Neuromodulation of central pattern generators and its role in the functional recovery of central pattern generator activity

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Abstract

Neuromodulators play an important role in how the nervous system organizes activity that results in behavior. Disruption of the normal patterns of neuromodulatory release or production are known to be related to the onset of severe pathologies such as Parkinson's Disease, Rett syndrome, Alzheimer's Disease and affective disorders. Some of these pathologies involve neuronal structures that are called central pattern generators, which are involved in the production of rhythmic activities throughout the nervous system. Here I discuss the interplay between CPGs and neuromodulatory activity, with particular emphasis on the potential role of neuromodulators on the recovery of disrupted neuronal activity. I refer to invertebrate and vertebrate model systems and some of the lessons we have learned from research on these systems and propose a few avenues for future research. I make one suggestion that may guide future research in the field: neuromodulators restrict the parameter landscape in which CPG components operate, and the removal of neuromodulators may enable a perturbed CPG in finding a new set of parameter values that can allow it to regain normal function.

Neuromodulators are substances that regulate neuronal activity by acting on a variety of targets, primarily by modifying second messenger pathways that act on ion channels as well as other neuromodulatory paths. Neuromodulators play important roles in how the nervous system generates and orchestrates the activity that drives behaviors, in particular behaviors involving rhythmic patterns. Disruption of the normal patterns of neuromodulatory release or production are known to be related to the onset of severe pathologies such as Parkinson's Disease (Viemari et al. 2005), Alzheimer's Disease (Severini et al. 2016), Rett syndrome (Dunn and MacLeod 2001) and affective disorders (Gu et al. 2016). In spite of the apparent importance of the roles that neuromodulators have in these pathologies, limited attention has been paid to their potential role in reconfiguring damaged neuronal networks leading towards compensatory recovery of function.

Central pattern generators (CPGs) are defined as central nervous system networks that generate periodic activity in the absence of periodic sensory input. Some form of input is often required to trigger or sustain the activity of a CPG, but that input activity does not need to be rhythmic. Transient or tonic inputs that enable or gate a CPG are common and examples include mechanical stimulation (Korta et al. 2007), chemical (e.g. O_2 deprivation in respiratory networks) (Lieske et al. 2000), and neuromodulatory input (Dickinson 2006; Kyriakatos et al. 2011). Tonic stimulation to enable CPG activity often comes in the form of tonic neuromodulatory input (Marder et al. 2014). **In this review, one of the focal points that I shall discuss is the role of neuromodulators on CPG activity, with particular emphasis on their effects on recovery from impaired rhythmic activity.**

Historically, the concept of central pattern generation was associated with the production of rhythmic motor activity. This is the case of systems such as the locust flight CPG (Wilson 1961), the crustacean stomatogastric ganglion (STG) pyloric and gastric mill network activity (Heinzel et al. 1993; Marder et al. 2005), the leech swimming and heart beat networks (Mullins et al. 2011; Norris et al. 2011), the gastropod feeding networks (Elliott and Susswein 2002) and the mammalian locomotion and respiration networks (Grillner and Manira 2015; Ramirez et al. 2012; Ramirez et al. 2004) (Fig. 1). More recently, that concept has been expanded to patterned cortical activities (Yuste et al. 2005).

The concept of CPG originated as a response to the claim by C. S. Sherrington that rhythmic patterns of activity could be generated solely on the basis of chains of reflexes (Sherrington 1910). The new paradigm was based on findings that deafferented networks could generate patterns of activity that produce behaviors resembling those observed in the intact animal (*i.e*. fictive behaviors). The first to suggest that a central mechanism could drive rhythmic motor activity was T. G. Brown working on decerebrated cats, who concluded that *These experiments show that the phasing of the acts of progression is determined neither by the peripheral skin stimuli nor by the self-generated proprioceptive stimuli of the muscles which take part in them* (Brown 1911). He further proposed that the central mechanism likely involved reciprocally inhibitory structures ("half-centers") whose inhibition can fatigue, allowing the partner center

to escape inhibition thanks to rebound properties previously shown to exist by Sherrington himself (Sherrington 1909). It was not until the early 1960s that the concept received unambiguously experimental evidence with the work of D. Wilson on the locust flight system (Wilson 1961). Additional, much less well studied, CPG networks have been identified in a number of vertebrate and invertebrate species (e.g. ventilation system in crustaceans (Dicaprio et al. 1997), micturition, ejaculation, defecation (see in (Guertin 2014)), mastication (Dellow and Lund 1971), and whisker movements in mammals (Gao et al. 2001), and vocalizations in frogs (Zornik and Yamaguchi 2012).

The concept of CPG, as it relates to the generation of rhythmic motor activity strictly generated by a central neuronal structure, has also been studied using a more integrative approach (Bässler 1986; Smith et al. 1991). In this view, rhythmic activity incorporates not only the CPG network but the key stabilizing and integrating inputs that the CPG receives from central as well as peripheral structures. This view is receiving a renewed attention and includes the role of motor neurons (Diekman et al. 2017; Falgairolle et al. 2017; Rotstein et al. 2017; Song et al. 2016) and sensory feedback (Bässler 1986; Li et al. 2017; Puhl et al. 2018). Consistent with this more expansive view of CPGs, recent attempts to design locomotion robots have expanded the use of concepts derived from the original CPG literature, to include either multiple coupled CPGs (Kiehn 2016; Ramirez and Baertsch 2018a) or layered CPGs (Grillner 2006a; Grillner and Manira 2015) integrated with sophisticated peripheral sensors and actuators that control stable and maneuverable robots.

While these ideas are of great interest, here I will focus on a number of relatively recent reports that center around the role of neuromodulation in the regulation of intrinsic and synaptic neuronal properties, which give rise to and regulate the generation and recovery of lost or disrupted rhythmic activity by CPGs.

Numerous reviews on the topic of central pattern generators have been published over the recent past that touch upon topics not discussed, or merely glanced upon here, which the reader may want to refer to, such as evolution of CPGs (Katz 2016), general principles of CPG function (Bucher et al. 2015; Marder and Calabrese 1996), the mammalian cortex as a putative CPG or ensemble of CPGs (Yuste et al. 2005), and sleep spindles as CPGs and their role in epilepsy (Beenhakker and Huguenard 2009).

Role of neuromodulators in the generation and regulation of CPG activity

Two basic types of CPG mechanisms have been described in most known systems: endogenous pacemakers (often active conditionally upon the effect of neuromodulators), which rely on intrinsic ionic currents to generate oscillatory activity by a given neuron, and network-based oscillators, which rely on synaptically connected sets of neurons (Fig. 1). A large number of ionic

currents have been found to be required to generate pacemaker activity in different systems (Amarillo et al. 2018; Bose et al. 2014; de Oliveira et al. 2010; Levitan et al. 1987; Mangoni et al. 2006; Mellon 2016; Zaza et al. 1997; Zhu et al. 2009), and still others to generate network-based oscillatory CPG activity (Daun et al. 2009; Sharp et al. 1996). Many of these currents are under neuromodulatory control. Pacemakers very often generate their activity through the activation of persistent inward currents, whether voltage-gated themselves or linear, but activated by another voltage-gated current. For example, in a population of inspiratory neurons of the pre-Bötzinger complex (preBötC), a mammalian breathing center found in the medulla (Fig. 1), a riluzole-sensitive persistent inward Na^+ current (I_{NaP}) is the dominant current for pacemaker activity generation, while in a different population of inspiratory neurons a non-inactivating (i.e. persistent) linear current (the calcium-activated nonspecific cation current, I_{CAN}), activated by Ca^{++} influx through synaptically-driven Ca^{++} channels, is the dominant one (Pena et al. 2004). An additional current, the non-selective, non-voltage gated, sodium-leak-channel (NaLCN), a member of the extended 4-domain NaV/CaV gene family, has more recently been added to the mix of currents involved in generating inspiratory pacemaker activity (Ramirez et al. 2012). These three currents are all expressed, in different combinations, and generating different levels of rhythmic activity among the various populations of inspiratory neurons in the preBötC that contribute to varying degrees to the generation of CPG activity in each (Carroll and Ramirez 2013; Ramirez and Baertsch 2018b) (Fig. 1). In the stomatogastric ganglion's (STG) pyloric network of crustaceans (Fig. 1), the pacemaker current is a persistent voltage-gated inward current carried mostly by Na^+ and activated by a variety of modulatory neuropeptides, the modulator-activated inward current, I_{MI} (Bose et al. 2014; Golowasch and Marder 1992). In both of these systems, large numbers of peptides, amines and other substances, acting upon a bewildering variety of receptors, target these pacemaker and other currents (Marder 2012; Ramirez et al. 2012), sometimes with each substance having different, even opposing, effects on currents from different target neurons or groups. This is the case in the preBötC, the post-inhibitory complex (PiCo) and the associated retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG), where modulators may have effects only on the activity of one of the nuclei, or have opposite effects on the same activity in each of them (Anderson et al. 2016; Doi and Ramirez 2008; Mellen et al. 2003). In the STG pyloric network, aminergic modulators show a similarly wide range of effects on different target currents depending on cell type. For example, dopamine (DA) in lobster STG enhances Ca^{++} currents in neurons PY, IC and LP, while it inhibits Ca^{++} currents in neurons PD, AB and VD (Harris-Warrick 2011), and depresses one inward current (I_{Ca}) while enhancing others (I_{NaP}, I_h) in the same neuron (Harris-Warrick 2011).

In gastropod mollusks, such as the sea hare *Aplysia*, the pond snail *Lymnaea* and others, the CPG networks that generate the feeding patterns have been extensively studied. While there are significant differences between these species in the behaviors and the underlying networks that generate these behaviors, the CPGs in both have a distributed organization, in which reciprocally connected neurons rather than truly endogenous oscillatory neurons generate the

rhythmic activity (Fig. 1) (Cropper et al. 2017; Elliott and Susswein 2002). This distributed character is something that they have in common with the rhythm generating networks in the mammalian respiratory network (Ramirez and Baertsch 2018b), but not the crustacean pyloric network (Fig. 1). In both gastropod species, the feeding CPG that controls the radula protraction and retraction can be activated by cerebral-buccal interneurons (CBIs), while other neurons form the core of the oscillator, such as the N1M, N2v and N3t interneurons in *Lymnaea* (Benjamin 2012), and the B63/B31/B32 and B64 in *Aplysia* (Cropper et al. 2017) (Fig. 1). Electrical coupling and, especially, chemical reciprocal synaptic inhibition are, like in many other CPGs, common (Sasaki et al. 2013), but some synapses generate feedforward excitation (e.g. excitation from N1 to N2 interneuron) that plays a role in the transitions to later phases of the behavior (Elliott and Susswein 2002) (in the mammalian respiratory network, excitatory connections appear to play a key synchronizing function (Carroll and Ramirez 2013)). Additionally, there are multiple neurons that are both members of the pattern generating networks as well as proprioceptors and/or exteroceptors (Elliott and Susswein 2002). In both species, the ability to generate rhythmic activity depends on intrinsic and synaptic properties that are regulated by neuromodulatory substances. Some well characterized modulators can be classified as intrinsic modulators, meaning that they are released by neurons that form the networks themselves, including motoneurons, while others are released by neurons outside the CPGs, and are thus regarded as extrinsic modulators (Benjamin 2012; Cropper et al. 2017; Elliott and Susswein 2002). In *Aplysia*, peptides released by inputs to the CPG are thought to regulate different properties of the various motor patterns of feeding behavior (Cropper et al. 2017). The modulators released by cerebro-buccal interneuron 2 (CBI-2), for instance, are known to reconfigure network activity to generate ingestive behavior (Dacks et al. 2012; Friedman and Weiss 2010; Koh and Weiss 2005; Morgan et al. 2000; Perkins et al. 2018; Proekt et al. 2004). As a consequence, and presumably by modifying the excitability and rhythm-generating properties of the ingestive CPG, a progressively stronger and more regular pattern typical of the repeating ingestive behavior is produced (Cropper et al. 2017). On the other hand, neurons and processes contained in the esophageal nerve (EN) are thought to reconfigure network activity to produce egestive behavior (Wu et al. 2010). In the *Lymnaea* feeding system there are complex interactions between extrinsic and intrinsic neuromodulation (Benjamin 2012; Elliott and Susswein 2002). Cerebral Giant Cells (CGC) and the slow oscillator interneuron (SO), for example, are not part of the feeding CPG and release 5-HT (Benjamin 2012) and ACh (Yeoman et al. 1993), respectively. Both also release the neuropeptide myomodulin (Santama et al. 1994). These neuromodulators regulate the intrinsic properties of core CPG interneurons (*e.g*. N1M and N2v neurons) to both excite them and activate plateau properties necessary for CPG activity. In addition, N2-type CPG interneurons (as well as several other buccal ganglion neurons) also express neuromodulatory peptides (myomodulin and small cardioactive peptide, SCP), and N1-type neurons express the neuromodulator buccalin (Santama et al. 1994), which function as intrinsic neuromodulators. However, what role these peptides play as intrinsic modulators released by these individual neurons is unclear.

Vertebrate locomotor systems, which are thought to be highly modular and based primarily on network driven CPGs typically requiring reciprocally inhibitory elements (Fig. 1), also receive substantial neuromodulatory input, both intrinsic and extrinsic, including peptides and other metabotropic receptor-activating substances that regulate frequency, regularity, etc. (Grillner 2006a; Grillner and Manira 2015; Sharples et al. 2014). In the crab STG, the gastric mill rhythm is also primarily driven by a network CPG (rather than by a pacemaker), which is heavily modulated and includes a modulatory neuron (the axon of the MCN1 projection neuron) as an integral part of the CPG itself (Fig. 1) (Coleman et al. 1995). Thus, as in the gastric network, neuromodulation by several amines of mammalian locomotor networks produce a broad range of (sometimes opposing) effects (Sharples et al. 2014).

The examples mentioned thus far indicate that a highly orchestrated and finely regulated organization of these neuromodulatory inputs and their effects must be at work so that functional CPG activity can be produced (*cf*. (Doi and Ramirez 2008)). One example of the orchestration that needs to take place at the cellular level is that one ionic current cannot be the sole current responsible for pacemaker activity because it needs to be balanced with appropriate counteracting currents to guarantee its oscillatory nature. Although this may appear obvious, few studies have addressed the balance between currents required to generate a stable and robust pattern of oscillatory activity. In the pyloric network of crustaceans, for example, a clear requirement for a balance between the levels of the abovementioned current I_{MI} and outward currents has been documented (Fig. 2) (Golowasch et al. 2017). Interestingly, only the pacemaker cells of the pyloric network express the appropriate balance between the I_{MI} and K^+ currents required to generate oscillatory activity (Fig. 2A, B), even though non-pacemaker (follower) cells in the same network also express I_{MI} (Swensen and Marder 2001; 2000). Follower cells overexpress a subset of high threshold K^+ currents to a degree that precludes the generation of pacemaker activity (Fig. 2C-D) (Golowasch et al. 2017). A further balancing act takes place in these cells: many pairs, and even larger subsets of ionic currents, appear to be "balanced", which has been shown to reveal itself as correlations of current or conductance amplitudes between these different current types in populations of identical neurons (Khorkova and Golowasch 2007; Temporal et al. 2012; Tran et al. 2019). Surprisingly, this is not restricted to "naturally" complementary currents such as the Na^+ and K^+ current that generate an action potential, or the abovementioned pacemaker I_{MI} and high threshold K^+ currents. It is also observed between various current pairs that are not naturally complementary in STG pyloric pacemaker cells, such as the inward current pair I_{Na} and I_h (Schulz et al. 2007), or the outward current pair I_A and I_{HTK} (Khorkova and Golowasch 2007; Temporal et al. 2012), or in mouse hippocampus granule cells, between the K^+ currents I_{Kd} and I_{Kir} (Tran et al. 2019). That this is not an artifact of electrophysiological recordings is confirmed by the fact that the same (plus additional) correlations are observed when measuring copy numbers of mRNA coding for these channels (Goaillard et al. 2009; Schulz et al. 2007; Temporal et al. 2012). This balancing of different currents likely serves a homeostatic or compensatory role in that it allows for individual currents to be slowly regulated to match others that may be acutely up- or down-

regulated by, for example, synaptic or sensory input (Fig. 3). In this manner, acute ionic current regulation is allowed to serve some immediate need. If some of these changes become long-lasting or permanent, the other conductances in a cell can slowly adjust their amplitudes or specific parameter values in order to ensure some basic overall stability of activity. This form of regulation has been shown theoretically to be useful to stabilize activity, at least within restricted parameter regions (Burdakov 2005; Franci et al. 2018; Hudson and Prinz 2010; Lamb and Calabrese 2013; Olypher and Calabrese 2007; Soofi et al. 2012; Taylor et al. 2009). Evidence also indicates that such a process of ionic current co-regulation likely involves the activation of a slow metabolic machinery (Ransdell et al. 2012). A consequence of such a co-regulation mechanism is the development of highly variable levels of the affected current's parameters as currents are slowly up- or down-regulated to more or less permanently compensate for changes in other currents. This has been shown to extend to all kinds of cell types, not only pacemaker neurons. Indeed, cells of a given (uniquely identified) type have been reported to express ionic currents parameters (maximum conductance, voltage-dependence parameters, as well as kinetic parameters) and the mRNA levels that code for these channels over a several-fold range of values (Amendola et al. 2012; Goldman et al. 2001; Golowasch 2014; Golowasch et al. 2002; Khorkova and Golowasch 2007; Li and Baccei 2011; Liu et al. 1998; McAnelly and Zakon 2000; Ransdell et al. 2013; Roffman et al. 2012; Schulz et al. 2006; Schulz et al. 2007; Swensen and Bean 2005; Tobin et al. 2009; Tran et al. 2019).

Interestingly, neuromodulators seem to be in part responsible for maintaining these correlations. When neuromodulators are removed in crab pyloric neurons, some of the maximum conductance correlations are lost, and this happens in a cell-type specific manner (Khorkova and Golowasch 2007; Temporal et al. 2012). However, the restitution of a single neuromodulatory peptide (proctolin) is sufficient to restore the lost correlations between three ionic currents in PD neurons of the pyloric network (Khorkova and Golowasch 2007), demonstrating that neuromodulators play an essential role in maintaining some of these correlations (a mechanism is suggested in Fig. 3). A similar role has been reported recently for nanomolar (tonic) concentrations of DA and serotonin (5-HT) in lobster neurons (Krenz et al. 2015).

Another well documented example of the balance required of pairs of currents to generate oscillatory activity is the generation of the electric organ discharge (EOD) of weakly electric fish electrocytes (McAnelly and Zakon 2000). Electrocytes express Na^+ and K^+ currents that generate action potentials responsible for the production of EODs and their characteristic frequency. The kinetics of these currents determine the duration of the action potentials, which in turn determines the frequency of the EOD. The EOD, which plays a crucial role in social communication, and its frequency can be regulated over a four-fold range thanks to large variations in the voltage-dependent activation and inactivation time constants of their Na^+ and K^+ currents across animals (McAnelly and Zakon 2000). Importantly, the time constants of activation of the two currents are coupled (or balanced), which allows the effective generation of action potentials of varying durations. Changes in these time constants modify EOD

frequencies, which can happen in real time, such as those that take place during social encounters. These are mediated by glutamate and GABA via ionotropic receptors. Over long times scales, regulation is dependent on the animals' age, the circadian period, as well as gender, and is mediated by a number of hormones including steroid and sex hormones, melatonin and prolactin (Zakon et al. 1999).

What mechanisms may ensure the balance of ionic current? As described above, neuromodulatory input appears to play a significant role in maintaining this balance (Khorkova and Golowasch 2007) (see Fig. 3). This can presumably happen via second messenger regulation of either transcription, translation and/or post-translational modifications, including channel insertion into the plasma membrane. Recently, Baro and collaborators showed that tonic low 5-HT concentrations enable the co-regulation of I_h and I_A levels in lobster pacemaker PD but not follower LP neurons, and low levels of DA do the same in LP but not PD neurons. As mentioned earlier, this leads to constant ratios of maximal conductances of these two currents (correlations) in populations of identical cells (Krenz et al. 2015). Krentz et al showed that this is mediated by an RNA interference silencing complex (RISC)-dependent process that is presumed to regulate microRNA effects on either 1) transcription of the channels, 2) transcription of regulators of channel transcription, or 3) translation of regulators of promoters of the Kv4 and HCN genes (which code for the A and h channels, respectively) (Krenz et al. 2015). Interestingly, an older study has shown that injection of Shal (Kv4) mRNA into the PD neurons led to the expected increase of I_A but also to an unexpected increase of I_h that resulted in a fixed conductance ratio of the two currents, and a conservation of the action potential latency of PD neurons on rebound from inhibition (MacLean et al. 2003). This co-regulation may be explained by regulation of the translation of the mRNA injected cells by residual 5-HT in the STG, consistent with the observations of Krentz et al (2015).

Neuronal activity is another factor that regulates ionic current levels, as seen in many different cell types and organisms, including pacemaker neurons (Campanac and Debanne 2007; Debanne et al. 1996; Golowasch et al. 1999; Turrigiano et al. 1994). Removing neuromodulatory inputs from a circuit disrupts the resulting intracellular signaling effects, which can also change the neural circuit activity by disrupting the effects of the neuromodulators on essential ionic channels. In the crustacean pyloric CPG, for example, removing all modulatory inputs often disrupts activity or makes it slow and irregular, because a number of neuromodulators activate the persistent inward current I_{MI}, believed to be the network's pacemaker current (Bose et al. 2014; Golowasch et al. 2017). Because neural activity could regulate ionic current expression levels, it is possible that changes in activity, rather than direct influence of neuromodulators, would have caused the decentralization-elicited disruption of correlations observed by Khorkova and Golowasch (2007). In that study, however, activity was ruled out as contributing to the generation of the correlated relationships between ionic currents by separating the effects of neuromodulators on activity from those on intracellular signaling. Tetrodotoxin (TTX), blocks both network activity and the endogenous release of neuromodulators in this system. Under those conditions, correlations are lost. However, the intracellular signaling effects of the

neuromodulators can be restored by applying one of the pepetides exogenously. Indeed, when the neuromodulatory peptide proctolin was bath applied in the presence of TTX, correlations were restored (Khorkova and Golowasch 2007) showing that neuromodulators alone can form ionic current correlations in some cells.

On the other hand, in a different study on the same system it was observed that pilocarpine, an acetylcholine muscarinic agonist that also activates I_{MI}, but that may act through a different intracellular signaling cascade, restored correlations via its effect on activity and not via its paracrine metabotropic effects (Temporal et al. 2014). Thus, it appears that both neuromodulation and neuronal activity can regulate long-term ionic current changes that can lead to correlations of ionic conductances in pacemaker neurons (see Fig. 3). Indeed, a modeling study showed that a number of experimental observations of STG pyloric activity could be well reproduced only if *both* neuromodulation and activity-dependent mechanisms are taken into account (Zhang et al. 2008).

Recently, O'Leary and collaborators reported a simple and elegant mechanism that can generate correlations of maximal conductances of virtually any pair of currents, as well as stable activity, using only an activity-dependent rule that regulates transcription or translation (O'Leary et al. 2013; O'Leary et al. 2014). In summary, while this model captures the existence of ionic current correlations, clearly other rules and mechanisms, such as direct metabotropic effects by neuromodulators, in addition to activity, must be included to account for the observations of the effects of proctolin on PD neuron conductance correlations (Khorkova and Golowasch 2007).

Another important aspect of neuromodulator actions on CPGs is that modulatory neurons can be active members of the network due to feedback from CPG network neurons. (cf. (Blitz 2017; Coleman et al. 1995; Dubuc and Grillner 1989; Frost and Katz 1996)). Thus, the modulatory actions of such neurons can be considered intrinsic neuromodulation (Katz and Frost 1996). For example, Nusbaum and collaborators demonstrated the role of a feedback circuit from a member of the crab STG gastric mill network onto a projection neuron (MCN1) (Fig. 1) (Coleman et al. 1995). While the neuromodulator released by MCN1 is essential to elicit and sustain the rhythmic activity of the gastric mill network, inhibitory feedback onto presynaptic terminals of MCN1 from one of the two rhythm-generating half-center pairs of neurons is key to producing the pattern of activity that characterizes the MCN1-evoked gastric mill rhythmic pattern (Bartos and Nusbaum 1997; Coleman et al. 1995). More recently, Blitz showed that a different feedback from the gastric mill CPG onto another modulatory projection neuron (CPN2) regulates the firing properties of CPN2, and does so in a manner that in turn depends on other modulatory and sensory inputs to the network (Blitz 2017). Blitz concluded that this modulation of CPN2 further affects the output properties of the target CPG, which indicates that the complexity of neuromodulatory regulation of CPGs is considerably higher than previously thought.

Role of neuromodulators in the recovery of CPG activity

Some level of recovery of function after injury occurs throughout the central nervous system in likely all animals (Herman et al. 2018; Luther et al. 2003; Martinez et al. 2011; Molinari 2009; Puhl et al. 2018; Sakurai and Katz 2009; Telgkamp et al. 2002). Considering the grave consequences that the loss of neural activity due to injury or disease has on the behavior and quality of life in humans, a large amount of research is devoted to it. Loss of activity is particularly serious if it involves CPG networks because nearly all rhythmic activities involve vital functions: heartbeat, respiration, locomotion, swallowing, mastication, gastric motility, childbirth, etc. Here I will concentrate only on recovery of activity of oscillatory systems that are likely to involve CPGs, focusing on a few (see Fig. 1) for which some solid experimental evidence exists. To aid in the recovery of function various approaches are employed, including surgery, electrical stimulation, pharmacological and behavioral treatments. Neuromodulators have the potential to play very important roles in the recovery of CPG activity but their role in vertebrates, and mammals in particular, has largely been underestimated, or at least not received much attention.

Several questions need to ultimately be addressed if rhythmic patterns of activity resembling normal patterns (sufficient to sustain a minimum level of normal function and behavior) are to be recovered after an initial insult that disrupts rhythmic activity: 1) Are the mechanisms of recovery dependent on the loss or modification of the neuromodulatory environment? 2) Are they dependent on the disruption of normal electrical activity? 3) Are they dependent on the loss of peripheral, sensory, or motor input? Intertwined with these issues are the exact cellular and molecular mechanisms that lead to the recovery of function in any of these cases. Work on invertebrates suggests that all these factors play an important role, which I will review, with particular emphasis on the role of neuromodulators.

Crustacean stomatogastric system

The decapod crustacean stomatogastric nervous system offers a revealing picture of what role neuromodulators may be playing in the maintenance and recovery of CPG function. Most of the studies so far have concentrated on the pyloric network of crabs and lobsters where it has been shown that most features of pyloric CPG activity recover after the network has been deprived of its neuromodulatory input for an extended period of time (Luther et al. 2003; Thoby-Brisson and Simmers 1998). Although some of these experiments have been recently repeated under somewhat different conditions and with partially different results (Hamood et al. 2015), this activity recovery suggests that neuromodulators are involved in sculpting and regulating rhythm generating capabilities that this (and perhaps other) CPGs naturally tend to express. This possible role of neuromodulators, in turn, suggests that manipulating the neuromodulatory environment of CPGs in general could be used to enhance or re-express rhythmic activity when it is lost.

Almost all neuromodulatory input to the STG arrives via neuromodulator-containing axons running along a single nerve. Conveniently, the study of the function of neuromodulators is facilitated by the fact that neuromodulator release can be stopped by blocking action potentials in these axons by simply cutting the nerve or otherwise blocking action potential conduction along it, which is referred to as 'decentralization'. When decentralized, pyloric CPG activity either slows down or ceases completely (Hamood et al. 2015; Luther et al. 2003; Nusbaum and Marder 1989; Thoby-Brisson and Simmers 1998). Hours later the pyloric CPG often recovers in frequency, typically to somewhat lower than, but sometimes to full, pre-decentralization levels (Luther et al. 2003; Thoby-Brisson and Simmers 1998). The timing of neuronal bursting of the different cells in the network relative to the onset and ending of a cycle of pyloric activity, is referred to as the phase or phase relationships of activity. The recovery of pyloric activity most clearly involves changes in the phase relationships of the different component neurons. These phase relationships before, immediately after decentralization, and during the early stages of recovery are very different from control, but they recover to values indistinguishable from those observed in intact preparations (Luther et al. 2003). These recovery experiments suggest that an internal rearrangement of cellular and molecular properties can take place during a critical period after neuromodulators have been removed. Remarkably, the reorganization of the pyloric network may include the replacement of the pacemaker neuron: recovery of full-blown pyloric CPG activity occurs even if the pacemaker neuron is ablated by photoinactivation (Luther et al. 2003; Thoby-Brisson and Simmers 1998).

It may be argued that recovery of activity simply involves the restoration of some level of neuromodulatory release from cut axon terminals of the neurons containing them. That this is unlikely was demonstrated by showing that photoinactivating these terminals cannot prevent the recovery of rhythmic activity (Luther et al. 2003; Thoby-Brisson and Simmers 1998). Thus, a profound reconfiguration of the network and its components must take place when neuromodulators are removed, but the mechanisms are not known. During the ensuing period, either neurons that only exhibit pacemaker activity in the presence of neuromodulators (conditional pacemakers) may turn into endogenous pacemakers of the network as suggested by Thoby-Brisson and Simmers (2002) or, alternatively, the system may develop network-based rhythmic activity (e.g. become members of a half-center oscillator). These observations suggest that the pyloric network has a broad repertoire of rhythm-generation mechanisms that could be tapped when the CPG loses activity due to injury to one or more of its components.

One important lesson from these experiments seems to be that one of the main roles of neuromodulators in pyloric neurons of the crustacean STG, but perhaps in other systems also, is to restrain most neurons of the network from developing certain properties, such as oscillatory capabilities, while allowing one or a restricted subset of neurons (the pacemaker or pacemaker kernel) to develop and maintain them. This restraint can then be released in their absence. That this may be part of the mechanism involved is supported by experiments with cultured neurons from the STG, both in lobsters and in crabs, where all neuromodulatory inputs were removed by the dissociation procedure. Newly dissociated cells lost their ability to generate both action

potentials and oscillatory activity. Nevertheless, while we know that the STG only has one pacemaker neuron (the pyloric network pacemaker AB neuron, (Hooper and Marder 1987)) over a few days in minimal culture conditions the vast majority of the cells developed oscillatory activity, with frequencies close to those observed in the pyloric network (Haedo and Golowasch 2006; Turrigiano et al. 1994), while retaining their ability to respond to acute application of neuromodulators (Golowasch et al. 1990; Turrigiano and Marder 1993). It is not known at this point if neuromodulator absence, by lifting a restraining effect on the development of oscillatory properties, is the sole driving force behind the recovery of oscillatory activity in these neurons. The change in activity of dissociated neurons (i.e. they all initially lose their ability to burst and most their ability to spike) may be part of the mechanism driving the recovery of oscillatory activity. This is suggested by the fact that rhythmic stimulation can revert bursting to tonic firing (Haedo and Golowasch 2006; Turrigiano et al. 1994), or sometimes accelerate the acquisition of bursting properties (Haedo and Golowasch 2006).

What are the molecular and cellular changes leading to the recovery of activity? One of them is the enhancement of neuromodulator sensitivity (a form of “denervation sensitization”), which can be attributed to the dramatic reduction of agonist concentration (Lett et al. 2017). Lett and collaborators tested the responsiveness of a pyloric (lateral pyloric, LP) neuron to crustacean cardioactive peptide (CCAP) after decentralization and found it to be enhanced when CCAP alone was removed, but further enhanced when additional neuromodulators were removed. The effects where observed at the level of the responsiveness to exogenous CCAP applications (it increases), the number of CCAP receptor RNA copy numbers (it increases), as well as RNA copy number changes of at least two of the voltage-gated channels expressed by LP neurons (Lett et al. 2017). These results again reflect a large reconfiguration of a number of molecular components in the continuous absence of the neuromodulators that normally bathe the pyloric neurons. It seems clear that neuromodulators control the expression levels of their own receptors but, importantly, also those of other receptors, as well as a diversity of ionic channels (Khorkova and Golowasch 2007; Lett et al. 2017; Mizrahi et al. 2001; Thoby-Brisson and Simmers 2002; 2000). Furthermore, this reconfiguration affects not only the protein expression levels (whether of receptors or ion channels), but also their distribution within the different neuronal compartments (Berger et al. 2001; Mizrahi et al. 2001).

Thus, recovery experiments suggest that neuromodulators play a crucial role in the generation and maintenance of pyloric CPG activity under normal (non-decentralized) conditions when they are continuously present, but can become unnecessary after a prolonged period of their absence. These observations suggest that the pyloric system, and perhaps other systems too, can configure, and reconfigure, itself to generate the same CPG activity in multiple different ways. Although the experiments described above and a number of others suggest that that may be the case, there are other possibilities that must be considered: 1) an increased sensitivity to circulating hormonally or locally released substances (Lett et al. 2017), 2) a renewed release of neuromodulators localized in surviving terminals within the ganglion,

perhaps aided by newly developing glia-neuron interactions (Parnas et al. 1998) (although recovery still occurs if all terminals are ablated as indicated before), 3) the expression of new or enhanced expression of existing neuromodulators (Fukamauchi and Kusakabe 1997), and/or 4) the constitutive activation of existing receptors or signaling pathways (Murray et al. 2010).

I suggest a fifth alternative: in the absence of neuromodulators the system is released from particular restraints, which lead to rapid changes of specific molecular components, allowing the system to wander in parameter space towards a new set of parameter values that permits it to generate CPG activity independent of the participation of neuromodulators (Fig. 4). As described before, neuromodulators are known to constrain the maximal conductances of various ionic currents (and of the mRNA levels that code for the channels that carry these currents) in populations of identified neurons to strict relationships (i.e. linear correlations) between different current types (Golowasch 2014; Khorkova and Golowasch 2007; Schulz et al. 2007). This has the consequence of reducing the global variability of ionic current levels mentioned before in that the variance of each ionic current is enslaved to the variance of other currents. The likely functional consequence of this is a reduction of physiological output variability (CPG frequency, phase relationships, etc.) as the relative conductance levels are kept constant (Golowasch 2014; Hudson and Prinz 2010; Prinz et al. 2004). In fact, the variability of the output of the pyloric network greatly increases in decentralized (but still rhythmic) preparations (Hamood et al. 2015). When neuromodulators are removed, some of these correlations are lost in a cell-type specific manner (Khorkova and Golowasch 2007; Temporal et al. 2012), and this may allow the system to find different regions in parameter space (and different mechanisms) that provide the same solution, i.e. the generation of pyloric activity (see Fig. 4) (Prinz et al. 2004). Thus, although theoretical (Hudson and Prinz 2010) and experimental work (Ransdell et al. 2012) indicates that the co-regulation and balance of conductances is important for the production of stable oscillatory activity in pacemaker cells and CPG networks, it is also possible that conductance correlations change (Temporal et al. 2012) or new ones are created during the process of recovery of activity. Furthermore, it is possible that, in the absence of neuromodulators, other mechanisms yet to be uncovered, which do not necessarily result in conductance correlations, can stabilize activity.

Another sign of deep restructuring of the pyloric network and its physiology following decentralization is the fact that after prolonged removal of neuromodulatory input, the network does not easily recover to its pre-decentralization responsiveness to neuromodulation (Nahar et al. 2012). This was tested thanks to the fact that decentralization can be performed reversibly. The authors conclude that either it is the reconfiguration of the pyloric network, or the networks of neuromodulator-containing neurons, which receive input from the target pyloric network itself (Blitz 2017; Wood et al. 2004), which may be more or less permanently modified (Nahar et al. 2012).

Finally, the fact that neuromodulatory input also regulates the levels and patterns of activity (Marder and Weimann 1992), requires that the effects of activity deprivation and

neuromodulator deprivation are carefully separated. In the lobster pyloric system this has been examined, and recovery, in fact, also occurs if oscillatory activity is kept high with high external K^+ concentration (Thoby-Brisson and Simmers 1998) suggesting that the absence of activity is not the main driving force behind this recovery but that what is key is the absence of neuromodulation.

Tritonia swimming

In the mollusk *Tritonia diomedea* a CPG that controls swimming crucially depends on a pedal ganglion interneuron (C2) synaptically exciting another interneuron (VSI) located on the contralateral pedal ganglion via axons running along pedal nerve 6 (PdN6) (Fig. 1). Fictive swimming can be elicited by exciting C2 (by stimulation of pedal nerve 3 (PdN3)) and it depends on the integrity of the axons connecting both sides that run along nerve PdN6 (Sakurai and Katz 2009). Thus, when PdN6 is cut or action potential transmission is blocked, swimming and also excitation of the contralateral VSI is disrupted because the swimming CPG now fails to become activated by PdN3 stimulation (Sakurai and Katz 2009). However, only a few hours later, both CPG and fictive swimming can be activated by brief stimulation of PdN3. How is this possible? C2 and VSI neurons make compound synaptic connections on both ipsi- and contra-lateral pedal ganglia, but the synapse in the ipsilateral ganglion is dominated by a primarily inhibitory component while that on the contralateral ganglion is dominated by an excitatory component. After separation of the two ganglia by transection of the PdN6 nerve, a fast reduction of the inhibitory synaptic component on the ipsilateral ganglion ensues, making the ipsilateral connection predominantly excitatory and capable of activating the swimming CPG (Sakurai and Katz 2009).

Although the authors of this study do not provide evidence for the molecular triggers that lead to these changes, they argue that changes in either activity or neuromodulation may be the leading factors (Sakurai and Katz 2009). As a model of the contribution(s) of these two factors to the full recovery of rhythmic activity it deserves to be carefully examined. This study also illustrates a distinct mechanism from that described for the pyloric network in that it is the change of synaptic properties and apparently not intrinsic properties in this case that leads to the restoration of oscillatory activity and swimming behavior. It is worth noting that in the pyloric network changes in synaptic strength as a consequence of neuromodulator removal have also been reported (Thoby-Brisson and Simmers 2002).

Gastropod feeding networks

Thus far, activity recovery observations in gastropods have focused on axonal regeneration. For instance, Sanchez et al (2000) have found that feeding activity in *Aplysia* recovers after the cerebral to buccal commissural nerves are crushed, which removes the modulatory (gating or

command) input from CBI-2 interneurons onto the feeding buccal ganglion network (see previous section and Fig. 1). However, it would be interesting to consider the effect of permanently eliminating some of these neuromodulatory neurons and ask if rhythmic activity can be recovered by some alternative compensatory mechanism. Another interesting cell in this regard is neuron B48 in *Aplysia*. This neuron is not an integral member of the core CPG. However, it contains two leukokinin peptides, which have a strong effect on one of the core neurons of the feeding CPG, neuron B64 (Fig. 1), enhancing its activity, and thus accelerating the termination of the protraction phase (Zhang et al. 2017), even though it is not known yet if these are direct effects of peptides released by the B48 neuron. On the other hand, the SPTR-Gene Family-Derived Peptides also have a similar accelerating effect in terminating protraction, but the sources of the modulatory peptides have been identified to be from CBI-12 interneuron (Zhang et al. 2018), and examining the role of eliminating this source should be interesting. In *Lymnaea*, the SO and N1L interneurons would be interesting to consider in this regard since SO is not typically considered to be an integral member of the core CPG in *Lymnaea*, while N1 neurons, especially N1L, are. Additionally, N1M, which is part of the core CPG (Fig. 1) releases the intrinsic neuromodulator buccalin. It would be interesting to test what role buccalin plays in maintaining the feeding rhythm or regulating the parameter space, and perhaps recovery from perturbations, of the feeding network. Removing these modulatory neurons using a cell inactivation method (e.g. photoinactivation) might yield interesting observations about the difference in homeostatic responses when intrinsic, or alternatively, extrinsic modulatory neurons to the feeding networks are ablated.

The mammalian respiratory system

As mentioned earlier, respiratory CPG activity in mammals is generated by a network of inspiratory neurons localized in the preBötC that express a combination of several inward non-linear currents, which in conjunction with synaptic excitation dynamically organizes its rhythmic activity (Anderson and Ramirez 2017; Ramirez and Baertsch 2018b). Two main groups, located in the preBötC and the PiCo, respectively (sometimes with a third group located in the RTN/pFRG, Fig. 1) are targets of neuromodulatory inputs (Anderson et al. 2016; Doi and Ramirez 2008; Mellen et al. 2003; Ramirez et al. 2012) that can change the properties of the respiratory activity. For example, the network can reconfigure during hypoxia to produce gasping, a rhythm that is more dependent on I_{NaP}, but which also requires serotonin (Pena et al. 2004; Tryba et al. 2006). As a consequence, disruption of neuromodulator-containing neurons that target inspiratory neurons can be expected to have profound effects on the quality of the breathing CPG and its recovery when disrupted. Here I will assume that disruption of eupneic activity, however transient or persistent, can be considered an insult to the breathing CPG, and will consider what role neuromodulators play in its recovery.

In the respiratory system the regularity of the respiratory pattern greatly depends on neuromodulatory input to the system. For example, blocking the substance P tachykinin

receptor NK1R found in the preBötC reduces the frequency as well as the regularity of eupneic respiratory activity via effects on the NaLCN channel (Hilaire et al. 2003; Telgkamp et al. 2002; Yeh et al. 2017), reminiscent of the effects of decentralization of the crustacean pyloric network. 5-HT acting on 5-HT_{2A} receptors (Pena and Ramirez 2002), and norepinephrine (NE) acting on both alpha1- (St-John and Leiter 2008) and alpha2-adrenergic receptors (Zanella et al. 2006) also contribute to the regularity of eupneic respiratory activity as revealed by neuromodulator deprivation experiments. Is there a difference in the effects of short-term versus long-term neuromodulator deprivation, perhaps comparable to the long-term effects of decentralization in the crustacean pyloric network? Indeed, Telgkamp and collaborators have shown that, while acute blockade of NK1Rs significantly slows down eupneic activity, chronic inhibition of the synthesis of the tachykinins substance P and neurokinin A (NKA) leads to what appears to be a compensatory reconfiguration of the respiratory network. This was examined thanks to the availability of a mutant mouse (PPT-A), which lacks the gene PPT-A that codes for the tachykinin precursor protein. It turns out that PPT-A mice express essentially normal eupneic activity, with frequency and variability indistinguishable from wild-type mice under normal oxygen levels, although PPT-A mice respond abnormally to anoxia, showing an increased irregularity of eupneic episodes and a significantly reduced capacity to generate autoresucitatory sighs compared to wildtype mice (Telgkamp et al. 2002). Thus, in the absence of a key neuromodulator, the system appears capable of reconfiguring itself to a new state in which it can generate respiratory activity comparable to that of normal animals. Interestingly, the new network that emerges in this homeostatic process is clearly different, as illustrated by their inability to respond to certain perturbations (*e.g.* anoxia) like the normal animal. Although the cellular and biophysical mechanisms have not been identified, these reports suggest that the network possesses mechanisms that are plastic enough to homeostatically engage and to compensate for the loss of neuromodulators or neuromodulator receptors necessary for the generation of normal respiratory activity (Doi and Ramirez 2008).

Gasping is a vital pattern of respiratory activity, typically evoked by hypoxia, which results in increased air intake and sometimes recovery of normal eupneic activity (autoresuscitation). This pattern appears to be strongly regulated by neuromodulators 5-HT (via 5-HT_2 receptors), and NE (via alpha-1 adrenergic receptors), which are required to sustain gasping after hypoxia-induced depression (St-John and Leiter 2008). This is likely mediated by modulatory effects of 5-HT and NE on riluzole-sensitive channels (thus on I_{NaP}) since during gasping cadmium-sensitive neurons are not involved in pattern generation (Koch et al. 2011). Gasping in patients at risk of Sudden Infant Death Syndrome (SIDS) is significantly reduced. This is suggested, for example, by the increased incidence of pathological signs (e.g. chronic hypoxia-induced gliosis) in patients who die of SIDS (Kinney et al. 2009). The risk of SIDS incidence appears to be associated with mutations in the promoter of the 5-HT transporter protein gene, as well as abnormalities in 5-HT receptor expression in the medulla (Kinney et al. 2009; Poets et al. 1991; Weese-Mayer et al. 2003). It is not known if compensatory mechanisms that can bypass the 5-HT regulatory pathway exist. However, it would be interesting to examine in experimental animals whether

manipulation of NE, 5-HT or other neuromodulatory paths can lead to protection from disruption of the 5-HT transporter protein or 5-HT receptor expression in the medulla and ultimately reduced risk of SIDS.

Rett syndrome patients and mouse Rett syndrome models (Mecp2-/y) have a mutated *Mecp2* gene, which encodes methyl-CpG-binding protein 2 (MECP2). These patients suffer from severe reductions in tyrosine hydroxylase (TH) and NE expressing neurons in the medulla (Viemari et al. 2005), reduced levels of 5-HT and DA (Koch et al. 2011), as well as substance P in the cerebrospinal fluid and brain stem (Dunn and MacLeod 2001). It is not currently known if any compensatory mechanisms similar to those described by Telgkamp et al (2002) are activated. Nevertheless, the existence of such compensatory mechanisms involving the regulation of neuromodulatory pathways in respiratory networks and elsewhere suggests that they could be induced or activated as a therapeutic approach to treat or reduce the risk of this disease, which ought to be further explored. One research path that could be examined is whether Rett syndrome patients or its mouse model, develop a phenotype similar to those of PPT-A mutant animals since they have brain stem deficiencies in substance P levels.

Recovery of locomotion CPG in vertebrates

The vertebrate locomotion CPG is thought to be a widely distributed network of interacting CPGs, all receiving descending projection inputs, for the most part neuromodulatory in nature, originating in the brain or supraspinal regions (Fig. 1) (Molinari 2009). A number of neuromodulators are involved in the activation of the mammalian locomotion CPG, with the main focus of research until now being on the role of aminergic modulators, NE and 5-HT, and a few exogenous peptides (Jordan and Slawinska 2011; Rossignol et al. 2011). 5-HT appears to control the excitability and activity mostly of inhibitory local spinal cord neurons (Jordan and Slawinska 2011). After spinal cord injury (SCI), 5-HT and NE hypersensitivity is observed that could drive some degree of functional recovery (Rossignol and Frigon 2011). However, the largest effort towards treating SCI cases have been devoted to understanding how to upregulate axon regeneration and identify the conditions for appropriate re-innervation are (Bradbury and McMahon 2006; Rossignol and Frigon 2011). Along this line of inquiry, it appears that peripheral input (both sensory and motor) may play an important role, and that seems to be at least partially under modulatory (*e.g*. DA) influence (Rossignol and Frigon 2011). Although not a vertebrate system, the leech locomotor system, which is also composed of a distributed network of CPG components that is driven in part by DA, provides an interesting example of functional recovery when devoid of descending signals. Recovery of crawling activity in leech (*i.e*. intersegmental coordination) occurs after full transection of the descending inputs. Interestingly, this involves regeneration of sensory axons that take over part of the coordination of activity between CPGs along the ventral cord (Puhl et al. 2018). In adult fish, generation of spinal motor neurons seems to be greatly influenced by dopaminergic projections, which occurs at the expense of interneurons both during development as well as in

the adult (Reimer et al. 2013). Such axonal regeneration seems to be sufficient for full recovery of swimming, which is also observed in lampreys (Herman et al. 2018).

Norepinephrine, which fully originates in the brain, is thought to be required to activate the mammalian locomotor CPG since the CPG can be activated by simple intraperitoneal or intrathecal injection of α_2AR agonists (*e.g*. clonidine) in acutely or chronically, partially or fully, spinalized cats, even though the exact details of the effects vary depending on the state of the preparation (Rossignol et al. 2011). Interestingly, in spite of the fact that NE clearly plays an important role in CPG activity, and that NE all but disappears from the spinal cord below a completely severed cord, it appears that the role of NE in the recovery from injury has not thoroughly been tested. If the work described above in decentralized pyloric networks and the respiratory network deprived of substance P are considered, it would be very interesting to examine the effect of depletion of NE or other neuromodulators *before* SCI. If one of the important long-term roles of modulators is to restrict the state that the networks can adopt, as I suggest here, removing them may then free the networks from some of its constraints and allow them to visit alternative states from which a recovery to a state somewhat similar to a pre-SCI may be a possibility. One important fact to consider, highlighted by the work of Telgkamp et al (2002) with the respiratory network, is their suggestion that depression of the tachykinin signaling pathways leads to a compensatory enhancement of other neuromodulator pathways. Although this suggestion still needs to be tested, it opens the possibility that in locomotor (or any other) networks, one should not necessarily expect to see an enhancement of one pathway (*e.g*. the NE pathway) when the levels of the modulator or receptors of that pathway are depressed (*e.g*. NE or NE receptor levels) as a result of SCI. Instead, other pathways may take over in compensation. It would be interesting, for example, to examine potential recovery of function (rates and degrees of recovery) in Rett syndrome patients (or model animals) in response to SCI. Since these patients have severely depressed neuromodulatory systems, they may be primed to recover faster if other neuromodulatory systems have been upregulated as a result of the disease prior to the SCI.

It is conceivable that proper integration of regenerating fibers in the injured spinal cord can happen only under the appropriate neuromodulatory environment. Thus, it would be important to test the effects of SCI on reinnervation (CPGs activity) in animals in which specific neuromodulator pathways have been manipulated (depleted or overexpressed) beforehand. This may prepare the networks to be in a more receptive state to receive the new innervations.

In general, it has been known for some time that a number of compensatory mechanisms in diverse systems are revealed by knockout experiments, some involving neuromodulatory systems (Fukamauchi and Kusakabe 1997; Marvel et al. 2018), some not (Chan et al. 2007; Kim et al. 2015). This body of evidence strongly suggests that the level of compensatory plasticity in the nervous system is great and that more needs to be done to understand it and to tap into it in malignancies involving neuromodulatory systems.

Neuromodulation, plasticity and recovery of function

Thus far I have made the claim that neuromodulators participate heavily in configuring networks involved in CPG activity. Most of the evidence presented comes from experiments in which neuromodulators are removed, resulting in CPG activity and neuromodulator tone loss, and subsequent network reconfiguration with resulting recovery of activity. Alternatively, of course, neuromodulators may be important to elicit the recovery of CPG activity. To my knowledge this later alternatively has not been shown to occur in CPG networks. The best and nearly exclusive evidence so far for such claim is a large body of literature claiming that neuronal plasticity is enhanced by neuromodulators. Because all the evidence to my knowledge is focused on synaptic plasticity, often in the context of learning and memory, I refer the reader to some of the most recent reviews on the subject (Creed 2018; Foncelle et al. 2018; Palacios-Filardo and Mellor 2018; Pawlak et al. 2010; Prince et al. 2016; Sebastiao and Ribeiro 2015). Nevertheless, the role of the presence of individual or subsets of neuromodulators on plastic processes that can lead to the recovery of lost CPG activity is of course an exciting avenue for research.

Concluding remarks

Several model systems, both vertebrates and invertebrates, have been used to examine the compensatory mechanisms activated by neuromodulators or their loss in rhythm-generating networks or CPGs. In particular, invertebrate systems afford networks with far fewer components (neurons and synapses), which make the understanding of the roles of these components significantly easier than vertebrate systems with their much larger numbers of such components. Given the crucial functions of CPGs in many vital functions, work on as many such model systems as possible should be pursued in order to understand possible ways in which CPGs are regulated, both by neuromodulators and by activity.

I have reviewed some principles highlighted by work primarily in the crustacean pyloric network, but also mammalian respiratory networks and others, which are heavily modulated. In particular, the pyloric network is modulated by numerous substances whose effects and, to some degree, mechanisms of action are known in some detail. I propose one general principle: neuromodulators over long stretches of time appear to constrain the parameter space in which CPGs operate. This restricts which neurons may behave as pacemakers, which synapses may be active and which not, what ionic currents are expressed in which cells and to what levels. I suggest that when neuromodulators are removed, together with the loss of function that often ensues, these parameter spaces are expanded. This then allows a CPG and its component elements to wander within these larger parameter spaces and sometimes land on a different region in this space – with a different combination of parameters – that allows it to perform a similar function to that which has been lost. The mechanisms that restrict these parameters spaces, and those that enable their relaxation, need to be much better understood.

I believe that a systematic approach to remove or alter the expression of specific neuromodulators from distinct regions of the nervous system in a carefully targeted manner should be undertaken to examine their roles in triggering compensatory mechanisms that may be useful in restoring disrupted neuronal CPG activity. New technologies, such as targeted expression of genes or gene inactivation and optogenetic tools should make this possible.

Figures Legends

Figure 1. *Connectivity diagrams of model systems used to study CPGs*. All diagrams are significantly simplified for illustration purposes. Common to all is the important role of neuromodulators, either as gating extrinsic elements in all the CPG networks (orange downward arrow) or as intrinsic to one of the members of the CPG (e.g. in the *Lymnaea* feeding network). Top row illustrates two networks based on the operation of pacemaker neurons, which are the main source of rhythmic activity (enclosed in gray circles with arrowhead symbolizing repetitive activity): one that uses a single pacemaker neuron (pyloric network) and the second (respiratory network) consisting of three neuronal populations with pacemaking properties of various strengths, which are organized dynamically cycle by cycle by the interplay of intrinsic properties and synchronizing excitatory synaptic connections (together with reciprocal inhibitory connections). In the bottom two rows are examples of fundamentally network-based CPGs, which normally rely on half-center reciprocally inhibiting pairs of neurons or populations of neurons. In the crustacean gastric mill network, two neurons (LG and Int1) form the core of the CPG (gray circle) but rely on modulatory input of neurons (MCN1) whose axons release modulators onto and receive chemical and electrical feedback from the core CPG. All other networks shown have a core CPG composed of more neurons than the key ones that are depicted. In the case of the vertebrate locomotion network, each limb is controlled by a large number of coupled interneurons (white circles) and several half-centers are thought to exist (gray circles), necessary to control the multiple antagonistic muscle groups. In the case of the *Tritonia* escape swim network, a crucial dual synapse between CPG neurons C2 and VSI occurs in ganglion (the pedal ganglion) different from where their cell bodies are located. Note that the respiratory network is also a network of interconnected neurons, but many of those can be considered pacemaker neurons. For nomenclature and general reference see: Crab pyloric and gastric mill networks (Marder and Bucher 2007), mammalian respiratory network (Ramirez and Baertsch 2018b), vertebrate locomotion network (Grillner 2006b), *Tritonia* escape swim network (Sakurai and Katz 2009), *Lymnaea* feeding network (Benjamin 2012), *Aplysia* feeding network (Sasaki et al. 2013). AB, Anterior Burster; PD, Pyloric Dialator; LP, Lateral Pyloric; PY, Pyloric Constrictor; Pre-BötC, Pre-Bötzinger Complex; PiCo, post-inhibitory complex; RTN/pFRG, retrotrapezoid nucleus/parafacial respiratory group; Int1, Interneuron 1; LG, Lateral Gastric; DSI, Dorsal Swim Interneuron; C2, Cerebral Neuron 2; VSI, Ventral Swim Interneuron; N1M, N1L, Medial, Lateral Interneuron 1; N2v, Ventral Interneuron 2; N3t, Tonic Interneuron 3; Bxx, Buccal neuron xx.

Figure 2. *Balance of ionic current levels is required for pacemaker activity*. **A**. PD neuron, a member of the crab pyloric network pacemaker kernel, oscillates readily when a pacemaker current is injected into it with dynamic clamp. **B**. Example of a follower neuron (LP neuron) injected with pacemaker current (same as in A) in dynamic clamp, showing that pyloric follower neurons are incapable of generating oscillations under similar conditions as the pacemaker neurons. **C**. Left shows the voltage-clamp measurement of the high threshold K^+ current (I_{HTK}) in

one PD neuron (voltage steps at the bottom). Right shows the average I-V curves from all the recorded PD neurons (black symbols and lines) and all the recorded LP neurons recorded (gray symbols and lines), showing the significantly smaller levels of I_{HTK} in PD than LP neurons. **D**. The LP neuron shown in B expresses oscillatory activity when the same amount of pacemaker current is injected with dynamic clamp but only after blocking part of I_{HTK} with tetraethylammonium, TEA. Top traces in A, B and D are membrane potential, bottom traces are dynamic clamp injected current. Details in (Golowasch et al. 2017) from which this figure has been modified.

Figure 3. *Both activity and neuromodulators can control the slow process of transcription that leads to correlated expression of sets of ionic current*. Activity, putatively via changes in intracellular Ca^{++} concentrations ($[Ca^{++}]$) due to modifications of plasma or intracellular compartment (ER) membrane Ca^{++} currents ($G_{Ca}(V_m)$, IP_3RCa, respectively) regulate activity-dependent signals (enzymes or regulatory sensors or factors, S_A) that can result in the parallel regulation of transcription (as shown here, but translation and even post-translational modifications can be envisioned also) of multiple ion channel genes (here only two, $G_1(V_m)$ and $G_2(V_m)$, are shown, but others including $G_{Ca}(V_m)$ and IP_3RCa themselves could be included). At the same time, activation of neuromodulatory receptors (R_{NMod}) can activate different signaling cascades (S_{NM}) that can regulate the transcription of sets of ionic channels, which may or not be the same as those activated by activity. These two types of regulation of transcription (Blue) have to be slow compared to other regulatory or activating signals (Black, Orange). Neuromodulator receptors can of course also rapidly activate specific ion channels, $G_{NMod}(V_m)$. Sensory or other input (e.g. synaptic) can modify the membrane potential (arrows pointing at V_m), which in turn can change the activation of additional voltage-gated ion channels. This process is assumed to be fast (centered on V_m of the right side of the diagram), and these conductance changes can move up and down relatively independently from the other slow processes. However, they are not disconnected since the activity changes thus induced can influence the slower transcription regulation processes via S_A (left side of the diagram). S_{NM}, Intracellular Neuromodulator Sensor; S_A, Intracellular Activity Sensor; IP_3RCa, IP_3 Receptor-activated Ca^{++} Current; ER, Endoplasmic Reticulum; mRNA(G_x), mRNA coding for conductance x; G_{syn}, Synaptic Conductances.

Figure 4. *Influence of neuromodulators on neuronal and network parameter space*. These diagrams illustrate the proposal that one function of neuromodulators in a neuronal network can be to restrict the parameter space in which its components operate. **A**. In the presence of neuromodulators, a neuron has an appropriate balance of parameters A and B (*e.g*. ionic conductances correlated along a positively sloped distribution), which enables it to act as a pacemaker (cell 1, shown as a circle with an arrowhead representing repetitive activity). Cells 2 and 3 also have restricted distributions of parameters C, D and E, F, respectively; synaptic strengths (shown as inhibitory but which could in principle also be excitatory) are indicated by the presence of solid lines and their thickness. This diagram is based on the core of the crab pyloric rhythm-generating network, but with appropriate modifications could be any network in

any system (e.g. Fig. 1). **B**. In the absence of neuromodulators, the linear distribution of parameters A and B in cell 1 has been lost and the cell has consequently also lost its ability to oscillate, a new synapse between cells 2 and 3 has been activated or enhanced, the parameter space occupied by parameters C-D (cell 2) and E-F (cell 3) have expanded, and cell 3 has lost the correlation of parameters E and F. However, the appearance of a new synapse between cells 2 and 3 is meant to illustrate the possible shift in the mechanism of generation of pacemaking activity from a pacemaker cell to a half-center oscillator (gray oval with arrowhead). Alternatively, with appropriate changes of conductance relationships, either cell 2 or 3 could become a pacemaker and rhythmically drive the entire network (not shown).

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