Astrocytes Coordinate Synaptic Networks: Balanced Excitation and Inhibition

Although neurons are essential for brain function, an emerging alternative view holds that astrocytes, the dominant glial cell type, coordinate synaptic networks. Through the release of glutamate, astrocytes locally excite neurons, and via adenosine, which accumulates due to the hydrolysis of released ATP, astrocytes suppress distant synapses.

Looking at the relative structure of cells in the nervous system, one would infer that neurons, with their long axonal projections, are preeminent in brain function. Their rapid conduction velocity logically suggests that no other cells play essential signaling roles. Why then is there an emerging school of thought that the slow-signaling astrocyte, the more numerous nonneuronal cell of the nervous system, is a critical regulator of neuronal function? What is the basis for this new idea of nervous system integration? In this discussion, we will bring together the results of a decade of work demonstrating that astrocytes listen and talk back to the synapse, work that leads us to conclude that astrocytes coordinate integration among neuronal and synaptic networks.

Glia cells are comprised of four subtypes: microglia are the resident macrophages of the brain, oligodendrocytes serve a myelination role, glial neuroprogenitors give rise to the neurons of the brain, and astrocytes, the focus of this discussion, regulate a diversity of functions including neurotransmitter signaling and recycling, the control of blood flow, synaptic transmission, synaptogenesis, and K+ homeostasis (15, 19, 39, 41).

In contrast to most neurons, astrocytes are small cells that are characterized by small cell somata (<10-μm diameter), numerous highly branched fine processes that extend for distances up to 100 μm (8) and that make contact with neuronal processes at the synapses. The astrocytic process is in intimate contact with both the pre- and postsynaptic terminal (FIGURE 1) (40). The majority of the synapses in the hippocampus (57%) are in close contact with an astrocytic process, and it has been proposed that the presence of the astrocytic process identifies this synapse as an active glutamate-releasing synapse. Although heterogeneity in astrocyte-to-neuron contact has been reported in different brain regions, the sheer magnitude of the number of astrocyte-synaptic contacts that are made by one astrocyte, estimated to be >100,000 (8), suggests important roles for these glia in synaptic regulation.

The first hint that the close proximity between the processes of astrocytes and the synapse might play important functional roles in the modulation of neuronal excitation was provided by the observations that these glial cells express neurotransmitter receptors. Because astrocytes express membrane receptors for almost all neurotransmitters (19), which are linked to IP3 production and release of Ca2+ from intracellular stores, the astrocytic process can sense neurotransmitter released at the synapse leading to astrocyte activation through the mobilization of their intracellular Ca2+ (see FIGURE 2A). Activated astrocytes have the ability to release a variety of neuroactive molecules including glutamate, ATP, nitric oxide, prostaglandins, atrial natriuretic peptide (ANP), and o-serine, which in turn influences neuronal excitability (4, 12, 30, 42).

This bi-directional signaling between astrocytes and neurons has led us to propose that the astrocyte represents a third active element of the synapse together with the pre- and postsynaptic terminals in what we have termed the “tripartite synapse” (2).

In the remainder of the discussion of the tripartite synapse, we will highlight some of the roles of two of these gliotransmitters, glutamate and ATP. By integrating experimental evidence provided by many laboratories, we will propose that the astrocyte serves a unique function in the nervous system and that by releasing two distinct transmitters the astrocyte provides spatially and temporally balanced excitation and inhibition to coordinate neuronal and synaptic networks.

**Glutamate Released from Astrocytes Modulates Both Excitatory and Inhibitory Synaptic Transmission**

After the discovery that cultured astrocytes express neurotransmitter receptors, a key observation demonstrated that, when an astrocytic Ca2+ signal confronts a co-cultured neuron, a delayed Ca2+ signal is detected in the neuronal element (27, 32). Since that time, the majority of the experimental evidence has supported a glutamate-mediated astrocyte-to-neuron signaling pathway. Although there has been debate about the
neurotransmitter release, the majority of the experimental evidence supports the presence of a Ca\(^{2+}\)-regulated exocytotic pathway of glutamate released from astrocytes (6, 44, 45).

Astrocytic glutamate has a variety of actions on neuronal systems. At the synaptic level, glial glutamate has been shown to act on neuronal metabotropic glutamate receptors (14), presynaptic kainate receptors (25), as well as postsynaptic extrasynaptic N-methyl-D-aspartate (NMDA) receptors (13). Through these actions, both amplitude and frequency of evoked IPSCs (21) can be augmented and the frequency of mEPSCs increased (14). Although it is clear that the astrocyte can modulate a synapse, an understanding

**FIGURE 1. The astrocyte is a morphologically complex cell**

Astrocyte labeling in a hippocampal section, using the astrocytic marker GFAP (red; left and middle) does not reveal the full extent of this glial cell’s processes, which becomes clear through the expression of EGFP (green; middle). Electron microscopy shows the tripartite nature of synaptic structures with astrocytic processes (green) associating with pre- and postsynaptic terminals (modified from Ref. 36a).

**FIGURE 2. Glutamate released from hippocampal astrocytes induces neuronal synchrony through the activation of extrasynaptic NR2B-containing NMDA receptors**

A: in the hippocampus, besides activating ionotropic glutamate receptors in the postsynaptic terminals, glutamate released from the presynaptic Schaffer collateral terminals activates metabotropic receptors in the plasma membrane of the nearby astrocytic processes. Activation of these receptors results in intracellular Ca\(^{2+}\) elevations in the astrocytes, which in turn lead to glutamate release from these glial cells through the fusion of tetanus toxin-sensitive vesicles. Astrocytic glutamate selectively acts on extrasynaptic, NR2B-containing NMDA receptors to trigger slow inward currents (SICs) in pyramidal CA1 neurons. B: Schematic representation of neuronal synchrony in which the release of glutamate from a single astrocyte (green cell) onto several dendrites of different neurons can lead to the synchronization of groups of neurons located within 100 \(\mu\)m of one another, which we refer to as neuronal domains (red cells).
of these actions in a conceptual view of synaptic function is lacking at this time.

**Astrocytic Glutamate: Functional Consequences on Neurons**

Early evidence of an effect of astrocytic glutamate on neurons in brain slice preparation came from confocal Ca²⁺ imaging studies. Activation of Ca²⁺ signaling in astrocytes leads to successive episodes of glutamate release, which causes repetitive Ca²⁺ increases in neurons through the activation of ionotropic glutamate receptors (5, 35). Electrophysiological evidence for a neuronal action of astrocytic glutamate was first described in Parri et al. (33). In this study, the authors showed that spontaneous Ca²⁺ astrocytic oscillations are associated with inward currents in the thalamic neurons, mediated exclusively by the activation of the NMDA receptors. These currents were characterized by similar slow kinetics to previously described slow inward currents (SICs) detected in cultured cells (3a).

In a series of recent studies focused on hippocampal

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**FIGURE 3.** Astrocytes coordinate the relative strength of adjacent synaptic pathways by the activity-dependent release of purines

A: recording configuration and cells of the inner retina. Electrophysiological traces showing adenosine inhibition recorded from a ganglion cell (a) when ATP₄S is applied through a pipette (b) to activate Ca²⁺ signals in Muller glial cells and astrocytes of the retina. Glial cell evoked neuronal outward currents are blocked by the A₁ antagonist DPCPX. Reproduced with permission from Ref. 29, copyright 2003 from the Society for Neuroscience. B: astrocytes are essential for heterosynaptic suppression. Tetanic stimulation (100 Hz, 1 s) of S₁, one of two independent presynaptic pathways that both act on common postsynaptic neurons, evokes long-term potentiation (LTP) of S₁ pathway while causing a suppression of the unstimulated pathway S₂. This suppression is due to the release of ATP from astrocyte, which is rapidly hydrolyzed to adenosine by extracellular ectonucleotidases. Adenosine then acts on presynaptic A₁ receptors to decrease the release of glutamate from the terminals of the S₂ pathway.
Astrocytes as a Source of ATP

Although neurons have been shown to be capable of releasing ATP during synaptic transmission, purinergic transmission is not a dominant form of signaling between neurons. In contrast, we now realize that the majority of astrocytes release ATP, which, through actions on P2Y receptors, serves a paracrine role regulating astrocytic Ca\(^{2+}\) oscillations and through hydrolysis to adenosine regulates synaptic suppression.

A role for ATP in mediating astrocyte-dependent signaling was initially identified in cell-culture studies where the Ca\(^{2+}\) waves that spread between adjacent astrocytes were shown to be regulated by a purinergic signal (17). The mechanisms mediating Ca\(^{2+}\) waves have been the subject of considerable speculation and debate: some evidence supports a role for the diffusion of a metabolite, such as IP3, through the gap junctions that interconnect astrocytes (20), whereas other evidence supports a role for released ATP acting on P2Y receptors (phospholipase C-coupled metabotropic receptors) to mobilize Ca\(^{2+}\) and induce further release of ATP from the activated astrocytes (24). Although there is still debate about the details of this mechanism, it is clear that astrocytes release ATP and that ATP can initiate glial Ca\(^{2+}\) signals. Beyond a role for ATP in mediating astrocytic Ca\(^{2+}\) signals, does it serve roles in regulating neuronal activity and synaptic transmission?

Purinergic Modulation of Neuronal Activity

Studies of the retina have provided considerable information on glial-mediated purinergic signaling. Dr. Newman has shown that calcium waves are transmitted from astrocytes to Müller cells, the principal retinal glial cell, and between Müller cells by the release of ATP (28). It is interesting to note that Müller cells have been described as the equivalent of hippocampal astrocytes in the retina (30). Further experiments demonstrated that Müller cells modulate neurons by the release of ATP. Released ATP is hydrolyzed to ADP, AMP, and adenosine by the action of extracellular ecto-nucleotidases. Adenosine then acts on adenosine A1 receptors to hyperpolarize retinal ganglion cells (FIGURE 3) (29, 31).

In the limbic system, ATP has been shown to have a direct excitatory effect on hippocampal interneurons through metabotropic P2Y1 receptors by producing a depolarization that triggers action potential generation (7, 22). As a consequence, both the frequency and amplitude of spontaneous IPSCs recorded from CA3 and CA1 neurons are increased by exogenous application of ATP (7, 22). Consequently, both in hippocampus and in the retina, ATP contributes to an inhibition or suppression of the neuronal network.

Astrocyte-Dependent, Purinergic Modulation of Synaptic Transmission

Purinergic signaling is an extremely complex process in which ATP, ADP, AMP, and adenosine can each have distinct actions. Thus the concentrations, rates of hydrolysis, and particular receptors expressed in a given region of the nervous system coordinately regulate the action of released ATP. For example, in culture, ATP inhibits hippocampal synaptic transmission through the direct activation of P2Y receptors (23, 43). However, in brain slices, the inhibitory effect of ATP on excitatory synaptic transmission is due to its conversion into adenosine by extracellular ecto-nucleotidases (10) and consequent activation of A1 adenosine receptors (11, 43).

Although our attention is frequently focused on the mechanisms underlying synaptic plasticity, of equivalent importance is how potentiated synapses achieve contrast with their neighbors. A classic example is provided by the retinal system where center-surround inhibition enhances contrast in the visual field. Over a decade ago, it was realized that activity in a synaptic pathway acts laterally to regulate the relative strength of neighboring synapses (26). Similar mechanisms occur in the hippocampus. Two independent presynaptic pathways were stimulated that both acted on common postsynaptic neurons. When pathway 1 was stimulated with a high-frequency train (100 Hz for 1 s), pathway 1 was potentiated through a process known as long-term potentiation (LTP). Of great interest was the observation that the unstimulated pathway (pathway 2) was depressed by activation of pathway 1 despite each pathway being independent of one another (26). How did these two pathways talk to one
another? Through pharmacological studies, it was determined that this process, called heterosynaptic suppression, was mediated by the accumulation of adenosine that acted through A1 receptors. However, the source was unclear. In 2003, Zhang et al. (43) suggested that the adenosine was derived from the astrocyte. Using lower frequency stimulation protocols, they showed a similar adenosine-mediated depression of pathway 2. To identify the astrocyte as providing this nucleoside, they used a metabolic poison, fluorocitrate, which is alleged to selectively inhibit the Krebs cycle of the astrocyte. Notwithstanding the concerns about this poison, this study raised the intriguing possibility that the astrocyte mediates heterosynaptic suppression of synaptic transmission.

Recently, our laboratory developed inducible, astrocyte-specific transgenic animals to directly address the role of the astrocyte in mediating heterosynaptic signaling (34). There is an emerging view that gliotransmitters can be released from astrocytes through an exocytotic mechanism (6, 9, 44, 45). We made use of this knowledge and developed transgenic mice that permitted the blockade of gliotransmission. Membrane fusion, and thus exocytosis of chemical transmitter, critically relies on the formation of an intermolecular complex consisting of three proteins; synaptobrevin, syntaxin, and SNAP-25 (or in the case of the astrocyte, SNAP-23). This complex forms through interactions of the so-called SNARE domains. To perturb gliotransmission, we conditionally expressed the cytosolic SNARE domain of one of the proteins, synaptobrevin II, where it has dominant negative actions. Selective expression of the SNARE domain selectively in astrocytes prevented the release of ATP from these glial cells, allowing a rigorous evaluation of the role of astrocytes in mediating heterosynaptic depression (34).

To asses the role of the astrocyte in mediating heterosynaptic suppression, we stimulated two independent pathways, S1 and S2. Delivery of a tetanic train to S1 caused homosynaptic potentiation (LTP) of the stimulated pathway and an adenosine-mediated depression of S2. Expression of the dominant negative SNARE domain in astrocytes, although permitting LTP

![FIGURE 4. Model of the spatio-temporal actions of gliotransmission mediated by glutamate and ATP. Activation of an astrocyte by synaptic activity leads to the release of two gliotransmitters: glutamate and ATP. Released glutamate acts rapidly through NMDA receptors to depolarize nearby neurons (time point 1). The distance and duration of action of glutamate action is constrained by the activity of glutamate transporters. In contrast, released ATP is not sufficiently concentrated to have immediate neuronal actions through P2 receptors. Instead, the action of this purine is delayed and requires hydrolysis to adenosine (~200 ms). As a result of this delay for hydrolysis, purines can diffuse to distant sites of action (time point 2) where, after hydrolysis to adenosine, they cause a suppression of excitatory synaptic transmission. After glutamate is cleared from the extracellular space, adenosine-mediated synaptic suppression persists until it slowly reequilibrates with cytosolic adenosine (time point 3) through the action of equilibrative nucleoside transporters. The time course of action of glutamate is derived from kinetics of neuronal actions of glial-derived glutamate (13), whereas that of adenosine is deduced from the studies of Zhang et al. (43).]
induction, prevented the heterosynaptic suppression of S2 (FIGURE 3). When these results are integrated with those of the previous studies, we conclude that activation of astrocytes by S1 causes the release of ATP from these glial cells, which accumulates as adenosine in the extracellular space and causes an A1 receptor-mediated depression of S2. The astrocyte, by releasing purines, is coordinating the strength of neighboring synaptic pathways (34), indicating that astrocytes regulate networks of neurons.

In addition to mediating heterosynaptic suppression, studies using these astrocyte-specific transgenic mice revealed some additional and surprising results. It has been known for some time that there is a tonic level of extracellular adenosine that persistently suppresses excitatory synaptic transmission in the hippocampus. Expression of the dominant negative SNARE domain in astrocytes relieved this suppression. Additionally, by changing the degree to which the synapse was suppressed, the dynamic range for LTD was consequently altered. Thus, by releasing ATP, the astrocyte tonically suppresses synaptic transmission, modulates the range over which a synapse may be plastic, and mediates activity-dependent heterosynaptic depression.

Through different mechanisms, in the paraventricular nucleus, ATP has been shown to potentiate the amplitude of glutamate-mediated mEPSCs (16). Although several details still remain to be determined, the results of this study are consistent with the idea that an extrinsic input to the astrocyte, mediated by norepinephrine, causes the release of ATP from these glial cells, which, by acting through postsynaptic P2X receptors, causes the insertion of postsynaptic AMPA receptors. Although these data suggest that an extrinsic input acts through an astrocytic intermediate to augment the strength of synaptic connections, it is not clear how a persistent elevation of ATP is achieved in the face of the activity of ectonucleotidases. Nonetheless, these exciting results point to a potential widespread role for an astrocytic source of ATP in the regulation of synaptic transmission.

**Feedback Versus Feedforward Control at the Tripartite Synapse**

After the initial conceptualization of the tripartite synapse, two notions were proposed: feedback and feedforward modulation of the synapse (12). There are now several examples where these signaling pathways have been described. Stimulation of presynaptic afferents, which leads both to synaptic activity and Ca2+ oscillations in the astrocyte, has now been shown to evoke an astrocyte-dependent feedback excitation of the same synaptic system. This feedback modulation is mediated by the release of glutamate from the astrocyte that acts through NMDA receptors to synchronously depolarize groups of neurons. The same afferent stimulation, by activating the astrocyte to release ATP, causes an adenosine-mediated heterosynaptic depression of neighboring unstimulated synaptic pathway: feedforward inhibition. It is not clear whether the glial-derived adenosine also causes a feedback inhibition of the stimulation pathway; however, it undergoes LTD, suggesting that this pathway is protected from the adenosine-mediated depression.

In summary, astrocytes, which are strategically positioned adjacent to synaptic terminals, integrate synaptic activity through the activation of their metabotropic receptors, which cause PLC-dependent release of gliotransmitters that have a range of spatiotemporal actions. By providing local feedback excitation mediated by glutamate, astrocytes provide a source of neuronal activation that may be critical in controlling the synchronous depolarization of groups of pyramidal neurons. At the same time, by providing
distant feedforward actions that are mediated by purines, the astrocyte suppresses synaptic transmission. Through these coordinated actions, the astrocyte provides balanced excitation and inhibition mediated by two distinct transmitter systems, which we propose cause a coordinated contrast enhancement between neighboring synaptic pathways. Any displacement of this equilibrium between excitation and inhibition has the potential to lead to disorders of the nervous system, including epilepsy and the negative symptoms of schizophrenia that might result from hyper- or hypo-activation of associated neurons.

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References


