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DYNAMIC SIGNALING BETWEEN ASTROCYTES AND NEURONS

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■ **Abstract** Astrocytes, a sub-type of glia in the central nervous system, are dynamic signaling elements that integrate neuronal inputs, exhibit calcium excitability, and can modulate neighboring neurons. Neuronal activity can lead to neurotransmitter-evoked activation of astrocytic receptors, which mobilizes their internal calcium. Elevations in astrocytic calcium in turn trigger the release of chemical transmitters from astrocytes, which can cause sustained modulatory actions on neighboring neurons. Astrocytes, and perisynaptic Schwann cells, by virtue of their intimate association with synapses, are strategically positioned to regulate synaptic transmission. This capability, that has now been demonstrated in several studies, raises the untested possibility that astrocytes are an integral element of the circuitry for synaptic plasticity. Because the highest ratio of glia-to-neurons is found at the top of the phylogenetic tree in the human brain, these recent demonstrations of dynamic bi-directional signaling between astrocytes and neurons leave us with the question as to whether astrocytes are key regulatory elements of higher cortical functions.

BACKGROUND

Since the original discovery and description of glia, these non-neuronal cells were largely ignored or believed to play roles in the nervous system that were subservient to those of neurons. The lack of understanding of their functional roles probably resulted from the inability of the available techniques to reveal dynamic properties of these cells. However, even during the early period of glial investigations, Cajal recognized that they were likely to have roles beyond passive functions.

> The prejudice that the relation between neuroglial fibers and neuronal cells is similar to the relation between connective tissue and muscle or gland cells, that is, a passive weft for merely filling and support (and in the best case, a gangue for taking nutritive juices), constitutes the main obstacle that the researcher needs to remove to get a rational concept about the activity of the neuroglia.
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> —*S Ramón y Cajal* (1)

During this initial period of documentation of the structural features of the nervous system, significant focus turned to neurons as a result of the recognition that their electrical excitability permitted them to convey relevant information in the nervous system. In contrast, as anyone who has recorded from glial cells knows, the membrane potential of glia is relatively stable, and although they can express voltage-gated channels (2–6), they exhibit little or no fluctuation in membrane potential. Indeed, early electrophysiological studies demonstrated that glial cells were electrically inexcitable and responded only passively to neuronal activity by sensing extracellular potassium levels (7). Only during the past 10 to 15 years, with the advent of molecular biology, the patch-clamp technique, and ion imaging methods have the potentially diverse dynamic roles of glial cells begun to be appreciated. Our objective in this review article is to focus attention on one of the most recently identified roles of glial cells—as integrators and modulators of neuronal activity and synaptic transmission.[4]

Glial cells of the central nervous system can be divided into two groups, micro- and macroglia. Microglia are macrophage-like cells that serve a phagocytic function. Macroglia are composed of two types of cell; oligodendrocytes, which are the central equivalent of the myelinating Schwann cell, and astrocytes, the glial cells, which are the focus of much of our discussion. Additionally we will include an examination of the properties of perisynaptic Schwann cells because they intimately interact with the nerve muscle junction in a manner akin to the astrocyte and the central synapse.

PROXIMITY OF ASTROCYTES TO NEURONS

Astrocytes are positioned to act as a conduit for the routing of signals between different cell types in the nervous system (Figure 1). Because the same astrocyte can make contact with a neuron and a capillary, it has the potential to shuffle nutrients and metabolites between the blood supply and the active neuron. Indeed, this structural association led to the initial ideas about astrocytes playing metabolic roles in the nervous system. Furthermore, because a single astrocyte can make contacts with multiple neurons, these non-neuronal cells are also positioned to

[4]Color images and digital movies that supplement this review are available on the World Wide Web in the supplemental section of the main Annual Reviews site (http://www.AnnualReviews.org).

provide information transfer between neighboring neurons, an intriguing potential role that has only been recently appreciated. Examination of the nervous system at the ultrastructural level has shown that astrocytes can be intimately associated with the synapse, literally enwrapping many pre- and postsynaptic terminals. In the hippocampus, 57% of the axon-spine interfaces are associated with astrocytes (8). In the cerebellum there are eight Bergmann glia for each Purkinje cell, and each Bergmann cell ensheaths between 2142 and 6358 synapses (9). It is likely that this close physical relationship provides an opportunity for many functional interactions between astrocytes and neurons. We discuss the potential role of astrocytes in integrating synaptic signals and providing feedback responses, in the form of the release of the transmitter glutamate, which regulates neuronal excitability and synaptic function (10). However, our focus on this area is not meant to diminish the importance of other roles of astrocytes, for example, in the clearance of elevated extracellular K^+ from the extracellular space (11), as well as in the uptake of neurotransmitters from the synapse (12, 13). However, since these functional roles, as well as other metabolic and structural functions, have been the topic of many review articles, our discussion of these issues is limited.

Astrocytes Possess a Form of Excitability Based on Variations of Intracellular Calcium

The development of optical probes and methods to study intracellular ions in living cells provided the technological capability to make breakthroughs in our understanding of astrocyte functions. In the late 1980s and early 1990s numerous studies demonstrated that astrocytes exhibit calcium excitability. Calcium-imaging studies changed our view of astrocytes. These investigations demonstrated that astrocytes, once thought to be relatively passive cells, express functional receptors for many different neurotransmitters that lead to changes in intracellular calcium levels and even to oscillations in internal calcium (14–23).

In many examples, neurotransmitters elevate astrocytic calcium levels as a result of the release of calcium from internal stores. For example, ATP, acting through $P2Y_1$ receptors, and presumably the GTP-binding protein G_q, activate phospholipase C, leading to the formation of inositol triphosphate (IP_3) (24). Subsequently IP_3 activates the IP_3 receptors of endoplasmic reticulum calcium stores, leading to the release of calcium into the cytosol. This IP_3-mediated release of calcium from internal stores is a mechanism that is shared by many neurotransmitters including glutamate, ATP, and acetylcholine (ACh) (14, 23).

Many of the initial studies demonstrating calcium excitability of astrocytes were performed in cell culture, which raised concerns about whether this property was representative of the physiological state of these cells in the intact nervous system (14). Studies using acutely isolated brain slices have supported the calcium excitability property of astrocytes and have demonstrated that transmitters including glutamate, γ-aminobutyric acid (GABA), norepinephrine, histamine, ATP, and ACh do regulate the internal calcium levels of astrocytes (14, 16, 23, 25–33).

Waves of Calcium Elevation Within and Between Astrocytes

Calcium elevations in astrocytes can propagate along the processes of the cell so that a localized elevation of internal calcium can eventually initiate calcium signals throughout the processes of that cell (Figure 2). This wave of intracellular calcium elevation may be important because, like the neuronal action potential, it might relay information to other regions of the cell about inputs located on distant processes.

In cell culture, intracellular calcium waves have frequently been shown to propagate between astrocytes and cause a wave of calcium elevation that can continue for hundreds of micrometers (20, 34–37). The mechanism of generation of the intercellularly propagating calcium wave has been the focus of significant debate. Several studies have suggested a role for gap junctions in mediating intercellular signaling in such calcium waves, whereas more recently, a role for an extracellular signal has become appreciated. Because addition of gap junction blockers can block calcium waves, it was initially hypothesized that gap junctions permit the intercellular spread of IP_3 between neighboring cells. In support of this hypothesis, it has been demonstrated that glia cell lines, which do not form gap junctions and do not exhibit calcium waves, can be made to do so by the expression of connexins, which permits the formation of gap junctions (38). While these data support a role of gap junctions in calcium wave propagation, increasing evidence supports the presence of an extracellular signal. For example, calcium waves were shown to cross cell-free gaps between cells (39), the direction of a wave could be affected by local perfusion of the extracellular saline (39), and waves were still present in cultured astrocytes of connexin 43 knockout mice (40, 41). Furthermore, purine receptor antagonists or extracellularly applied Apyrase (an ATPase) decreased the propagation of the calcium wave (36, 42). Additionally, studies using luciferin and luciferase demonstrated that ATP can be released from astrocytes during a calcium wave, suggesting that ATP is the extracellular signal involved in the calcium wave propagation (36, 42).

Thus evidence supports the possibility of an important role for gap junctions, as well as extracellular ATP, in mediating calcium waves. Although these data seem contradictory, the expression of connexins increases the release of ATP from astrocytes (42, 43). How the connexins couple to ATP release is unknown, but its expression helps put together a rational explanation for data that had seemed contradictory. Despite this convergence of data, intercellular diffusion of messengers may also, under certain conditions, mediate some forms of calcium wave.

Recently, the role for ATP in mediating calcium waves has been further supported by the use of ATP imaging techniques (44). In these studies photon-counting imaging was used to detect the presence of ATP in the saline solution around astrocytes. Astrocytes were bathed in a saline solution containing luciferin and luciferase. Stimulation of a calcium wave was shown to be correlated with a wave of extracellular ATP. By chopping between chemiluminescence measurements of extracellular ATP and fluorescence detection of calcium levels, this study showed

the appropriate correlated timing of the calcium and ATP waves. Additionally, the application of the ATP antagonist, suramin, blocked the ATP and calcium waves. Taken together, these studies indicate that ATP causes an elevation of astrocytic internal calcium and an additional release of ATP. Presumably this ATP-induced ATP release is critical for the spread of the calcium waves in cell cultures. In addition to ATP acting as an extracellular signal critical for calcium wave propagation, a recent study has demonstrated that nitric oxide might also regulate calcium waves (45).

Although waves of elevated internal calcium have been repeatedly observed in cell cultures, their presence in the CNS is less clear. An intracellular calcium wave can propagate throughout the processes of a given astrocyte (Figure 2) (46); however, intercellular waves have not been seen in acutely isolated preparations of hippocampus (25, 46). In the retina, however, concentrically propagating waves of elevated calcium can be initiated in astrocytes and Müller cells, the two principal glial cells of the retina (47). In contrast to the lack of calcium waves in hippocampal slices, organotypic cultures of the hippocampus do exhibit long-range calcium signals (15, 18, 48). Perhaps the presence of the calcium wave in slice cultures represents a change in property that is associated with injury and the transformation to reactive astrocytes. This is an intriguing possibility because it might indicate that reactive astrocytes are hyperexcitable with a lower threshold for calcium oscillations. The functional impact of such enhanced excitability is not yet clear.

ASTROCYTES INTEGRATE NEURONAL INPUTS

Astrocytes express many neurotransmitter receptors and transporters that have the potential to be activated by synaptically released neurotransmitters (49–51). In addition, as discussed above, astrocytes possess a number of neurotransmitter receptors coupled to intracellular calcium mobilization. Therefore, a potential functional consequence of the ability of neurotransmitters to mobilize astrocytic calcium is that neuronal activity could regulate astrocytic calcium levels. Indeed, several laboratories have demonstrated that astrocytes and perisynaptic Schwann cells do respond to synaptic activity through the activation of glial receptors.

The perisynaptic Schwann cells that loosely wrap the nerve terminal at the neuromuscular junctions play different functions and express different molecules than myelinating Schwann cells. Measurements of intracellular calcium in these perisynaptic Schwann cells have shown that, just as with astrocytes, they can respond to neurotransmitters with elevations of their internal calcium level (52, 53). Stimulation of the presynaptic axons can, in addition to causing the end plate potential, evoke a substantial elevation of perisynaptic Schwann cell calcium (52). It has been difficult to unequivocally determine which neurotransmitter evokes these calcium changes. Two candidates are ATP and ACh, which are co-released from the nerve terminal. Experimental data are consistent with roles for activity-dependent ATP release contributing to the induced calcium elevation in the perisynaptic Schwann cell (29). ACh might also be involved in such responses although this possibility

has been difficult to test because of the lack of availability of good muscarinic antagonists for this system (28).

Studies with hippocampal slices have demonstrated that neuronal activity causes glutamate-dependent astrocytic calcium elevations. Organotypic cultures of hippocampal slices were initially utilized to test the hypothesis that neuronal activity could regulate astrocyte calcium levels. Indeed following stimulation of mossy fibers, calcium levels were elevated in cells, located in area CA3 of the hippocampus, that were later shown to be glial fibrillary acidic protein (GFAP) positive (18). Studies using acutely isolated slices have also demonstrated the presence of this neuron-to-astrocyte pathway. In the hippocampus, as well as in the visual cortex, high (but not low) frequency stimulation of neuronal afferents triggers repetitive intracellular calcium elevations in astrocytes (25, 46). In the presence of either tetrodotoxin (TTX) or the metabotropic glutamate receptor (mGluR) antagonist α-methyl-4-carboxyphenylglycine (MCPG), the astrocyte response is abolished, thus providing evidence that glutamate released from active synaptic terminals can activate astrocytic mGluRs and trigger intracellular calcium oscillations. The additional observation that an increase in neuronal firing results in an increase in the frequency of intracellular calcium oscillations in astrocytes suggests, on the one hand, that the frequency of these intracellular calcium oscillations is under dynamic control by neuronal activity and, on the other hand, that astrocytes possess the remarkable capacity to discriminate between different levels and patterns of synaptic activity. The frequency of intracellular calcium oscillations may ultimately represent the code of neuron-astrocyte signaling (46).

Glutamate is not the only neuronally released transmitter that controls astrocytic calcium. Stimulation of GABAergic interneurons in hippocampal slices can elevate the calcium level of nearby astrocytes, an action that is blocked by the $GABA_B$ receptor antagonist CGP55845A and mimicked by baclofen (31). Application of norepinephrine (16, 33, 54), histamine, or acetylcholine (32) mobilizes astrocytic calcium in hippocampal slices, and stimulation of either the molecular or granule cell layer of the cerebellum causes an α_1 adrenoreceptor-dependent elevation of Bergmann glial calcium (54). Because electron micrographs have shown putative noradrenergic terminals making synaptic-like contacts with astrocytes (55, 56), these data suggest that extrinsic axon pathways can act on target astrocytes to regulate astrocytic calcium.

The elaborate ramified structure of astrocytes, which individually make contact with numerous neurons and synapses, raises questions about the relative autonomy of distinct regions of a given astrocyte. Put another way, does an individual site of neurotransmitter action on an astrocyte lead to a global change in the astrocytic calcium, or can individual sites act as functionally isolated regions? Stimulation of parallel fibers causes a calcium elevation in Bergmann glia of the cerebellum (57). These neuronally evoked calcium elevations can be highly localized to distinct domains in a glial cell, suggesting that glia can be functionally compartmentalized (57). Imaging studies also clearly show that in hippocampal astrocytes a calcium elevation can either propagate along the astrocytic process to promote

coherent activity of the processes of that cell or remain restricted to that process (Figure 2) (46). Because one astrocyte makes many intimate contacts with numerous synapses, an understanding of the conditions that lead to the switch between local and global calcium signals in an astrocyte is likely to guide our understanding of the roles of these calcium signals in astrocyte function.

NEURONAL ACTIVITY CAN INDUCE ASTROCYTIC PLASTICITY

In the adult nervous system, we now know that the synaptic connections between neurons are subject to reorganization, a process that underlies learning and memory. Indeed, it was recently established that individual dendritic spines extend during the induction of long-term potentiation (LTP) (58, 59). Whether astrocytes exhibit similar structural plasticity and whether such a process would be important for synaptic plasticity are not clear. However, testosterone treatment causes a rapid stellation of astrocytes in the arcuate nucleus (60–62), and in cell culture the application of glutamate causes the extension of filopodia from astrocytes (63). While we do not know whether neural activity triggers structural change in astrocytes, it is clear that these non-neuronal cells can integrate neural information and respond with long-term changes in certain properties. For example, multiple applications of glutamate, or the delivery of multiple afferent stimuli, lead to prolonged changes in the frequency of oscillations of astrocytic calcium levels (46, 64). Axonal activity can regulate GFAP expression in perisynaptic Schwann cells (65). Finally, the activity-dependent nonsynaptic release of ATP from sensory neurons regulates Schwann cell internal calcium levels in culture and the expression of the immediate early genes c-*fos* and *krox-24* (66). Because astrocytes have been shown to be important regulators of synaptogenesis (67, 68), the relation between astrocytes, astrocytic structural dynamics, and the formation of new synapses during plasticity is an exciting area for future study.

BI-DIRECTIONAL GLUTAMATE-MEDIATED SIGNALING BETWEEN ASTROCYTES AND NEURONS

Astrocytes not only integrate neurotransmitter inputs but also can release their own transmitters that act on neighboring neurons (10). Thus there is a bi-directional signaling pathway between astrocytes and neurons, which opens the possibility for a rich information exchange in the nervous system. Our initial insights into the presence of an astrocyte-to-neuron signaling pathway that is mediated by the release of chemical transmitters were obtained from cell culture studies and later confirmed and extended using more intact preparations (Figure 3).

Starting in 1994 there was a sequence of four reports demonstrating that elevations of astrocytic calcium lead to delayed elevations in neuronal calcium

(17, 35, 69, 70). One problem in this type of study, however, is in providing selective stimuli that would lead to the activation of only the astrocyte. To overcome this obstacle, either mechanical or focal electrical stimulation was provided. Such stimuli raised calcium in an astrocyte that then spread as a radially propagating wave of elevated calcium among the astrocytic network. As the calcium wave passed underneath co-cultured neurons, a delayed neuronal calcium elevation was detected (17, 35, 69, 70). Initially, the mechanism underlying this astrocyte-to-neuron signal was not clear, and electrotonic coupling between astrocytes and neurons was suggested to mediate the pathway (69). However, with no initial evidence supporting gap junctions connecting astrocytes and neurons, this hypothesis fell into disfavor, and the presence of a chemical signal that was released from the astrocyte became more popular.

The addition of neuroligands that elevate astrocytic internal calcium was shown to cause the calcium-dependent release of glutamate. When neurons were co-cultured with astrocytes, either mechanical stimulation of an astrocyte to raise its calcium level (17, 35), or application of the neuroligand, bradykinin (17), which elevates astrocyte calcium, caused a delayed neuronal calcium elevation that was sensitive to glutamate receptor antagonists. Further support for glutamate-mediated signaling from astrocytes to neurons was obtained by electrophysiological studies demonstrating that calcium elevations in astrocytes induce a glutamate-dependent slow inward current, or a depolarization, in neurons (35, 71, 72).

These cell culture studies paved the way for many subsequent exciting studies about the bi-directional signaling between astrocytes and neurons. However, because they were performed in culture, it was essential that similar experiments were performed in more intact preparations in order to determine whether this phenomenon represents a physiological signaling pathway or merely a curiosity of cell culture. Confocal imaging studies performed using hippocampal and visual cortex slices provided the first demonstration of the presence of bi-directional signaling between astrocytes and neurons (46). When mGluR agonists were applied to slices, astrocytic calcium was elevated, which was followed by a delayed neuronal calcium spike that was sensitive to the ionotropic glutamate receptor antagonists 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(f)-quinoxalinedione (NBQX) and D-2-amino-5-phosphonopentanoic acid (D-AP5) (46). Taken together with the results from enzymatic glutamate assays (37, 73), these data provided the first compelling demonstration of the presence of not only neurotransmitter-mediated neuron-to-astrocyte signaling but also of chemical transmitter-mediated astrocyte-to-neuron modulation.

Although several lines of evidence support the notion that glutamate is a chemical transmitter that mediates astrocyte-neuron signaling, other transmitters might also underlie signaling in this pathway. Recent work has reported that D-serine is an endogenous ligand of the glycine site of *N*-methyl-D-aspartate (NMDA) receptors responsible for modulating NMDA receptor-mediated synaptic transmission in cultured hippocampal neurons (74). Because the serine racemase responsible for the biosynthesis of D-serine from L-serine is highly expressed by glial cells, and

astrocytes possess high levels of D-serine (75) that can be released from astrocytes upon stimulation with glutamate (76), D-serine might represent a newly identified messenger for astrocyte-neuron signaling. Thus it would appear that D-serine (76), ATP (44), secretogranin II (77), and glutamate (17) are chemical transmitters that can be released from astrocytes in a regulated manner. It will be intriguing to determine the numbers of different chemical transmitters that different astrocytes release and to ask whether astrocytes release as large a variety of transmitters as neurons.

MECHANISM OF GLUTAMATE RELEASE FROM ASTROCYTES

In order to unravel the functional consequences of glutamate-mediated astrocyte-to-neuron signaling, it is essential that a mechanistic understanding of the release pathway is gained. Three prominent mechanisms have been suggested as potential pathways to underlie the release of glutamate from astrocytes (78): reverse operation of glutamate transporters, swelling-induced release, and calcium-dependent exocytosis. Glial cells are known to express high levels of glutamate transporters that normally function to clear glutamate from the extracellular space, especially in the vicinity of the synapse. Under conditions of depolarization or when the Na^+/K^+ electrochemical gradient used by these transporters has been reversed, glutamate can be released through these transporters (79). This does not underlie calcium-dependent glutamate release because ligands that cause glutamate release do not depolarize astrocytes, and transport inhibitors do not attenuate the magnitude of ligand-induced glutamate release (17, 72, 73, 80). Although swelling can lead to significant liberation of glutamate from astrocytes (81), imaging studies designed to monitor cell volume have demonstrated an absence of volumetric change during or after addition of ligands that cause calcium-dependent glutamate release (73, 82).

The calcium-dependent release of neurotransmitters is caused, in neurons, by the exocytosis of quanta of neurotransmitter-filled vesicles from the nerve terminal. While this property was initially thought to be a unique property of neuronal and exocrine tissues, there are several examples of quantal transmitter release from atypical preparations. For example, during patch recordings from myocytes, ACh quanta are detected when the cell is dialyzed with this transmitter (83). Similarly patch recordings from fibroblasts have demonstrated the presence of calcium-dependent quantal transmitter release (83, 84). To test the possibility that the release of glutamate from astrocytes is mediated by a vesicular mechanism, a variety of studies have been performed. First, the potent secretagogue, α-latrotoxin, which induces the exocytosis of vesicles at nerve terminals, also stimulates glutamate release from astrocytes (82). Second, the SNARE proteins, syntaxin, synaptobrevin II (VAMP) (85), and SNAP-23 (a homolog of SNAP-25) (86), which are known to be essential for vesicle fusion, are present in astrocytes. A member of the ras

family of GTPases, rab3, which is associated with synaptic vesicles and is likely involved in mediating docking and fusion events in neurons, is also expressed in astrocytes (87, 88). Third, treatment of astrocytes with clostridial toxins, which are highly selective proteases that cleave SNARE proteins (89–92), causes a blockade of calcium-dependent glutamate release from these cells (73, 80, 93). Fourth, glutamate release from astrocytes is attenuated after treatment with bafilomycin A1 (80). This toxin inhibits the V-ATPase of vesicles, which pumps protons into the vesicle lumen. Because the filling of vesicles with glutamate requires this proton gradient, an attenuation of glutamate release by bafilomycin is consistent with glutamate being stored in and then released from a vesicular compartment.

The final piece of evidence supporting vesicle-mediated glutamate release from astrocytes derives from results obtained through a novel experimental approach that used HEK cells transfected with the cDNA for the green fluorescent protein (GFP) and the NMDA receptor 1 and 2A subunits (94). After transfection, HEK cells were plated onto cultured astrocytes and used as sensors for glutamate release. Activation in astrocytes of AMPA receptors and mGluRs caused calcium signaling and glutamate release, thus leading in HEK cells to repetitive NMDA-mediated inward currents. These currents were similar in kinetics to those activated by quantal release of glutamate from synaptic terminals. Cleavage of astrocytic synaptobrevin by tetanus toxin or inhibition of the V-ATPase of astrocytic vesicles by bafilomycin greatly reduced glutamate release from astrocytes.

While all these results provide strong support for the existence in astrocytes of an exocytotic mechanism for transmitter release similar to that in neurons, ultrastructural evidence for the presence of transmitter-containing, small clear vesicles in astrocytes has not been found. However, immunoelectron microscopic analysis revealed that synaptobrevin II and synaptophysin are associated with the membrane of vesicular organelles in cultured astrocytes (88). Dense-core and clear vesicles have also been detected in a subpopulation of hippocampal astrocytes in culture, and a calcium-dependent regulated release of secretogranin II-containing vesicles has been demonstrated (77). Although further experiments need to be performed, a vesicle-mediated release process is the most plausible, perhaps the only, mechanism that could account for calcium-dependent glutamate release from astrocytes.

GLUTAMATE RELEASED FROM ASTROCYTES MODULATES SYNAPTIC TRANSMISSION AND NEURONAL EXCITABILITY

Astrocytes are intimately associated with neurons, especially at synaptic sites. Consequently, after demonstrating calcium-dependent glutamate release from astrocytes a highly logical question concerns the functional consequences of this transmitter release pathway upon synaptic transmission. To address such a question is highly challenging because it is necessary to have independent experimental control over the calcium levels in astrocytes, as well as voltage control of the pre- and postsynaptic neurons. Thus an interplay of experiments have been performed

in cell culture, where the system offers exquisite experimental control, and in slice preparations, which are less controlled but offer insights into the physiological relevance of the identified signaling pathways.

Cell culture studies of synaptic transmission between rat hippocampal neurons have shown that the elevation of astrocytic calcium causes a modulation of synaptic transmission mediated by released glutamate. Initiation of a calcium wave between astrocytes causes a transient depression of evoked synaptic transmission at hippocampal synapses that is sensitive to mGluR antagonists (71). Furthermore, in some cases, astrocytic calcium elevations lead to glutamate-dependent NMDA receptor-mediated increase in the frequency of miniature postsynaptic currents (mPSCs) (95). A modulation of synaptic transmission has also been demonstrated by recording from pyramidal neurons in hippocampal slices (31). Depolarization of astrocytes, or activation of GABAergic interneurons, elevates calcium in these glial cells and causes an AP5 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) -sensitive modulation of the frequency of mPSCs in pyramidal neurons. Of particular interest is the observation that this increase in miniature synaptic current frequency is of sustained duration, lasting in excess of 15 min, suggesting that glutamate released from astrocytes can contribute to synaptic plasticity.

Further support for a role for synaptically associated glia in regulating synaptic transmission has been provided by studies performed at frog neuromuscular junction. As discussed earlier, perisynaptic Schwann cells are intimately associated with the nerve muscle junction, and activity in the presynaptic axon can cause calcium elevations in these cells (52). To test the potential role of these synaptically associated non-neuronal cells in modulating synaptic transmission, guanine nucleotide analogs were directly injected into the perisynaptic Schwann cell. Activation of GTP-binding proteins by injection of GTPγS caused a dramatic reduction in the magnitude of the nerve-evoked end-plate potential, supporting the concept that the synaptically associated glial cell can modulate synaptic transmission (96). To determine whether this glial cell might be recruited into a modulatory role by neuronal activity, glial GTP-binding proteins were inactivated through the microinjection of GTPγS. In an unperturbed preparation, high-frequency activity in the axon causes a gradual decrease in the amplitude of the end-plate potential. GTPγS microinjection into the glial cell caused a surprising result. Although GTP-binding protein inactivation did not affect the magnitude of spike-to-spike transmission, it largely blocked the frequency-dependent depression of the end-plate potential (96). Prior to this experiment it was thought that this activity-dependent reduction in the magnitude of transmitter release resulted from an intrinsic property of the nerve terminal. On the contrary, these results clearly demonstrate a role for glia in integrating inputs from neurons, and then, in providing feedback modulation to the synapse.

In addition to modulating synaptic transmission, glia can also regulate neuronal excitability. As discussed above, calcium elevations in astrocytes in cell culture can induce glutamate-dependent slow inward currents in neurons that depolarize the membrane potential, thus regulating membrane excitability (35, 71, 72). Astrocytic regulation of neuronal activity has also been demonstrated in an in situ

preparation. Initiation of a calcium wave in Müller cells and astrocytes in the retina can modulate the output of retinal ganglion cells that is initiated by light activation of the photoreceptors (97). When the glial calcium wave approaches the region of the retina containing the recorded ganglion cell, either an excitation or inhibition of ganglion cell activity is detected. The glial-evoked inhibition was studied pharmacologically. Addition of either the glutamate receptor antagonists, NBQX (AMPA receptors) and D-2-amino-7-phosphonoheptanoic acid (D-AP7) (NMDA receptors), or the $GABA_A$ and glycine antagonists, bicuculine and strychnine, blocked the glial-evoked inhibition of activity. It is not known whether calcium elevations in these glia lead to transmitter release. However, given that several studies have demonstrated a calcium-dependent release of glutamate from astrocytes (10), one potential interpretation of the retinal studies is that glutamate released from retinal glia, acting indirectly through GABAergic interneurons, causes the modulation of the ganglion cell electrical activity. Indeed, Newman & Zahs (97) suggest that glutamate is released from the glia onto amacrine cells, which in turn release GABA and glycine to inhibit retinal ganglion cell activity. Despite the uncertainty about mechanism, studies at the frog neuromuscular junction (96) and in the retina (97) clearly demonstrate that glial modulation of synaptic transmission and neuronal excitability are properties of glia in intact systems, not just properties of astrocytes in cell culture.

Work in several preparations now indicates that astrocytes and perisynaptic Schwann cells can produce a variety of forms of neuronal modulation. As discussed above, astrocytes can cause a mGluR-dependent inhibition of hippocampal synaptic transmission (10, 71), and activation of perisynaptic Schwann cells suppresses transmission at the neuromuscular junction (96). In contrast, however, in the hippocampal slice preparation, activation of GABAergic interneurons elevates astrocytic calcium, which causes an ionotropic glutamate receptor-mediated increase in frequency of mIPSCs detected in pyramidal neurons (31). It is likely that glutamate release from astrocytes can indeed cause facilitatory or inhibitory effects on the same synapse. Although elevation of astrocytic calcium causes an mGluR-mediated suppression of synaptic transmission between hippocampal pyramidal neurons in culture (71), removal of Mg^{2+} from the saline selects for an NMDA receptor-dependent elevation of miniature synaptic current frequency following the elevation of astrocytic calcium (95). It is not yet clear which physiological conditions select for astrocyte-mediated facilitation or depression of the synapse, but it is tempting to speculate that co-activation of the synapse and the astrocyte would lead to a facilitation of the synaptic interaction.

Where is the Target of Action of Transmitter that is Released from Astrocytes?

Until the sites of transmitter release from astrocytes are identified it will be difficult to determine the target(s) of glutamate action, although physiological studies do provide at least a clue to these locations. Because glutamate that is released from

astrocytes can modulate synapses, it is likely that a target of action is in the vicinity of the synapse. However, it is unlikely that this glutamate can access the receptors located in the synapse because even low concentrations of glutamate would desensitize these receptors, an action that we do not detect (95). Additionally, studies using MK-801 have demonstrated that glutamate released from astrocytes acts on extrasynaptic NMDA receptors to enhance the frequency of mPSCs (95). In this work, hippocampal cultures were incubated in the presence of MK-801, which causes an open channel block of NMDA receptors in order to block synaptically activated NMDA receptors. Despite demonstrating a blockade of synaptic NMDA receptors, subsequent elevation of astrocytic calcium levels was still able to cause an AP5-sensitive increase in mPSC frequency. The location of these extrasynaptic NMDA receptors is unknown. However, an interesting possibility is that they are located extrasynaptically on the presynaptic terminal. Certainly, NMDA receptors have been demonstrated in this location in some preparations (98, 99). Consistent with an extrasynaptic target for glutamate action is ultrastructural data that have demonstrated the presence of mGluRs at extrasynaptic sites in presynaptic terminals (100). Perhaps these receptors mediate the mGluR-mediated depression of the synaptic transmission, which can be induced by glutamate that is released from astrocytes.

PHYSIOLOGICAL ROLES OF GLUTAMATE-MEDIATED ASTROCYTE-TO-NEURON SIGNALING

With the variety of information that is now available about astrocyte-induced neuromodulation we are approaching a period in which we can begin to speculate about the physiological roles for this signaling pathway. It should first be stated that neurons are obviously critical for nervous system function. Without the neuron we would be forced to rely on a calcium signaling pathway that propagates at rates of only 20 μm/s instead of the action potential, which can propagate at rates in excess of 20 m/s. Phylogeny provides a clue about the potential role of glia. In *Caenorhabditis elegans* there are 302 neurons and only 56 glial and associated support cells (101). As one rises through phylogeny, the ratio of glia to neurons increases. The human brain contains the greatest numbers of glia and the highest ratio of glia to neurons (at least 10:1). Consequently, one is tempted to suggest that glial cells, and particularly astrocytes, play roles in higher cognitive functions that are normally associated with higher species.

One area related to these higher functions that could potentially be investigated is the role of astrocytes in learning and memory. There is little information about this relationship. However, the elevation of astrocytic calcium can cause a sustained increase in the frequency of mPSCs recorded in hippocampal pyramidal neurons (31). GFAP-deficient mice exhibit an enhancement of LTP (102), and impaired LTD (103). However, until a mechanistic understanding of these changes is identified, it is not clear how they should be interpreted. Nonetheless, our knowledge

of the thresholds for activation of calcium signaling in astrocytes indicates that they might play a role in activity-dependent synaptic plasticity because astrocytes are typically activated by elevated stimulation frequencies (15, 18, 25, 46, 52, 53) that are frequently used to induce synaptic plasticity. While this possibility has not yet been systematically evaluated, perturbation of astrocytic calcium signaling in transgenic mice can affect synaptic plasticity. Mice in which the astrocyte-specific calcium-binding protein S-100 has been over-expressed show altered synaptic plasticity and impaired spatial learning (104). Thus data support the possible role of astrocytes in contributing to synaptic plasticity; however, carefully designed experiments are required to thoroughly test this hypothesis.

We have entered into a new era of physiological studies in the neurosciences in which it is now appreciated that astrocytes are dynamic signaling elements that integrate neuronal inputs, exhibit calcium excitability, and can modulate neighboring neurons through the calcium-dependent release of the chemical transmitter glutamate. Astrocytes, and perisynaptic Schwann cells, by virtue of their intimate association with synapses, are strategically positioned to regulate synaptic transmission. This capability, which has been demonstrated in several studies, raises the possibility that astrocytes are an integral element of the circuitry for synaptic plasticity. Because the highest ratio of glia to neurons is found at the top of the phylogentic tree in the human brain, these recent demonstrations of dynamic bidirectional signaling between astrocytes and neurons leave us with the question as to whether astrocytes are key regulatory elements of higher cortical functions.

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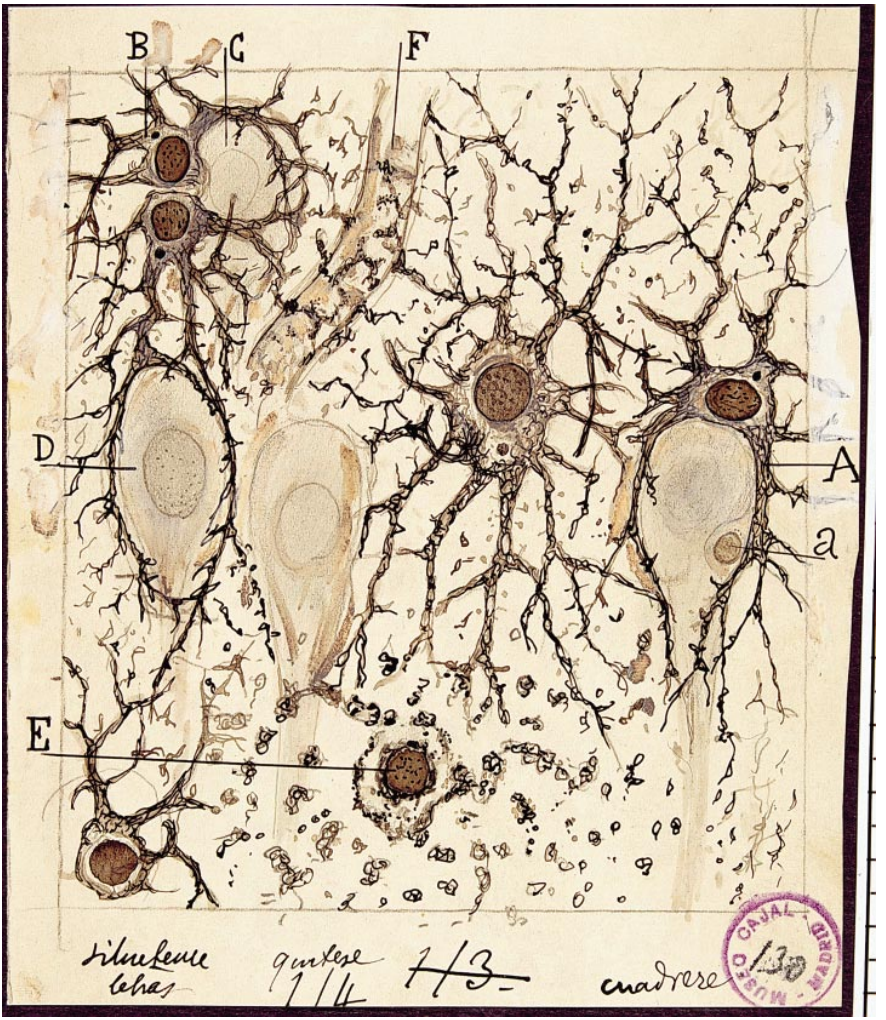

Figure 1 "Neuroglia of the pyramidal layer and stratum radiatum of the Ammon horn. Adult man autopsied three hours after death. Chloride of gold. (*A*) big astrocyte embracing a pyramidal neuron. (*B*) twin astrocytes forming a nest around a cell (*C*), while one of them sends two branches forming another nest (*D*). (*E*) cell with signs of autolysis" (1). Figure reproduced from original drawing with permission of Legado Cajal.

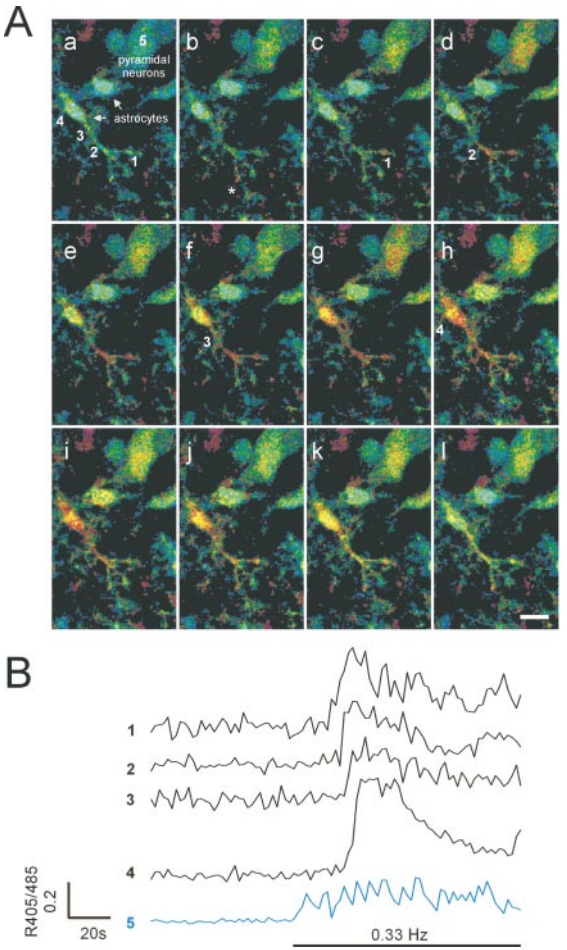

Figure 2 Propagation of internal calcium changes along astrocytic processes. (*A*) Time series of pseudocolor images illustrating the spreading of an internal calcium elevation along the process of an astrocyte that is located in area CA1 of the hippocampus. The calcium response in the astrocyte was triggered by stimulation of Schaffer collaterals at 0.33 Hz, i.e. train of pulses delivered at 30 Hz for 100 ms that were applied every 3s. Slices were obtained from a nine-day old rat and loaded with the calcium indicator Indo-1. Labels 1–4 indicate discrete portions of the astrocyte processes; label 5 indicates a pyramidal neuron. The time interval between images a–h is 2 s; between h–l is 12 s. Scale bar 10 mm. (*B*) Kinetics of the internal calcium changes as measured at the level of different portions of the astrocytic process and soma shown in *A* upon neuronal stimulation. The kinetics of the calcium change in neuron 5 is also reported. It is noteworthy that in image *A*b, the process indicated by the asterisk displayed a transient and localized calcium elevation that remained restricted to the process.

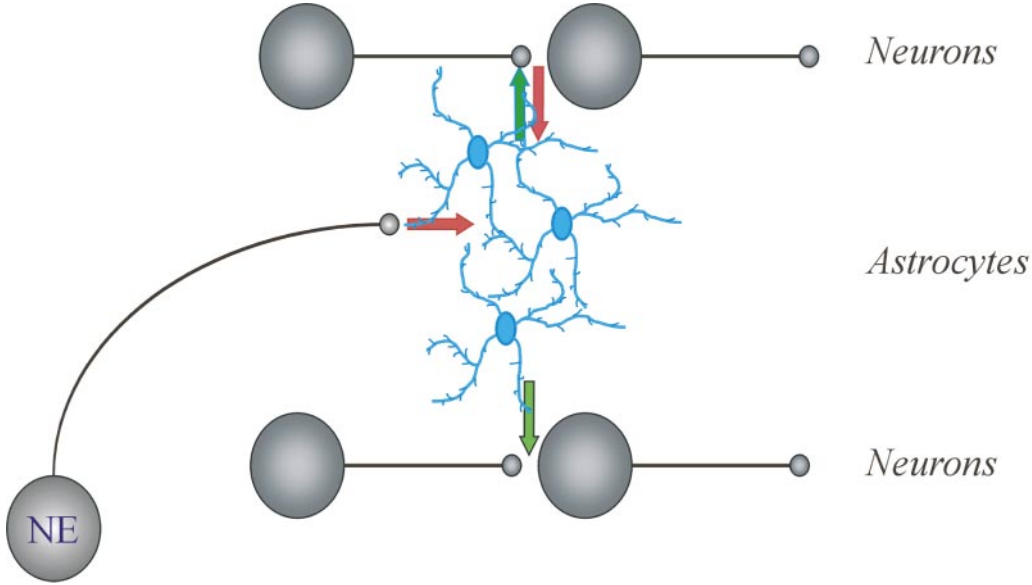

Figure 3 Bi-directional signaling between astrocytes and neurons. Activity at synaptic connections (*top*) can lead to neurotransmitter signaling from neurons to astrocytes (red arrow), which mobilizes calcium in the adjacent astrocytes (blue). Calcium elevations in astrocytes can then initiate feedback signals (green arrows) in the form of the release of chemical transmitters, which modulate either the initiatally active synapse, or more distant synapses (green arrow, *bottom*). In addition to intrinsic synapses leading to the activation of astrocytes, extrinsic axonal inputs, which can form apparent release sites directly with astrocytes, can also mobilize astrocytic calcium and potentially lead to the calcium-dependent release of chemical transmitters from astrocytes onto neighboring neurons and synapses. In this example, NE represents the neurotransmitter norepinephrine.