosphorylated substrates, and upstream kinases, impede the reversal of LTP. Moreover, although D1 receptors are certainly implicated (Picconi et al., 2003), an altered function of glutamate receptors is likely to play a crucial role through pathways that remain to be established.

Despite our lack of mechanistic information at the molecular level, the robust evidence of altered glutamatereceptor trafficking and abnormal corticostriatal synaptic plasticity in LID provide a rationale for evaluating the antidyskinetic potential of glutamate receptor antagonists (Chase and Oh, 2000; Brotchie, 2005; Schapira et al., 2006). The clinical efficacy of amantadine in reducing the severity of LID has been attributed to its action as a noncompetitive NMDA receptor antagonist (Blanchet et al., 2003). The feasibility of reducing LID by antagonizing glutamate transmission is further supported by the tight functional interactions that have been documented to exist between D1 and ionotropic glutamate receptors (reviewed in Cenci and Lundblad, 2006). The prophylactic and/or acute antidyskinetic efficacy of ionotropic glutamate-receptor antagonists seems to depend on the specific properties of the compounds and animal models used. Several drugs that antagonize NMDA or AMPA receptors have yielded promising antidyskinetic effects in animal models (Konitsiotis et al., 2000; Hadj Tahar et al., 2004). Studies using selective NR2Bselective NMDA antagonists have provided conflicting results (reviewed in Cenci, 2007), but a recent trial in PD patients has reported reductions of peak-dose LID upon treatment with a NR2B antagonist (Nutt et al., 2008).

Among the glutamate receptor ligands so far tested in rodent LID models, antagonists of metabotropic glutamate receptors type 5 (mGluR5) have proven particularly effective in reducing the severity of the AIMs, and in inhibiting their development (Mela et al., 2007). Moreover, mGluR5 antagonists are able to prevent maladaptive molecular changes in striatal neurons, such as the upregulation of phospho-ERK1/2 (Rylander et al., 2009), FosB (Levandis et al., 2007), and prodynorphin mRNA (Mela et al., 2007) (these changes and their interrelationship are illustrated in Fig. 36.1). Metabotropic glutamate receptor type 5 are abundantly expressed in striatal neurons, where they modulate postsynaptic signaling by coupling to intracellular signal transduction pathways (Voulalas et al., 2005). Interestingly, the striatal expression of mGluR5 is increased in parkinsonian rat and monkey affected by dyskinesia (Konradi et al., 2004; Samadi et al., 2008), further pointing to a potentially important role of this receptor in LID.

## C. Changes in Striatal Gene and Protein Expression: Hypothesis-Driven Studies

While ERK1/2 phosphorylation is a rapid response that subsides within 2 hours (Westin et al., 2007), the induction



**FIGURE 36.1** The molecular response to L-DOPA in striatal medium spiny neurons is conditional on the presence or absence of dyskinesia. A. Photomicrographs of striatal sections were taken from rats with 6-OHDA lesions that exhibited (*Dyskinetic*, left column) or did not exhibit (*Non-dyskinetic*, right column) abnormal involuntary movements in response to the treatment. Dyskinetic rats displayed a marked induction of phosphory-lated (active) ERK1/2, FosB/ $\Delta$ FosB-like immunoreactivity, and prodynorphin mRNA. The time course of these upregulations is given in brackets. Prodynorphin mRNA was detected by radioactive in situ hybridization histochemistry, and photomicrographs were taken under dark-field optics using a 20x objective. B. Schematic drawing of a medium spiny neuron represents the hypothetical signaling pathway that links the above molecular events. References to relevant original articles are given in the text. *Abbreviations in B*: AP1, CRE, and SRE are enhancer elements in the promoter regions of affected genes; Elk-1 and MSK-1 are nuclear targets of ERK1/2 (a transcription factor and a histone kinase, respectively).

of genes and proteins by L-DOPA has a more protracted time course, leading to long-lasting adaptations in dopaminoceptive neurons. An abundance of studies have examined changes in striatal gene expression in 6-OHDA-lesioned animals treated with L-DOPA, and it would not be possible to review all these studies in the limited space here available. The following review will therefore focus on prominent examples of hypothesis-driven investigations in this area.

The most extensively studied genes in DA-denervated and L-DOPA-treated animals are those coding for the opioid precursors preproenkephalin-A (PPE, commonly referred to as "enkephalin") and prodynorphin (preproenkephalin-B, PPE-B), which are abundantly expressed in striatal neurons of the indirect and direct pathway, respectively (Gerfen, 1992). Interestingly, these two genes are modulated in an opposite manner by DA denervation, which promptly elevates the expression of PPE mRNA, while causing a mild downregulation of prodynorphin mRNA (reviewed in Steiner and Gerfen, 1998). The relationship between LID and PPE gene expression in the striatum is far from straightforward. Several studies in both rat and non-human primate models have found a positive correlation between LID severity and striatal levels of PPE mRNA (Herrero et al., 1995; Cenci et al., 1998; Zeng et al., 2000; Tel et al., 2002), but other studies have not (Quik et al., 2002). It is difficult to understand how L-DOPA could enhance PPE gene expression in the striatum, given that pharmacological stimulation of D2 receptors has been shown to reduce PPE mRNA levels (Chen et al., 1993), while D2 receptor antagonists cause a pronounced upregulation of the same transcript (reviewed in Steiner and Gerfen, 1998; see Chapter 29). In most studies thus far, the expression of opioid precursor mRNAs was examined following at least one day of L-DOPA treatment washout. Only one study has examined striatal gene expression shortly after the administration of L-DOPA (i.e., one hour post dosing) (Aubert et al., 2007). Interestingly, this study has reported a significant reduction in PPE mRNA levels in dyskinetic animals (Aubert et al., 2007). Overall, the available data thus indicate that PPE gene transcription would decrease shortly after the administration of L-DOPA, while increasing at longer post-dosing intervals (>24 hours), the latter effect being greater in dyskinetic subjects. The pathophysiological significance of these changes is presently unknown, and the specific contribution of D2/PPE-positive striatal neurons to LID remains an unresolved issue.

By contrast, evidence from different animal models points to a strong association between LID and prodynorphin gene expression in the striatum (see also Chapter 29, for a discussion of the role of dynorphin in basal ganglia function). In mouse, rat and macaque models of LID, the L-DOPA-induced AIM scores are positively and tightly correlated with the levels of prodynorphin mRNA expression in the striatum (Cenci et al., 1998; Winkler et al., 2002; Lundblad et al., 2004; Aubert et al., 2007). At least in rodents, L-DOPA-induced AIM scores show a similar, positive correlation with the striatal levels of  $\Delta$ FosB-like proteins (Cenci et al., 1998; Andersson et al., 1999; Lundblad et al., 2004; Sgambato-Faure et al., 2005; Pavon et al., 2006; Levandis et al., 2007), which are colocalized with prodynorphin mRNA at the regional and cellular level (Andersson et al., 1999; Sgambato-Faure et al., 2005). In the "dyskinetic striatum",  $\Delta$  FosB-like transcription factors bind with great affinity to both cyclic AMP-responsive elements (CRE) and activator protein 1 (AP-1) enhancers from the prodynorphin promoter (Andersson et al., 2001), driving the upregulation of prodynorphin mRNA during chronic L-DOPA treatment (Andersson et al., 1999). The striatal upregulation of FosB/ $\Delta$ FosB immunoreactivity (Westin et al., 2007) or prodynorphin mRNA by L-DOPA (St-Hilaire et al., 2005) is totally prevented by selective D1-like receptor antagonists. Antiparkinsonian medications that stimulate D2-class receptors, such as bromocriptine, ropinirole or lisuride, do not induce  $\Delta$  FosB (Westin et al., 2007) nor prodynorphin mRNA in the striatum (Henry et al., 1999; Westin et al., 2001; Tel et al., 2002; Ravenscroft et al., 2004) (Fig. 36.1). Not surprisingly, these drugs have low dyskinesiogenic potential, and they do not induce AIMs in rodents (Lundblad et al., 2002; Lundblad et al., 2005).

While immediate-early genes (including fosB) show an attenuated induction following repeated exposure to L-DOPA (Asin et al., 1995; Valastro et al., 2007a), FosB-like proteins and prodynorphin mRNA show a strikingly protracted upregulation. The expression of both markers rises gradually during a 2-week course of L-DOPA administration (Andersson et al., 2001; Valastro et al., 2007a), maintaining stable, high levels of expression for up to one year, which is the longest period of L-DOPA administration so far examined in this type of studies (Westin et al., 2001). Following discontinuation of L-DOPA treatment, the striatal expression of FosB and prodynorphin mRNA remains significantly elevated for weeks (Andersson et al., 2003) (longer periods of treatment withdrawal were not examined). Conceivably, these sustained changes in gene expression are instrumental to the development and maintenance of a "dyskinesia-primed" state upon dopaminergic drug treatment (discussed and reviewed in Cenci and Lundblad, 2006; Cenci and Lindgren, 2007; Nadjar et al., 2008).

It is presently unknown how many additional genes and proteins show expression kinetics as sustained as those of prodynorphin and  $\Delta$ FosB in the "dyskinetic" striatum. Detailed time course analyses are rarely found in studies addressing gene expression changes following L-DOPA treatment. One gene showing a sustained striatal upregulation at 2 to 24 hours following chronic L-DOPA treatment is *arc* (activity-regulated cytoskeletal-associated gene) (Sgambato-Faure et al., 2005), which may participate in cytoskeletal rearrangements during synaptic plasticity (Steward and Worley, 2001). In dyskinetic rats, Arc mRNA and protein levels are upregulated in prodynorphin-positive neurons within the same striatal regions that show induction of  $\Delta$ FosB-like proteins (Sgambato-Faure et al., 2005). These regions are somatotopically related to the specific profile of AIMs exhibited by the animals (Andersson et al., 1999; Sgambato-Faure et al., 2005). Another gene showing gradual and sustained upregulation upon chronic treatment with L-DOPA is p11, coding for an adaptor protein that promotes surface expression of 5-HT<sub>1B</sub> receptors. The *p11* gene is induced by L-DOPA in striatonigral neurons, and its upregulation has been proposed to serve as a negativefeedback mechanism to counteract the hyperactivity in these neurons in LID (Zhang et al., 2008). Indeed, stimulation of 5-HT<sub>1B</sub> receptors inhibits cAMP formation (Bouhelal et al., 1988). In addition to identifying p11 as a gene induced by L-DOPA, the study by Zhang et al. (Zhang et al., 2008) highlights the role of striatal 5-HT receptors as modulators of L-DOPA-mediated actions. Several 5-HT receptors are highly expressed in the striatum (Barnes and Sharp, 1999), and may provide targets for antidyskinetic therapies (Brotchie, 2005; Schapira et al., 2006). Interestingly, 5-HT1A agonists can normalize L-DOPAinduced prodynorphin gene expression and produce behavioral improvements via a direct action in the striatum (Bishop et al., 2008; Dupre et al., 2008).

## D. Changes in Striatal Gene and Protein Expression: Discovery-Based Studies

The changes in gene and protein expression described above were identified using a hypothesis-driven approach. Discovery-based approaches, such as DNA microarrays or proteomics methods, also have been applied to animal models of LID in a search for unheralded molecular cues and new therapeutic targets. In a seminal gene-chip microarray study (Konradi et al., 2004), Konradi and collaborators compared the patterns of striatal mRNA expression between 6-OHDA-lesioned rats that were chronically treated with a therapeutic dose of L-DOPA or with saline. Some L-DOPA-treated rats developed AIMs, while others remained free from dyskinesia. Gene expression was studied at one single time point (18 hours) after the last injection. Data were visualized on maps representing either biological pathways or particular neurotransmitter systems. The most salient features of the mRNA expression profile associated with dyskinesia indicated increased transcriptional activity of GABAergic neurons, structural and synaptic plasticity, altered calcium homeostasis and calcium-dependent signaling, and an imbalance between metabolic demands and capacity for energy production in the striatum. Some of these microarray data prompted ideas for new antidyskinetic treatments, which were later successfully pursued in the rat model (see e.g., Mela et al., 2007; Valastro et al., 2009). A very recent microarray study in 6-OHDA-lesioned rats focused on the comparison between acutely and chronically L-DOPA-treated rats (Atifi-Borel et al., 2009). Here, the L-DOPA dose was much above threshold for the induction of dyskinesia in all animals. Acute and chronic L-DOPA treatment were found to regulate a common set of genes involved in signal-transduction, transcription, translation, exocytosis and synaptic transmission. Genes involved in neurite outgrowth, synaptogenesis and cell proliferation were, however, more prominently affected in the chronically L-DOPA-treated rats, pointing to an association between repeated exposure to L-DOPA and structural and synaptic remodeling in the striatum (cf. Section IVB).

Striatal protein changes induced by L-DOPA have been investigated both in 6-OHDA-lesioned rats (Valastro et al., 2007b) and MPTP-treated monkeys (Scholz et al., 2008) using two-dimensional difference in-gel electrophoresis (2D-DIGE) and mass spectrometry. In both animal models, the protein expression patterns suggested an association between LID and altered activity of specific metabolic pathways. Some interesting and unexpected clues emerged from a comparison between de novo and long-term L-DOPA treatment in the monkey model (Scholz et al., 2008). Noticeably, acute L-DOPA appeared to induce irreversible post-translational modifications of proteins, which were not further modified by chronic L-DOPA treatment. These findings have been interpreted as indicating that repeated exposure to L-DOPA is not essential to prime the brain for dyskinesia, and that denervation-induced changes preexistent to the treatment are likely to play a much more important role, at least in the animal models (discussed in Nadjar et al., 2008).

## IV. SYSTEM-LEVEL ADAPTATIONS AND STRUCTURAL PLASTICITY IN THE BASAL GANGLIA

# A. Increased Activity in Striatofugal GABAergic Pathways

Several GABA-related genes are expressed at very high levels in striatal neurons in dyskinetic rats (Konradi et al., 2004). The gene coding for glutamic acid decarboxylase isoform 67 (GAD67), the main GABA synthetizing enzyme, is upregulated in direct pathway neurons (Nielsen and Soghomonian, 2004), and this change is positively correlated with the severity of LID (Cenci et al., 1998). Moreover, 6-OHDA-lesioned and L-DOPA-treated rats show upregulations of GABA-A receptors at the level of mRNA expression and/or radioligand binding density in the basal ganglia output stations, i.e., the entopeduncular nucleus (rodent equivalent of the internal globus pallidus, GPi) and the substantia nigra pars reticulata (SNr) (Nielsen and Soghomonian, 2004; Katz et al., 2005). Also in human PD patients and non-human primate models, LID is associated with upregulated radioligand binding densities at GABA-A receptors in the GPi (Calon et al., 1995; Calon et al., 2003). These molecular adaptations are likely to depend on, and contribute to, an increased GABA transmission in the basal ganglia output structures. Increased GABA transmission at this level has been documented in the rat LID model using the in vivo microdialysis technique. With this approach, a peripheral injection of L-DOPA was found to cause a large increase in the extracellular levels of GABA in the SNr, but not the GPe, during the expression of AIMs (Mela et al., 2007). Such an increase reflects GABA efflux from striatonigral ("direct pathway") axons, as it is largely prevented by intrastriatal influsion of D1-like receptor antagonists (Mela et al., unpublished). Neurons in the SNr are very sensitive to phasic alterations in GABA afferent activity. For example, changes in GABA input are the primary factor in determining fluctuations in the activity states of SNr neurons (Windels and Kiyatkin, 2004). An increased GABA input to GPi/SNr may contribute to the altered firing patterns that accompany the expression of dyskinetic movements, consisting in a decreased average firing rate, associated with the occurrence of synchronized

activities and slow (<10Hz) oscillations of the local field potential (reviewed in Cenci, 2007). These altered patterns of activity have been documented to occur in the SNr in rat models of LID (Meissner et al., 2006) as well as in the subthalamic nucleus in dyskinetic PD patients (Alonso-Frech et al., 2006).

## **B.** Structural Plasticity

The clinical experience has indicated that LID is extremely difficult to reduce or reverse after it has appeared. Once developed, LID is promptly elicited by L-DOPA even after long periods of treatment discontinuation, and it is also induced by non-dyskinesiogenic drugs (e.g., long-acting DA agonists), or by stress (reviewed in (Linazasoro, 2005; Cenci and Lundblad, 2006; Jenner, 2008). The persistence of the movement disorder suggests an association with virtually permanent plastic reorganizations in the brain. This suggestion is supported by several studies performed in animals that received repeated (as opposed to acute) administration of L-DOPA.

Microarray studies of striatal mRNAs from chronically L-DOPA-treated rats have revealed upregulation of genes involved in extracellular matrix remodeling, myelin growth, neurite extension and endothelial and cellular proliferation (Konradi et al., 2004; Atifi-Borel et al., 2009: Ferrario, 2004). These results point to extensive structural remodeling of the cellular microenvironment. Proteomics analyses of striatal samples from dyskinetic rats support this suggestion (Valastro et al., 2007b). Two different aspects of L-DOPA-induced structural plasticity have been described thus far. One is a hypertrophy of the neuropile in the entopeduncular nucleus and SNr (Tomiyama et al., 2004). The other aspect consists in microvascular remodeling. Indeed, 6-OHDA-lesioned and L-DOPA-treated rats exhibit endothelial proliferation, upregulation of immature endothelial markers, and downregulation of blood-brain barrier proteins in the basal ganglia to an extent that is linearly related to the severity of LID (Westin et al., 2006). These microvascular changes are seen in the striatum and all its projection targets, but they are most pronounced in the entopeduncular nucleus and the SNr, where they are accompanied by a significant increase in total microvessel length (Westin et al., 2006; Lindgren et al., 2009a). Treatment of 6-OHDA-lesioned rats with bromocriptine, an antiparkinsonian agent producing motor activation without AIMs, does not induce angiogenic activity in the basal ganglia (Lindgren et al., 2009a). Similar to



FIGURE 36.2 Summary of the pathophysiological cascade of LID and potential therapeutic approaches.

the activation of ERK1/2 signaling and the associated molecular changes (cf. Section IIIC), L-DOPA-induced angiogenesis depends on the stimulation of D1 receptors, being completely blocked by D1- but not D2-like receptor antagonists, and reproduced by treatment with selective D1-like receptor agonists at doses that induce AIMs (Lindgren et al., 2009a). Interestingly, histological changes suggestive of angiogenesis have been found upon post-mortem examination of basal ganglia tissue from PD patients (Faucheux et al., 1999; Wada et al., 2006). These findings were however regarded as a facet of the underlying disease pathology, not as consequence of L-DOPA pharmacotherapy. The recent observations from L-DOPA-treated dyskinetic rats call for a reappraisal of the mechanisms and significance of brain angiogenesis in PD. Since the passage of L-DOPA from blood to brain is tightly regulated at the level of the brain endothelium (reviewed in Cenci and Lundblad, 2006), angiogenic microvessels may create foci of high L-DOPA concentrations in the brain following peripheral drug treatment. If verified with suitable methods, this hypothesis can add an exacerbating mechanism to the pathophysiological cascade of LID (summarized in Fig. 36.2).

#### V. CONCLUDING REMARKS

In the past few years, LID has attracted growing interest as a model of altered basal ganglia plasticity sustaining abnormal patterns of movement. A body of recent studies have contributed to uncovering presynaptic abnormalities in DA release and clearance, molecular and synaptic adaptations of striatal neurons, and the involvement of serotonergic, glutamatergic and GABAergic mechanisms in LID. From all these studies, a multifaceted pathophysiological cascade is emerging, which is partly summarized in Fig. 36.2. Several crucial questions remain unanswered, only a few of which have been highlighted in this chapter. The interplay between different neurotransmitter systems, and cellular and nuclear components of the basal ganglia in generating LID is only partially understood. Further work also is required to shed light on the interrelationship between presynaptic and postsynaptic abnormalities in LID, and on their relative contribution to different forms of dyskinesia and motor fluctuations. The knowledge gained from the study of LID will contribute to our basic understanding of DA-dependent movement control in the basal ganglia.

#### REFERENCES

- Abercrombie ED, Bonatz AE, Zigmond MJ (1990) Effects of L-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopaminetreated rats. Brain Res 525:36–44.
- Ahlskog JE, Muenter MD (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord 16:448–458.
- Alonso-Frech F, Zamarbide I, Alegre M, et al. (2006) Slow oscillatory activity and levodopa-induced dyskinesias in Parkinson's disease. Brain 129:1748–1757.
- Andersson M, Hilbertson A, Cenci MA (1999) Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis 6:461–474.
- Andersson M, Konradi C, Cenci MA (2001) cAMP response elementbinding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. J Neurosci 21:9930–9943.

- Andersson M, Westin JE, Cenci MA (2003) Time course of striatal DeltaFosB-like immunoreactivity and prodynorphin mRNA levels after discontinuation of chronic dopaminomimetic treatment. Eur J Neurosci 17:661–666.
- Asin KE, Bednarz L, Nikkel A, Perner R (1995) Rotation and striatal c-fos expression after repeated, daily treatment with selective dopamine receptor agonists and levodopa. J Pharmacol Exp Ther 273:1483–1490.
- Atifi-Borel EM, Buggia-Prevot V, Platet N, Benabid AL, Berger F, Sgambato-Faure V (2009) De novo and long-term L-Dopa induce both common and distinct striatal gene profiles in the hemiparkinsonian rat. Neurobiol Dis 34:340–350.
- Aubert I, Guigoni C, Li Q, Dovero S, Bioulac BH, Gross CE, Crossman AR, Bloch B, Bezard E (2007) Enhanced preproenkephalin-B-derived opioid transmission in striatum and subthalamic nucleus converges upon globus pallidus internalis in L-3,4-dihydroxyphenylalanineinduced dyskinesia. Biol Psychiatry 61:836–844.
- Aubert I, Guigoni C, Hakansson K, et al. (2005) Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann Neurol 57:17–26.
- Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152.
- Berthet A, Porras G, Doudnikoff E, Stark H, Cador M, Bezard E, Bloch B (2009) Pharmacological analysis demonstrates dramatic alteration of D1 dopamine receptor neuronal distribution in the rat analog of L-DOPA-induced dyskinesia. J Neurosci 29:4829–4835.
- Bezard E, Ferry S, Mach U, Stark H, Leriche L, Boraud T, Gross C, Sokoloff P (2003) Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nat Med 9:762–767.
- Bishop C, Taylor JL, Kuhn DM, Eskow KL, Park JY, Walker PD (2006) MDMA and fenfluramine reduce L-DOPA-induced dyskinesia via indirect 5-HT1A receptor stimulation. Eur J Neurosci 23:2669–2676.
- Bishop C, Krolewski DM, Eskow KL, Barnum CJ, Dupre KB, Deak T, Walker PD (2008) Contribution of the striatum to the effects of 5-HT1A receptor stimulation in L-DOPA-treated hemiparkinsonian rats. J Neurosci Res 87:1645–1658.
- Blanchet PJ, Metman LV, Chase TN (2003) Renaissance of amantadine in the treatment of Parkinson's disease. Adv Neurol 91:251–257.
- Bouhelal R, Smounya L, Bockaert J (1988) 5-HT1B receptors are negatively coupled with adenylate cyclase in rat substantia nigra. Eur J Pharmacol 151:189–196.
- Brotchie JM (2005) Nondopaminergic mechanisms in levodopa-induced dyskinesia. Mov Disord 20:919–931.
- Cai G, Zhen X, Uryu K, Friedman E (2000) Activation of extracellular signal-regulated protein kinases is associated with a sensitized locomotor response to D(2) dopamine receptor stimulation in unilateral 6-hydroxydopamine-lesioned rats. J Neurosci 20:1849–1857.
- Calon F, Morissette M, Rajput AH, Hornykiewicz O, Bedard PJ, Di Paolo T (2003) Changes of GABA receptors and dopamine turnover in the postmortem brains of parkinsonians with levodopa-induced motor complications. Mov Disord 18:241–253.
- Calon F, Goulet M, Blanchet PJ, Martel JC, Piercey MF, Bedard PJ, Di Paolo T (1995) Levodopa or D2 agonist induced dyskinesia in MPTP monkeys: correlation with changes in dopamine and GABAA receptors in the striatopallidal complex. Brain Res 680:43–52.
- Carta AR, Pinna A, Morelli M (2006) How reliable is the behavioural evaluation of dyskinesia in animal models of Parkinson's disease?. Behav Pharmacol 17:393–402.
- Carta M, Carlsson T, Kirik D, Bjorklund A (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in Parkinsonian rats. Brain 130:1819–1833.

- Cenci MA (2007) Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. Trends Neurosci 30:236–243.
- Cenci MA, Lundblad M (2006) Post- versus presynaptic plasticity in L-DOPA-induced dyskinesia. J Neurochem 99:381–392.
- Cenci MA, Lindgren HS (2007) Advances in understanding L-DOPAinduced dyskinesia. Curr Opin Neurobiol 17:665–671.
- Cenci MA, Lundblad M (2007) Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. Curr Protoc Neurosci Chapter 9:Unit 9 25.
- Cenci MA, Lee CS, Bjorklund A (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci 10:2694–2706.
- Cenci MA, Whishaw IQ, Schallert T (2002) Animal models of neurological deficits: how relevant is the rat? Nat Rev Neurosci 3:574–579.
- Chase TN, Oh JD (2000) Striatal dopamine- and glutamate-mediated dysregulation in experimental parkinsonism. Trends Neurosci 23:S86–S91.
- Chen JF, Aloyo VJ, Weiss B (1993) Continuous treatment with the D2 dopamine receptor agonist quinpirole decreases D2 dopamine receptors, D2 dopamine receptor messenger RNA and proenkephalin messenger RNA, and increases mu opioid receptors in mouse striatum. Neuroscience 54:669–680.
- Corvol JC, Muriel MP, Valjent E, Feger J, Hanoun N, Girault JA, Hirsch EC, Herve D (2004) Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. J Neurosci 24:7007–7014.
- Day M, Wang Z, Ding J, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259.
- De Vries L, Zheng B, Fischer T, Elenko E, Farquhar MG (2000) The regulator of G protein signaling family. Annu Rev Pharmacol Toxicol 40:235–271.
- Dekundy A, Lundblad M, Danysz W, Cenci MA (2007) Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. Behav Brain Res 179:76–89.
- Delfino M, Kalisch R, Czisch M, et al. (2007) Mapping the effects of three dopamine agonists with different dyskinetogenic potential and receptor selectivity using pharmacological functional magnetic resonance imaging. Neuropsychopharmacology 32:1911–1921.
- Deutch AY (2006) Striatal plasticity in parkinsonism: dystrophic changes in medium spiny neurons and progression in Parkinson's disease. J Neural Transm(Suppl):67–70.
- Dunah AW, Sirianni AC, Fienberg AA, Bastia E, Schwarzschild MA, Standaert DG (2004) Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. Mol Pharmacol 65:121–129.
- Dunah AW, Wang Y, Yasuda RP, Kameyama K, Huganir RL, Wolfe BB, Standaert DG (2000) Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. Mol Pharmacol 57:342–352.
- Dupre KB, Eskow KL, Barnum CJ, Bishop C (2008) Striatal 5-HT1A receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. Neuropharmacology 55:1321–1328.
- Errico F, Santini E, Migliarini S, et al. (2008) The GTP-binding protein Rhes modulates dopamine signaling in striatal medium spiny neurons. Mol Cell Neurosci 37:335–345.

- Fabbrini G, Brotchie JM, Grandas F, Nomoto M, Goetz CG (2007) Levodopa-induced dyskinesias. Mov Disord 22:1379–1389 quiz 1523.
- Faucheux BA, Bonnet AM, Agid Y, Hirsch EC (1999) Blood vessels change in the mesencephalon of patients with Parkinson's disease. Lancet 353:981–982.
- Fiorentini C, Rizzetti MC, Busi C, Bontempi S, Collo G, Spano P, Missale C (2006) Loss of synaptic D1 dopamine/N-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesia in the rat. Mol Pharmacol 69:805–812.
- Gardoni F, Picconi B, Ghiglieri V, et al. (2006) A critical interaction between NR2B and MAGUK in L-DOPA induced dyskinesia. J Neurosci 26:2914–2922.
- Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285–320.
- Gerfen CR, Miyachi S, Paletzki R, Brown P (2002) D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 22:5042–5054.
- Geurts M, Maloteaux JM, Hermans E (2003) Altered expression of regulators of G-protein signaling (RGS) mRNAs in the striatum of rats undergoing dopamine depletion. Biochem Pharmacol 66:1163–1170.
- Geurts M, Hermans E, Cumps J, Maloteaux JM (1999) Dopamine receptor-modulated [35S]GTPgammaS binding in striatum of 6-hydroxydopamine-lesioned rats. Brain Res 841:135–142.
- Gold SJ, Hoang CV, Potts BW, et al. (2007) RGS9-2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. J Neurosci 27:14338–14348.
- Guigoni C, Doudnikoff E, Li Q, Bloch B, Bezard E (2007) Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic nonhuman primates. Neurobiol Dis 26:452–463.
- Hadj Tahar A, Gregoire L, Darre A, Belanger N, Meltzer L, Bedard PJ (2004) Effect of a selective glutamate antagonist on L-dopa-induced dyskinesias in drug-naive parkinsonian monkeys. Neurobiol Dis 15:171–176.
- Hallett PJ, Dunah AW, Ravenscroft P, et al. (2005) Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Neuropharmacology 48:503–516.
- Harrison LM, LaHoste GJ (2006) Rhes, the Ras homolog enriched in striatum, is reduced under conditions of dopamine supersensitivity. Neuroscience 137:483–492.
- Harrison LM, Lahoste GJ, Ruskin DN (2008) Ontogeny and dopaminergic regulation in brain of Ras homolog enriched in striatum (Rhes). Brain Res 1245:16–25.
- Hauge C, Frodin M (2006) RSK and MSK in MAP kinase signaling. J Cell Sci 119:3021–3023.
- Henry B, Crossman AR, Brotchie JM (1998) Characterization of enhanced behavioral responses to L-DOPA following repeated administration in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Exp Neurol 151:334–342.
- Henry B, Crossman AR, Brotchie JM (1999) Effect of repeated L-DOPA, bromocriptine, or lisuride administration on preproenkephalin-A and preproenkephalin-B mRNA levels in the striatum of the 6-hydroxydopamine-lesioned rat. Exp Neurol 155:204–220.
- Herrero MT, Augood SJ, Hirsch EC, Javoy-Agid F, Luquin MR, Agid Y, Obeso JA, Emson PC (1995) Effects of L-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. Neuroscience 68:1189–1198.
- Herve D, Levi-Strauss M, Marey-Semper I, Verney C, Tassin JP, Glowinski J, Girault JA (1993) G(olf) and Gs in rat basal ganglia:

possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. J Neurosci 13:2237–2248.

- Hurley MJ, Stubbs CM, Jenner P, Marsden CD (1996a) D3 receptor expression within the basal ganglia is not affected by Parkinson's disease. Neurosci Lett 214:75–78.
- Hurley MJ, Jolkkonen J, Stubbs CM, Jenner P, Marsden CD (1996b) Dopamine D3 receptors in the basal ganglia of the common marmoset and following MPTP and L-DOPA treatment. Brain Res 709:259–264.
- Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res 503:334–338.
- Ingham CA, Hood SH, van Maldegem B, Weenink A, Arbuthnott GW (1993) Morphological changes in the rat neostriatum after unilateral 6-hydroxydopamine injections into the nigrostriatal pathway. Exp Brain Res 93:17–27.
- Jenner P (2003) The MPTP-treated primate as a model of motor complications in PD: primate model of motor complications. Neurology 61:S4–S11.
- Jenner P (2008) Molecular mechanisms of L-DOPA-induced dyskinesia. Nat Rev Neurosci 9:665–677.
- Kannari K, Yamato H, Shen H, Tomiyama M, Suda T, Matsunaga M (2001) Activation of 5-HT(1A) but not 5-HT(1B) receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. J Neurochem 76:1346–1353.
- Katz J, Nielsen KM, Soghomonian JJ (2005) Comparative effects of acute or chronic administration of levodopa to 6-hydroxydopaminelesioned rats on the expression of glutamic acid decarboxylase in the neostriatum and GABAA receptors subunits in the substantia nigra, pars reticulata. Neuroscience 132:833–842.
- Klawans HL, Goetz C, Nausieda PA, Weiner WJ (1977) Levodopainduced dopamine receptor hypersensitivity. Trans Am Neurol Assoc 102:80–83.
- Konitsiotis S, Blanchet PJ, Verhagen Metman L, Lamers E, Chase TN (2000) AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. Neurology 54:1589–1595.
- Konradi C, Westin JE, Carta M, Eaton ME, Kuter K, Dekundy A, Lundblad M, Cenci MA (2004) Transcriptome analysis in a rat model of L-DOPA-induced dyskinesia. Neurobiol Dis 17:219–236.
- Lee J, Zhu WM, Stanic D, et al. (2008) Sprouting of dopamine terminals and altered dopamine release and uptake in Parkinsonian dyskinaesia. Brain 131:1574–1587.
- Levandis G, Bazzini E, Armentero MT, Nappi G, Blandini F (2007) Systemic administration of an mGluR5 antagonist, but not unilateral subthalamic lesion, counteracts L-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. Neurobiol Dis.
- Linazasoro G (2005) New ideas on the origin of L-dopa-induced dyskinesias: age, genes and neural plasticity. Trends Pharmacol Sci 26:391–397.
- Lindgren HS, Ohlin KE, Cenci MA (2009a) Differential involvement of D1and D2 receptors in L-DOPA-induced angiogenic activity in a rat model of Parkinson's disease. Neuropsychopharmacology 34:2477–2488.
- Lindgren HS, Rylander D, Ohlin KE, Lundblad M, Cenci MA (2007) The "motor complication syndrome" in rats with 6-OHDA lesions treated chronically with L-DOPA: relation to dose and route of administration. Behav Brain Res 177:150–159.
- Lindgren HS, Andersson DR, Lagerkvist S, Nissbrandt H, Cenci MA (2009b) L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: temporal and quantitative relationship to the expression of dyskinesia. J Neurochem, accepted pending revision.

- Lundblad M, Picconi B, Lindgren H, Cenci MA (2004) A model of L-DOPA-induced dyskinesia in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 16:110–123.
- Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA (2002) Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur J Neurosci 15:120–132.
- Lundblad M, Usiello A, Carta M, Hakansson K, Fisone G, Cenci MA (2005) Pharmacological validation of a mouse model of L-DOPAinduced dyskinesia. Exp Neurol 194:66–75.
- Luquin MR, Scipioni O, Vaamonde J, Gershanik O, Obeso JA (1992) Levodopa-induced dyskinesias in Parkinson's disease: clinical and pharmacological classification. Mov Disord 7:117–124.
- Manson A, Schrag A (2006) Levodopa-induced dyskinesias, the clinical problem: clinical features, incidence, risk factors, management and impact on quality of life. In: Recent Breakthroughs in Basal Ganglia Research (Bezard E ed), pp. 369–380. New York, NY: Nova Science Publishers Inc.
- Marconi R, Lefebvre-Caparros D, Bonnet AM, Vidailhet M, Dubois B, Agid Y (1994) Levodopa-induced dyskinesias in Parkinson's disease phenomenology and pathophysiology.. Mov Disord 9:2–12.
- Marin C, Rodriguez-Oroz MC, Obeso JA (2006) Motor complications in Parkinson's disease and the clinical significance of rotational behavior in the rat: have we wasted our time? Exp Neurol 197:269–274.
- Meissner W, Ravenscroft P, Reese R, et al. (2006) Increased slow oscillatory activity in substantia nigra pars reticulata triggers abnormal involuntary movements in the 6-OHDA-lesioned rat in the presence of excessive extracellular striatal dopamine. Neurobiol Dis 22:586–598.
- Mela F, Marti M, Dekundy A, Danysz W, Morari M, Cenci MA (2007) Antagonism of metabotropic glutamate receptor type 5 attenuates L-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. J Neurochem 101:483–497.
- Mishra RK, Gardner EL, Katzman R, Makman MH (1974) Enhancement of dopamine-stimulated adenylate cyclase activity in rat caudate after lesions in substantia nigra: evidence for denervation supersensitivity. Proc Natl Acad Sci USA 71:3883–3887.
- Munoz A, Li Q, Gardoni F, et al. (2008) Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of L-DOPA-induced dyskinesia. Brain 131:3380–3394.
- Muriel MP, Orieux G, Hirsch EC (2002) Levodopa but not ropinirole induces an internalization of D1 dopamine receptors in parkinsonian rats. Mov Disord 17:1174–1179.
- Nadjar A, Gerfen CR, Bezard E (2008) Priming for L-dopa-induced dyskinesia in Parkinson's disease: A feature inherent to the treatment or the disease? Prog Neurobiol 87:1–9.
- Nicholas AP, Lubin FD, Hallett PJ, Vattem P, Ravenscroft P, Bezard E, Zhou S, Fox SH, Brotchie JM, Sweatt JD, Standaert DG (2008) Striatal histone modifications in models of levodopa-induced dyskinesia. J Neurochem 106:486–494.
- Nielsen KM, Soghomonian JJ (2004) Normalization of glutamate decarboxylase gene expression in the entopeduncular nucleus of rats with a unilateral 6-hydroxydopamine lesion correlates with increased GABAergic input following intermittent but not continuous levodopa. Neuroscience 123:31–42.
- Nowak SJ, Corces VG (2000) Phosphorylation of histone H3 correlates with transcriptionally active loci. Genes Dev 14:3003–3013.

- Nutt JG (1990) Levodopa-induced dyskinesia: review, observations, and speculations. Neurology 40:340–345.
- Nutt JG, Gunzler SA, Kirchhoff T, Hogarth P, Weaver JL, Krams M, Jamerson B, Menniti FS, Landen JW (2008) Effects of a NR2B selective NMDA glutamate antagonist, CP-101,606, on dyskinesia and Parkinsonism. Mov Disord 23:1860–1866.
- Pavon N, Martin AB, Mendialdua A, Moratalla R (2006) ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol Psychiatry 59:64–74.
- Peter D, Liu Y, Sternini C, de Giorgio R, Brecha N, Edwards RH (1995) Differential expression of two vesicular monoamine transporters. J Neurosci 15:6179–6188.
- Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci 6:501–506.
- Picconi B, Paille V, Ghiglieri V, et al. (2008) L-DOPA dosage is critically involved in dyskinesia via loss of synaptic depotentiation. Neurobiol Dis 29:327–335.
- Pifl C, Nanoff C, Schingnitz G, Schutz W, Hornykiewicz O (1992) Sensitization of dopamine-stimulated adenylyl cyclase in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated rhesus monkeys and patients with idiopathic Parkinson's disease. J Neurochem 58:1997–2004.
- Quik M, Police S, Langston JW, Di Monte DA (2002) Increases in striatal preproenkephalin gene expression are associated with nigrostriatal damage but not L-DOPA-induced dyskinesias in the squirrel monkey. Neuroscience 113:213–220.
- Quik M, Police S, He L, Di Monte DA, Langston JW (2000) Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia nigra after nigrostriatal degeneration: effect of levodopa treatment. Neuroscience 98:263–273.
- Quinn NP (1998) Classification of fluctuations in patients with Parkinson's disease. Neurology 51:S25–S29.
- Rascol O, Payoux P, Ory F, Ferreira JJ, Brefel-Courbon C, Montastruc JL (2003) Limitations of current Parkinson's disease therapy. Ann Neurol 53(Suppl 3):S3–S12 discussion S12-15.
- Rascol O, Nutt JG, Blin O, et al. (2001) Induction by dopamine D1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. Arch Neurol 58:249–254.
- Ravenscroft P, Chalon S, Brotchie JM, Crossman AR (2004) Ropinirole versus L-DOPA effects on striatal opioid peptide precursors in a rodent model of Parkinson's disease: implications for dyskinesia. Exp Neurol 185:36–46.
- Reszka AA, Seger R, Diltz CD, Krebs EG, Fischer EH (1995) Association of mitogen-activated protein kinase with the microtubule cytoskeleton. Proc Natl Acad Sci USA 92:8881–8885.
- Rinne JO, Laihinen A, Lonnberg P, Marjamaki P, Rinne UK (1991) A post-mortem study on striatal dopamine receptors in Parkinson's disease. Brain Res 556:117–122.
- Rylander D, Recchia A, Mela F, Dekundy A, Danysz W, Cenci MA (2009) Pharmacological modulation of glutamate transmission in a rat model of L-DOPA-induced dyskinesia: effects on motor behavior and striatal nuclear signaling. J Pharmacol Exp Ther 330:227–235.
- Samadi P, Gregoire L, Morissette M, et al. (2008) mGluR5 metabotropic glutamate receptors and dyskinesias in MPTP monkeys. Neurobiol Aging 29:1040–1051.
- Santini E, Valjent E, Usiello A, et al. (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J Neurosci 27:6995–7005.

- Schapira AH, Bezard E, Brotchie J, et al. (2006) Novel pharmacological targets for the treatment of Parkinson's disease. Nat Rev Drug Discov 5:845–854.
- Scholz B, Svensson M, Alm H, et al. (2008) Striatal proteomic analysis suggests that first L-dopa dose equates to chronic exposure. PLoS ONE 3:e1589.
- Schuster S, Nadjar A, Guo JT, Li Q, Ittrich C, Hengerer B, Bezard E (2008a) The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor lovastatin reduces severity of L-DOPA-induced abnormal involuntary movements in experimental Parkinson's disease. J Neurosci 28:4311–4316.
- Schuster S, Doudnikoff E, Rylander D, Berthet A, Aubert I, Ittrich C, Bloch B, Cenci MA, Surmeier DJ, Hengerer B, Bezard E (2008b) Antagonizing L-type Ca(2+) channel reduces development of abnormal involuntary movement in the rat model of L-3,4-dihydroxyphenylalanine-induced dyskinesia. Biol Psychiatry 65:518–526.
- Seger R, Krebs EG (1995) The MAPK signaling cascade. Faseb J 9:726–735.
- Sgambato V, Vanhoutte P, Pages C, Rogard M, Hipskind R, Besson MJ, Caboche J (1998) In vivo expression and regulation of Elk-1, a target of the extracellular-regulated kinase signaling pathway, in the adult rat brain. J Neurosci 18:214–226.
- Sgambato-Faure V, Buggia V, Gilbert F, Levesque D, Benabid AL, Berger F (2005) Coordinated and spatial upregulation of arc in striatonigral neurons correlates with L-dopa-induced behavioral sensitization in dyskinetic rats. J Neuropathol Exp Neurol 64:936–947.
- St-Hilaire M, Landry E, Levesque D, Rouillard C (2005) Denervation and repeated L-DOPA induce complex regulatory changes in neurochemical phenotypes of striatal neurons: implication of a dopamine D1dependent mechanism. Neurobiol Dis 20:450–460.
- Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 123:60–76.
- Steward O, Worley PF (2001) Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. Neuron 30:227–240.
- Stowe R, Ives N, Clarke C, van Hilten J, Ferreira J, Hawker R, Shah L, Wheatley K, Gray R (2008) Dopamine agonist therapy in early Parkinson's disease. Cochrane Database Syst Rev:CD006564.
- Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P (2004) DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol 44:269–296.
- Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 76:1–10.
- Swope SL, Moss SJ, Blackstone CD, Huganir RL (1992) Phosphorylation of ligand-gated ion channels: a possible mode of synaptic plasticity. Faseb J 6:2514–2523.
- Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. Neuroreport 10:631–634.
- Tel BC, Zeng BY, Cannizzaro C, Pearce RK, Rose S, Jenner P (2002) Alterations in striatal neuropeptide mRNA produced by repeated administration of L-DOPA, ropinirole or bromocriptine correlate with dyskinesia induction in MPTP-treated common marmosets. Neuroscience 115:1047–1058.
- Tomiyama M, Mori F, Kimura T, Ichinohe N, Wakabayashi K, Matsunaga M, Baba M (2004) Hypertrophy of medial globus pallidus and sub-

stantia nigra reticulata in 6-hydroxydopamine-lesioned rats treated with L-DOPA: implication for L-DOPA-induced dyskinesia in Parkinson's disease. Neuropathology 24:290–295.

- Tong J, Fitzmaurice PS, Ang LC, Furukawa Y, Guttman M, Kish SJ (2004) Brain dopamine-stimulated adenylyl cyclase activity in Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy. Ann Neurol 55:125–129.
- Turjanski N, Lees AJ, Brooks DJ (1997) In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. Neurology 49:717–723.
- Valastro B, Andersson M, Lindgren HS, Cenci MA (2007a) Expression pattern of JunD after acute or chronic L-DOPA treatment: comparison with deltaFosB. Neuroscience 144:198–207.
- Valastro B, Dekundy A, Danysz W, Quack G (2009) Oral creatine supplementation attenuates L-DOPA-induced dyskinesia in 6-hydroxydopamine-lesioned rats. Behav Brain Res 197:90–96.
- Valastro B, Dekundy A, Krogh M, Lundblad M, James P, Danysz W, Quack G, Cenci MA (2007b) Proteomic analysis of striatal proteins in the rat model of L-DOPA-induced dyskinesia. J Neurochem 102:1395–1409.
- Vargiu P, De Abajo R, Garcia-Ranea JA, Valencia A, Santisteban P, Crespo P, Bernal J (2004) The small GTP-binding protein, Rhes, regulates signal transduction from G protein-coupled receptors. Oncogene 23:559–568.
- Voulalas PJ, Holtzclaw L, Wolstenholme J, Russell JT, Hyman SE (2005) Metabotropic glutamate receptors and dopamine receptors cooperate to enhance extracellular signal-regulated kinase phosphorylation in striatal neurons. J Neurosci 25:3763–3773.
- Wada K, Arai H, Takanashi M, Fukae J, Oizumi H, Yasuda T, Mizuno Y, Mochizuki H (2006) Expression levels of vascular endothelial growth factor and its receptors in Parkinson's disease. Neuroreport 17:705–709.
- Westin JE, Andersson M, Lundblad M, Cenci MA (2001) Persistent changes in striatal gene expression induced by long-term L-DOPA treatment in a rat model of Parkinson's disease. Eur J Neurosci 14:1171–1176.
- Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA (2007) Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol Psychiatry 62:800–810.
- Westin JE, Lindgren HS, Gardi J, Nyengaard JR, Brundin P, Mohapel P, Cenci MA (2006) Endothelial proliferation and increased blood-brain barrier permeability in the basal ganglia in a rat model of 3,4-dihydroxyphenyl-L-alanine-induced dyskinesia. J Neurosci 26:9448–9461.
- Windels F, Kiyatkin EA (2004) GABA, not glutamate, controls the activity of substantia nigra reticulata neurons in awake, unrestrained rats. J Neurosci 24:6751–6754.
- Winkler C, Kirik D, Bjorklund A, Cenci MA (2002) I-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 10:165–186.
- Zaja-Milatovic S, Milatovic D, Schantz AM, et al. (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. Neurology 64:545–547.
- Zeng BY, Pearce RK, MacKenzie GM, Jenner P (2000) Alterations in preproenkephalin and adenosine-2a receptor mRNA, but not preprotachykinin mRNA correlate with occurrence of dyskinesia in normal monkeys chronically treated with L-DOPA. Eur J Neurosci 12:1096–1104.

- Zhang X, Andren PE, Greengard P, Svenningsson P (2008) Evidence for a role of the 5-HT1B receptor and its adaptor protein, p11, in L-DOPA treatment of an animal model of Parkinsonism. Proc Natl Acad Sci USA 105:2163–2168.
- Zhen X, Torres C, Cai G, Friedman E (2002) Inhibition of protein tyrosine/mitogen-activated protein kinase phosphatase activity is associated with D2 dopamine receptor supersensitivity in a rat model of Parkinson's disease. Mol Pharmacol 62:1356–1363.

# Compensatory Mechanisms in Experimental and Human Parkinsonism: Potential for New Therapies

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mechanisms continue to take place after clinical features

become noticeable; however the interaction between ongo-

ing compensatory mechanisms and the effect of standard

symptomatic treatment, i.e., L-DOPA, has not been stud-

ied and is not necessarily synergistic. It is also likely that compensatory mechanisms are implicated in the onset of

VII. Conclusions – Compensation vs. Sensing Dopamine Depletion References

## I. INTRODUCTION

Parkinson's disease (PD) is a widespread neurodegenerative disorder, the major pathologic feature of which is the profound loss of pigmented dopamine (DA) neurons, mainly in the pars compacta of the substantia nigra (SNc) (Hassler, 1938; Ehringer and Hornykiewicz, 1960) (see also Chapter 34). The cardinal features of PD, that is, tremor, rigidity and bradykinesia (Singh et al., 2007), typically arise when DA neuronal death reaches a critical threshold: 70-80% of striatal nerve terminals and 50-60% of SNc perikarya (Bernheimer et al., 1973). This dissociation between the onset of parkinsonian motor features and the presence of large DA depletions is understood as a consequence of compensatory mechanisms (Zigmond et al., 1990; Bezard and Gross, 1998; Bezard et al., 2003). Thus, compensatory mechanisms can delay the clinical onset of PD and could also play a role in the progression of motor deficits. Indeed, it is quite possible that compensatory

cz, 1960) clinical manifestations associated with advanced PD such as cognitive impairment, autonomic disturbances or dise-quilibrium. Currently, the available data is limited to motor symptoms and signs.
d 50–60% The model commonly accepted to explain the compension that follows nigral lesion was proposed by Zigmond and co-workers (Zigmond et al., 1990; Zigmond, 1997) as a con- and indicates that surviving DA neurons undergo func-

tional changes aimed at preserving DA release in the striatum (Zigmond et al., 1990). However, the sole importance of such a mechanism has been questioned on the basis of findings in the MPTP monkey model (Bezard et al., 1997c). In this model, repeated administration of low doses of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to non-human primate initiates a process of neurodegeneration reminiscent of that seen in PD (Bezard et al., 2001c). The novelty of the model accrues from the fact that the protocol produces a reproducible, progressive DA cell loss over a time course of approximately one month (Bezard et al., 2001c). Findings in the MPTP monkey model have revealed compensatory mechanisms beyond the nigrostriatal DA system.

In this review, we describe these different compensatory mechanisms, discuss the evidence sustaining their relative importance and examine the practical implications of these findings for defining conceptually novel approaches to the diagnosis and treatment of PD.

## II. OVERVIEW OF COMPENSATION, CLASSIC CONCEPTS

The appearance of parkinsonian signs is supposed to closely reflect the breakdown of striatal DA homeostasis. In patients this process occurs over several years (mean estimation 7 years) and post-mortem analysis of parkinsonian brains indicates that extensive loss of DA in the putamen and in the caudate nucleus can still be accompanied by only minor clinical manifestations (Bernheimer et al., 1973; Agid, 1991). In animal models of PD even higher degrees of striatal DA depletion are tolerated (Zigmond and Stricker, 1973; Bezard et al., 1997a; Bezard et al., 1997c).

At the beginning of DA cell loss, DA efficiency is such that modest reduction of SNc neurones does not necessitate any adaptive response (Abercrombie et al., 1990; Garris et al., 1997). Discrete compensation of striatal DA could occur because the profuse DA striatal innervations has some degree of redundancy or, more likely, because DA diffuse by volume transmission (Fuxe and Agnati, 1991) from lesser affected areas. However, once a critical threshold of neurodegeneration has been reached, it becomes essential to regulate DA release. This regulation of DA activity is homeostatically controlled and implies adaptive changes in the synthesis and release of DA, and in the response of the striatal neurones (Zigmond, 1993). Increased neuronal firing and DA turnover, once believed to be a major homeostatic mechanism, has recently been shown to occur only to a modest degree and only when DA depletion is substantial (Bezard et al., 2001c; Bezard et al., 2003; Rodriguez et al., 2003). Indeed, in the early stages of SNc degeneration, other compensatory mechanisms have been identified, intrinsic and extrinsic to the basal ganglia,

that are not directly related to modifications in levels of extracellular DA and do not actively compensate for DA loss in the classically understood manner. In the following sections we review findings regarding *striatal mechanisms*, including evidence in favour and against changes in DA availability, and other *basal ganglia and thalamo-cortical mechanisms* also possibly involved in compensating DA reduction in PD.

### **III. STRIATAL MECHANISMS**

# A. Pre- and Postsynaptic Changes in Dopaminergic Activity

There are several putative mechanisms by which the nigrostriatal system may adapt to restore or maintain DA activity within limits compatible with an apparently normal motor performance (Fig. 37.1). These include the following: (i) increased SNc neuronal activity leading to increase DA release and turnover (Zigmond et al., 1990); (ii) reduced DA transporter (DAT) expression to augment DA synaptic availability; (iii) increased DA receptor sensitivity.

#### 1. Increased DA Activity

The preferential implication of the nigrostriatal DA pathway in the compensatory preservation of DA function has been supported by studies showing an increase in DA release, DA turnover, DA uptake and tyrosine hydroxylase activity/protein after nigrostriatal damage in animal models and in the brain of patients with PD (Bernheimer et al., 1973; Onn et al., 1986; Snyder et al., 1990; Zigmond et al., 1990; Hornykiewicz, 1998; Pifl and Hornykiewicz, 2006; Perez et al., 2008) (Fig. 37.1). Indeed, it was the seminal work of Hornykiewicz in the brain of a few PD patients where it was first shown that the ratio of DA to its striatal metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was increased in patients with mild symptoms (Bernheimer et al., 1973; Zigmond et al., 1990). It was suggested that such up-regulation of DA transmission might be a possible mechanism operative in the preclinical stages of the disease attempting to compensate for DA loss in PD (Hornykiewicz, 1993; Pifl and Hornykiewicz, 2006) However, in the progressive MPTP-lesioned primate model of PD discussed above, the up-regulation of DA metabolism is unchanged in the presymptomatic period and only occurs at the very end stage of the symptomatic part of the progression. The finding



FIGURE 37.1 Schematic summary of basal ganglia compensatory mechanisms in Parkinson's disease. (A) Main basal ganglia connections in the "normal" state. Black arrows correspond to the dopaminergic nigrostriatal and nigro-subthalamic projections. Red and green arrows indicate inhibitory GABAergic and excitatory glutamatergic projections, respectively. The thickness of the arrows indicates relative functional activity. Black dots symbolize released dopamine (DA). (B) Proposed compensatory mechanisms in the presymptomatic stage of Parkinson's disease. (1) Nigrostriatal compensatory mechanisms: increased DA release and turnover, increased receptor sensitivity, sprouting, and reduced DA re-uptake by DAT. (2) Increased PPE-A mRNA expression: increased enkephalin release in GPe may reduce GABA release through enhanced activation of delta opioid receptors and keep activity of GPe within normal limits. (3) Loss of dopaminergic projections may lead to hyperactivity of the STN, before DA depletion in the putamen reaches an extent that alters the putamen-GPe projection, leading to increased GPe activity which in turn inhibits GPi/SNr, thus maintaining normal output activity. (C) Further dopamine loss in the putamen reaches a level that cannot be compensated. This causes decreased inhibitory activity in the "direct" strio-GPi/SNr projection and excessive inhibitory activity in the "indirect" projection and GPe hypoactivity. The latter leads to further hyperactivity of the STN and GPi, accounting for the onset of parkinsonian motor features. DAT, dopamine transporter; PPE, pre-proenkephalin A; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; GPe, globus pallidus external segment; GPi, globus pallidus internal segment. To view a color version of this image please visit http://www.elsevierdirect.com/companion/9780123747679

that most of the remaining SNc neurons of PD patients exhibit lower rather than high tyrosine hydroxylase mRNA expression (Javoy-Agid et al., 1990) further challenges the effectiveness of this compensatory mechanism.

Zigmond et al. (1990) posited that surviving neurons and terminals compensate by releasing more DA following denervation. Complementary views have been proposed (Bergstrom and Garris, 2003; Bezard et al., 2003) on the basis of both rodent and primate investigations. However, it is noteworthy that experimental findings on this area are variable and may even be contradictory. Thus, on the one hand, the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease demonstrates that dialysate DA levels collected in the striatum are normal until the loss of DA terminals is nearly complete (Zhang et al., 1988; Abercrombie et al., 1990; Robinson et al., 1994). Furthermore, the fact that endogenous DA release was not increased in severely lesioned mice suggests that augmented DA release does not constitute a pre-synaptic compensatory mechanism (Bezard et al., 2000). On the other hand, a 300% increment in endogenous striatal DA release was encountered in moderately lesioned MPTP monkeys (Perez et al., 2008) and nicotineevoked [3H]DA release was maintained at or near control levels in the ventromedial striatum after MPTP treatment, despite >50% declines in DA. Moreover, although DA release was reduced to about 10% of control in the dorsolateral striatum with denervation, it was still greater than the DA levels (<1% of control) in this region. These results contrast with other studies using real-time voltammetry to probe neurotransmitter dynamics which did not find changes in pre-synaptic DA release, but additionally suggested a downregulation of DA uptake (Garris et al., 1997; Rothblat and Schneider, 1999; Dentresangle et al., 2001).

#### 2. Reduced DAT Expression

The absence of up-regulated release is striking, but reduced DA uptake by the DA transporter (DAT) could as well be compensatory (Fig. 37.1). Earlier post-mortem studies in PD patients described down-regulation of DAT mRNA (Uhl et al., 1994; Joyce et al., 1997), after symptoms have appeared, but the relevance for functional compensation in the preclinical phase was not clear and in most instances patients have received dopaminergic drugs, which probably modifies DAT expression. In models of preclinical phase, DAT was not found down-regulated (Bezard et al., 2000; Bezard et al., 2001c; Dentresangle et al., 2001). However, a number of more recent findings using positron emission tomography (PET) do suggest a very important role for DAT regulation in early PD. A PET study with three radioligands to assess vesicular monoamine transport, plasma membrane DA transport and synthesis of DA in patients with early and advanced PD were consistent with increased activity of aromatic L-aminoacid decarboxylase and down-regulation of DAT (Lee et al., 2000; Adams et al., 2005). A similar approach has recently been applied by the same study group in the 6-OHDA rat's model, where a very positive correlation was found between reduced labeling for DAT (by PET) and denervation (Sossi et al., 2009). DAT down-regulation appears to be functionally capable of maintaining fairly constant and normal DA synaptic levels up to 75% of DA lost. In the same way, as low as 5% of normal striatal DA

levels is sufficient for normal motor performance in nonhuman primates (Elsworth et al., 2000). It is therefore, quite conceivable that reduction in uptake mechanism associated with moderate denervation could lead to longer-range diffusion of DA away from its site of release, increasing tonic DA activity in a modest but sufficient amount to maintain striatal physiology within normal levels.

#### 3. DA Receptor Changes

The role of changes in DA receptors has also been explored as a possible compensatory mechanism (Fig. 37.1). Thus, increased affinity of D2 receptors for DA could mask an effect of neurotransmitter loss on binding. Primate studies suggest that increases in D2 receptors might act as adaptive mechanisms in the early stages of disease progression (Bezard et al., 2001c). The relationship between D2-like receptor binding and both DAT binding and DA content is not linear but instead is best represented by equations combining the synergistic actions of two processes (Bezard et al., 2001c). D2-like receptors are located on both the presynaptic DA terminals and the postsynaptic striatal neurons. Thus, the quadratic correlation can be explained by the initial decrease in D2 receptor binding reflecting only disappearance of the DA terminals while the subsequent increase represents a compensatory response occurring postsynaptically. The transition between these two phenomena occurs in the middle of the presymptomatic period, These findings suggest not only that presymptomatic increase in D2 receptor binding might be a compensatory mechanism but also that the breakdown of striatal DA homeostasis occurs earlier than expected. In addition, findings in the rat and monkey models indicate that DA depletion is associated with an increase in the fraction of D2-like receptors in a high-affinity state (Seeman et al., 2005; Chefer et al., 2008). Another possible mechanism leading to an increase in D2 receptor affinity for DA could be a translocation of these receptors from the intracellular pool to the membrane surface (Chefer et al., 2008). Confirmation of early changes in D2 receptors number or affinity state in patients could lead to a presymptomatic diagnosis of PD.

#### **B.** Re-Innervation

Regeneration or sprouting of terminals from unaffected DA cell groups is a potential compensatory mechanism (Fig. 37.1). Certainly, in animal models sprouting has been shown to occur in animals with partial lesions, and several

neurotrophic agents act through this mechanism (Blanchard et al., 1996; Song and Haber, 2000). Extensive work has been carried out with beautiful morphological analyses of nigrostriatal DA axons in rats with different degrees of DA depletion by 6-OHDA; such studies reported significant axonal sprouting in the DA terminal arbors (Finkelstein et al., 2000; Stanic et al., 2003), an effect that would normally be inhibited by a tonic stimulation of D2 autoreceptors (Parish et al., 2002). This homeostatic mechanism is overcome when >70% of nigral DA neurons are lost (Finkelstein et al., 2000; Stanic et al., 2003). Re-innervation is accompanied by behavioral recovery in MPTP monkeys, marked increases in DA levels and modest elevations of metabolic activity (HVA/DA ratio) (Elsworth et al., 2000). However, PD is a progressive degenerative process where the capacity for healthy neurons to sprout is probably diminished. In this regard, it is interesting that one study in human brains showed non-melanised neurons in the ventral tegmental area displaying a significantly higher expression of TH mRNA than melanised neurons in the same PD brains and control subjects (Tong et al., 2000), consistent with up-regulation of TH expression in these neurons.

#### C. Serotonin Compensation

Monkeys intoxicated with MPTP systemically show a clear tendency to recover spontaneously unless treatment is continued until a large enough lesion is achieved. Using this model, it has been shown that recovered monkeys displayed more DA and serotoninergic fibres than those with stable motor symptoms in sensorimotor and associative territories of striatum and more DA fibres in the GPi (Mounayar et al., 2007). Such serotonergic sprouting (Gaspar et al., 1993) could be seen as related with competition due to reduced number of DA fibres or could be a real compensatory mechanism. Studies in 6-OHDA lesion rats have produced inconsistent results, reporting either increased or decreased serotonergic striatal innervations (Takeuchi et al., 1991; Zhou et al., 1991). Recently however, more direct data by microdialysis in MPTP-treated monkeys indicate that recovery of motor symptoms is associated with increased serotonin striatal levels (~370%) (Boulet et al., 2008).

# D. Volume Transmission and Passive Stabilization

There are two principal mechanisms of striatal DA release (see also Chapter 17): (i) "*phasic release*", which is associated with abrupt increases in SNc neuron firing leading to activation of D1 and D2 receptors located within the synapse; such DA is subjected to intense uptake and metabolic degradation (Parent et al., 1995); and (ii) *tonic release*, which is independent of SNc firing, primarily excites extra-synaptically located D1 receptors on neurons of the "direct" pathway (see Chapter 1) by non-synaptic diffusion transmission (Onn et al., 2000). This "*volume transmission*" (Zoli et al., 1999) would appear to play a far more important role in pathological conditions. In animal models of PD, DA transmission is in part restored by volume transmission of the neurotransmitter from intact to denervated regions (Bezard and Gross, 1998; Zoli et al., 1999).

These well established mechanisms have recently been challenged by analysis of steady-state levels of extracellular DA, which mimics the normal dynamics of DA-mediated tone (Garris et al., 1997; Bergstrom and Garris, 2003). In these studies, kinetic analysis demonstrated that DA transmission was maintained without plasticity of release or clearance mechanisms suggesting that the primary mechanisms controlling extracellular DA levels were not actively altered. This so-called "passive sta*bilization*" is mediated by the simple physical principles of diffusion and steady state, and forms the basis for a new compensation model of preclinical parkinsonism. Would the regulation of uptake be not necessary, the observation that only 5% of normal DA levels are able to restore normal motor performance in non-human primates (Elsworth et al., 2000) would support the "passive stabilization" theory and would not be in support of the down-regulation of DA uptake. This provocative theory would actually provide an elegant explanation to both the high redundancy in the system and the sharpness and brutality of the threshold for symptom appearance.

# IV. BASAL GANGLIA-MEDIATED COMPENSATION

According to the classic pathophysiological model of the basal ganglia (Alexander and Crutcher, 1990), DA deficiency leads to reduced inhibition of gamma-aminobutyric acid (GABA)-ergic striatal neurons in the indirect pathway and decreased facilitation of GABA-ergic neurons in the direct pathway (Albin et al., 1989; DeLong, 1990) (see Chapter 1). Reduced inhibition of neurons in the indirect pathway would lead sequentially to over-inhibition of the globus pallidus pars externalis (GPe), disinhibition of the subthalamic nucleus (STN), and thus, overactivity of basal ganglia outputs from the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) (Mitchell et al., 1989; DeLong, 1990). Similarly, decreased activation of neurons in the direct pathway reduces its inhibitory influence on GPi/SNr and contributes to the excessive basal ganglia output activity.

The striato-GPe projection uses enkephalins, derived from the precursor pre-proenkephalin-A (PPE-A), as cotransmitters with GABA (see also Chapter 29). A wealth of evidence suggests that the activity of both the GABAergic and enkephalinergic components of this pathway is increased in the parkinsonian MPTP-treated monkey (Asselin et al., 1994; Herrero et al., 1995; Levy et al., 1995; Morissette et al., 1999). However, both the role of this co-transmission in the generation of parkinsonian symptoms and the nature of any functional interaction between GABA and enkephalin are not clear. It has recently been shown that PPE-A mRNA levels are elevated before the appearance of parkinsonian motor features in the progressive MPTP model of degeneration in the primate (Bezard et al., 2001b). Importantly, this up-regulation is restricted to motor regions of the basal ganglia circuitry. The increased PPE-A mRNA expression observed in asymptomatic, but DA-depleted animals provide further support for the hypothesis that the breakdown of striatal DA homeostasis is dissociated from the clinical signs appearance (Fig. 37.1).

The functional significance of this presymptomatic up-regulation of enkephalinergic transmission remains subject to debate. Increased PPE-A mRNA levels could represent an endogenous mechanism attempting to reduce an overactive inhibitory GABAergic input from the striatum to the GPe (Maneuf et al., 1994) (see also Chapter 29). Enhanced enkephalin release in GPe may thus reduce GABA release through enhanced activation of delta-opioid receptors by met-enkephalin and keep activity of GPe normal. This compensatory mechanism, intrinsic to the basal ganglia circuitry, but outside the nigrostriatal pathway, may be responsible of the absence of changes in GPe neuronal activity at any stage of parkinsonism (Herrero et al., 1996; Vila et al., 1997; Bezard et al., 1999). When looking at the relationship between DAT binding and the expression level of PPE-A mRNA, it appears that up-regulation of enkephalinergic transmission starts early in the degenerative process, at a stage comparable to the D2 receptor up-regulation. Further support for the concept that an elevation of PPE-A expression might be a presymptomatic compensatory mechanism is provided by the finding that, once symptoms appear, the up-regulation of PPE-A does not remain indefinitely (Schneider et al., 1999).

While current imaging technologies might have difficulty imaging an up-regulation of PPE-A expression, the idea of using enhanced enkephalin transmission as a biomarker for presymptomatic PD is not without possibilities. For instance, a potential non-imaging approach might involve the administration of an opioid antagonist to elicit parkinsonian symptoms in presymptomatic PD. A short acting antagonist combined with sensitive electromyographic measurement of rigidity and tremor might permit the development of a test that would cause minimal discomfort. Such a strategy would however never be accepted without a cure available (or at least a significantly neuroprotective drug) for obvious ethical reasons.

Although the notion that levels of gene expression are tightly coupled to levels of physiological activity is simplistic, the presymptomatic increase in PPE-A expression level is nonetheless suggestive of changes in the activity of striatal medium spiny neurons before symptoms appear. The question of presymptomatic changes in the electrophysiological activity of basal ganglia nuclei has thus emerged.

Changes in activity in the STN and in GPi have been assessed, using multiunit electrophysiological recordings, in monkeys intoxicated with MPTP according to the progressive regimen (Bezard et al., 1999, 2002) (see also Chapters 25 and 38 for reviews of changes in basal ganglia circuit activities in MPTP models). In this model, both overactivity of STN and GPi actually occur before the appearance of symptoms and thus might be related to presymptomatic compensation (Bezard et al., 1999, 2002). Similar results were reported in the 6-OHDA-treated rat model of PD where early changes in the firing frequency of STN neurons occur before it is possible to induce rotations in those animals (Vila et al., 2000).

The increase in the activity of GPi, in the latter stages of the presymptomatic period, has recently been confirmed using multi-channel single unit recordings in the same MPTP monkey model (Boraud et al., 2001; Leblois et al., 2006; Leblois et al., 2007). These results are suggestive of dissociation between the changes in the basal ganglia activity and the appearance of the parkinsonian motor abnormalities. Thus, there appears to be very early striatal DA homeostasis breakdown highlighted by the early upregulation of both DA D2 receptor and enkephalin precursor expression and a comparatively later increase in the activity of the basal ganglia output structures. Indeed, the breakdown of the DA homeostasis occurs before the changes in the basal ganglia electrophysiological activity, further supporting the existence of non-DA compensatory mechanisms intrinsic to the basal ganglia.

Though STN and GPi are both overactive before the appearance of symptoms, both changes in neural activity are not necessarily presymptomatic compensatory mechanisms. However, overactivity of the STN could be a compensatory mechanism initially (Fig. 37.1). Blockade of the subthalamic excitation of surviving DA neurons in the presymptomatic period can provoke the emergence of parkinsonian symptoms (Bezard et al., 1997a, b; Obeso et al., 2004). Thus, it appears that by increasing the firing rate of surviving DA neurons the overactive STN might be able to enhance remaining DA transmission and delay the appearance of symptoms until relatively late in the progression. An alternative view regarding the functional modulation of the STN after DA depletion has been recently proposed (Obeso et al., 2004). The hyperactivity of the STN and related glutamatergic nuclei arises early in the course of PD, before striatal DA depletion has become significant (Vila et al., 2000; Bezard et al., 2003). At this time (in the presymptomatic state), DA loss in the caudal putamen is still not large enough (<50%) to increase activity in the striatal inhibitory projection to the GPe. As a result, increased STN activity exerts a powerful excitatory effect on the GPe that leads to increased inhibition of the GPi and maintains the output of the motor circuit within normal limits. In that fashion, the "internal" STN-GPe-GPi circuit compensates itself (Obeso et al., 2000). Increased STN activity could increase GPi firing by its direct excitatory projection (Whone et al., 2003), but this can be compensated by the still-normal inhibitory projection to the GPi (i.e., "direct" pathway). In PD, where the changes occur slowly, excessive STN drive onto the GPi might also be compensated by way of the nigro-pallidal DA projection, which has been shown to increase during the presymptomatic phase, by fluorodopa positron emission tomography (PET) (Shink et al., 1996).

In humans it has been suggested that GPi in particular could be implicated in compensatory mechanisms (Whone et al., 2003). The 18F-dopa uptake increases in early phase of PD in the GPi and additionally a modification of tyrosine hydroxylase (TH) labeling has been noticed in GPi of MPTP-intoxicated monkeys (Jan et al., 2000; Mounayar et al., 2007). Although this change in TH labeling is less marked in the pallidal complex than in the striatum, suggesting that the pallidal omplex could contribute to compensation, there is no evidence, or theoretical basis, for a mechanism by which overactive GPi (driven by overactive STN) could represent a presymptomatic compensatory mechanism.

## V. THALAMO-CORTICAL-MEDIATED COMPENSATION

Overactivation of lateral premotor areas has also been reported in PD patients, generally interpreted as a compensation for deficiencies in the midline motor system during internally generated tasks (Cunnington et al., 1999; Berardelli et al., 2001). Recent studies in MPTP-treated monkey have revealed the involvement of non-basal ganglia structures, throwing light upon putative cortical compensatory mechanisms (Bezard et al., 2001a; Escola et al., 2003; Huang et al., 2007; Mounayar et al., 2007). Although it is likely that many cortical or subcortical regions are involved in the pathophysiology of motor signs (Hirsch et al., 2000; Pahapill and Lozano, 2000; Berardelli et al., 2001), the pivotal position of the supplementary motor area (SMA) between the prefrontal and motor cortical areas makes it probable that these structures play an essential role, particularly in the origin of bradykinesia, in the pathophysiology of PD.

The 2-deoxyglucose (2-DG) metabolic mapping technique has been applied to identify changes in neuronal metabolic activity that occur before and after the appearance of parkinsonian motor abnormalities, in the MPTP model that recapitulates the progression of the disease (Mitchell et al., 1989; Bezard et al., 2001a). 2-DG levels were assessed in the whole basal ganglia, the motor thalamic nuclei, and the SMA (Alexander and Crutcher, 1990; DeLong, 1990). It was shown that decreased metabolic rate in the SMA only appears when MPTP-treated monkeys become fully parkinsonian (Bezard et al., 2001a). These changes occur after the early changes in the electrophysiological activity described above. Therefore, neural activity in SMA is normal in presymptomatic animals but decreased in parkinsonian monkeys (Escola et al., 2003). The decrease in metabolic activity in SMA accompanying motor symptoms of fully parkinsonian animals was similar to that reported in previous in vivo and ex vivo functional imaging studies in PD patients (Palombo et al., 1990; Jenkins et al., 1992; Rascol et al., 1992; Playford et al., 1993; Eidelberg et al., 1995; Rascol et al., 1998). Altogether these observations suggest that PD symptoms are closely linked to changes in SMA activity. In itself, overactivity of basal ganglia outputs does not necessarily lead to the generation of symptoms while impairment of SMA metabolic activity does. It thus appears that compensatory mechanisms outside the basal ganglia exist to prevent the appearance of symptoms even though the basal ganglia are in a parkinsonian-like state.

Other territories could be implicated in the phenomenon of recovery and, furthermore, in the compensatory mechanisms in early phases of PD, as well. The parafascicular-centromedian complex of the thalamus (Orieux et al., 2000) and the pedunculopontine nucleus (PPN) in the brainstem (Pahapill and Lozano, 2000) are known to be hyperactive in the 6-OHDA rat model, (Orieux et al., 2000), while the motor cortex projection is hypoactive (Orieux et al., 2002). However, how hyperactivity ensues in these motor-related glutamatergic nuclei associated with STN is not understood (Hirsch et al., 2000; Obeso et al., 2000; Vila et al., 2000). If the PD process begins with the loss of DA cells in the SNc, as is the case in the animal models induced by neurotoxins, it appears likely that the glutamatergic hyperactivity would arise as a direct consequence of reduced DA input. In this regard, there is now considerable evidence indicating that DA exerts a modulatory effect upon STN activity, and therefore it can be assumed that the effect of DA drugs is not limited to the striatum. One should not forget that degeneration in PD is multisystemic and affects among many other nuclei, the thalamus (Henderson, 2000; Jellinger, 1999). Therefore, we could not rule out this widespread lesioning as one of the many important factors that may counteract the defects associated with the DA denervation.

Finally, regarding the thalamo-cortical compensation mechanism, it has been suggested that the major dysfunction after DA depletion at the early stages of PD could be the loss of functional segregation within cortico-basal ganglia circuits. This dysfunction appears to affect only the pallidal-nigral thalamus in the asymptomatic state, and to extend to the cerebellar thalamus coinciding with the appearance of PD motor features (Pessiglione et al., 2005). As the DA depletion becomes more severe, motor deficits characteristic of parkinsonism may be superimposed upon pre-existing executive deficits (Schneider and Kovelowski, 1990; Schneider and Pope-Coleman, 1995). In this context, the loss of functional segregation may first induce interferences within the motor or associative circuit and then between motor and cognitive areas.

## VI. DOPAMINE COMPENSATION REAPPRAISED

Until recently, compensatory mechanisms associated with recovery from PD motor symptoms were thought to result principally from the adaptive properties of DA neurons (Zigmond, 1997). Studies on MPTP monkeys with motor features of varying degrees of severity have shown that a limited DA loss can, in fact, be counterbalanced by an increase in DA release from the remaining fibers. "Passive stabilization" of the DA system may thus represents an active mechanism that maintains DA homeostasis with denervation (Garris et al., 1997; Bezard et al., 2000; Bergstrom and Garris, 2003) and contribute to presynaptic DA compensation. Also, a large body of evidence suggests that presynaptic DA-mediated compensation delays the appearance of parkinsonian symptoms with moderate nigrostriatal damage. This increased DA release from spared DA terminals could help to maintain DA homeostasis at the early stages of neuronal degeneration. With further degenerative changes, the continued loss of DA nerve terminal integrity may no longer support increased DA function (Pifl and Hornykiewicz, 2006; Perez et al., 2008).

The question thus remains: what compensates for DA degeneration? Our lack of understanding of the compensation process itself may have been hampered because of a widely held belief in concepts for which solid support was not available, that is:

- the late appearance of parkinsonian motor features is due to failure of compensatory mechanisms thought to reside solely within the nigrostriatal pathway; and
- the appearance of parkinsonian signs closely reflects the breakdown of the striatal DA homeostasis, i.e., the failure of the above-mentioned DA compensatory mechanisms.

These views imply an intimate and causative relationship between the breakdown of the striatal DA homeostasis, changes in striatal activity (as well as in the other components of the basal ganglia) and the appearance of motor abnormalities.

Until now, it has proved difficult to ascertain the true nature of these relationships on the basis of changes in neurotransmission, DA or otherwise, observed in PD. DA depletion alters expression of multiple neurotransmitters, particularly the serotonergic system as recently suggested (Mounayar et al., 2007), neuropeptides and receptors present in the different compartments of the striato-pallidal and striato-nigral output pathways (see also Chapters 28 and 36). A combination of pre- and postsynaptic compensatory mechanisms most likely accounts for the delayed development of the motor symptoms characteristic of PD.

Consequently, it is reasonable to assume that the disease process and aging in DA and non-DA structures involve a biologic interaction. Thus, studies about the pathogenic abnormalities implicated in PD should further account not only for the relative selectivity of the disease process to the SN, but also for the widespread involvement of the whole brain in late clinical stages of the disease.

# VII. CONCLUSIONS – COMPENSATION VS. SENSING DOPAMINE DEPLETION

Consensus arises for stating that compensation is multifactorial, involving several neuromodulators and neurotransmitter systems as well as occurring at several levels of the cortio-basal ganglia-thalamo-cortical loop. Considering compensation only under the angle of the nigrostriatal pathway is clearly not sufficient, although its paramount importance is recognized. As a last example, we would like to emphasize the marked plasticity of the SNc itself. Taking advantage of the progressive non-human primate model, we monitored transcriptional fluctuations in the SN using affymetrix microarrays in control (normal), saline-treated (normal), 6 day-treated (asymptomatic with 20% cell loss), 12 day-treated (asymptomatic with 40% cell loss) and 25 day-treated animals (fully parkinsonian with 85% cell loss) (Bassilana et al., 2005). Surprisingly, different profiles of gene regulation were defined using a hierarchical clustering algorithm. Such profiles are likely to represent activation/deactivation of mechanisms of different nature. The main conclusion of this work was the demonstration of (i) the existence of yet unknown compensatory mechanisms within the SN itself in the course of the degeneration; (ii) the putative triggering of a developmental program in the mature brain in reaction to progressing degeneration and finally; (iii) the activation of mechanisms leading eventually to death in final stage (Bassilana et al., 2005). Thus, the SN itself is undergoing plastic, likely compensatory changes in the course of the degenerative process.

We would, however, like to conclude on a more conceptual note. Most, if not all previous studies on compensation have related changes in given markers, whatever the marker is, and a given level of nigrostriatal degeneration. The compensatory nature of these changes is merely hypothetical as almost none of them have been causally demonstrated as being compensatory. To do so, one should block or enhance the hypothesized mechanism resulting into worsening/appearance of motor symptoms or reduction/masking of those symptoms, respectively. While the true compensatory nature of the glutamatergic inputs of the SNc was shown by blocking glutamatergic activity and revealing parkinsonian features earlier in the intoxication process (Bezard et al., 1997b), similar approaches have not been formally undertaken for other markers/parameters. It is even possible that most of these "compensatory mechanisms" are not compensatory but simply represent the consequences of DA depletion or the network dysfunction. Manipulating these would thus bring new knowledge on the compensatory mechanisms at work. What is critically needed now is to reappraise the compensatory nature of the number of hypothesized mechanisms by formally testing the impact of their modulation upon symptom appearance or severity. Such a step constitutes the missing link before embarking on developing new strategies aiming at promoting compensatory mechanisms in a therapeutically relevant manner for PD patients.

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#### REFERENCES

- Abercrombie ED, Bonatz AE, Zigmond MJ (1990) Effects of I-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. Brain Res 525:36–44.
- Adams JR, van Netten H, Schulzer M, et al. (2005) PET in LRRK2 mutations: comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation. Brain 128:2777–2785.
- Agid Y (1991) Parkinson's disease: pathophysiology. Lancet 337:1321–1324.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.
- Asselin MC, Soghomonian JJ, Cote PY, Parent A (1994) Striatal changes in preproenkephalin mRNA levels in parkinsonian monkeys. Neuroreport 5:2137–2140.
- Bassilana F, Mace N, Li Q, Stutzmann JM, et al. (2005) Unraveling substantia nigra sequential gene expression in a progressive MPTPlesioned macaque model of Parkinson's disease. Neurobiol Dis 20:93–103.
- Berardelli A, Rothwell JC, Thompson PD, Hallett M (2001) Pathophysiology of bradykinesia in Parkinson's disease. Brain 124:2131–2146.
- Bergstrom BP, Garris PA (2003) "Passive stabilization" of striatal extracellular dopamine across the lesion spectrum encompassing the

presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. J Neurochem 87:1224–1236.

- Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (1973) Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations. J Neurol Sci 20:415–455.
- Bezard E, Gross CE (1998) Compensatory mechanisms in experimental and human parkinsonism: towards a dynamic approach. Prog Neurobiol 55:93–116.
- Bezard E, Gross CE, Brotchie JM (2003) Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. Trends Neurosci 26:215–221.
- Bezard E, Boraud T, Bioulac B, Gross CE (1997a) Presymptomatic revelation of experimental parkinsonism. Neuroreport 8:435–438.
- Bezard E, Boraud T, Bioulac B, Gross CE (1997b) Compensatory effects of glutamatergic inputs to the substantia nigra pars compacta in experimental parkinsonism. Neuroscience 81:399–404.
- Bezard E, Boraud T, Bioulac B, Gross CE (1999) Involvement of the subthalamic nucleus in glutamatergic compensatory mechanisms. Eur J Neurosci 11:2167–2170.
- Bezard E, Crossman AR, Gross CE, Brotchie JM (2001a) Structures outside the basal ganglia may compensate for dopamine loss in the presymptomatic stages of Parkinson's disease. FASEB J 15:1092–1094.
- Bezard E, Boraud T, Bioulac B, Gross CE (2002) Evolution of the multiunit activity of the basal ganglia in the course of experimental parkinsonism. In: The Basal Ganglia VI (Graybiel A, Delang M, Kitaï S, eds), pp. 107–116. New York: Kluwer Academic Publishers.
- Bezard E, Imbert C, Deloire X, Bioulac B, Gross CE (1997c) A chronic MPTP model reproducing the slow evolution of Parkinson's disease: evolution of motor symptoms in the monkey. Brain Res 766:107–112.
- Bezard E, Ravenscroft P, Gross CE, Crossman AR, Brotchie JM (2001b) Upregulation of striatal preproenkephalin gene expression occurs before the appearance of parkinsonian signs in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys. Neurobiol Dis 8:343–350.
- Bezard E, Jaber M, Gonon F, Boireau A, Bloch B, Gross CE (2000) Adaptive changes in the nigrostriatal pathway in response to increased 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegeneration in the mouse. Eur J Neurosci 12:2892–2900.
- Bezard E, Dovero S, Prunier C, et al. (2001c) Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. J Neurosci 21:6853–6861.
- Blanchard V, Anglade P, Dziewczapolski G, Savasta M, Agid Y, Raisman-Vozari R (1996) Dopaminergic sprouting in the rat striatum after partial lesion of the substantia nigra. Brain Res 709:319–325.
- Boraud T, Bezard E, Bioulac B, Gross CE (2001) Dopamine agonistinduced dyskinesias are correlated to both firing pattern and frequency alterations of pallidal neurones in the MPTP-treated monkey. Brain 124:546–557.
- Boulet S, Mounayar S, Poupard A, et al. (2008) Behavioral recovery in MPTP-treated monkeys: neurochemical mechanisms studied by intrastriatal microdialysis. J Neurosci 28:9575–9584.
- Chefer SI, Kimes AS, Matochik JA, et al. (2008) Estimation of D2-like receptor occupancy by dopamine in the putamen of hemiparkinsonian Monkeys. Neuropsychopharmacology 33:270–278.
- Cunnington R, Iansek R, Bradshaw JL (1999) Movement-related potentials in Parkinson's disease: external cues and attentional strategies. Mov Disord 14:63–68.

- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13:281–285.
- Dentresangle C, Le Cavorsin M, Savasta M, Leviel V (2001) Increased extracellular DA and normal evoked DA release in the rat striatum after a partial lesion of the substantia nigra. Brain Res 893:178–185.
- Ehringer H, Hornykiewicz O (1960) Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems/ [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system.]. Klin Wochenschr 38:1236–1239.
- Eidelberg D, Moeller JR, Ishikawa T, et al. (1995) Assessment of disease severity in parkinsonism with fluorine-18-fluorodeoxyglucose and PET. J Nucl Med 36:378–383.
- Elsworth JD, Taylor JR, Sladek JR Jr., Collier TJ, Redmond DE Jr., Roth RH (2000) Striatal dopaminergic correlates of stable parkinsonism and degree of recovery in old-world primates one year after MPTP treatment. Neuroscience 95:399–408.
- Escola L, Michelet T, Macia F, Guehl D, Bioulac B, Burbaud P (2003) Disruption of information processing in the supplementary motor area of the MPTP-treated monkey: a clue to the pathophysiology of akinesia? Brain 126:95–114.
- Finkelstein DI, Stanic D, Parish CL, Tomas D, Dickson K, Horne MK (2000) Axonal sprouting following lesions of the rat substantia nigra. Neuroscience 97:99–112.
- Fuxe K, Agnati LF (1991) Two principal modes of electrochemical communication in the brain: volume versus wiring transmission. In: Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission (Fuxe K, Agnati LF, eds), pp. 1–9. New-York: Raven Press.
- Garris PA, Walker QD, Wightman RM (1997) Dopamine release and uptake rates both decrease in the partially denervated striatum in proportion to the loss of dopamine terminals. Brain Res 753:225–234.
- Gaspar P, Febvret A, Colombo J (1993) Serotonergic sprouting in primate MTP-induced hemiparkinsonism. Exp Brain Res 96:100–106.
- Hassler R (1938) Zur Pathologie der Paralysis Agitans und des postenzephalitischen Parkinsonismus. J Psychol Neurol 48:387–476.
- Herrero MT, Augood SJ, Hirsch EC, Javoy-Agid F, Luquin MR, Agid Y, Obeso JA, Emson PC (1995) Effects of L-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. Neuroscience 68:1189–1198.
- Herrero MT, Levy R, Ruberg M, et al. (1996) Consequence of nigrostriatal denervation and l-dopa therapy on the expression of glutamic acid decarboxylase messenger RNA in the pallidum. Neurology 47:219–224.
- Hirsch EC, Perier C, Orieux G, et al. (2000) Metabolic effects of nigrostriatal denervation in basal ganglia. Trends Neurosci 23:S78–S85.
- Hornykiewicz O (1993) Parkinson's disease and the adaptive capacity of the nigrostriatal dopamine system: possible neurochemical mechanisms. Adv Neurol 60:140–147.
- Hornykiewicz O (1998) Biochemical aspects of Parkinson's disease. Neurology 51:S2–S9.
- Huang C, Tang C, Feigin A, Lesser M, Ma Y, Pourfar M, Dhawan V, Eidelberg D (2007) Changes in network activity with the progression of Parkinson's disease. Brain 130:1834–1846.
- Jan C, Francois C, Tande D, Yelnik J, Tremblay L, Agid Y, Hirsch E (2000) Dopaminergic innervation of the pallidum in the normal state, in MPTP-treated monkeys and in parkinsonian patients. Eur J Neurosci 12:4525–4535.

- Javoy-Agid F, Hirsch EC, Dumas S, Duyckaerts C, Mallet J, Agid Y (1990) Decreased tyrosine hydroxylase messenger RNA in the surviving dopamine neurons of the substantia nigra in Parkinson's disease: an in situ hybridization study. Neuroscience 38:245–253.
- Jenkins IH, Fernandez W, Playford ED, Lees AJ, Frackowiak RS, Passingham RE, Brooks DJ (1992) Impaired activation of the supplementary motor area in Parkinson's disease is reversed when akinesia is treated with apomorphine. Ann Neurol 32:749–757.
- Joyce JN, Smutzer G, Whitty CJ, Myers A, Bannon MJ (1997) Differential modification of dopamine transporter and tyrosine hydroxylase mRNAs in midbrain of subjects with Parkinson's, Alzheimer's with parkinsonism, and Alzheimer's disease. Mov Disord 12:885–897.
- Leblois A, Meissner W, Bezard E, Bioulac B, Gross CE, Boraud T (2006) Temporal and spatial alterations in GPi neuronal encoding might contribute to slow down movement in Parkinsonian monkeys. Eur J Neurosci 24:1201–1208.
- Leblois A, Meissner W, Bioulac B, Gross CE, Hansel D, Boraud T (2007) Late emergence of synchronized oscillatory activity in the pallidum during progressive Parkinsonism. Eur J Neurosci 26:1701–1713.
- Lee CS, Samii A, Sossi V, et al. (2000) In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease. Ann Neurol 47:493–503.
- Levy R, Herrero MT, Ruberg M, et al. (1995) Effects of nigrostriatal denervation and l-dopa therapy on the GABAergic neurons in the striatum in MPTP-treated monkeys and Parkinson's disease: an in situ hybridization study of GAD67 mRNA. Eur J Neurosci 7:1199–1209.
- Maneuf YP, Mitchell IJ, Crossman AR, Brotchie JM (1994) On the role of enkephalin cotransmission in the GABAergic striatal efferents to the globus pallidus. Exp Neurol 125:65–71.
- Mitchell IJ, Clarke CE, Boyce S, Robertson RG, Peggs D, Sambrook MA, Crossman AR (1989) Neural mechanisms underlying parkinsonian symptoms based upon regional uptake of 2-deoxyglucose in monkeys exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neuroscience 32:213–226.
- Morissette M, Grondin R, Goulet M, Bedard PJ, Di Paolo T (1999) Differential regulation of striatal preproenkephalin and preprotachykinin mRNA levels in MPTP-lesioned monkeys chronically treated with dopamine D1 or D2 receptor agonists. J Neurochem 72:682–692.
- Mounayar S, Boulet S, Tande D, et al. (2007) A new model to study compensatory mechanisms in MPTP-treated monkeys exhibiting recovery. Brain 130:2898–2914.
- Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Lanciego JL, Artieda J, Gonzalo N, Olanow CW (2000) Pathophysiology of the basal ganglia in Parkinson's disease. Trends in Neurosciences 23:S8–S19.
- Obeso JA, Rodriguez-Oroz M, Marin C, et al. (2004) The origin of motor fluctuations in Parkinson's disease: importance of dopaminergic innervation and basal ganglia circuits. Neurology 62:S17–S30.
- Onn SP, West AR, Grace AA (2000) Dopamine-mediated regulation of striatal neuronal and network interactions. Trends Neurosci 23:S48–S56.
- Onn SP, Berger TW, Stricker EM, Zigmond MJ (1986) Effects of intraventricular 6-hydroxydopamine on the dopaminergic innervation of striatum: histochemical and neurochemical analysis. Brain Res 376:8–19.
- Orieux G, Francois C, Feger J, Hirsch EC (2002) Consequences of dopaminergic denervation on the metabolic activity of the cortical

neurons projecting to the subthalamic nucleus in the rat. J Neurosci 22:8762-8770.

- Orieux G, Francois C, Feger J, Yelnik J, Vila M, Ruberg M, Agid Y, Hirsch EC (2000) Metabolic activity of excitatory parafascicular and pedunculopontine inputs to the subthalamic nucleus in a rat model of Parkinson's disease. Neuroscience 97:79–88.
- Pahapill PA, Lozano AM (2000) The pedunculopontine nucleus and Parkinson's disease. Brain 123(Pt 9):1767–1783.
- Palombo E, Porrino LJ, Bankiewicz KS, Crane AM, Sokoloff L, Kopin IJ (1990) Local cerebral glucose utilization in monkeys with hemiparkinsonism induced by intracarotid infusion of the neurotoxin MPTP. J Neurosci 10:860–869.
- Parent A, Cote PY, Lavoie B (1995) Chemical anatomy of primate basal ganglia. Prog Neurobiol 46:131–197.
- Parish CL, Finkelstein DI, Tripanichkul W, Satoskar AR, Drago J, Horne MK (2002) The role of interleukin-1, interleukin-6, and glia in inducing growth of neuronal terminal arbors in mice. J Neurosci 22:8034–8041.
- Perez XA, Parameswaran N, Huang LZ, O'Leary KT, Quik M (2008) Pre-synaptic dopaminergic compensation after moderate nigrostriatal damage in non-human primates. J Neurochem 105:1861–1872.
- Pessiglione M, Guehl D, Rolland AS, Francois C, Hirsch EC, Feger J, Tremblay L (2005) Thalamic neuronal activity in dopamine-depleted primates: evidence for a loss of functional segregation within basal ganglia circuits. J Neurosci 25:1523–1531.
- Pifl C, Hornykiewicz O (2006) Dopamine turnover is upregulated in the caudate/putamen of asymptomatic MPTP-treated rhesus monkeys. Neurochem Int 49:519–524.
- Playford ED, Jenkins IH, Passingham RE, Frackowiak RS, Brooks DJ (1993) Impaired activation of frontal areas during movement in Parkinson's disease: a PET study. Adv Neurol 60:506–510.
- Rascol O, Sabatini U, Chollet F, Celsis P, Montastruc JL, Marc-Vergnes JP, Rascol A (1992) Supplementary and primary sensory motor area activity in Parkinson's disease. Regional cerebral blood flow changes during finger movements and effects of apomorphine. Arch Neurol 49:144–148.
- Rascol O, Sabatini U, Brefel C, et al. (1998) Cortical motor overactivation in parkinsonian patients with l-dopa-induced peak-dose dyskinesia. Brain 121(Pt 3):527–533.
- Robinson TE, Mocsary Z, Camp DM, Whishaw IQ (1994) Time course of recovery of extracellular dopamine following partial damage to the nigrostriatal dopamine system. J Neurosci 14:2687–2696.
- Rodriguez M, Gonzalez J, Sabate M, Obeso J, Pereda E (2003) Firing regulation in dopaminergic cells: effect of the partial degeneration of nigrostriatal system in surviving neurons. Eur J Neurosci 18:53–60.
- Rothblat DS, Schneider JS (1999) Regional differences in striatal dopamine uptake and release associated with recovery from MPTPinduced parkinsonism: an in vivo electrochemical study. J Neurochem 72:724–733.
- Schneider JS, Kovelowski CJ 2nd (1990) Chronic exposure to low doses of MPTP. I. Cognitive deficits in motor asymptomatic monkeys. Brain Res 519:122–128.
- Schneider JS, Pope-Coleman A (1995) Cognitive deficits precede motor deficits in a slowly progressing model of parkinsonism in the monkey. Neurodegeneration 4:245–255.
- Schneider JS, Decamp E, Wade T (1999) Striatal preproenkephalin gene expression is upregulated in acute but not chronic parkinsonian monkeys: implications for the contribution of the indirect striatopallidal circuit to parkinsonian symptomatology. J Neurosci 19:6643–6649.

- Seeman P, Weinshenker D, Quirion R, et al. (2005) Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci USA 102:3513–3518.
- Shink E, Bevan MD, Bolam JP, Smith Y (1996) The subthalamic nucleus and the external pallidum: two tightly interconnected structures that control the output of the basal ganglia in the monkey. Neuroscience 73:335–357.
- Singh N, Pillay V, Choonara YE (2007) Advances in the treatment of Parkinson's disease. Progress in Neurobiology 81:29–44.
- Snyder GL, Keller RW Jr., Zigmond MJ (1990) Dopamine efflux from striatal slices after intracerebral 6-hydroxydopamine: evidence for compensatory hyperactivity of residual terminals. J Pharmacol Exp Ther 253:867–876.
- Song DD, Haber SN (2000) Striatal responses to partial dopaminergic lesion: evidence for compensatory sprouting. J Neurosci 20:5102–5114.
- Sossi V, Dinelle K, Topping GJ, et al. (2009) Dopamine transporter relation to levodopa-derived synaptic dopamine in a rat model of Parkinson's: an in-vivo imaging study. J Neurochem 109(1):85–92.
- Stanic D, Finkelstein DI, Bourke DW, Drago J, Horne MK (2003) Timecourse of striatal re-innervation following lesions of dopaminergic SNpc neurons of the rat. Eur J Neurosci 18:1175–1188.
- Takeuchi Y, Sawada T, Blunt S, Jenner P, Marsden CD (1991) Effects of 6-hydroxydopamine lesions of the nigrostriatal pathway on striatal serotonin innervation in adult rats. Brain Res 562:301–305.
- Tong ZY, Kingsbury AE, Foster OJ (2000) Up-regulation of tyrosine hydroxylase mRNA in a sub-population of A10 dopamine neurons in Parkinson's disease. Brain Res Mol Brain Res 79:45–54.
- Uhl GR, Walther D, Mash D, Faucheux B, Javoy-Agid F (1994) Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons. Ann Neurol 35:494–498.

- Vila M, Levy R, Herrero MT, Ruberg M, Faucheux B, Obeso JA, Agid Y, Hirsch EC (1997) Consequences of nigrostriatal denervation on the functioning of the basal ganglia in human and nonhuman primates: an in situ hybridization study of cytochrome oxidase subunit I mRNA. J Neurosci 17:765–773.
- Vila M, Perier C, Feger J, et al. (2000) Evolution of changes in neuronal activity in the subthalamic nucleus of rats with unilateral lesion of the substantia nigra assessed by metabolic and electrophysiological measurements. Eur J Neurosci 12:337–344.
- Whone AL, Moore RY, Piccini PP, Brooks DJ (2003) Plasticity of the nigropallidal pathway in Parkinson's disease. Ann Neurol 53:206–213.
- Zhang WQ, Tilson HA, Nanry KP, Hudson PM, Hong JS, Stachowiak MK (1988) Increased dopamine release from striata of rats after unilateral nigrostriatal bundle damage. Brain Res 461:335–342.
- Zhou FC, Bledsoe S, Murphy J (1991) Serotonergic sprouting is induced by dopamine-lesion in substantia nigra of adult rat brain. Brain Res 556:108–116.
- Zigmond MJ (1997) Do compensatory processes underlie the preclinical phase of neurodegenerative disease? Insights from an animal model of parkinsonism. Neurobiol Dis 4:247–253.
- Zigmond MJ, Stricker EM (1973) Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. Science 182:717–720.
- Zigmond MJ, Abercrombie ED, Berger TW, Grace AA, Stricker EM (1990) Compensations after lesions of central dopaminergic neurons: some clinical and basic implications. Trends Neurosci 13:290–296.
- Zigmond SH (1993) Recent quantitative studies of actin filament turnover during cell locomotion. Cell Motil Cytoskeleton 25:309–316.
- Zoli M, Jansson A, Sykova E, Agnati LF, Fuxe K (1999) Volume transmission in the CNS and its relevance for neuropsychopharmacology. Trends Pharmacol Sci 20:142–150.

# Pathological Synchrony of Basal Ganglia-Cortical Networks in the Systemic MPTP Primate Model of Parkinson's Disease

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# I. INTRODUCTION: PARKINSON'S DISEASE – PREVALENCE, SYMPTOMS AND THERAPY

Parkinson's disease (PD) affects tens of millions of people worldwide, and the prevalence and associated socioeconomic burden of PD are set to rise as the elderly population continues to grow (Van Den Eeden et al., 2003). The motor symptoms of the disease include poverty and slowness of movement (akinesia and bradykinesia, respectively), muscle rigidity, tremor at rest and postural instability (see also Chapter 34). The core, but not exclusive, pathology of PD is degeneration of the dopamine neurons in the midbrain and the resulting depletion of striatal dopamine. The striatum is the major input stage of the basal ganglia, receiving input from the cerebral cortex and thalamus, and projecting directly and indirectly to the output stages of the basal ganglia – the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) (see Chapter 1).

The dopamine precursor L-DOPA remains the gold standard for the treatment of PD. However, long-term use of L-DOPA (over 5 to 10 years in most patients) is associated with the development of motor complications (e.g., dyskinesia) (see also Chapters 36 and 37). As a result, the last decade has seen a resurgence of interest in functional neurosurgery - in particular, high-frequency stimulation of the subthalamic nucleus (STN) or the GPi (Benabid et al., 2006) (see Chapter 39). These procedures are grouped herein as deep brain stimulation, DBS procedures. The multi-stage therapy of PD, from dopamine replacement methods to modulation of the activity of the basal ganglia structures using DBS, reinstates interest in identifying the critical features of abnormal basal ganglia activity that follow striatal dopamine depletion and lead to the symptoms of PD (Rivlin-Etzion et al., 2006; Hammond et al., 2007). In this chapter we will summarize the main physiological findings in the MPTP primate model of PD (see also Chapter 34), and compare them to the recent physiological findings in human patients.

# II. ANATOMICAL AND PHYSIOLOGICAL ORGANIZATION OF THE BASAL GANGLIA

The operation of the basal ganglia-cortical network in health and disease is heavily determined by its network (connectivity) and cellular properties. The striatum is the main input nucleus of the basal ganglia (see Chapter 1). It receives glutamatergic input from all functional subdivisions of the cortex and from several thalamic nuclei. This information is processed by a striatal network composed of about 5% interneurons (GABAergic and cholinergic) (see Chapters 7 and 8) and about 95% GABAergic projection neurons, the medium spiny neurons (MSNs) (see Chapter 5). The MSNs provide the sole striatal output, thus the striatal network can be modelled as a one-layer network. The efficacy of cortico-striatal transmission (e.g., the gain of information transfer from the cortex to the projection neurons of the striatum) is modulated mainly by the dopaminergic and cholinergic systems (Wickens, 2008) (see also Chapter 6). Nevertheless, other neurotransmitters and modulators [e.g., 5-HT, adenosine (see Chapter 11) and endocannabinoids (see Chapter 9)] affect cortico-striatal efficacy as well. The high level of striatal plasticity enables the basal ganglia network to play a major role in reinforcement, procedural or implicit learning (Schultz et al., 1997; Sutton and Barto, 1998). The striatal network is further characterized by feed-forward, feed-back and lateral GABAergic inhibitions generated by fast spiking GABA interneurons (see Chapter 8) and the MSN axon collaterals (Wilson, 2007; Tepper et al., 2007) (see Chapter 5). The consequence of this organization and of the cellular properties of striatal neurons is that MSNs seldom fire during period of rest. Action potential discharge of MSNs occurs probably only in response to synchronized activity in many converging cortico-striatal and/or thalamo-striatal afferents (Bennett and Wilson, 2000) (see Chapter 19). This has led to the suggestion that MSNs shape their input-output relationship by triple Hebbian learning rules, according to the precise synchronization of the discharge of the cortical and thalamic inputs, the striatal postsynaptic neurons and the striatal neuromodulators (Arbuthnott and Wickens, 2007).

The subthalamic nucleus (STN) is the second input nuclei of the basal ganglia (see Chapter 15) STN neurons are the only glutamatergic neurons in the basal ganglia network. They receive direct projections from the cortex (termed as the hyper-direct pathway (Nambu et al., 2002). However, their cortical input is more limited than that of the striatum and comes mainly from somato-motor cortical areas. The number of neurons in the STN is much smaller than in the striatum. However, in contrast to the very low spontaneous discharge rate of the striatum, STN neurons exhibit a tonic discharge rate of 20–30 spikes/s in the normal condition (Wichmann et al., 1994). The STN and the striatum thus provide a balanced excitation and inhibition of the subsequent stations of the basal ganglia – the external segment of the globus pallidus (GPe) (see Chapter 13) and the output nuclei of the basal ganglia – GPi and SNr.

The anatomy and physiology of striatal and STN neurons stands in contrast to that of neurons in GPe, GPi and SNr. All striatal and STN neurons project to the GPe. In addition, a significant fraction of striatal neurons (those with D1 dopamine receptors and substance P as cotransmitter; see Chapter 1) as well as all STN and GPe neurons project to GPi and SNr. Each of these three structures (GPe, GPi and SNr) is composed of a homogenous population of GABA output neurons. The long dendrites of these neurons are densely covered with synaptic boutons. The majority of these boutons represent striatopallidal GABAergic terminals and the rest are mainly STN glutamatergic terminals. Most of the GPe, GPi and SNr neurons are characterized by their spontaneous highfrequency (50-80 spikes/s) discharge (HFD) rate. The high-frequency discharge of many of the GPe neurons, but not the GPi and SNr neurons, is also interrupted by spontaneous and uncorrelated long intervals of total silence or pauses (Elias et al., 2007).

## III. THE MPTP PRIMATE MODEL OF PARKINSON'S DISEASE

Early animal models of PD were based on lesions of midbrain areas in monkeys. These anatomical lesions mainly produced rigidity, but only rarely resulted in a spontaneous sustained tremor. More modern animal models of PD have shifted from anatomical to chemical lesions. Early chemical (e.g., the 6-hydroxydopamine rodent) models of PD were limited to dopaminergic damage. These models mainly reproduced PD akinesia; however, rigidity and tremor were seldom observed in these rodent models. The primate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD (see Chapter 34) better mimics the broad clinical and the pathological picture of human PD. Most monkeys treated with MPTP exhibit mainly the

akinetic-rigid symptoms of PD; low-frequency (4-7 Hz) resting tremor is not readily replicated in MPTP-treated macaque monkeys. Nevertheless, some species, notably the vervet (African green) monkey, often develop a prominent low-frequency tremor following MPTP injections, whereas Macaques usually develop short intermittent episodes of high (10-12Hz) tremor. Unlike human PD patients, the tremor of both species seem to be more action/postural and of axial rather than distal distribution. Dopamine replacement therapy (DRT) is very effective for both vervet and macaque monkeys following MPTP treatment, and as in human PD, DRT often leads to dyskinesia. Finally, postmortem examination of the brains of MPTP-treated primates reveals that the primary damage is indeed to the dopaminergic system. However, as in human PD, other neuromodulators are also affected.

## IV. EXCESSIVE SYNCHRONY AND OSCILLATIONS IN PARKINSON'S DISEASE

#### A. Results from Animal Models

Monkeys treated with the neurotoxin MPTP show an increase in the fraction of basal ganglia neurons that discharge in bursts (Bergman et al., 1998) (see also Chapter 25). These bursts are either irregular or oscillatory and have been found in the striatum (cholinergic tonically active interneurons, TANs), STN, GPe, GPi and SNr. In most cases the bursts tend to follow higher harmonics of the tremor frequency. The average frequency of tremor in the MPTP treated vervet monkey is 5Hz (Heimer et al., 2002), and the majority of GPi neurons oscillate at 10 Hz, while oscillations of STN neurons are at 15 Hz (Bergman et al., 1994). Both STN inactivation and dopamine replacement therapy significantly ameliorate the MPTP tremor and other motor PD symptoms and reduce 8-20Hz oscillations in GPi, suggesting a critical role for oscillations in this frequency band in the patho-physiology of PD symptoms. Finally, basal ganglia oscillations are also seen in non-tremulous animals. Thus, the correlation between the neural oscillations and the tremor does not imply simple causal relations. Basal ganglia oscillation can reflect peripheral feedback to central nervous system, and the high-frequency oscillations seems to be correlated more with the PD akinetic rigid symptoms rather than with the tremor phenomena.

Physiological studies of simultaneously recorded neurons in the pallidum demonstrate that their oscillatory bursts are also often synchronized after MPTP treatment (Raz et al., 2000). This is also the case among striatal cholinergic tonically active interneurons (TANs) and between TANs and pallidal neurons in MPTP-treated monkeys. In most cases, the maximal power of the synchronous oscillations was found to be at 10 Hz (i.e., at double the tremor frequency). As in the studies of oscillations in single neurons, the abnormal pallidal oscillatory synchronization between neurons decreases in response to dopamine replacement therapy.

Synchronicity in the nervous system is commonly seen among oscillating units, and therefore might be assumed to share the same pathophysiological mechanisms. Indeed, many experimental and theoretical studies have revealed that increased neuronal coupling can lead to synchronous oscillations. However, mechanisms like subthreshold cellular resonance phenomena and increased inhibitory coupling may differentially contribute to synchronization and oscillation, and it is of note that non-oscillatory synchronization has been found to coexist with oscillatory synchronization in the basal ganglia of MPTP treated monkeys. Thus synchronization and oscillation may occur together or separately within the dopamine depleted basal ganglia, likely reflecting biases in a variety of pathophysiological mechanisms in the dopamine-depleted parkinsonian state.

The studies of pair-wise correlations between neurons discussed above might actually tend to underestimate the extent of synchrony present in the neuronal population as a whole. Even weak pair-wise correlation can imply a highly synchronized network state (Schneidman et al., 2006). Indeed, studies of local field potential (LFP) activity in the basal ganglia are in agreement with this conclusion. Studies of LFP and spiking activity in the cortex and the basal ganglia concluded that, in the parkinsonian condition, cortex-basal ganglia networks are more tightly related to global modes of brain dynamics that are echoed by the cortex and basal ganglia LFP. Chronic and acute dopaminergic denervation in the rodent leads to excessive oscillatory activity in basal ganglia LFPs that can be suppressed by DRT. Interestingly, the frequency of synchronization tends to be slightly higher in the parkinsonian rodent (Mallet et al., 2008) than in the MPTP-treated primate and similar to that seen in LFP studies of patients with PD. Whether this relates to real biological (e.g., species) or methodological differences (e.g., a bias introduced by different measures of synchrony, or high pass filtering of the LFP) remains to be established.

### **B.** Observations in Human Patients

Surgical treatment for advanced PD in human patients includes DBS of the STN or GPi, which has proven to be safe and beneficial over time (see Chapter 39). During surgery for implanting a DBS macro-electrode, microelectrode recording is often utilized to verify localization of the implanted electrode in the target nucleus. These recordings can be used for physiological studies of the neural properties in the human disease condition.

Several groups looking at data from human PD patients have reported a broad range of abnormal oscillations, such as the low-frequency (4-8Hz) tremor-related ones, the alpha (8-13 Hz), beta (15-30 Hz), gamma (30-100 Hz) and even at higher frequency bands (Hammond et al., 2007; Chen et al., 2007; Foffani et al., 2003; Weinberger et al., 2009). Further support for a relationship between these oscillations and the pathological state comes from studies showing a reduction in the amplitude of these oscillations in conjunction with reduced tremor scores following pharmacological intervention and active movement of the limbs (Amirnovin et al., 2004). Our recent study of STN activity in human patients revealed two groups of oscillating units. The first group oscillated at the tremor frequency (5Hz) and the second group oscillated at 15Hz. Only the higher frequency group was synchronized with the STN background activity (Moran et al., 2008).

In some treatment centers, LFPs are more readily recorded from the basal ganglia of PD patients than the activity of single neurons because the former recordings can also be made in the interval between surgical implantation of the STN or GPi and connection of the DBS macroelectrodes to a subcutaneous stimulator a few days later (Brown and Eusebio, 2008). LFP recordings in patients withdrawn from their anti-parkinsonian medication have consistently revealed prominent beta oscillations of 8 to 30 Hz. Like the pathological synchronization in animal models of PD, the oscillatory LFP activity in the beta band in PD patients is suppressed by treatment with dopaminer-gic drugs and this suppression is correlated with clinical improvement (Hammond et al., 2007).

## V. HOW MIGHT EXCESSIVE SYNCHRONY IMPAIR BASAL GANGLIA PROCESSING?

Information theory studies reveal that the information encoded by the simultaneous activity of neurons can be independent, redundant or synergistic (Schneidman et al., 2003). These activity modes are related to the level of pair-wise correlations between the neuronal elements of the network. Usually, we can assume that a correlated network is redundant. In that case, the information encoded by the neuronal population will be smaller than the sum of information encoded by its single elements.

Neuronal networks consist of millions of weakly coupled elements (neurons), with a rich plethora of reciprocal, feed-forward and feedback connections. Any physical system with such an architecture faces a huge tendency to synchronize. It is therefore not surprising that synchrony of neural networks characterizes many disease states (e.g., epilepsy). Indeed, our previous theoretical models have suggested that the computational goal of the basal ganglia networks is to maximize the representation of the cortical information by using decorrelation mechanisms controlled by dopamine reinforcement signals (Bar-Gad et al., 2003). We therefore suggest that the consequence of striatal dopamine depletion is the loss of basal ganglia-independent activity and the development of synchronization between neurons as well as synchronous oscillations. As in many physical systems the neuronal synchronization phenomenon can be the result of phase transition. Accordingly, the amount of synchronization in the network might not be a linear function of the striatal dopamine level, and at some critical point, the number of synchronous neurons in the basal ganglia network can increase exponentially.

A growing amount of evidence suggests that there is no direct coupling between the basal ganglia synchronous oscillations and PD tremor, or other symptoms (see also Chapter 25). Several studies have reported that basal ganglia oscillations appear after the development of the PD symptoms (Leblois et al., 2007). Similarly, synchronous oscillations are not always detected in the basal ganglia of MPTP-treated monkeys. Finally, basal ganglia oscillations might be evoked by the peripheral tremor and the afferent information from the muscles to the central nervous system (Rivlin-Etzion et al., 2006). Another confounding property is the difference in oscillation frequencies encountered at different parts of the system (5Hz in the peripheral tremor, 5 and 10Hz in the cortex and the GPi, and 5 and 15Hz in the STN). We therefore suggest that the PD symptoms and the basal ganglia oscillations reflect a complex, non-linear feed-forward, lateral and feed-back system. Such a system could produce the 5Hz peripheral tremor out of the GPi 10Hz and the STN 15Hz oscillatory activity. As shown in Figure 38.1, 5Hz oscillations could be the result of merging of 10 and 15Hz oscillations.



**FIGURE 38.1** Two to three synchronization of 10 and 15 Hz rhythms can lead to 5 Hz oscillations. A schematic representation of 10 Hz bursts (black bars) in the GPi and 15 Hz bursts in the STN. The common frequency of these two rhythms is the "tremor" 5 Hz rhythm.

This merging of the 10 and the 15 Hz oscillations may drive down-stream neural activity at their common mode – 5 Hz rhythm (Fig. 38.1).

# VI. CONCLUSIONS AND FUTURE DIRECTIONS

There is an accumulation of data linking excessive synchrony at low frequencies in basal ganglia-thalamo-cortical loops to impaired motor processing in PD. Whether synchronization is an epiphenomenon or truly pathogenic in PD (Eusebio and Brown, 2009), it provides a clear *biological marker* for the disease process. Recent studies indicate the differential roles or correlates of the distinctive bands of oscillatory activity in the pathogenesis of PD (Eusebio and Brown, 2009; Kuhn et al., 2009). We therefore suggest that that amelioration of specific domains of basal gangliacortical synchronized oscillatory activity could form the basis for future closed-loop stimulation regimes (Tass, 2003; Feng et al., 2007a; Feng et al., 2007b) for human PD patients.

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#### REFERENCES

- Amirnovin R, Williams ZM, Cosgrove GR, Eskandar EN (2004) Visually guided movements suppress subthalamic oscillations in Parkinson's disease patients. J Neurosci 24:11302–11306.
- Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30:62–69.

- Bar-Gad I, Morris G, Bergman H (2003) Information processing, dimensionality reduction and reinforcement learning in the basal ganglia. Prog Neurobiol 71:439–473.
- Benabid AL, Deuschl G, Lang AE, Lyons KE, Rezai AR (2006) Deep brain stimulation for Parkinson's disease. Mov Disord 21(Suppl 14):S168–S170.
- Bennett BD, Wilson CJ (2000) Synaptology and physiology of neostriatal neurons. In: Brain Dynamics and the Strital Complex (Miller R, Wickens JR, eds), pp 111–140. Australia: Taylor and Francis.
- Bergman H, Feingold A, Nini A, Raz A, Slovin H, Abeles M, Vaadia E (1998) Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. Trends Neurosci 21:32–38.
- Bergman H, Wichmann T, Karmon B, DeLong MR (1994) The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol 72:507–520.
- Brown P, Eusebio A (2008) Paradoxes of functional neurosurgery: clues from basal ganglia recordings. Mov Disord 23:12–20.
- Chen CC, Litvak V, Gilbertson T, et al. (2007) Excessive synchronization of basal ganglia neurons at 20 Hz slows movement in Parkinson's disease. Exp Neurol 205(1):214–221.
- Elias S, Joshua M, Goldberg JA, Heimer G, Arkadir D, Morris G, Bergman H (2007) Statistical properties of pauses of the high-frequency discharge neurons in the external segment of the globus pallidus. J Neurosci 27:2525–2538.
- Eusebio A, Brown P (2009) Synchronisation in the beta frequencyband – The bad boy of parkinsonism or an innocent bystander? Exp Neurol. 217(1):1–3.
- Feng XJ, Greenwald B, Rabitz H, Shea-Brown E, Kosut R (2007a) Toward closed-loop optimization of deep brain stimulation for Parkinson's disease: concepts and lessons from a computational model. J Neural Eng 4(2):L14–L21.
- Feng XJ, Shea-Brown E, Greenwald B, Kosut R, Rabitz H (2007b) Optimal deep brain stimulation of the subthalamic nucleus-a computational study. J Comput Neurosci.
- Foffani G, Priori A, Egidi M, Rampini P, Tamma F, Caputo E, Moxon KA, Cerutti S, Barbieri S (2003) 300-Hz subthalamic oscillations in Parkinson's disease. Brain 126:2153–2163.
- Hammond C, Bergman H, Brown P (2007) Pathological synchronization in Parkinson's disease: networks, models and treatments. Trends Neurosci 30(7):357–364
- Heimer G, Bar-Gad I, Goldberg JA, Bergman H (2002) Dopamine replacement therapy reverses abnormal synchronization of pallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of parkinsonism. J Neurosci 22:7850–7855.
- Kuhn AA, Tsui A, Aziz T, Ray N, Brucke C, Kupsch A, Schneider GH, Brown P (2009) Pathological synchronisation in the subthalamic nucleus of patients with Parkinson's disease relates to both bradykinesia and rigidity. Exp Neurol 215:380–387.
- Leblois A, Meissner W, Bioulac B, Gross CE, Hansel D, Boraud T (2007) Late emergence of synchronized oscillatory activity in the pallidum during progressive parkinsonism. Eur J Neurosci 26:1701–1713.
- Mallet N, Pogosyan A, Marton LF, Bolam JP, Brown P, Magill PJ (2008) Parkinsonian beta oscillations in the external globus pallidus and their relationship with subthalamic nucleus activity. J Neurosci 28:14245–14258.
- Moran A, Bergman H, Israel Z, Bar-Gad I (2008) Subthalamic nucleus functional organization revealed by parkinsonian neuronal oscillations and synchrony. Brain 131(Pt 12):3395–3409.

- Nambu A, Tokuno H, Takada M (2002) Functional significance of the cortico-subthalamo-pallidal "hyperdirect" pathway. Neurosci Res 43:111–117.
- Raz A, Vaadia E, Bergman H (2000) Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine vervet model of parkinsonism. J Neurosci 20:8559–8571.
- Rivlin-Etzion M, Marmor O, Heimer G, Raz A, Nini A, Bergman H (2006) Basal ganglia oscillations and pathophysiology of movement disorders. Curr Opin Neurobiol 16:629–637.
- Schneidman E, Berry MJ, Segev R, Bialek W (2006) Weak pairwise correlations imply strongly correlated network states in a neural population. Nature 440:1007–1012.
- Schneidman E, Bialek W, Berry MJ (2003) Synergy, redundancy, and independence in population codes. J Neurosci 23:11539–11553.
- Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599.
- Sutton RS, Barto AG (1998) Reinforcement Learning An Introduction. Cambridge, Massachsetts: The MIT Press.

- Tass PA (2003) A model of desynchronizing deep brain stimulation with a demand-controlled coordinated reset of neural subpopulations. Biol Cybern 89:81–88.
- Tepper JM, Abercrombie ED, Bolam JP (2007) Basal ganglia macrocircuits. Prog Brain Res 160:3–7.
- Van Den Eeden SK, Tanner CM, Bernstein AL, Fross RD, Leimpeter A, Bloch DA, Nelson LM (2003) Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol 157:1015–1022.
- Weinberger M, Hutchison WD, Lozano AM, Hodaie M, Dostrovsky JO (2009) Increased gamma oscillatory activity in the subthalamic nucleus during tremor in Parkinson's disease patients. J Neurophys 101:789–802.
- Wichmann T, Bergman H, DeLong MR (1994) The primate subthalamic nucleus. I. Functional properties in intact animals. J Neurophysiol 72:494–506.
- Wickens JR (2008) Synaptic plasticity in the basal ganglia. Behav Brain Res 199(1):119–128.
- Wilson CJ (2007) GABAergic inhibition in the neostriatum. Prog Brain Res 160:91–110.

# Deep-Brain Stimulation for Neurologic and Psychiatric Disorders

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## I. INTRODUCTION

Ablative procedures, such as thalamotomy and pallidotomy for the treatment of Parkinson's disease, were commonplace in the 1950s and 1960s. However, treatment for patients with Parkinson's disease radically changed with the introduction of L-DOPA therapy in the 1960s. L-DOPA therapy was rapidly adopted by patients and physicians, and the use of stereotactic surgery decreased greatly. Subsequently, it became clear, however that L-DOPA and related drugs frequently induce long-term side effects, such as drug-induced involuntary movements, motor fluctuations, and non-motor complications, which limit the usefulness of these drug therapies, especially in patients with advanced Parkinson's disease. The need for novel treatments led to renewed interest in neurosurgical approaches. Initially, neurosurgeons revisited the use of ablative functional surgeries (such as pallidotomy), but,

Handbook of Basal Ganglia Structure and Function Copyright © 2010 Elsevier B.V. All rights reserved. over the past decade deep-brain stimulation (DBS) has emerged as the treatment of choice, because of its reversibility, adjustability, and less invasive nature.

DBS was introduced as treatment for patients with tremor and Parkinson's disease in the 1990s. Since then, DBS has been successively extended to other movement disorders, such as dystonia. Based on the success of DBS for movement disorders, the use of this therapy has now been extended to neuropsychiatric conditions, including Tourette's syndrome (TS), obsessive compulsive disorder (OCD), and treatment-refractory depression (TRD). All of these conditions are associated with disturbances in basal ganglia function. In this chapter we will briefly review the functional organization and general pathophysiology of basal ganglia disorders, discuss the concept of "circuit disorders" involving the basal ganglia, and then review the use and rationale of DBS for these conditions. This review will not discuss the numerous other disorders for which DBS is currently being investigated (e.g., epilepsy, pain, cluster headaches, obesity, brain injury and minimally conscious states), which involve structures outside of the basal ganglia circuitry.

## II. BASAL GANGLIA-THALAMOCORTICAL CIRCUITS

### A. Circuit Anatomy

Anatomically, the basal ganglia are components of a family of parallel re-entrant largely closed cortical-subcortical circuits, in which information is sent from individual cortical areas to the basal ganglia, processed, and then returned to the respective frontal lobe target via the thalamus (Alexander et al., 1986; Alexander and Crutcher, 1990; Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000) (see Chapter 1). The different circuits share anatomical features, supporting the view that they may work as processing modules whose specific functions are determined by the cortical regions they are centered upon. Based on the presumed function of the cortical region involved (Fig. 39.1), the circuits are commonly designated as "motor," "oculomotor," "prefrontal," (or "associative") and "limbic" (Alexander et al., 1986; Alexander and Crutcher, 1990; Alexander et al., 1990; Parent, 1990; Haber et al., 1995). Each of these broad divisions is comprised of numerous subcircuits, centered on identified cortical sub-regions of the larger circuits. Abnormal neuronal activities within specific circuits may lead to signs and symptoms that differ, depending on the circuit involved and the nature of the change within the given circuit.

In addition to participating in the cortex-basal gangliathalamocortical loops, the basal ganglia also send direct projections to brainstem areas, such as the superior colliculus and the pedunculopontine nucleus (PPN). These projections are discussed below.

The anatomical segregation of the basal ganglia circuits is exploited in focal neurosurgical interventions, such as DBS, which is aimed at modulating brain activity patterns in a highly circumscribed manner within a specific circuit or portions thereof, while leaving the activity in the other circuits or sub-circuits unchanged.

Parkinson's disease and most other hypo- and hyperkinetic movement disorders, result from disturbances in the "motor" circuit, which takes origin in pre- and post-central sensorimotor areas, including the primary motor cortex (M1), the supplementary motor area (SMA), the premotor



FIGURE 39.1 "Motor" and "limbic" cortex-basal ganglia-thalamocortical circuits. The targets of current DBS treatments are labeled with asterisks (\*). Abbreviations: ACA, anterior cingulate area; M1, primary motor cortex; MD, mediodorsal nucleus of thalamus; MOFC, medial orbitofrontal cortex; VApc, ventral anterior nucleus of thalamus, pars parvocellularis; VAmc, ventral anterior nucleus of thalamus, pars magnocellularis; VLm, ventrolateral nucleus of thalamus, pars medialis; VLo, ventrolateral nucleus of thalamus, pars medialis; VLo, ventrolateral nucleus of thalamus, pars oralis. See text for other abbreviations.

cortex (PMC), the cingulate motor area (CMA), and related post-central sensory cortical areas. Output from these cortical areas is conveyed through the circuit by virtue of topographic connections between specific "motor" portions in each of the basal ganglia structures and thalamus. By contrast with movement disorders, the majority of neuropsychiatric disorders amenable to DBS result from disturbances within the limbic circuit, which takes origin from limbic cortices, including the anterior cingulate and medial orbitofrontal cortices and project to the ventral striatum (VS), which includes the nucleus accumbens.

### **B.** Basal Ganglia Anatomy and Circuitry

In the following we summarize the major features of the cortico-basal ganglia- thalamocortical circuits, as covered in detail in Chapter 1. The striatum and subthalamic nucleus (STN) are the main entry points for cortical and thalamic inputs to the basal ganglia, while the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) provide basal ganglia output to the thalamus and brainstem. The striatum is linked to the output structures via two distinct pathways, the so-called "direct" and "indirect" striato-pallidal pathways (Fig. 39.1). The direct pathway arises from sets of striatal medium spiny neurons (MSNs) that project monosynaptically to neurons in GPi and SNr. These neurons also contain the neuropeptides substance P and dynorphin, and preferentially express D1 dopamine receptors. The indirect pathway arises from a separate set of striatal MSNs, which project to the external segment of the globus pallidus (GPe) (Shink et al., 1996; Smith et al., 1998). The striatal neurons that give rise to the indirect pathway preferentially express enkephalin and D2 dopamine receptors (Gerfen et al., 1990). The level of separation between the two pathways remains a matter of debate, as single-cell labeling studies of striatal MSNs have shown that some MSNs send collaterals to both segments of the globus pallidus (Parent et al., 2000).

The STN (see also Chapter 15) is viewed as a key component of the indirect pathway, receiving major output from the GPe and projecting to the GPi/SNr as well as back to the GPe (Fig. 39.1). As does the striatum, the STN receives segregated projections from motor, oculomotor, associative and limbic cortical areas and from corresponding functional divisions of the thalamic intralaminar nuclei (the centromedian [CM] and parafascicular nuclei [Pf]). The cortical projection to the STN, which arises from collaterals of corticobulbar and corticospinal fibers and the subsequent STN projections to the GPi/SNr, has been referred to as the "hyperdirect" pathway, in order to emphasize its potential importance as a faster route for cortical input to modulate the activity in the output nuclei of the basal ganglia.

The organization of cortical projections to the striatum and STN is maintained by highly topographic connections in the direct and indirect pathways. For instance, GPe neurons which receive functionally specific input from the striatum (thus giving rise to sensorimotor, associative or limbic territories within the nucleus), and populations of GPe neurons within these territories, are reciprocally connected with functionally similar neurons in the STN. Together with the segregated cortical inputs to the STN, these anatomical relationships define a dorsolateral motor region, a ventromedial associative region, and a rostromedial limbic region in the STN. Neurons in each of these regions, in turn, innervate the corresponding functionally specific territories in GPi/SNr (Shink et al., 1996; Smith et al., 1998). Likewise, the direct pathway projections from the striatum to GPi/SNr are topographically arranged, in registry with the terminations of the indirect pathway in these territories.

### C. Basal Ganglia Output

The "motor" territories of GPi and SNr receive movement-related inputs via the direct and indirect pathways and projects to the anterior part of the ventrolateral nucleus (VL) which then sends projections towards M1 and the premotor areas (Schell and Strick, 1984; Hoover and Strick, 1993; Inase and Tanji, 1995). The more rostromedial associative areas of GPi project preferentially to the parvocellular part of the ventral anterior (VA) nucleus and the dorsal VL (DeVito and Anderson, 1982; Sidibe et al., 1997). These thalamic areas are preferentially connected to prefrontal cortex (Goldman-Rakic and Porrino, 1985; Middleton and Strick, 1994). Collaterals from the pallido- and nigrofugal projections also reach the CM/Pf. The latter projections are part of a system of segregated basal ganglia-thalamostriatal feedback projections (Sidibe et al., 1997; Smith et al., 2004) (see Chapter 22). In primates, CM receives input from motor areas in GPi and SNr and projects to the motor portions of putamen and STN, whereas Pf inputs and output are related to associative and limbic territories of the basal ganglia (Smith and Parent, 1986; Sadikot et al., 1992).

Basal ganglia output also reaches the PPN (see Harnois and Filion, 1982; Rye et al., 1988) which, in turn, gives rise to ascending projections to basal ganglia, thalamus and basal forebrain and to descending projections to pons, medulla and spinal cord (Inglis and Winn, 1995; Mena-Segovia et al., 2004) (see Chapter 23). In addition, the SNr projects to the superior colliculus and may influence saccadic and head/neck orienting movements (Hikosaka, 2007; Kaneda et al., 2008; Liu and Basso, 2008; May et al., 2009).

#### D. Normal Functions of the Motor Circuit

The view that the anatomically and physiologically defined basal ganglia "motor" areas are involved in movement is strongly supported by electrophysiologic and metabolic mapping studies in animals and functional imaging data in humans (Grafton and DeLong, 1997; Turner et al., 1998; Eidelberg and Edwards, 2000; Ghilardi et al., 2000). According to the classic model of basal ganglia motor functions, activation of MSNs that give rise to the direct pathway reduces inhibitory basal ganglia output from targeted neurons with subsequent disinhibition of related thalamocortical projection neurons. This may lead to increased activity in appropriate cortical neurons and facilitation of the movement. By contrast, activation of MSNs that give rise to the indirect pathway would lead to increased (inhibitory) basal ganglia output onto thalamocortical neurons, and to suppression of movement. The opposing actions of the direct and indirect pathways on GPi/SNr neurons have been interpreted in different ways. For instance, according to the "scaling" hypothesis, the balance between direct and indirect pathway activity would regulate the overall amount of movement, while according to the "focusing/action selection" hypothesis specific activation patterns (for instance, a center-surround type of activation involving direct and indirect pathways) would limit the extent or duration of ongoing movements. Activation of the indirect pathway has been proposed, by some, as inhibiting "unwanted" or competing movements (Albin et al., 1989; Mink and Thach, 1993; Gurney et al., 2001; Nambu et al., 2004). However, although single cell recordings in behaving primates have revealed highly specific relations to movements of individual body parts and to specific parameters of movement, such as direction and amplitude/velocity of movement, there is little experimental support for a primary or critical role of the basal ganglia in the online-control of movement. The fact that the timing of changes in neuronal activity in the basal ganglia in relation to movement onset lags that in the cerebellum and cortex in reaction time tasks (e.g., Mitchell et al., 1987; Wichmann et al., 1994) and that lesions of the basal ganglia motor output (the sensorimotor territory of GPi) have little or no immediate or long-term effects on posture or movement initiation or execution in normal animals and improves movement in patients with Parkinson's disease, argues against a prominent role of the motor circuit in on-line motor control. The available evidence would suggest that the motor circuit has more general functions in the control of movement, such as motor learning (Bar-Gad and Bergman, 2001; Pisani et al., 2005; Doyon, 2008; Graybiel, 2008; Moustafa et al., 2008).

It may seem paradoxical to suggest that the basal ganglia do not normally participate in the initiation or execution of movement or in the control of posture, since disturbances in movement and posture are fundamental features of Parkinson's disease and other movement disorders involving the basal ganglia. It is, however, clear that neither lesions nor electrical stimulation of the sensorimotor portion of GPi, the output nucleus of the motor circuit, have obvious effects on movement or posture or produces involuntary movements. The fact that disturbances of neuronal activity solely within the basal ganglia, such as focal lesions or inactivation of the STN (Hamada and DeLong, 1992), injection of the GABA-A receptor antagonist bicuculline into GPe (Grabli et al., 2004) and electrical stimulation of the primate putamen (Alexander and DeLong, 1985a,b) can produce involuntary movements, makes it clear that abnormal activity in the basal ganglia can, however, generate movements in abnormal states, most often of a stereotyped or repetitive nature. These findings suggest that neuronal representations of learned or fixed behavioral patterns may be stored in the basal ganglia and released by cortical inputs or abnormal activation within the receiving areas of the basal ganglia. It is conceivable that disruption of the motor circuit's involvement in normal motor learning contributes to the alterations of voluntary movement in these disorders (particularly dystonia), or that disordered basal ganglia output simply disrupts otherwise normal activity in thalamus and cortex (see below). The fact that pallidotomy and chronic GPi stimulation (DBS) restore normal movement and posture in these disorders (see below), while interrupting or overriding abnormal output is testimony to the fact that the basal ganglia are not necessary for online motor control.

In all models of basal ganglia function, the modulatory effects of dopamine on striatal transmission play a central role. Dopamine is released in the striatum from terminals of projections from the substantia nigra pars compacta (SNc) and regulates the activity of the basal ganglia output neurons in GPi and SNr by facilitating corticostriatal transmission upon the direct pathway and inhibiting corticostriatal transmission upon medium spiny neurons that give rise to the indirect pathway (see Chapter 6). These opposing actions are likely mediated via D1 and D2 receptors (Gerfen, 1995; Wilson and Kawaguchi, 1996; Tseng et al., 2001; Murer et al., 2002). The net effect of striatal dopamine release appears to be to reduce basal ganglia output to the thalamus and other targets, and, according to the classic circuit model, thereby, to result in increased overall movement. Dopamine may also strongly influence discharge patterns and rates throughout the basal ganglia, and may have a role in motor learning. Activation of dopamine receptors on MSNs has been shown to be involved in the induction of long-term potentiation and depression at glutamatergic (presumably corticostriatal) synapses (Reynolds et al., 2001; Gerdeman et al., 2002; Calabresi et al., 2007; Kreitzer and Malenka, 2007; Singla et al., 2007; Tozzi et al., 2007; Shen et al., 2008) (see Chapter 12).

## III. "CIRCUIT DISORDERS" INVOLVING THE BASAL GANGLIA

It is clear that dysfunction within the "motor", "oculomotor", "limbic" and "associative" circuits may lead to a wide spectrum of motor and non-motor behavioral abnormalities. Although details of the circuit dysfunction have been characterized in considerable detail for movement disorders, such as Parkinson's disease and dystonia, such details are largely lacking for neuropsychiatric conditions. We will focus in this section on two movement disorders, Parkinson's disease and dystonia. Two other movement disorders, Huntington's disease and hemiballismus, will be briefly discussed in the section on the use of DBS in movement disorders.

## A. Parkinsonism

The cardinal motor features of Parkinson's disease, i.e., the triad of akinesia/bradykinesia, tremor at rest and muscular rigidity, result from decreased dopaminergic transmission in the motor portions of the basal ganglia, in particular the putamen, due to progressive loss of innervation from dopaminergic neurons in the SNc. Other features of the disease such as depression, autonomic dysfunction, sleep disorders, cognitive impairment, and gait/balance problems appear to result from widespread progressive pathologic changes outside of the dopaminergic system, starting in the lower brainstem and ascending to the midbrain, amygdala, thalamus and cerebral cortex (Braak et al., 2003). We will focus here on those aspects of the disease that result from dopamine deficiency, since very little is known about the pathophysiology of the non-dopaminergic symptoms and signs. The study of parkinsonism-related changes in basal ganglia-thalamocortical circuits has been greatly facilitated by the availability of animal models of dopamine depletion, in which dopaminergic toxins such as 6-hydroxy-dopamine (6-OHDA) or 1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine (MPTP) are used to produce permanent and selective loss of dopamine neurons (see also Chapter 34).

Early studies of MPTP-treated primates emphasized the importance of activity alterations in the striato-pallidal pathways. Such changes were suggested by studies of metabolic activity in the basal ganglia of MPTP treated primates (Crossman et al., 1985; Schwartzman and Alexander, 1985) which indicated increased synaptic activity in GPe and GPi. Subsequent microelectrode recording studies in MPTP-treated primates showed a reduction of neuronal discharge in GPe, and increased firing in STN, GPi and SNr (Miller and DeLong, 1987; Filion et al., 1988; Bergman et al., 1994; Wichmann et al., 1999), and high neuronal discharge rates in GPi in most parkinsonian patients undergoing functional neurosurgical procedures (Vitek et al., 1993; Dogali et al., 1994; Lozano et al., 1996) (see Chapter 38). It is postulated that striatal dopamine depletion leads to increased activity in striatal neurons of the indirect pathway, resulting in inhibition of GPe, and subsequent disinhibition of STN and GPi/SNr. Local dopamine loss in the extrastriatal structures (specifically in STN, GPi and SNr) may play a role as well, specifically with regard to the emergence of abnormal firing patterns in these nuclei. It is likely that other structures and feedback loops, such as those involving the PPN and the CM contribute to the abnormalities of discharge that are found in the basal ganglia output nuclei. For instance, it is believed that reduced or abnormal PPN activity contributes to akinesia (Kojima et al., 1997; Munro-Davies et al., 1999; Kringelbach et al., 2007). In contrast to the parkinsonian state, dyskinesias are associated with decreased neuronal activity in the GPi in both the MPTP primate model and in patients undergoing microelectrode mapping during DBS surgery. This has led to the notion that dyskinesias result from the lowering of inhibition in the motor thalamus.

It is now widely believed that changes in discharge patterns are of equal or greater importance than the aforementioned rate changes in GPi output (see Chapter 25), since lesions of the thalamus do not result in bradykinesia and since lesions of GPi do not result in dyskinesias. One of the most discussed changes in the basal ganglia of parkinsonian subjects is the development of neuronal oscillations (Gatev et al., 2006; Hammond et al., 2007). Abnormal oscillations have been identified in the activity of single neurons in GPi, SNr and STN in animals and patients, and, more recently, in recordings of local field potentials (LFPs) in patients which may reflect activities of more extensive networks of connections. The latter LFP recordings were made by using implanted DBS macro-electrodes as recording probes during the time immediately following the implantation surgery. Recordings in the STN and other basal ganglia areas with this method have demonstrated the presence of LFP oscillations in the 10-25 Hz (beta) range in STN, GPi and cortex in unmedicated PD patients. The studies have also shown that beta range oscillations give way to a more normal pattern of oscillations in the 60-80 Hz
(gamma) range when the patients are treated with L-DOPA or when the STN is stimulated at high frequencies (see below, and Brown and Williams, 2005; Hammond et al., 2007). The prominence of beta-band activity is apparent throughout the basal ganglia-thalamocortical circuitry. For example, the desynchronization of EEG oscillations that normally precedes movement was shown to be abnormally small in parkinsonian patients, perhaps interfering with the initiation of movement (Wang et al., 1999; Williams et al., 2002; Klostermann et al., 2007).

Another parkinsonism-related abnormality in spontaneous discharge is the emergence of abnormal synchrony between neurons. Under physiologic conditions, basal ganglia activity is highly specific in its relation to movement parameters and body part, and appears to be segregated even at the cellular level, where neighboring neurons are rarely found to fire in synchrony (Wichmann et al., 1994). In parkinsonism, this level of segregation is lost and the discharge of neighboring neurons is often found to be correlated and abnormally synchronized (Bergman et al., 1994; Hammond et al., 2007).

Finally, the proportion of cells in STN, GPi and SNr which discharge in bursts (oscillatory or non-oscillatory) is greatly increased in parkinsonism (Miller and DeLong, 1987; Filion and Tremblay, 1991; Bergman et al., 1994; Wichmann and DeLong, 2006; Hammond et al., 2007). Oscillatory burst discharge patterns are also seen in conjunction with tremor, which may reflect tremor-related proprioceptive input or a more active participation of basal ganglia in the generation of tremor.

The relative importance of the different changes in basal ganglia activity for the development of the behavioral signs of parkinsonism is unclear. While bursty, synchronized oscillatory activity in the basal ganglia-thalamocortical circuits is associated with parkinsonism (Brown, 2003), direct links between oscillatory activity and specific parkinsonian deficits have not been established. In fact, recent studies in monkeys that underwent a gradual MPTP-treatment protocol that slowly induced parkinsonism have cast doubt on the notion that synchronous oscillatory firing contributes strongly to (early) parkinsonism (Leblois et al., 2007). In these single-neuron recording studies, synchrony and oscillations in neuronal spiking activity were detected only after the development of bradykinesia and akinesia. Similarly, recent rodent experiments have suggested that abnormal oscillations in the basal ganglia do not result simply from (acute) lack of dopaminergic stimulation, but may rather be due to the chronic absence of dopamine (Degos et al., 2008; Mallet et al., 2008). Such 'late'

changes could be related to anatomic alterations secondary to dopamine depletion, such as the loss of dendritic spines of MSNs (Chan et al., 2007; Peterson and Surmeier, 2007; Villalba et al., 2009) (see also Chapter 38).

# B. Dystonia

In patients with dystonia, normal movements are disrupted by co-contraction of agonist and antagonist muscles, and by excessive activation of inappropriate muscle groups (overflow), leading to abnormal movements and postures. Dystonias are classified as either primary, in which there are no other findings (other than tremor in some cases) or secondary, in which dystonia is a sequel to focal damage to the putamen, thalamus, cortex or cerebellum, exposure to neuroleptics (in the form of acute dystonic reactions or tardive dystonia), or other factors. Dystonia, as a neurologic sign, is often seen in the setting of other disorders such as Parkinson's disease, Huntington's disease, mitochondrial disorders, or metabolic disturbances. Dystonia can also be characterized as generalized, segmental, focal, or hemi-dystonia.

In adults, dystonia most commonly occurs in a focal manner, involving the neck (cervical dystonia), eyes (blepharospasm), or vocal apparatus (spasmodic dysphonia). In children, generalized forms of dystonia are more common. The earliest manifestation of limb dystonia in the childhood primary (genetic) forms is often an "action dystonia", occurring only with attempted voluntary movement such as in-turning of the foot with walking. At present, 18 dystonia-related genes have been identified. One of the main forms of generalized dystonia in children and young adults, idiopathic torsion dystonia (DYT1), is caused by a genetic defect of the TOR1A gene on chromosome 9 (Ozelius et al., 1997).

While a number of animal models for DYT1 and other types of dystonia have been described, these models for the most part do not replicate the phenotype of the disease in a convincing manner (Raike et al., 2005). Consequently, little is known about the brain abnormalities that underlie the dystonic phenotype. It seems clear, however, that dystonia is not a neurodegenerative disorder (such as Parkinson's disease), but that the activity changes seen in dystonia are due to dysfunction secondary to abnormal biology, and are, thus, potentially correctable through pharmacological or neurosurgical treatments.

Dystonia is often associated with disturbances in dopaminergic transmission (Wichmann, 2008). For instance, dystonia may develop acutely in normal individuals treated with dopamine-receptor blocking agents, or appear after long-term treatment with these drugs (tardive dystonia). In Parkinson's disease patients, dystonia develops usually as the result of exposure to dopaminergic drugs, but, especially in young-onset cases, may appear early, independent of medications. Dystonia is also present in patients with familial dystonia and parkinsonian features who respond to treatment with low-dose L-DOPA (DOPA-responsive dystonia, or DRD). Many of these patients suffer from genetic defects of tyrosine hydroxylase function that results in abnormal dopamine synthesis (Ichinose et al., 1994; Ichinose and Nagatsu, 1997; Nagatsu and Ichinose, 1997; Sato et al., 2008).

Evidence for altered dopamine metabolism was also found in human autopsy material from DYT1 patients (Augood et al., 2002), and recent studies in genetic mouse models of DYT1 dystonia have indicated that striatal dopamine release (but not dopamine levels) may be altered (Balcioglu et al., 2007; Zhao et al., 2008). Electrophysiologic studies in mice overexpressing mutant torsinA (a model of DYT1), have indicated that the dopaminergic control of cholinergic and GABAergic transmission in the striatum may be affected in the disease, leading to a renewed interest in the cholinergic system in dystonia (Pisani et al., 2006; Pisani et al., 2007; Sciamanna et al., 2009).

Details of brain activity changes (including changes in the basal ganglia) in dystonia remain sketchy. Metabolic studies in primate models have suggested that dystonia may be associated with a reduction of activity along the putamen-GPe connection, and increased inhibition of STN and GPi by GPe efferents (Hantraye et al., 1990; Mitchell et al., 1990). An involvement of the direct and indirect pathways is also supported by pharmacologic studies, suggesting that a relative increase in the activity of striatal neurons of the direct pathway over those that give rise to the indirect pathway contributes to dystonia (Casey, 1992; Gerlach and Hansen, 1997).

Positron emission tomography (PET) and single-cell recording studies in human patients with dystonia have also found changes in the direct/indirect pathway systems of the basal ganglia. Single-cell recording studies in patients undergoing functional neurosurgical treatments have found that discharge rates in GPe and GPi are low (Lenz et al., 1998; Vitek et al., 1999; Vitek, 2002; Zhuang et al., 2004; Starr et al., 2005; Tang et al., 2007). At least with regard to the proposed overactivity of the (inhibitory) direct pathway, these data are in line with the aforementioned animal studies. Oscillatory activity in the basal ganglia may also be involved in the pathophysiology of dystonia. Studies have shown the emergence of low-frequency oscillations in single-cell activities of neurons in the basal ganglia or thalamus (Lenz et al., 1999; Zhuang et al., 2004; Starr

et al., 2005), and have found prominent 4–10Hz activity in local field potential recordings from DBS electrodes in GPi (Silberstein et al., 2003; Chen et al., 2006).

Consistent with the concept that dystonia is a circuit disorder similar to Parkinson's disease, there is substantial evidence for altered cortical functioning in dystonia, specifically from imaging and electrophysiologic studies that have used transcranial magnetic stimulation and other methods. For instance, PET studies in dystonic patients (Carbon et al., 2004; Asanuma et al., 2005) have demonstrated widespread changes in the activity of prefrontal areas (Karbe et al., 1992; Eidelberg et al., 1995; Galardi et al., 1996; Playford et al., 1998), specifically involving the SMA, anterior cingulate and dorsolateral prefrontal motor areas (Eidelberg et al., 1995; Galardi et al., 1996; Playford et al., 1998). In focal limb dystonia, abnormal somatotopic maps were demonstrated in M1 (Byrnes et al., 1998; Rona et al., 1998). In addition, intracortical excitability in motor areas may be increased (Deuschl et al., 1995; Kaji et al., 1995; Ikeda et al., 1996; Hamano et al., 1999; Sommer et al., 2002). Patients with writer's cramp were also shown to have an abnormally small degree of beta-band EEG desynchronization (Toro et al., 2000). Considerable evidence now indicates a disturbance in motor learning and abnormal plasticity (Ghilardi et al., 2003; Sharma et al., 2005; Byl, 2007; Breakefield et al., 2008; Doyon, 2008).

Sensory abnormalities may also play a role in dystonia. There is convincing evidence for reduced cortico-cortical inhibition in the sensory system (Ridding et al., 1995; Chen et al., 1997; Filipovic et al., 1997; Butefisch et al., 2005), and increased and improperly modulated precentral sensory evoked potentials (N30) (Reilly et al., 1992; Kanovsky et al., 1997; Berardelli et al., 1998; Hallett, 1998; Kanovsky et al., 1999; Tinazzi et al., 1999; Murase et al., 2000). In addition, single cell recording and imaging studies have suggested altered somatotopic representation at the cortical (Byl et al., 1996; Bara-Jimenez et al., 1998; Elbert et al., 1998), putamen (Delmaire et al., 2005) and thalamic levels (Lenz and Byl, 1999; Lenz et al., 1999), although such changes were not seen in recent GPi recording studies (Chang et al., 2007; Tang et al., 2007).

# **IV. DEEP-BRAIN STIMULATION**

# A. Historical Aspects

The use of DBS for movement disorders was pioneered in the 1980s by Benabid and others, who adapted the technology from the fields of pain and epilepsy and applied it first to the thalamus for treatment of treatment-resistant essential and parkinsonian tremor. The STN was subsequently targeted for Parkinson's disease (Patel et al., 2003; Alvarez et al., 2005), following the finding that STN lesions dramatically reversed the cardinal motor signs of Parkinsonism in the MPTP primate model (Bergman et al., 1990; Aziz et al., 1991). Following decades of experience with pallidal lesioning as treatment for movement disorders, GPi-DBS was also used for treatment of parkinsonism and, subsequently, dystonia. At the present time, the US Food and Drug Administration (FDA) has approved DBS only for use in patients with tremor or Parkinson's disease. However, the application of DBS has been extended to dystonia through a Humanitarian Device Exemption, a limited form of approval for the use of devices in the treatment of rare diseases that lack adequate conventional treatments. DBS has now largely replaced ablation for nearly all neurologic and neuropsychiatric disorders.

# **B.** Technical Aspects

DBS therapy delivers chronic focal stimulation to discrete targets within the brain in order to modulate neuronal circuits. The DBS system consists of an implantable electrode (i.e., four insulated wires which terminate in ring electrodes that are spaced 0.5-1.5 mm apart along the tip of the electrode), and a small constant-voltage (or constant current) pulse generator which is also implanted subcutaneously, typically in the clavicular region, and can be telemetrically controlled. During the DBS lead implantation procedures, the electrode is stereotactically placed into the brain, under neuroimaging and varying degrees of electrophysiologic guidance. The extra-cranial portion of the electrode is connected to the pulse generator by an extension wire that is subcutaneously tunneled and attached to the pulse generator. Subsequently, electrical stimulation is administered continuously, except for some patients with tremor who may turn off the stimulator at night in order to extend the life of the battery in the implanted pulse generator. Many patients require bilateral DBS leads; in these cases, independent single-channel or a dual-channel stimulator may be implanted. Multi-site DBS lead implantations can be done during single surgical sessions or as staged procedures.

The stimulation system can be remotely (telemetrically) programmed to provide a wide range of stimulation parameters, so that DBS can be optimized. Both monoand bipolar stimulation may be delivered. For monopolar stimulation, i.e., the application of negative voltage steps to a single ring electrode, with reference to the pulse generator (ground) a relatively large area around the electrode is stimulated. Much smaller stimulation volumes can be accomplished by using bipolar stimulation, where the voltage step pulses are applied to one or more (–) electrode(s) and referenced to another (+) electrode. The electrodes chosen for either of these modes of stimulation can be freely chosen from among the four available electrodes. The electrical characteristics of the stimulation, such as the frequency, amplitude and duration of the voltage steps can be adjusted to optimize clinical benefit. Parameters of stimulation vary in various targets for different disorders, but typically pulses are delivered at 60–185 Hz, with less than 4 V, and pulse widths from 60 to 200  $\mu$ s.

DBS treatment requires a dedicated DBS team with a strong commitment to team-work and to sharing of information. One of the key functions of the team is to select appropriate patients to rule out comorbidities that would reduce the success rate of the procedure. Patients should be screened by a neurologist experienced in treating patients with movement disorders, a neuropsychologist, and a psychiatrist, and undergo magnetic resonance imaging (MRI) of the brain before referral to the neurosurgeon. It is also critical for the success of a DBS program that the neurosurgeon has sufficient training and experience with functional and stereotactic surgical procedures. Trained programmers, speech and physical/occupational therapists are essential for postoperative management and programming of patients.

# C. Mechanism of Action

When DBS was first introduced, the clinical similarities between the effects of DBS and lesioning of the same target prompted the belief that DBS works similar to lesioning procedures, i.e., by inactivating the stimulated tissue, through mechanisms such as depolarization block, or the local release of inhibitory transmitters. However, electrophysiologic recording studies in primates and patients demonstrated that DBS therapy has, in fact, multiple actions on cell bodies and fibers, both afferent and efferent, and that such actions may differ with distance from the stimulation lead (Kringelbach et al., 2007). Modeling studies have shown that STN-DBS may inhibit cell bodies of STN neurons through activation of local GABA release from GPe afferents, while directly activating axons of nearby neurons (McIntyre et al., 2004). Stimulation in the STN has been demonstrated to evoke complex excitatory effects in the GPi, one of the primary recipients of STN efferents, and may alter oscillatory resonance characteristics of the STN-GPi network. In addition, STN stimulation may have prominent effects on nearby pallidothalamic fibers, thereby directly affecting thalamic activities. Finally, recent electrophysiological and optogenetic studies in rodents suggest that STN stimulation may influence cortical activity via antidromic activation of the hyperdirect cortico-subthalamic pathway (Li et al., 2007; Gradinaru et al., 2009). Stimulation of GPi may directly activate the axons of GPi cells and incoming striatal afferents.

DBS in STN or GPi has been shown to alter firing patterns in the associated thalamocortical circuitry (Anderson et al., 2003; Guo et al., 2008). In further support of this idea, STN-DBS has been shown to normalize intracortical inhibitory mechanisms in transcranial magnetic stimulation studies (Cunic et al., 2002; Tisch et al., 2007), and functional imaging studies have shown that STN-DBS induces widespread normalization of activity in frontal motor areas both at rest and with movement tasks (e.g., Grafton et al., 2006).

Although the effects of DBS are incompletely understood, it appears that a major mechanism whereby high frequency stimulation works is to override and replace the abnormal basal ganglia output patterns with a more tolerable output on the targets of basal ganglia output. The fact that patients with disorders as varied clinically and pathophysiologically as Parkinson's disease, dystonia, ballismus and dyskinesias respond to stimulation of the same DBS target suggests that the effect of this treatment on cortical activity patterns is not disease- or symptom-specific, but *circuit*-specific. Of course, disturbances within individual sub-circuits of the larger motor circuit may play specific roles in different disorders. Because of the close proximity of the subcircuits, DBS generally affects multiple subcircuits.

Both ablation or stimulation therapies may lead to the removal of noisy basal ganglia activity, acting as an "informational lesion" (Grill et al., 2004), and replacing or overriding whatever abnormal pattern is present in the network. A recent study of changes in neuronal activity recorded in the thalamus of monkeys undergoing DBS in the STN indicated that the entropy was decreased in thalamic neurons, reflecting an increased signal to noise ratio (Guo et al., 2008).

# D. Ablation vs. DBS

It is worthwhile to compare DBS treatment with ablative therapies, since in general, they have comparable clinical efficacy and perioperative risk. Perhaps the most important difference is that, compared to ablation, DBS is less invasive and, should a better form of treatment become available, the device can be either turned off or can be removed without permanent effect. Another advantage of DBS therapy over lesioning strategies is that the stimulation sites and parameters are adjustable. Furthermore, DBS therapy can be applied bilaterally, while bilateral lesioning carries a higher risk of permanent side effects. Finally, inaccurate lesion placement may result in permanent neurologic damage.

On the other hand, with ablative therapies the postoperative risk for infections is low, lead or device failure is nonexistent, and patient compliance is not an issue. Also, lesions do not carry a risk for stimulation-induced side effects, such as paresthesias or cognitive/emotional changes. One of the most significant advantages of ablation over DBS is that only routine post-operative care is needed, while patients undergoing DBS require repeated time-consuming stimulator adjustment sessions. One advantage of DBS over ablation is that the effects of DBS can also be rigorously tested in double-blinded clinical trials, something that is nearly impossible to accomplish with lesioning procedures. Finally, the cost of the DBS surgery and follow-up patient visits for adjusting DBS settings is much higher than the overall cost for lesion therapy. The high up-front cost and the lack of availability of trained personnel for post-operative programming limit the usablility of DBS in some (rural) areas in developed countries, and especially in developing areas of the world. Pallidotomy and other ablative procedures remain a viable alternative in these situations (Gross, 2008).

# V. DBS TREATMENT OF MOVEMENT DISORDERS

# A. Parkinson's Disease

The most common indications for surgery in patients with Parkinson's disease are the presence of intractable tremor, or severe and disabling drug-induced motor fluctuations and dyskinesias. A favorable outcome is generally seen in patients who show a good response to L-DOPA and in whom signs or symptoms of atypical Parkinson's disease, dementia or other psychiatric diseases are absent. Based on empiric evidence, DBS targets in the motor portions of either GPi or STN are currently preferred for the treatment of Parkinson's disease (Volkmann, 2004; Deuschl et al., 2006; Ostergaard and Sunde, 2006; Rodrigues et al., 2007; Wider et al., 2008). In advanced patients, DBS is commonly performed bilaterally, although unilateral DBS can be highly effective for asymmetric cases. Although there are no published blinded comparisons of the effects of GPi- and STN-DBS, the STN target is currently preferred by most neurosurgeons because of its perceived greater antiparkinsonian effect. A number of smaller comparative trials comparing STN and GPi, however, have not found significant differences, and two large controlled doubleblinded trials are now completed and data will be available shortly on the relative benefits of the two targets. DBS in the STN or GPi alleviates parkinsonian motor signs, particularly during "off" periods, and reduces drug-induced side effects such as dyskinesia, dystonia or motor fluctuations. In patients with advanced Parkinson's disease, DBS strongly improves the patient's quality of life and is more effective in this regard than medical management (e.g., Ostergaard and Sunde, 2006; Weaver et al., 2009). In contrast to patients with GPi-DBS, those with STN-DBS are often able to substantially reduce their medications, which may, in part, account for its anti-dyskinetic effects (Rodriguez-Oroz et al., 2004).

For both surgical targets, the basal ganglia motor circuit is targeted in each nucleus. In GPi, the sensorimotor territory is contained within the postero-lateral part of the nucleus, while for STN, the dorsolateral portion of the nucleus has been identified as the optimal DBS target. DBS at this location is likely to reach a larger proportion of the motor circuit within the much smaller STN, than DBS within the larger GPi sensorimotor area. Stimulation at the "ideal" STN-DBS target may, nonetheless, spread to involve portions of the dorsally adjacent fields of Forel and the Zona incerta (Yelnik et al., 2003).

The surgical risk of significant surgical complications with DBS such as intracerebral hemorrhage is small (1-2%). However, stimulation, particularly of the STN, may have side effects, including the induction of paresthesias, involuntary movements, worsening of gait or speech, gaze deviation or paralysis, as well as cognitive and mood side effects. Many of these side effects can be eliminated by adjusting the stimulation parameters. Cognitive side effects, specifically reduced verbal fluency and declines in executive functions (Troster et al., 1997; Parsons et al., 2006), and postoperative depression, mania, anxiety, and apathy (Temel et al., 2006) are reported more with STN-DBS than with GPi-DBS (Anderson et al., 2005). These side effects may be caused by inadvertent stimulation of limbic circuit elements in portions of the nearby zona incerta, the ventral STN or the SNr (e.g., Bejjani et al.,

1999; Stefurak et al., 2003). The true incidence of mood changes or anxiety with STN-DBS remains debated, however. A recent study found no significant differences for mood or anxiety scores between the DBS group and a control group, and suggested that obsessive-compulsive traits scores may be lower in DBS patients (Castelli et al., 2008). In contrast, a recent multicenter retrospective study of 5,000 surgically treated patients with Parkinson's disease, STN-DBS was found to increase the risk of suicide (Voon et al., 2008). The only factor that predicted completed suicide was postoperative depression. Factors associated with attempted suicides included a history of impulse control disorders, compulsive medication use, postoperative depression or apathy, and single marital status. This study points out the importance of careful pre- and postoperative screening for psychiatric risk factors and aggressive treatment of both pre- and postoperative depression.

Besides the STN and GPi, there are other DBS targets which may be useful in specific patient populations. For instance, parkinsonian tremor (but not other parkinsonian signs) can be effectively treated with thalamic DBS at the border between the thalamic nucleus ventralis oralis (Vop) and the nucleus ventralis intermedius (Vim, see, e.g., Obeso et al., 1997; Ondo et al., 1998; Kumar et al., 2003). Stimulation of the PPN has also been explored in patients with Parkinson's disease who experience worsening of gait and balance, despite treatment with L-DOPA (and in some cases with STN- or GPi-DBS). The PPN is a major target of GPi output and projects back to the STN as well as downstream (Mena-Segovia et al., 2004). The PPN-DBS target overlaps or is part of what has been described as the "locomotion center" in the pons (Jahn et al., 2008). Positive effects of low-frequency stimulation of the PPN have been described in MPTP-treated monkeys (Nandi et al., 2002), and, recently, in preliminary studies, also in parkinsonian patients (Plaha and Gill, 2005; Stefani et al., 2007; Lozano and Snyder, 2008), where beneficial effects on gait were described. The PPN differs from the other DBS targets, in that inactivation of this area worsens the symptoms in experimental animals, whereas for all other targets both lesions and DBS have similar effects. Also different is the fact that low frequency DBS (30Hz) is most effective (rather than conventional high-frequency DBS at 130 Hz in the other targets). Questions remain as to the exact position of the electrodes and the degree of improvement and lasting benefit. Finally, stimulation of the region of the caudal Zona incerta, just posterior and medial to the STN, has significant effects on parkinsonian tremor, as well as rigidity

and akinesia, reportedly rivaling those of STN-DBS (Kitagawa et al., 2005; Plaha et al., 2006). At this location, DBS may exert its antiparkinsonian activity through effects on pallidofugal as well as cerebellar afferents to the thalamus which pass through the stimulated area.

In summary, DBS can have a major effect on a range of symptoms, in patients with advanced Parkinson's disease. Unfortunately, however, patients with dementia, psychosis, and L-DOPA-refractory freezing and balance problems are not suitable candidates for conventional targets. DBS has only a symptomatic effect on the dopamine deficiency signs and symptoms and does not appear to alter progression of the disease. Gait, balance, speech and cognition and behavior may deteriorate significantly over time in many patients. New targets, such as the PPN, or different approaches are needed to address these and those of the majority of patients who do not meet the basic requirements for DBS.

# **B.** Dystonia

The insight that dystonia may arise from abnormalities in circuit elements that are similar to those affected by parkinsonism, and the past experience with ablative procedures in dystonia, have lead to a number of trials of GPi-DBS in cases of advanced intractable dystonia. GPi-DBS generally works well in cases of primary generalized dystonia (Coubes et al., 2004; Eltahawy et al., 2004; Vidailhet et al., 2005), but is less effective for the secondary dystonias (e.g., Cif et al., 2003; Kupsch et al., 2006). Other indications for GPi-DBS are treatment (botulinum toxin) resistant severe cervical and other focal dystonias, and tardive dystonia (Loher et al., 2000; Eltahawy et al., 2004; Houser and Waltz, 2005; Trottenberg et al., 2005; Pretto et al., 2008; Sako et al., 2008). DBS at other locations, particularly in the STN has also recently been explored (Pastor-Gomez et al., 2003; Sun et al., 2007). STN-DBS may offer an advantage of immediate improvement for dystonia as well as longer battery life, due to lower voltage requirement. Although thalamotomy was a favored target in the past for dystonia, the results of thalamic DBS have been disappointing thus far, although the targets and patient types have been limited. One exception is writer's cramp, a focal occupational form of dystonia that appears to be uniquely sensitive to both thalamotomy and thalamic DBS (Taira and Hori, 2003; Fukaya et al., 2007). There is growing evidence that not all dystonia results from basal ganglia disturbances and that cerebellar output may play a role in some forms (Jinnah and Hess,

2006; Neychev et al., 2008). Such differences may account for the failure of GPi stimulation in some types of dystonia.

For unclear reasons, the beneficial effects of GPi-DBS are often delayed in dystonia patients, often by weeks or months (Krauss et al., 2002). While it can be speculated that these delays involve anatomic or functional remodeling of neuronal interactions, i.e., a change in neuronal plasticity within the basal ganglia-thalamocortical circuitry, no specific information regarding this point is available. GPi-DBS for dystonia has few (if any) cognitive side effects (Pillon et al., 2006). The ability of GPi-DBS, administered to the motor portions of GPi to modulate the activity in the basal ganglia-thalamocortical motor circuit has been shown in PET activation studies, which normalize motor cortical activity in dystonia (Detante et al., 2004). Electrophysiologic studies have demonstrated that GPi-DBS may enhance motor cortex excitability through modulation of thalamocortical projections (Kuhn et al., 2003).

Postoperative programming for dystonia has proven to be more challenging than for patients with Parkinson's disease or tremor, because, as mentioned, benefits may take weeks to become evident and 6-12 months to reach peak, although early improvement (days to weeks) after programming is sometimes seen with pain and mobile (as opposed to fixed) dystonia (Kiss et al., 2007; Ostrem et al., 2007; Vidailhet et al., 2007) Also, in the past, higher voltages and wider pulse widths were used for GPi-DBS in dystonia than used for the treatment of Parkinson's disease, resulting in a shortening of battery life. It now appears that this may have been the result of too frequent adjustments in stimulation parameters because of the unappreciated delayed response to stimulation. Currently, stimulation parameters are chosen that are more similar to those used for patients with Parkinson's disease (Hung et al., 2007). A recent study failed to show any difference in outcome when using short, medium, and long pulse-width durations were compared (Vercueil et al., 2007). Recently, lower frequency stimulation (60 Hz), has been shown to be effective in primary generalized dystonia (Alterman et al., 2007b; Alterman et al., 2007a).

As for Parkinson's disease, GPi-DBS provides significant benefit for patients with treatment resistant primary generalized and tardive dystonia as well as some forms of focal dystonia, but does not appear to offer consistent benefit for the large number of patients with secondary dystonias. Further exploration of alternative targets, such as the STN and the thalamus, in particular the pallidal receiving portion, is needed.

# VI. DBS TREATMENT OF OTHER HYPERKINETIC DISORDERS

# A. Hemiballism

Hemiballism, a syndrome of involuntary movements, sometimes violent, of the arm and leg on one side of the body, is perhaps the most dramatic of the hyperkinetic disorders. This remarkable disorder results most often from a small vascular lesion confined to the STN. Hemiballism most often resolves spontaneously or responds to neuroleptics. Pallidotomy has been performed successfully for intractable cases.

In animal models, hemiballism has been observed after destructive or reversible neurotoxin lesions of the STN (Whittier and Mettler, 1949; Hamada and DeLong, 1992). Neuronal recordings in GPi in primates and in patients suffering from intractable hemiballism (Suarez et al., 1997; Vitek et al., 1999; Slavin et al., 2004) have revealed a marked decrease in the discharge frequency of GPi neurons, likely because of the loss of glutamatergic drive of GPi from the STN. Reduced discharge in GPi would act to lower the (inhibitory) output to the thalamus and brainstem, suggesting that disinhibition of the thalamus might be the explanation for the involuntary movements. The finding, however, that pallidotomy abolishes the involuntary movements of ballism, is clear evidence that reduced GPi output is not the explanation for involuntary movements. Although pallidal DBS has not been reported for the treatment of hemiballism, thalamic DBS has been performed successfully (Tsubokawa et al., 1995).

# B. Huntington's Chorea

Huntington's chorea, a progressive neurologic disorder, is characterized by a combination of chorea, dementia and behavioral disturbances. The underlying genetic defect is the expansion of a trinucleotide repeat sequence within a gene on chromosome 4 coding for the protein huntingtin. The expression of abnormal huntingtin is responsible for widespread neurodegeneration involving most of the brain, with heavy involvement of the basal ganglia. Only a small number of patients have undergone pallidotomy or GPi DBS for intractable chorea in Huntington's disease (Hebb et al., 2006; Fasano et al., 2008). A reduction of both chorea and dystonia has been a consistent finding in these studies.

These findings in two of the hyperkinetic disorders are illustrative of the potential use of DBS for a broad range of hyperkinetic disorders. The fact that pallidotomy or DBS of the motor pallidum, the same target used for Parkinson's disease and dystonia, is also effective for these hyperkinetic disorders, lends strong support to the notion that DBS is not a specific form of therapy for a particular disorder, but rather acts nonspecifically to override and remove abnormal activity in the motor circuit, whatever its nature. This same observation will be seen in the following discussion of DBS for neuropsychiatric disorders.

# VII. DBS TREATMENT OF NEUROPSYCHIATRIC DISORDERS

The previous era of "psychosurgery," as performed in the 1950s and 1960s, ended in the 1970s in disgrace amidst mounting evidence and outcry that the available procedures were inadequately studied, indiscriminately applied and largely ineffective. However, given the success of DBS in movement disorders, physicians and their patients are now exploring the use DBS to treat severe psychiatric disorders, such as the TS, OCD and depression. This change in public and physician attitude arises from a number of factors including increased awareness of the enormous burden of these disorders on patients and caregivers, the significant shortcomings of existing therapies and the less invasive and reversible nature of DBS. Different from the past era is the greater scrutiny and protection of patient rights, the better clinical characterization of the individual disorders and the development of validated clinical rating scales. However, it needs to be emphasized that DBS procedures for neuropsychiatric conditions remain strictly experimental at this point. However, the FDA has recently granted a Human Device Exemption for the treatment of intractable OCD.

As a starting point for discussing DBS for psychiatric disorders, it is important to keep in perspective the long history of empirical and serendipitous findings of neurosurgical lesioning approaches to these disorders and the record of success with various targets. This is, comparable to the early history of DBS for movement disorders, where the thalamic and pallidal targets used for ablation were similarly used and found effective for thalamic and pallidal stimulation. The optimal targets for DBS in psychiatric disorders in this still early phase of exploration, however, remain unclear.

The use of DBS in neuropsychiatric diseases and the selection of DBS targets for these disorders can be understood within the frame work of the circuit disorders concept, i.e., that certain behavioral and neuropsychiatric disorders result from disturbances of neural activity within the non-motor (associative and/or limbic) basal ganglia circuits, in a manner analogous to the mechanisms whereby movement disorders arise from disturbances in the motor circuit. The primary targets of basal ganglia DBS for these conditions are the nodes of the "limbic" circuit which originates from the anterior cingulate and medial orbitofrontal cortices, the VS, the ventral and rostromedial GPi and rostrodorsal SNr, and continues to the paramedian portion of the mediodorsal nucleus of the thalamus, which projects back to the same limbic cortices. The Pf contains the limbic portions of the intrinsic GPi/SNr-thalamic loop. The "limbic" circuit is believed to play a role in the regulation of motivation, reward and mood, and may provide reinforcing stimuli to dopaminergic cells in the ventral tegmental area and the SNc.

Although DBS is less invasive, more versatile and "forgiving" than lesioning procedures, and although the preliminary results for the treatment of psychiatric disorders are encouraging, it should be emphasized that patients with long-standing psychiatric disorders present additional and more complex problems in management than patients with movement disorders. Psychiatric disorders differ fundamentally from movement disorders in that the patient's social and family interactions have often been disturbed over a long period and any reversal of mood or behavior may take a long period of time to show clear improvements in symptom severity, functioning and quality of life. On the other hand, as for patients with movement disorders, patients who, after a long period of disability, suddenly become more functional and independent, often face a disruption to the relationships between patient and spouse and other caregivers. Furthermore, the adverse effects of undetected stimulation failure in psychiatric patients are potentially far more severe than for movement disorders, because of the risk of severe mood changes and potential suicide attempts. As for movement disorders, an experienced and dedicated psychiatrist, psychologist or social worker, neuropsychologist, trained functional neurosurgeon and surgical team is critical. An ethicist may also be needed because of difficulties with informed consent in adults with psychiatric diseases, and in minors.

The exploration of DBS for these disorders should be undertaken only by experienced teams with a methodical approach, and a rush to conclusions about treatment effects, optimal targets and stimulation parameters should be avoided. As for dystonia, and unlike Parkinson's, the clinical benefits of DBS are generally delayed and develop progressively over weeks and months. Finally, the premature focus on single "best" targets would be a mistake since it appears that TS, OCD and TRD can all be modulated by DBS at several of the cortical, basal ganglia and thalamic nodes of the limbic circuit. In the following paragraphs we will briefly summarize the experience of treating these conditions with DBS.

# A. Tourette's Syndrome

TS is a familial, neurologic disorder characterized by childhood onset of motor and vocal tics. Tics are typically rapid, stereotyped movements with eye blinking, head and facial movements, as well as vocalizations such as throat clearing, coughing, grunting, or more complex behavioral acts and utterances. In addition, a high percentage of patients suffer from OCD, attention-deficit hyperactivity disorder, depression and psychosocial difficulties which are often as or even more disabling than the tics. The symptoms of TS typically peak in preadolescence and decline in the later teens. Treatment of the tics with neuroleptics and the newer antipsychotic medications are often only modestly effective, and their use runs the risk of inducing tardive dyskinesia. The other components of the disease may require antidepressant and other therapies. Based on neuroimaging and other studies (Kopell and Greenberg, 2008), TS may involve abnormalities in the limbic and motor circuitry, accounting for the complex constellation of non-motor and motor signs.

In a small proportion of TS patients, severe symptoms persist into adulthood in spite of all therapeutic efforts. Recently, reports of single cases or small case series have explored the use of DBS in such patients. Several targets have been used, including the region of the midline caudal intralaminar nuclei of the thalamus, i.e., the CM/PF (Visser-Vandewalle et al., 2003; Temel and Visser-Vandewalle, 2004; Visser-Vandewalle et al., 2004; Houeto et al., 2005; Ackermans et al., 2006; Visser-Vandewalle et al., 2006; Bajwa et al., 2007; Maciunas et al., 2007; Ackermans et al., 2008; Servello et al., 2008; Shields et al., 2008), based on earlier lesioning studies by Hassler and Dieckmann (Hassler and Dieckmann, 1970, 1973, 1997). In the largest series to date of medial thalamic DBS, Servello and colleagues recently reported on 18 cases of treatment refractory TS who underwent bilateral DBS in the region of the CM/Pf and ventralis oralis complex of the thalamus (Servello et al., 2008). The authors found that tics, as well as OCD, self-injurious behaviors and anxiety, with time, decreased with DBS. Other targets include the motor and limbic portions of GPi (Diederich et al., 2005; Houeto et al., 2005; Ackermans et al., 2006), and the anterior limb of the internal capsule (IC), close to the VS (Nuttin et al., 1999; Nuttin et al., 2003; Flaherty et al., 2005). The rationale for using the GPi-DBS in TS was that stimulation of the sensorimotor territory of GPi had been shown to ameliorate hyperkinetic states (Diederich et al., 2005; Ackermans et al., 2006). Interestingly, DBS in both the motor and limbic areas of GPi have been found to have benefits. Reports of a small number of patients with electrodes placed simultaneously in the pallidal target and the medial thalamus suggest that comparable effects can be obtained from both, with no additional benefit from simultaneous stimulation (Ackermans et al., 2006).

Although the preliminary results from early DBS trials are promising it is clear that for most patients with TS a specific challenge for the implementation of DBS is the existence of obsessive compulsive behavior in these patients. For example, patients given the option to vary the stimulation parameters themselves may engage in compulsive repeated adjustments of stimulation parameters. Others may cause lead breakage due to compulsive picking or manipulation of the skin over the stimulators, or the subcutaneous portion of the wires connecting the DBS leads to the stimulators. Before the use of these procedures can be recommended for routine treatment in TS patients, careful exploration of the various targets and the development of patient selection guidelines and the development of postoperative management plans are mandatory (Mink, 2006).

# **B.** Obsessive Compulsive Disorder

OCD is a relatively common disorder, characterized by the presence of intrusive thoughts, compulsive behaviors and rituals. Although selective serotonin reuptake inhibitors and behavioral therapies are effective in the majority of patients, some cases are refractory and may be considered for surgical treatments. Neurosurgical treatments of OCD have been carried out for many years, with lesions of empirically defined targets, e.g., the cingulate gyrus and the anterior limb of the IC. The early DBS targets for OCD focused on the region of the anterior limb of the IC and the nearby VS (Hodgkiss et al., 1995; Jenike, 1998; Lippitz et al., 1999; Cosgrove, 2000; Dougherty et al., 2002; Montoya et al., 2002; Greenberg et al., 2003; Sturm et al., 2003). There is growing evidence that the benefits may result from involvement of afferent and efferent fibers of the VS which receives afferents from multiple limbic areas, such as the amygdala, orbitofrontal/medial prefrontal cortex, caudate, and pallidum, and sends efferents to multiple mesolimbic and prefrontal areas as well as the cingulate cortex, striatum, pallidum, and thalamus (Nauta and Domesick, 1984; Haber et al., 2000; Heimer, 2003). A DBS lead spanning this region is capable of activating any of these structures, depending on the lead geometry and stimulation parameters used.

While lesions such as anterior capsulotomy may benefit a significant proportion (35–70%) of these patients, the destructive character and irreversibility of lesions are not acceptable to some patients. Case reports and several small case series have shown that the anterior limb of the IC can also be used successfully as a target for DBS (Nuttin et al., 2003). The benefits from the procedure are long-lasting (Greenberg et al., 2006) and, in most patients, few side effects are seen.

Functional imaging studies in OCD patients have demonstrated abnormalities in the activity of limbic basal ganglia-thalamocortical projection systems. Similar studies have suggested that anterior capsule stimulation works in OCD by influencing the activity in the nearby limbic VS (e.g., Nuttin et al., 2003). In fact, in one OCD patient treated with direct VS stimulation, long lasting benefits were seen (Aouizerate et al., 2004). Recent studies of DBS targeted directly at the VS have shown clear benefits (Greenberg et al., 2006). An analysis of the combined results of a multi-center trial of DBS targeting the VS region in OCD patients showed functional improvements in two-thirds of the treated patients and DBS was well tolerated. The study identified the junction of the anterior capsule, anterior commissure and posterior VS as the most effective DBS target (Greenberg et al., 2008).

A recent PET imaging study has demonstrated that brain activity during DBS of the VS/IC target is enhanced predominately in limbic areas of cortex, basal ganglia and thalamus (Rauch et al., 2006). In another study it was found, serendipitously that chronic stimulation in the STN in Parkinson's patients with co-existent OCD reduced obsessive-compulsive symptoms (Mallet et al., 2002; Fontaine et al., 2004). In these patients the medial (limbic) portion of the STN was inadvertently stimulated Accordingly, the medial portion of the STN is now being studied as a potential target for OCD (Baup et al., 2008).

# C. Treatment-Resistant Depression

A small proportion of patients with chronic major depression fail to respond to traditional antidepressants, behavioral therapy, vagal nerve stimulation or electroconvulsive therapy. Clues to the potential for DBS to modulate mood and behavior have come from observations in a small number of parkinsonian patients in whom severe, but reversible, depression was reported in response to STN-DBS (Temel et al., 2006). In these cases the cause of the mood change appeared to be inadvertent stimulation of the underlying SNr. Other effects such as hypomania, merriment, and laughter have been reported with stimulation in the STN, GPi, and in the zona incerta. These studies show that focal stimulation of portions of the basal ganglia can profoundly alter mood and emotional expression, and suggest that DBS of nodes of the limbic circuitry could potentially be used to treat depression in TRD patients.

Only recently have the potential surgical targets for depression been explored more systematically. In reports of single cases, DBS of mesothalamic targets or of the inferior thalamic peduncle was found to be effective against depression in patients with chronic pain or TRD. In a more recent case series of TRD patients, DBS of the white matter in the subgenual cingulate region (area 25) produced significant clinical benefits in four of six patients (Mayberg et al., 2005). The area 25 target was chosen based on results of a series of PET studies in patients with depression in whom clinical improvements coincided with a decrease in activity in area 25 (Mayberg et al., 1999; Mayberg, 2003; Cohen et al., 2007; Schlaepfer et al., 2008). More recently the same investigators reported on the results from a study of 20 patients with TRD treated with the same intervention (Lozano et al., 2008). Six months after initiation of DBS, 60% of patients responded, and 35% met criteria for remission. These benefits were sustained at 12 months. In PET studies on these same patients, DBS was associated with specific changes in the metabolic activity localized to cortical and limbic circuits implicated in the pathogenesis of depression. The number of serious adverse effects was small with no patient experiencing permanent deficits. Obviously, a careful double-blind appraisal is required before the procedure can be recommended for use on a wider scale. In addition to area 25, a region in the rostral cingulate gyrus has also been proposed as a possible target for DBS in depression.

Another treatment target for TRD is the ventral caudate/ VS region, based on its position within the limbic circuitry of the brain, its role in pleasure and reward processing, and the experience with depression in the course of DBS treatment for OCD. There have been several careful studies of bilateral DBS in these regions, demonstrating substantial antidepressant effects, as well as depression rebound with cessation of therapy (e.g., Malone et al., 2009). DBS was, overall, well-tolerated.

It is premature to judge which of the different targets might be best for TRD, due to the preliminary nature of the studies. It will be important in future studies to compare not only the overall antidepressant qualities of these interventions, but also the nature and duration of the remissions and the long-term outcomes. It is noteworthy, in light of the evidence that depression may be lateralized, that to date there has been no systematic testing to determine whether bilateral DBS is necessary.

# D. Lesch–Nyhan Disease

Lesch–Nyhan Disease (LND) is a neurodevelopmental disorder with a characteristic neurobehavioral syndrome with behavioral, cognitive and motor components that include self-injurious behavior (SIB), cognitive disability of varying degree, and severe generalized dystonia. SIB is a lifelong problem that can lead to tissue damage with substantial disfigurement. Most patients cannot walk and require assistance with basic activities of daily living. The clinical features of the disease eventually reflect a static developmental defect rather than an ongoing degenerative process. Because there are no effective medical treatments for LND, considerable effort is devoted to supportive care, including physical restraints to prevent serious injuries.

LND is caused by congenital deficiency of the enzyme, hypoxanthine-guanine phosphoribosyl transferase (HPRT), causing a disruption of purine metabolism. Among other metabolic changes, the enzyme defect leads to dysfunction of dopaminergic neurons during early development which may be central to the genesis of the neurobehavioral problems.

The experience with DBS in TS and OCD is particularly relevant to LND, since some patients with TS have tics with a compulsive self-injurious quality similar to SIB in LND. Because, DBS of the limbic GPi in TS has been found to markedly reduce self-injurious tics, a similar approach has been used in LND in several preliminary studies. In the first patient to undergo DBS for LND, both motor and limbic circuits of the basal ganglia were targeted bilaterally with a single electrode into GPi (Taira et al., 2003). Substantial improvements in dystonia and a complete elimination of SIB were observed. These findings were subsequently confirmed in two patients in whom electrodes were placed bilaterally into both the motor and limbic GPi (Pralong et al., 2005; Cif et al., 2007).

# VIII. CONCLUSIONS

A number of movement and neuropsychiatric disorders are now recognized as "circuit disorders", resulting from disturbances arising within the basal ganglia-thalamocortical networks. The success with DBS for movement disorders has led to its application to more complex neuropsychiatric disorders. The choice of targets for lesioning procedures or DBS is strongly influenced by our understanding of the anatomy and function of the basal ganglia-thalamocortical circuits. The clear therapeutic effects of these procedures, directed at multiple nodes of the motor and limbic circuits provides further support for the notion that both hypo- and hyper-kinetic disorders result from specific disturbances within the motor circuit, while TS, OCD, TRD, and elements of LND appear to result from disturbances involving, at least in large part, the limbic basal ganglia-thalamocortical circuitry. The seemingly indiscriminate effectiveness of stimulation procedure disorders across a wide spectrum of disorders with greatly varying phenotypes and underlying pathophysiologies argues against disease-specific effects, such as antiparkinsonian, antidystonic or anti-compulsive effects, of DBS. Instead, it is more likely that these interventions act to override and replace abnormal subcortical or cortical signals whatever their nature, allowing the otherwise relatively intact systems to function more normally. It might be said that the net result of both the earlier ablative and the newer DBS therapies have a similar effect, i.e., reducing abnormal activity in the basal gangliathalamo-cortical network. Studies of these interventions have not only helped to confirm hypotheses based on animal experimentation, but led to new insights into the structure and "mysterious functions of the basal ganglia" (Marsden, 1982).

# REFERENCES

- Ackermans L, Temel Y, Visser-Vandewalle V (2008) Deep brain stimulation in Tourette's Syndrome. Neurotherapeutics 5:339–344.
- Ackermans L, Temel Y, Cath D, et al. (2006) Deep brain stimulation in Tourette's syndrome: two targets? Mov Disord 21:709–713.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375.
- Alexander GE, DeLong MR (1985a) Microstimulation of the primate neostriatum. I. Physiological properties of striatal microexcitable zones. J Neurophysiol 53:1401–1416.
- Alexander GE, DeLong MR (1985b) Microstimulation of the primate neostriatum. II. Somatotopic organization of striatal microexcitable zones and their relation to neuronal response properties. J Neurophysiol 53:1417–1430.
- Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271.

- Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381.
- Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119–146.
- Alterman RL, Shils JL, Miravite J, Tagliati M (2007a) Lower stimulation frequency can enhance tolerability and efficacy of pallidal deep brain stimulation for dystonia. Mov Disord 22:366–368.
- Alterman RL, Miravite J, Weisz D, Shils JL, Bressman SB, Tagliati M (2007b) Sixty hertz pallidal deep brain stimulation for primary torsion dystonia. Neurology 69:681–688.
- Alvarez L, Macias R, Lopez G, et al. (2005) Bilateral subthalamotomy in Parkinson's disease: initial and long-term response. Brain 128:570–583.
- Anderson ME, Postupna N, Ruffo M (2003) Effects of high-frequency stimulation in the internal globus pallidus on the activity of thalamic neurons in the awake monkey. J Neurophysiol 89:1150–1160.
- Anderson VC, Burchiel KJ, Hogarth P, Favre J, Hammerstad JP (2005) Pallidal vs subthalamic nucleus deep brain stimulation in Parkinson disease. Arch Neurol 62:554–560.
- Aouizerate B, Cuny E, Martin-Guehl C, et al. (2004) Deep brain stimulation of the ventral caudate nucleus in the treatment of obsessivecompulsive disorder and major depression. Case report. J Neurosurg 101:682–686.
- Asanuma K, Carbon-Correll M, Eidelberg D (2005) Neuroimaging in human dystonia. J Med Invest 52(Suppl):272–279.
- Augood SJ, Hollingsworth Z, Albers DS, et al. (2002) Dopamine transmission in DYT1 dystonia: a biochemical and autoradiographical study. Neurology 59:445–448.
- Aziz TZ, Peggs D, Sambrook MA, Crossman AR (1991) Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1,2,3,6tetrahydropyridine (MPTP)-induced parkinsonism in the primate. Mov Disord 6:288–292.
- Bajwa RJ, de Lotbiniere AJ, King RA, Jabbari B, Quatrano S, Kunze K, Scahill L, Leckman JF (2007) Deep brain stimulation in Tourette's syndrome. Mov Disord 22:1346–1350.
- Balcioglu A, Kim MO, Sharma N, Cha JH, Breakefield XO, Standaert DG (2007) Dopamine release is impaired in a mouse model of DYT1 dystonia. J Neurochem 102:783–788.
- Bar-Gad I, Bergman H (2001) Stepping out of the box: information processing in the neural networks of the basal ganglia. Curr Opinion Neurobiol 11:689–695.
- Bara-Jimenez W, Catalan MJ, Hallett M, Gerloff C (1998) Abnormal somatosensory homunculus in dystonia of the hand. Ann of Neurol 44:828–831.
- Baup N, Grabli D, Karachi C, Mounayar S, Francois C, Yelnik J, Feger J, Tremblay L (2008) High-frequency stimulation of the anterior subthalamic nucleus reduces stereotyped behaviors in primates. J Neurosci 28:8785–8788.
- Bejjani BP, Damier P, Arnulf I, et al. (1999) Transient acute depression induced by high-frequency deep-brain stimulation. N Engl J Med 340:1476–1480.
- Berardelli A, Rothwell JC, Hallett M, Thompson PD, Manfredi M, Marsden CD (1998) The pathophysiology of primary dystonia. Brain 121:1195–1212.
- Bergman H, Wichmann T, DeLong MR (1990) Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 249:1436–1438.

- Bergman H, Wichmann T, Karmon B, DeLong MR (1994) The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol 72:507–520.
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211.
- Breakefield XO, Blood AJ, Li Y, Hallett M, Hanson PI, Standaert DG (2008) The pathophysiological basis of dystonias. Nat Rev Neurosci 9:222–234.
- Brown P (2003) Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson's disease. Mov Disord 18:357–363.
- Brown P, Williams D (2005) Basal ganglia local field potential activity: character and functional significance in the human. Clin Neurophysiol 116:2510–2519.
- Butefisch CM, Boroojerdi B, Chen R, Battaglia F, Hallett M (2005) Taskdependent intracortical inhibition is impaired in focal hand dystonia. Mov Disord 20:545–551.
- Byl NN (2007) Learning-based Animal Models: Task-specific Focal Hand Dystonia. Ilar J 48:411–431.
- Byl NN, Merzenich MM, Jenkins WM (1996) A primate genesis model of focal dystonia and repetitive strain injury: I. Learning-induced dedifferentiation of the representation of the hand in the primary somatosensory cortex in adult monkeys. Neurology 47:508–520.
- Byrnes ML, Thickbroom GW, Wilson SA, Sacco P, Shipman JM, Stell R, Mastaglia FL (1998) The corticomotor representation of upper limb muscles in writer's cramp and changes following botulinum toxin injection. Brain 121:977–988.
- Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30:211–219.
- Carbon M, Trost M, Ghilardi MF, Eidelberg D (2004) Abnormal brain networks in primary torsion dystonia. Adv Neurol 94:155–161.
- Casey DE (1992) Dopamine D1 (SCH 23390) and D2 (haloperidol) antagonists in drug-naive monkeys. Psychopharmacol (Berlin) 107:18–22.
- Castelli L, Zibetti M, Rizzi L, Caglio M, Lanotte M, Lopiano L (2008) Neuropsychiatric symptoms three years after subthalamic DBS in PD patients: a case-control study. J Neurol 255:1515–1520.
- Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ (2007) "Rejuvenation" protects neurons in mouse models of Parkinson's disease. Nature 447:1081–1086.
- Chang EF, Turner RS, Ostrem JL, Davis VR, Starr PA (2007) Neuronal responses to passive movement in the globus pallidus internus in primary dystonia. J Neurophysiol 98:3696–3707.
- Chen CC, Kuhn AA, Hoffmann KT, et al. (2006) Oscillatory pallidal local field potential activity correlates with involuntary EMG in dystonia. Neurology 66:418–420.
- Chen R, Wassermann EM, Canos M, Hallett M (1997) Impaired inhibition in writer's cramp during voluntary muscle activation. Neurology 49:1054–1059.
- Cif L, El Fertit H, Vayssiere N, Hemm S, Hardouin E, Gannau A, Tuffery S, Coubes P (2003) Treatment of dystonic syndromes by chronic electrical stimulation of the internal globus pallidus. J Neurosurg Sci 47:52–55.
- Cif L, Biolsi B, Gavarini S, Saux A, Robles SG, Tancu C, Vasques X, Coubes P (2007) Antero-ventral internal pallidum stimulation improves behavioral disorders in Lesch–Nyhan disease. Mov Disord 22:2126–2129.

- Cohen OS, Hassin-Baer S, Spiegelmann R (2007) Deep brain stimulation of the internal globus pallidus for refractory tardive dystonia. Parkinsonism Relat Disord 13:541–544.
- Cosgrove GR (2000) Surgery for psychiatric disorders. CNS Spectr 5:43-52.
- Coubes P, Cif L, El Fertit H, et al. (2004) Electrical stimulation of the globus pallidus internus in patients with primary generalized dystonia: long-term results. J Neurosurg 101:189–194.
- Crossman AR, Mitchell IJ, Sambrook MA (1985) Regional brain uptake of 2-deoxyglucose in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. Neuropharmacology 24:587–591.
- Cunic D, Roshan L, Khan FI, Lozano AM, Lang AE, Chen R (2002) Effects of subthalamic nucleus stimulation on motor cortex excitability in Parkinson's disease. Neurology 58:1665–1672.
- Degos B, Deniau JM, Chavez M, Maurice N (2009) Chronic but not acute dopaminergic transmission interruption promotes a progressive increase in cortical beta frequency synchronization: relationships to vigilance state and akinesia. Cerebr Cortex 19:1616–1630.
- Delmaire C, Krainik A, Tezenas du Montcel S, et al. (2005) Disorganized somatotopy in the putamen of patients with focal hand dystonia. Neurology 64:1391–1396.
- Detante O, Vercueil L, Thobois S, et al. (2004) Globus pallidus internus stimulation in primary generalized dystonia: a H215O PET study. Brain 127:1899–1908.
- Deuschl G, Toro C, Matsumoto J, Hallett M (1995) Movement-related cortical potentials in writer's cramp. Ann Neurol 38:862–868.
- Deuschl G, et al. (2006) A randomized trial of deep-brain stimulation for Parkinson's disease. N Engl J Med 355:896–908.
- DeVito JL, Anderson ME (1982) An autoradiographic study of efferent connections of the globus pallidus in *Macaca mulatta*. Exp Brain Res 46:107–117.
- Diederich NJ, Kalteis K, Stamenkovic M, Pieri V, Alesch F (2005) Efficient internal pallidal stimulation in Gilles de la Tourette syndrome: A case report. Mov Disord 20:1496–1499.
- Dogali M, Beric A, Sterio D, et al. (1994) Anatomic and physiological considerations in pallidotomy for Parkinson's disease. Stereotact Funct Neurosurg 62:53–60.
- Dougherty DD, Baer L, Cosgrove GR, et al. (2002) Prospective longterm follow-up of 44 patients who received cingulotomy for treatment-refractory obsessive-compulsive disorder. Am J Psychiatry 159:269–275.
- Doyon J (2008) Motor sequence learning and movement disorders. Curr Opin Neurol 21:478–483.
- Eidelberg D, Edwards C (2000) Functional brain imaging of movement disorders. Neurol Res 22:305–312.
- Eidelberg D, Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Przedborski S, Fahn S (1995) The metabolic topography of idiopathic torsion dystonia. Brain 118:1473–1484.
- Elbert T, Candia V, Altenmuller E, Rau H, Sterr A, Rockstroh B, Pantev C, Taub E (1998) Alteration of digital representations in somatosensory cortex in focal hand dystonia. Neuroreport 9: 3571–3575.
- Eltahawy HA, Saint-Cyr J, Poon YY, Moro E, Lang AE, Lozano AM (2004) Pallidal deep brain stimulation in cervical dystonia: clinical outcome in four cases. Can J Neurol Sci 31:328–332.
- Fasano A, Mazzone P, Piano C, Quaranta D, Soleti F, Bentivoglio AR (2008) GPi-DBS in Huntington's disease: results on motor function and cognition in a 72-year-old case. Mov Disord 23:1289–1292.

- Filion M, Tremblay L (1991) Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res 547:142–151.
- Filion M, Tremblay L, Bedard PJ (1988) Abnormal influences of passive limb movement on the activity of globus pallidus neurons in parkinsonian monkeys. Brain Res 444:165–176.
- Filipovic SR, Ljubisavljevic M, Svetel M, Milanovic S, Kacar A, Kostic VS (1997) Impairment of cortical inhibition in writer's cramp as revealed by changes in electromyographic silent period after transcranial magnetic stimulation. Neurosci Lett 222:167–170.
- Flaherty AW, Williams ZM, Amirnovin R, Kasper E, Rauch SL, Cosgrove GR, Eskandar EN (2005) Deep brain stimulation of the anterior internal capsule for the treatment of Tourette syndrome: technical case report. Neurosurgery 57:E403 discussion E403.
- Fontaine D, Mattei V, Borg M, von Langsdorff D, Magnie MN, Chanalet S, Robert P, Paquis P (2004) Effect of subthalamic nucleus stimulation on obsessive-compulsive disorder in a patient with Parkinson disease. Case report. J Neurosurg 100:1084–1086.
- Fukaya C, Katayama Y, Kano T, Nagaoka T, Kobayashi K, Oshima H, Yamamoto T (2007) Thalamic deep brain stimulation for writer's cramp. J Neurosurg 107:977–982.
- Galardi G, Perani D, Grassi F, Bressi S, Amadio S, Antoni M, Comi GC, Canal N, Fazio F (1996) Basal ganglia and thalamo-cortical hypermetabolism in patients with spasmodic torticollis. Acta Neurol Scand 94:172–176.
- Gatev P, Darbin O, Wichmann T (2006) Oscillations in the basal ganglia under normal conditions and in movement disorders.. Mov Disord 21:1566–1577.
- Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451.
- Gerfen CR (1995) Dopamine receptor function in the basal ganglia. Clin Neuropharmacol 18:S162–S177.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons.. Science 250:1429–1432.
- Gerlach J, Hansen L (1997) Clozapine and D1/D2 antagonism in extrapyramidal functions. Br J Pychiatr 17(Suppl):34–37.
- Ghilardi M, Ghez C, Dhawan V, Moeller J, Mentis M, Nakamura T, Antonini A, Eidelberg D (2000) Patterns of regional brain activation associated with different forms of motor learning. Brain Res 871:127–145.
- Ghilardi MF, Carbon M, Silvestri G, Dhawan , Tagliati M, Bressman S, Ghez C, Eidelberg D (2003) Impaired sequence learning in carriers of the DYT1 dystonia mutation. Ann Neurol 54:102–109.
- Goldman-Rakic PS, Porrino LJ (1985) The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. J Comp Neurol 242:535–560.
- Grabli D, McCaim K, Hirsch EC, Agid Y, Feger J, Francois C, Tremblay L (2004) Behavioural disorders induced by external globus pallidus dysfunction in primates: I. Behavioural study. Brain 127:2039–2054 2004 Sep.
- Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K (2009) Optical deconstruction of parkinsonian neural circuitry. Science 324:354–359.
- Grafton ST, DeLong M (1997) Tracing the brain's circuitry with functional imaging. Nat Med 3:602–603.
- Grafton ST, Turner RS, Desmurget M, Bakay R, Delong M, Vitek J, Crutcher M (2006) Normalizing motor-related brain activity:

Subthalamic nucleus stimulation in Parkinson disease. Neurology 66:1192–1199.

- Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31:359–387.
- Greenberg BD, Price LH, Rauch SL, SA, et al. (2003) Neurosurgery for intractable obsessive-compulsive disorder and depression: critical issues. Neurosurg Clin N Am 14:199–212.
- Greenberg BD, Malone DA, Friehs GM, et al. (2006) Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder. Neuropsychopharm 31:2384–2393.
- Greenberg BD, Gabriels LA, Malone DA Jr., et al. (2008) Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience. Mol Psychiatry:1–16.
- Grill WM, Snyder AN, Miocinovic S (2004) Deep brain stimulation creates an informational lesion of the stimulated nucleus. Neuroreport 15:1137–1140.
- Gross RE (2008) What happened to posteroventral pallidotomy for Parkinson's disease and dystonia? Neurotherapeutics 5:281–293.
- Guo Y, Rubin JE, McIntyre CC, Vitek JL, Terman D (2008) Thalamocortical relay fidelity varies across subthalamic nucleus deep brain stimulation protocols in a data-driven computational model. J Neurophysiol 99:1477–1492.
- Gurney K, Prescott TJ, Redgrave P (2001) A computational model of action selection in the basal ganglia. II. Analysis and simulation of behaviour. Biol Cybernet 84:411–423.
- Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
- Haber SN, Kunishio K, Mizobuchi M, Lynd-Balta E (1995) The orbital and medial prefrontal circuit through the primate basal ganglia. J Neurosci 15:4851–4867.
- Hallett M (1998) The neurophysiology of dystonia. Arch Neurol 55:601–603.
- Hamada I, DeLong MR (1992) Excitotoxic acid lesions of the primate subthalamic nucleus result in transient dyskinesias of the contralateral limbs. J Neurophysiol 68:1850–1858.
- Hamano T, Kaji R, Katayama M, Kubori T, Ikeda A, Shibasaki H, Kimura J (1999) Abnormal contingent negative variation in writer's cramp. Clin Neurophysiol 110:508–515.
- Hammond C, Bergman H, Brown P (2007) Pathological synchronization in Parkinson's disease: networks, models and treatments. Trends Neurosci 30:357–364.
- Hantraye P, Riche D, Maziere M, Isacson O (1990) A primate model of Huntington's disease: behavioral and anatomical studies of unilateral excitotoxic lesions of the caudate-putamen in the baboon. Exp Neurol 108:91–104.
- Harnois C, Filion M (1982) Pallidofugal projections to thalamus and midbrain: a quantitative antidromic activation study in monkeys and cats. Exp Brain Res 47:277–285.
- Hassler R, Dieckmann G (1970) Stereotaxic treatment of tics and inarticulate cries or coprolalia considered as motor obsessional phenomena in Gilles de la Tourette's disease. Rev Neurol (Paris) 123:89–100.
- Hassler R, Dieckmann G (1973) Relief of obsessive-compulsive disorders, phobias and tics by stereotactic coagulations of the rostral intralaminar and medial-thalamic nuclei. In: Surgical approaches in psychiatry. Proceedings of the Third International Congress of Psychosurgery (Laitinen LV, Livingston K, Eds.), pp. 206–212. Cambridge, UK: Garden City Press.

- Hassler R, Dieckmann G (1997) Stereotaxic treatment of tics and inarticulate cries or coprolalia considered as motor obsessional phenomena in Gilles de la Tourette's disease. Rev Neurol (Paris) 123:89–100.
- Hebb MO, Garcia R, Gaudet P, Mendez IM (2006) Bilateral stimulation of the globus pallidus internus to treat choreathetosis in Huntington's disease: technical case report. Neurosurgery 58:E383 discussion E383.
- Heimer L (2003) The legacy of the silver methods and the new anatomy of the basal forebrain: implications for neuropsychiatry and drug abuse. Scand J Psychol 44:189–201.
- Hikosaka O (2007) Basal ganglia mechanisms of reward-oriented eye movement. Ann NY Acad Sci 1104:229–249.
- Hodgkiss AD, Malizia AL, Bartlett JR, Bridges PK (1995) Outcome after the psychosurgical operation of stereotactic subcaudate tractotomy, 1979–1991. J Neuropsychiatry Clin Neurosci 7:230–234.
- Hoover JE, Strick PL (1993) Multiple output channels in the basal ganglia. Science 259:819–821.
- Houeto JL, Karachi C, Mallet L, et al. (2005) Tourette's syndrome and deep brain stimulation. J Neurol Neurosurg Psychiatry 76:992–995.
- Houser M, Waltz T (2005) Meige syndrome and pallidal deep brain stimulation. Mov Disord 20:1203–1205.
- Hung SW, Hamani C, Lozano AM, et al. (2007) Long-term outcome of bilateral pallidal deep brain stimulation for primary cervical dystonia. Neurology 68:457–459.
- Ichinose H, Nagatsu T (1997) Molecular genetics of hereditary dystoniamutations in the GTP cyclohydrolase I gene. Brain Res Bull 43:35–38.
- Ichinose H, Ohye T, Takayashi E, et al. (1994) Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. Nature Genetics 8:236–242.
- Ikeda A, Shibasaki H, Kaji R, Terada K, Nagamine T, Honda M, Hamano T, Kimura J (1996) Abnormal sensorimotor integration in writer's cramp: study of contingent negative variation. Mov Disord 11:683–690.
- Inase M, Tanji J (1995) Thalamic distribution of projection neurons to the primary motor cortex relative to afferent terminal fields from the globus pallidus in the macaque monkey. J Comp Neurol 353:415–426.
- Inglis WL, Winn P (1995) The pedunculopontine tegmental nucleus: where the striatum meets the reticular formation. Prog Neurobiol 47:1–29.
- Jahn K, Deutschlander A, Stephan T, Kalla R, Wiesmann M, Strupp M, Brandt T (2008) Imaging human supraspinal locomotor centers in brainstem and cerebellum. Neuroimage 39:786–792.
- Jenike MA (1998) Neurosurgical treatment of obsessive-compulsive disorder. Br J Psychiatry(Suppl):79–90.
- Jinnah HA, Hess EJ (2006) A new twist on the anatomy of dystonia: the basal ganglia and the cerebellum? Neurology 67:1740–1741.
- Kaji R, Ikeda A, Ikeda T, et al. (1995) Physiological study of cervical dystonia. Task-specific abnormality in contingent negative variation. Brain 118:511–522.
- Kaneda K, Isa K, Yanagawa Y, Isa T (2008) Nigral inhibition of GABAergic neurons in mouse superior colliculus. J Neurosci 28:11071–11078.
- Kanovsky P, Streitova H, Dufek J, Rektor I (1997) Lateralization of the P22/N30 component of somatosensory evoked potentials of the median nerve in patients with cervical dystonia. Mov Disord 12:553–560.
- Kanovsky P, Streitova H, Dufek J, Znojil V, Daniel P, Rektor I (1999) Lateralization of the P22/N30 precentral cortical component of the

median nerve somatosensory evoked potentials is different in patients with a tonic or tremulous form of cervical dystonia. Mov Disord 14:642–651.

- Karbe H, Holthoff VA, Rudolf J, Herholz K, Heiss WD (1992) Positron emission tomography demonstrates frontal cortex and basal ganglia hypometabolism in dystonia. Neurology 42:1540–1544.
- Kiss ZH, Doig-Beyaert K, Eliasziw M, Tsui J, Haffenden A, Suchowersky O (2007) The Canadian multicentre study of deep brain stimulation for cervical dystonia. Brain 130:2879–2886.
- Kitagawa M, Murata J, Uesugi H, Kikuchi S, Saito H, Tashiro K, Sawamura Y (2005) Two-year follow-up of chronic stimulation of the posterior subthalamic white matter for tremor-dominant Parkinson's disease. Neurosurgery 56:281–289 discussion 281-289.
- Klostermann F, Nikulin VV, Kuhn AA, et al. (2007) Task-related differential dynamics of EEG alpha- and beta-band synchronization in cortico-basal motor structures. Eur J Neurosci 25:1604–1615.
- Kojima J, Yamaji Y, Matsumura M, Nambu A, Inase M, Tokuno H, Takada M, Imai H (1997) Excitotoxic lesions of the pedunculopontine tegmental nucleus produce contralateral hemiparkinsonism in the monkey. Neurosci Lett 226:111–114.
- Kopell BH, Greenberg BD (2008) Anatomy and physiology of the basal ganglia: Implications for DBS in psychiatry. Neurosci Biobehav Rev 32:408–422.
- Krauss JK, Loher TJ, Pohle T, Weber S, Taub E, Barlocher CB, Burgunder JM (2002) Pallidal deep brain stimulation in patients with cervical dystonia and severe cervical dyskinesias with cervical myelopathy. J Neurol Neurosurg Psychiatry 72:249–256.
- Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647.
- Kringelbach ML, Jenkinson N, Owen SL, Aziz TZ (2007) Translational principles of deep brain stimulation. Nat Rev Neurosci 8:623–635.
- Kuhn AA, Meyer BU, Trottenberg T, Brandt SA, Schneider GH, Kupsch A (2003) Modulation of motor cortex excitability by pallidal stimulation in patients with severe dystonia. Neurology 60:768–774.
- Kumar R, Lozano AM, Sime E, Lang AE (2003) Long-term follow-up of thalamic deep brain stimulation for essential and parkinsonian tremor. Neurology 61:1601–1604.
- Kupsch A, et al. (2006) Pallidal deep-brain stimulation in primary generalized or segmental dystonia. N Engl J Med 355:1978–1990.
- Leblois A, Meissner W, Bioulac B, Gross CE, Hansel D, Boraud T (2007) Late emergence of synchronized oscillatory activity in the pallidum during progressive Parkinsonism. Eur J Neurosci 26:1701–1713.
- Lenz FA, Byl NN (1999) Reorganization in the cutaneous core of the human thalamic principal somatic sensory nucleus (Ventral caudal) in patients with dystonia. J Neurophysiol 82:3204–3212.
- Lenz FA, Jaeger CJ, Seike MS, Lin YC, Reich SG, DeLong MR, Vitek JL (1999) Thalamic single neuron activity in patients with dystonia: dystonia-related activity and somatic sensory reorganization. J Neurophysiol 82:2372–2392.
- Lenz FA, Suarez JI, Metman LV, Reich SG, Karp BI, Hallett M, Rowland LH, Dougherty PM (1998) Pallidal activity during dystonia: somatosensory reorganisation and changes with severity. J Neurol Neurosurg Psychiatry 65:767–770.
- Li S, Arbuthnott GW, Jutras MJ, Goldberg JA, Jaeger D (2007) Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. J Neurophysiol 98:3525–3537.
- Lippitz BE, Mindus P, Meyerson BA, Kihlstrom L, Lindquist C (1999) Lesion topography and outcome after thermocapsulotomy or gamma

knife capsulotomy for obsessive-compulsive disorder: relevance of the right hemisphere. Neurosurgery 44:452–458; discussion 458-460.

- Liu P, Basso MA (2008) Substantia nigra stimulation influences monkey superior colliculus neuronal activity bilaterally. J Neurophysiol 100:1098–1112.
- Loher TJ, Hasdemir MG, Burgunder JM, Krauss JK (2000) Long-term follow-up study of chronic globus pallidus internus stimulation for posttraumatic hemidystonia. J Neurosurg 92:457–460.
- Lozano A, Hutchison W, Kiss Z, Tasker R, Davis K, Dostrovsky J (1996) Methods for microelectrode-guided posteroventral pallidotomy. J Neurosurg 84:194–202.
- Lozano AM, Snyder BJ (2008) Deep brain stimulation for parkinsonian gait disorders. J Neurol 255(Suppl 4):30–31.
- Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH (2008) Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. Biol Psychiatry 64:461–467.
- Maciunas RJ, Maddux BN, Riley DE, et al. (2007) Prospective randomized double-blind trial of bilateral thalamic deep brain stimulation in adults with Tourette syndrome. J Neurosurg 107:1004–1014.
- Mallet L, Mesnage V, Houeto JL, et al. (2002) Compulsions, Parkinson's disease, and stimulation. Lancet 360:1302–1304.
- Mallet N, Pogosyan A, Sharott A, Csicsvari J, Bolam JP, Brown P, Magill PJ (2008) Disrupted dopamine transmission and the emergence of exaggerated beta oscillations in subthalamic nucleus and cerebral cortex. J Neurosci 28:4795–4806.
- Malone DA Jr., Dougherty DD, Rezai AR, et al. (2009) Deep brain stimulation of the ventral capsule/ventral striatum for treatment-resistant depression. Biol Psychiatry 65:267–275.
- Marsden CD (1982) The mysterious motor function of the basal ganglia: the Robert Wartenburg lecture. Neurology 32:514–539.
- May PJ, McHaffie JG, Stanford TR, et al. (2009) Tectonigral projections in the primate: a pathway for pre-attentive sensory input to midbrain dopaminergic neurons. Eur J Neurosci 29:575–587.
- Mayberg HS (2003) Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. Br Med Bull 65:193–207.
- Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45:651–660.
- Mayberg HS, Liotti M, Brannan SK, et al. (1999) Reciprocal limbiccortical function and negative mood: converging PET findings in depression and normal sadness. Am J Psychiatry 156:675–682.
- McIntyre CC, Savasta M, Walter BL, Vitek JL (2004) How does deep brain stimulation work? Present understanding and future questions. J Clin Neurophysiol 21:40–50.
- Mena-Segovia J, Bolam JP, Magill PJ (2004) Pedunculopontine nucleus and basal ganglia: distant relatives or part of the same family? Trends Neurosci 27:585–588.
- Middleton FA, Strick PL (1994) Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. Science 266:458–461.
- Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Brain Res Rev 31:236–250.
- Miller WC, DeLong MR (1987) Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism. In: The Basal Ganglia II (Carpenter MB, Jayaraman A, eds), pp. 415–427. New York: Plenum Press.
- Mink JW (2006) Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. Adv Neurol 99:89–98.

- Mink JW, Thach WT (1993) Basal ganglia intrinsic circuits and their role in behavior. Curr Opinion Neurobiol 3:950–957.
- Mitchell IJ, Luquin R, Boyce S, Clarke CE, Robertson RG, Sambrook MA, Crossman AR (1990) Neural mechanisms of dystonia: evidence from a 2-deoxyglucose uptake study in a primate model of dopamine agonist-induced dystonia. Mov Disord 5:49–54.
- Mitchell SJ, Richardson RT, Baker FH, DeLong MR (1987) The primate globus pallidus: Neuronal activity related to direction of movement. Exp Brain Res 68:491–505.
- Montoya A, Weiss AP, Price BH, et al. (2002) Magnetic resonance imaging-guided stereotactic limbic leukotomy for treatment of intractable psychiatric disease. Neurosurgery 50:1043–1049 discussion 1049-1052.
- Moustafa AA, Sherman SJ, Frank MJ (2008) A dopaminergic basis for working memory, learning and attentional shifting in Parkinsonism. Neuropsychologia 46:3144–3156.
- Munro-Davies LE, Winter J, Aziz TZ, Stein JF (1999) The role of the pedunculopontine region in basal-ganglia mechanisms of akinesia. Exp Brain Res 129:511–517.
- Murase N, Kaji R, Shimazu H, et al. (2000) Abnormal premovement gating of somatosensory input in writer's cramp. Brain 123:1813–1829.
- Murer MG, Tseng KY, Kasanetz F, Belluscio M, Riquelme LA (2002) Brain oscillations, medium spiny neurons, and dopamine.[erratum appears in Cell Mol Neurobiol. 2003 Jun;23(3):449]. Cell Mol Neurobiol 22:611–632.
- Nagatsu T, Ichinose H (1997) GTP cyclohydrolase I gene, dystonia, juvenile parkinsonism, and Parkinson's disease. J Neural Trans(Suppl 49):203–209.
- Nambu A, Mori S, Stuart DG, Wiesendanger M (2004). A new dynamic model of the cortico-basal ganglia loop. In Progress in Brain Research, pp. 461–466. Oxford: Elsevier.
- Nandi D, Liu X, Winter JL, Aziz TZ, Stein JF (2002) Deep brain stimulation of the pedunculopontine region in the normal non-human primate. J Clin Neurosci 9:170–174.
- Nauta WJ, Domesick VB (1984) Afferent and efferent relationships of the basal ganglia. Ciba Foundation Symposium 107:3–29.
- Neychev VK, Fan X, Mitev VI, Hess EJ, Jinnah HA (2008) The basal ganglia and cerebellum interact in the expression of dystonic movement. Brain 131:2499–2509.
- Nuttin B, Cosyns P, Demeulemeester H, Gybels J, Meyerson B (1999) Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. Lancet 354:1526.
- Nuttin BJ, Gabriels LA, Cosyns PR, HG, et al. (2003) Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. Neurosurgery 52:1263–1272; discussion 1272-1264.
- Obeso JA, Rodriguez MC, Gorospe A, Guridi J, Alvarez L, Macias R (1997) Surgical treatment of Parkinson's disease. Bailliere's Clin Neurol 6:125–145.
- Ondo W, Jankovic J, Schwartz K, Almaguer M, Simpson RK (1998) Unilateral thalamic deep brain stimulation for refractory essential tremor and Parkinson's disease tremor. Neurology 51:1063–1069.
- Ostergaard K, Sunde NA (2006) Evolution of Parkinson's disease during 4 years of bilateral deep brain stimulation of the subthalamic nucleus. Mov Disord 21:624–631.
- Ostrem JL, Marks WJ Jr., Volz MM, Heath SL, Starr PA (2007) Pallidal deep brain stimulation in patients with cranial-cervical dystonia (Meige syndrome). Mov Disord 22:1885–1891.
- Ozelius LJ, Hewett J, Kramer P, et al. (1997) Fine localization of the torsion dystonia gene (DYT1) on human chromosome 9q34: YAC map and linkage disequilibrium. Genome Res 7:483–494.

- Parent A (1990) Extrinsic connections of the basal ganglia. Trends Neurosci 13:254–258.
- Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 20:91–127.
- Parent A, Sato F, Wu Y, Gauthier J, Levesque M, Parent M (2000) Organization of the basal ganglia: the importance of axonal collateralization. Trends Neurosci 23:S20–S27.
- Parsons TD, Rogers SA, Braaten AJ, Woods SP, Troster AI (2006) Cognitive sequelae of subthalamic nucleus deep brain stimulation in Parkinson's disease: a meta-analysis. Lancet Neurol 5:578–588.
- Pastor-Gomez J, Hernando-Requejo V, Luengo-Dos Santos A, Pedrosa-Sanchez M, Sola RG (2003) [Treatment of a case of generalised dystonia using subthalamic stimulation]. Rev Neurol 37:529–531.
- Patel NK, Heywood P, O'Sullivan K, McCarter R, Love S, Gill SS (2003) Unilateral subthalamotomy in the treatment of Parkinson's disease. Brain 126:1136–1145.
- Peterson JD, Surmeier DJ (2007) Dopamine depletion results in differential alterations in corticostriatal and thalamostriatal glutamatergic synapses onto striatonigral and striatopallidal neurons. Soc Neurosci Abstr 590.516.
- Pillon B, Ardouin C, Dujardin K, et al. (2006) Preservation of cognitive function in dystonia treated by pallidal stimulation. Neurology 66:1556–1558.
- Pisani A, Centonze D, Bernardi G, Calabresi P (2005) Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. Mov Disord 20:395–402.
- Pisani A, Bernardi G, Ding J, Surmeier DJ (2007) Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30:545–553.
- Pisani A, Martella G, Tscherter A, Bonsi P, Sharma N, Bernardi G, Standaert DG (2006) Altered responses to dopaminergic D2 receptor activation and N-type calcium currents in striatal cholinergic interneurons in a mouse model of DYT1 dystonia. Neurobiol Dis 24:318–325.
- Plaha P, Gill SS (2005) Bilateral deep brain stimulation of the pedunculopontine nucleus for Parkinson's disease. Neuroreport 16:1883–1887.
- Plaha P, Ben-Shlomo Y, Patel NK, Gill SS (2006) Stimulation of the caudal zona incerta is superior to stimulation of the subthalamic nucleus in improving contralateral parkinsonism. Brain 129:1732–1747.
- Playford ED, Passingham RE, Marsden CD, Brooks DJ (1998) Increased activation of frontal areas during arm movement in idiopathic torsion dystonia. Mov Disord 13:309–318.
- Pralong E, Pollo C, Coubes P, Bloch J, Roulet E, Tetreault MH, Debatisse D, Villemure JG (2005) Electrophysiological characteristics of limbic and motor globus pallidus internus (GPI) neurons in two cases of Lesch-Nyhan syndrome. Neurophysiol Clin 35:168–173.
- Pretto TE, Dalvi A, Kang UJ, Penn RD (2008) A prospective blinded evaluation of deep brain stimulation for the treatment of secondary dystonia and primary torticollis syndromes. J Neurosurg 109:405–409.
- Raike RS, Jinnah HA, Hess EJ (2005) Animal models of generalized dystonia. NeuroRx 2:504–512.
- Rauch SL, Dougherty DD, Malone D, et al. (2006) A functional neuroimaging investigation of deep brain stimulation in patients with obsessive-compulsive disorder. J Neurosurg 104:558–565.
- Reilly JA, Hallett M, Cohen LG, Tarkka IM, Dang N (1992) The N30 component of somatosensory evoked potentials in patients with dystonia. Electroencephalogr Clin Neurophysiol 84:243–247.
- Reynolds JN, Hyland BI, Wickens JR (2001) A cellular mechanism of reward-related learning. Nature 413:67–70.

- Ridding MC, Sheean G, Rothwell JC, Inzelberg R, Kujirai T (1995) Changes in the balance between motor cortical excitation and inhibition in focal, task specific dystonia. J Neurol Neurosurg Psychiatry 59:493–498.
- Rodrigues JP, Walters SE, Watson P, Stell R, Mastaglia FL (2007) Globus pallidus stimulation improves both motor and nonmotor aspects of quality of life in advanced Parkinson's disease. Mov Disord 22:1866–1870.
- Rodriguez-Oroz MC, Zamarbide I, Guridi J, Palmero MR, Obeso JA (2004) Efficacy of deep brain stimulation of the subthalamic nucleus in Parkinson's disease 4 years after surgery: double blind and open label evaluation. J Neurol Neurosurg Psychiatry 75:1382–1385.
- Rona S, Berardelli A, Vacca L, Inghilleri M, Manfredi M (1998) Alterations of motor cortical inhibition in patients with dystonia. Mov Disord 13:118–124.
- Rye DB, Lee HJ, Saper CB, Wainer BH (1988) Medullary and spinal efferents of the pedunculopontine tegmental nucleus and adjacent mesopontine tegmentum in the rat. J Comp Neurol 269:315–341.
- Sadikot AF, Parent A, Francois C (1992) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a PHA-L study of subcortical projections. J Comp Neurol 315:137–159.
- Sako W, Goto S, Shimazu H, et al. (2008) Bilateral deep brain stimulation of the globus pallidus internus in tardive dystonia. Mov Disord 23:1929–1931.
- Sato K, Sumi-Ichinose C, Kaji R, et al. (2008) Differential involvement of striosome and matrix dopamine systems in a transgenic model of dopa-responsive dystonia. Proc Natl Acad Sci USA 105:12551–12556.
- Schell GR, Strick PL (1984) The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. J Neurosci 4:539–560.
- Schlaepfer TE, Cohen MX, Frick C, et al. (2008) Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. Neuropsychopharm 33:368–377.
- Schwartzman RJ, Alexander GM (1985) Changes in the local cerebral metabolic rate for glucose in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model of Parkinson's disease. Brain Res 358:137–143.
- Sciamanna G, Bonsi P, Tassone A, et al. (2009) Impaired striatal D2 receptor function leads to enhanced GABA transmission in a mouse model of DYT1 dystonia. Neurobiol Dis 34:133–145.
- Servello D, Porta M, Sassi M, Brambilla A, Robertson MM (2008) Deep brain stimulation in 18 patients with severe Gilles de la Tourette syndrome refractory to treatment: the surgery and stimulation. J Neurol Neurosurg Psychiatry 79:136–142.
- Sharma N, Baxter MG, Petravicz J, Bragg DC, Schienda A, Standaert DG, Breakefield XO (2005) Impaired motor learning in mice expressing torsinA with the DYT1 dystonia mutation. J Neurosci 25:5351–5355.
- Shen W, Flajolet M, Greengard P, Surmeier DJ (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. Science 321:848–851.
- Shields DC, Cheng ML, Flaherty AW, Gale JT, Eskandar EN (2008) Microelectrode-guided deep brain stimulation for Tourette syndrome: within-subject comparison of different stimulation sites. Stereotact Funct Neurosurg 86:87–91.
- Shink E, Bevan MD, Bolam JP, Smith Y (1996) The subthalamic nucleus and the external pallidum: two tightly interconnected structures that control the output of the basal ganglia in the monkey. Neurosci 73:335–357.
- Sidibe M, Bevan MD, Bolam JP, Smith Y (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: I. Topography

and synaptic organization of the pallidothalamic projection. J Comp Neurol 382:323–347.

- Silberstein P, Kuhn AA, Kupsch A, P, et al. (2003) Patterning of globus pallidus local field potentials differs between Parkinson's disease and dystonia. Brain 126:2597–2608.
- Singla S, Kreitzer AC, Malenka RC (2007) Mechanisms for synapse specificity during striatal long-term depression. J Neurosci 27:5260–5264.
- Slavin KV, Baumann TK, Burchiel KJ (2004) Treatment of hemiballismus with stereotactic pallidotomy. Case report and review of the literature. Neurosurg Focus 17:E7.
- Smith Y, Parent A (1986) Differential connections of caudate nucleus and putamen in the squirrel monkey (*Saimiri sciureus*). Neurosci 18:347–371.
- Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neurosci 86:353–387.
- Smith Y, Raju DV, Pare JF, Sidibe M (2004) The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 27:520–527.
- Sommer M, Ruge D, Tergau F, Beuche W, Altenmuller E, Paulus W (2002) Intracortical excitability in the hand motor representation in hand dystonia and blepharospasm. Mov Disord 17:1017–1025.
- Starr PA, Rau GM, Davis V, Marks WJ Jr., Ostrem JL, Simmons D, Lindsey N, Turner RS (2005) Spontaneous pallidal neuronal activity in human dystonia: comparison with Parkinson's disease and normal macaque. J Neurophysiol 93:3165–3176.
- Stefani A, Lozano AM, Peppe A, et al. (2007) Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. Brain 130:1596–1607.
- Stefurak T, Mikulis D, Mayberg H, Lang AE, Hevenor S, Pahapill P, Saint-Cyr J, Lozano A (2003) Deep brain stimulation for Parkinson's disease dissociates mood and motor circuits: a functional MRI case study. Mov Disord 18:1508–1516.
- Sturm V, Lenartz D, Koulousakis A, Treuer H, Herholz K, Klein JC, Klosterkotter J (2003) The nucleus accumbens: a target for deep brain stimulation in obsessive-compulsive- and anxiety-disorders. J Chem Neuroanat 26:293–299.
- Suarez JI, Metman LV, Reich SG, Dougherty PM, Hallett M, Lenz FA (1997) Pallidotomy for hemiballismus: efficacy and characteristics of neuronal activity. Ann Neurol 42:807–811.
- Sun B, Chen S, Zhan S, Le W, Krahl SE (2007) Subthalamic nucleus stimulation for primary dystonia and tardive dystonia. Acta Neurochir Suppl 97:207–214.
- Taira T, Hori T (2003) Stereotactic ventrooralis thalamotomy for taskspecific focal hand dystonia (writer's cramp). Stereotact Funct Neurosurg 80:88–91.
- Taira T, Kobayashi T, Hori T (2003) Disappearance of self-mutilating behavior in a patient with Lesch–Nyhan syndrome after bilateral chronic stimulation of the globus pallidus internus. Case report. J Neurosurg 98:414–416.
- Tang JK, Moro E, Mahant N, Hutchison WD, Lang AE, Lozano AM, Dostrovsky JO (2007) Neuronal firing rates and patterns in the globus pallidus internus of patients with cervical dystonia differ from those with Parkinson's disease. J Neurophysiol 98:720–729.
- Temel Y, Visser-Vandewalle V (2004) Surgery in Tourette syndrome. Mov Disord 19:3–14.
- Temel Y, Kessels A, Tan S, Topdag A, Boon P, Visser-Vandewalle V (2006) Behavioural changes after bilateral subthalamic stimulation in

advanced Parkinson disease: a systematic review. Parkinsonism Relat Disord 12:265–272.

- Tinazzi M, Frasson E, Polo A, Tezzon F, Bovi P, Deotto L, Mauguiere F, Fiaschi A, Ferrari G (1999) Evidence for an abnormal cortical sensory processing in dystonia: selective enhancement of lower limb P37-N50 somatosensory evoked potential. Mov Disord 14:473–480.
- Tisch S, Rothwell JC, Bhatia KP, et al. (2007) Pallidal stimulation modifies after-effects of paired associative stimulation on motor cortex excitability in primary generalised dystonia. Exp Neurol 206:80–85.
- Toro C, Deuschl G, Hallett M (2000) Movement-related electroencephalographic desynchronization in patients with hand cramps: evidence for motor cortical involvement in focal dystonia. Ann Neurol 47:456–461.
- Tozzi A, Tscherter A, Belcastro V, et al. (2007) Interaction of A2A adenosine and D2 dopamine receptors modulates corticostriatal glutamatergic transmission. Neuropharmacology 53:783–789.
- Troster AI, Fields JA, Wilkinson SB, Pahwa R, Miyawaki E, Lyons KE, Koller WC (1997) Unilateral pallidal stimulation for Parkinson's disease: neurobehavioral functioning before and 3 months after electrode implantation. Neurology 49:1078–1083.
- Trottenberg T, Volkmann J, Deuschl G, Kuhn AA, Schneider GH, Muller J, Alesch F, Kupsch A (2005) Treatment of severe tardive dystonia with pallidal deep brain stimulation. Neurology 64:344–346.
- Tseng KY, Kasanetz F, Kargieman L, Riquelme LA, Murer MG (2001) Cortical slow oscillatory activity is reflected in the membrane potential and spike trains of striatal neurons in rats with chronic nigrostriatal lesions. J Neurosci 21:6430–6439.
- Tsubokawa T, Katayama Y, Yamamoto T (1995) Control of persistent hemiballismus by chronic thalamic stimulation. Report of two cases. J Neurosurg 82:501–505.
- Turner RS, Grafton ST, Votaw JR, Delong MR, Hoffman JM (1998) Motor subcircuits mediating the control of movement velocity: a PET study. J Neurophysiol 80:2162–2176.
- Vercueil L, Houeto JL, Krystkowiak P, et al. (2007) Effects of pulse width variations in pallidal stimulation for primary generalized dystonia. J Neurol.
- Vidailhet M, Vercueil L, Houeto JL, et al. (2005) Bilateral deep-brain stimulation of the globus pallidus in primary generalized dystonia. N Engl J Med 352:459–467.
- Vidailhet M, Vercueil L, Houeto JL, et al. (2007) Bilateral, pallidal, deepbrain stimulation in primary generalised dystonia: a prospective 3 year follow-up study. Lancet Neurol 6:223–229.
- Villalba RM, Lee H, Smith Y (2009) Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. Exp Neurol 215:220–227.
- Visser-Vandewalle V, Temel Y, van der Linden C, Ackermans L, Beuls E (2004) Deep brain stimulation in movement disorders. The applications reconsidered. Acta Neurol Belg 104:33–36.
- Visser-Vandewalle V, Temel Y, Boon P, et al. (2003) Chronic bilateral thalamic stimulation: a new therapeutic approach in intractable Tourette syndrome. Report of three cases. J Neurosurg 99:1094–1100.
- Visser-Vandewalle V, Ackermans L, van der Linden C, et al. (2006) Deep brain stimulation in Gilles de la Tourette's syndrome. Neurosurgery 58:E590.
- Vitek JL (2002) Pathophysiology of dystonia: a neuronal model. Mov Disord 17(Suppl 3):S49–S62.
- Vitek JL, Kaneoke Y, Turner R, Baron M, Bakay R, DeLong M (1993) Neuronal activity in the internal (GPi) and external (GPe) segments

of the globus pallidus (GP) of parkinsonian patients is similar to that in the MPTP-treated primate model of parkinsonism. Soc Neurosci Abstr 19:1584.

- Vitek JL, Chockkan V, Zhang JY, et al. (1999) Neuronal activity in the basal ganglia in patients with generalized dystonia and hemiballismus. Ann Neurol 46:22–35.
- Volkmann J (2004) Deep brain stimulation for the treatment of Parkinson's disease. J Clin Neurophysiol 21:6–17.
- Voon V, Krack P, Lang AE, et al. (2008) A multicentre study on suicide outcomes following subthalamic stimulation for Parkinson's disease. Brain 131:2720–2728.
- Wang HC, Lees AJ, Brown P (1999) Impairment of EEG desynchronisation before and during movement and its relation to bradykinesia in Parkinson's disease. J Neurol Neurosurg Psychiatry 66:442–446.
- Weaver FM, et al. (2009) Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA 301:63–73.
- Whittier JR, Mettler FA (1949) Studies of the subthalamus of the rhesus monkey. II. Hyperkinesia and other physiologic effects of subthalamic lesions with special references to the subthalamic nucleus of Luys. J Comp Neurol 90:319–372.
- Wichmann T (2008) Commentary: Dopaminergic dysfunction in DYT1 dystonia. Exp Neurol 212:242–246.
- Wichmann T, DeLong MR (2006) Basal ganglia discharge abnormalities in Parkinson's disease. J Neural Transm(Suppl):21–25.

- Wichmann T, Bergman H, DeLong MR (1994) The primate subthalamic nucleus. I. Functional properties in intact animals. J Neurophysiol 72:494–506.
- Wichmann T, Bergman H, Starr PA, Subramanian T, Watts RL, DeLong MR (1999) Comparison of MPTP-induced changes in spontaneous neuronal discharge in the internal pallidal segment and in the substantia nigra pars reticulata in primates. Exp Brain Res 125:397–409.
- Wider C, Pollo C, Bloch J, Burkhard PR, Vingerhoets FJ (2008) Long-term outcome of 50 consecutive Parkinson's disease patients treated with subthalamic deep brain stimulation. Parkinsonism Relat Disord 14:114–119.
- Williams D, Tijssen M, Van Bruggen G, et al. (2002) Dopamine-dependent changes in the functional connectivity between basal ganglia and cerebral cortex in humans. Brain Cogn 125:1558–1569.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 16:2397–2410.
- Yelnik J, Damier P, Demeret S, et al. (2003) Localization of stimulating electrodes in patients with Parkinson disease by using a three-dimensional atlas-magnetic resonance imaging coregistration method. J Neurosurg 99:89–99.
- Zhao Y, Decuypere M, Ledoux MS (2008) Abnormal motor function and dopamine neurotransmission in DYT1 DeltaGAG transgenic mice. Exp Neurol 210:719–730.
- Zhuang P, Li Y, Hallett M (2004) Neuronal activity in the basal ganglia and thalamus in patients with dystonia. Clin Neurophysiol 115:2542–2557.

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