By forming close contacts with synapses, astrocytes secrete neuroactive substances and remove neurotransmitters, thus influencing the processing of information by the nervous system. Here, we review recent work on astrocytes and their roles in regulating neuronal function and synaptic plasticity. Astrocytes are organized as networks and communicate with each other, thereby affecting larger neural circuits. They also provide a link between neurons and the vasculature, potentially changing the cerebral microcirculation. Recent work has provided insights into the relative contributions of specific astrocytic cues and transporters to synaptic transmission, plasticity, and animal behavior.

Introduction

A single protoplasmic astrocyte of the gray matter extends thousands of fine membranous processes that enwrap synapses. At this site, astrocytes exchange information with the synaptic neuronal elements, both responding to the neuron and regulating synaptic transmission, thereby forming the so-called ‘tripartite synapse’ [1]. Signaling between neurons and astrocytes at the tripartite synapse is reciprocal, where astrocytes not only respond to neuronal activity but also actively modulate synaptic activity. Several studies have shown that astrocytes can release a variety of different molecules upon activation by neurotransmitters, which has led to the concept of ‘gliotransmission’ [2]. Termination of neurotransmission and prevention of neurotransmitter spreading into the extrasynaptic space is achieved by active transport of transmitter molecules, such as glutamate, glycine and γ-aminobutyric acid (GABA), into the surrounding neuronal and glial cells through complex transport systems. Glial transport plays a crucial role in shaping synaptic transmission. Astrocytes also form networks and communicate with each other through waves of Ca2+, propagated through specialized channels, so-called gap junctions. These astroglial networks can spread information over large distances and are therefore well positioned to influence larger neuronal circuits. Astrocytes link neurons and blood capillaries, and the three elements together are sometimes termed the neurovascular unit. This close coupling of neurons, glia, and blood vessels appears to control cognitive functions [3]. Here we review progress in this rapidly emerging field of neuron–astrocyte communication and the regulation of synaptic plasticity. Roles of other types of glia, including white matter glia, microglia, and polydendrocytes are not discussed and we refer the reader to other recent review articles [4–6]. Summaries of astrocyte functions during synaptogenesis and disease can also be found elsewhere [1,7,8].

Astrocyte glutamate release

Glutamate serves as the principal excitatory neurotransmitter in most regions of the CNS and its release from astrocytes has been shown to modulate synaptic transmission. Glutamate released from neurons activates metabotropic glutamate receptors on astrocytes, leading to astrocytic Ca2+ increase and a subsequent release of glial transmitters; however, it has also become clear that glial transport plays a crucial role in shaping synaptic transmission. Astrocytes also form networks and communicate with each other through waves of Ca2+, propagated through specialized channels, so-called gap junctions. These astroglial networks can spread information over large distances and are therefore well positioned to influence larger neuronal circuits. Astrocytes link neurons and blood capillaries, and the three elements together are sometimes termed the neurovascular unit. This close coupling of neurons, glia, and blood vessels appears to control cognitive functions [3]. Here we review progress in this rapidly emerging field of neuron–astrocyte communication and the regulation of synaptic plasticity. Roles of other types of glia, including white matter glia, microglia, and polydendrocytes are not discussed and we refer the reader to other recent review articles [4–6]. Summaries of astrocyte functions during synaptogenesis and disease can also be found elsewhere [1,7,8].

Astrocyte D-serine release

Among the many gliotransmitters released by astrocytes [2], D-serine may be the most interesting. D-serine serves as a coagonist with glutamate at NMDA receptors (NMDARs) and is primarily synthesized by astrocytes. Previous studies had suggested that astrocytes can regulate NMDAR activation through Ca2+-dependent release of D-serine in the supraoptic nucleus [15]. More recent work has addressed the role of D-serine release in NMDAR-dependent long-term potentiation (LTP) in the Schaffer collateral (SC)–CA1 synapses [16**]. Blocking Ca2+ elevations in astrocytes, by buffering intracellular Ca2+ concentrations with Ca2+ chelators, abolished LTP induction and decreased NMDAR currents at nearby synapses. These effects could be rescued by the addition of D-serine. Inhibiting D-serine synthesis, by blocking its synthetic enzyme serine racemase in astrocytes, concomitantly with the depletion of intracellular pools of D-serine, by applying high frequency stimulation, suppressed LTP induction, demonstrating that
Astrocytes are the direct source of "D-serine" in the hippocampus (Figure 1b).

**Astrocyte ATP and adenosine release**

ATP and its hydrolysis product adenosine have also been shown to be released from astrocytes and to control synaptic transmission (Figure 1b). In a study using combined two-photon Ca\(^{2+}\) imaging, photolysis of caged compounds and electrophysiology, astrocytic ATP was shown to act on postsynaptic hypothalamic neurons to scale glutamate synapses in a multiplicative manner [17].

Synaptic activity increased Ca\(^{2+}\) signals in the soma and processes of astrocytes and increased quantal synaptic current amplitude in neighboring magnocellular neurosecretory cells (MNCs), via activating purinergic P2X receptors. The same effect was obtained by uncaging glutamate or inositol 1,4,5-triphosphate (IP\(_3\)) onto an astrocyte. This process is dependent on the activation of group I mGluRs in astrocytes and the release of Ca\(^{2+}\) from internal stores through the generation of IP\(_3\). To support the role of astrocytes in this process, the authors performed the same experiments in the presence of tetanus toxin without perturbing the increase in synaptic efficacy upon glutamate uncaging. Instead, reducing astrocyte coverage by dehydration failed to increase sEPSC amplitude upon glutamate uncaging. This analysis concluded that all active synapses within one MNC are scaled multiplicatively in response to astrocyte activation.

Adenosine accumulates in the extracellular space, where it can act on synaptic adenosine 1 receptors (A\(_1\)R). In the SC, activation of A\(_1\)R suppresses glutamate release from presynaptic terminals, thereby increasing the dynamic range for LTP. This also provides a mechanism for crosstalk to distant synapses: ATP can diffuse to distant sites, where adenosine heterosynaptically depresses synaptic transmission [18]. Adenosine has recently been shown to regulate sleep homeostasis [19]. This study used inducible transgenic animals that express a dominant-negative (dn) SNARE domain in astrocytes to impair gliotransmission. dnSNARE transgenic animals had reduced sleep pressure, that is, the brain’s urge to sleep was reduced, which positively correlates with decreased slow wave activity during non-rapid eye movement (NREM) sleep. These animals failed in the compensatory response to sleep deprivation and the cognitive impairments associated with sleep deprivation were
Astrocyte TNF-α release

Glial is also the source of the cytokine tumor-necrosis factor-α (TNF-α). TNF-α contributes to homeostatic synaptic scaling in the hippocampus, a mechanism that adjusts synaptic strength globally in response to prolonged changes in activity [20]. More recently, it was shown that TNF-α is required for experience-dependent plasticity in the developing visual cortex. Monocular visual deprivation in mice deficient in TNF-α revealed normal initial loss of deprived eye responses, but a lack of the subsequent increase in open eye responses [21]. These results suggest that the two phases are mechanistically distinct and that the second phase is a form of homeostatic synaptic scaling.

Debating gliotransmission

In spite of accumulating data supporting gliotransmitter release and its role in synaptic plasticity, this view is still under debate [1,22]. Arguments against the concept of gliotransmission focus on the fact that the cytoplasmic levels of glutamate in astrocytes are much lower than in neurons. Glutamate that is taken up by astrocytes is converted to glutamine by the enzyme glutamine synthetase, raising the question of whether a sufficiently high concentration of glutamate could be released to activate neuronal receptors. Also, studies using genetically modified mice to either selectively activate or abolish astrocytic Ca^{2+} signaling mediated by G_{q}-linked G-protein coupled receptors (GPCRs) have failed to find evidence that astrocytic Ca^{2+} affects glutamate release and neuronal transmission at the SC–CA1 synapse [23,24]. Another argument concerns the mechanism of astrocyte transmitter release. Although Ca^{2+}-dependent transmitter release has been proposed to occur via exocytosis, astrocytes in vivo do not seem to express vesicular glutamate transporters nor components of regulated vesicular release found in neurons [25]. Additional work will be needed to resolve these uncertainties [26].

Role of glial glutamate uptake in synaptic transmission and plasticity

Glutamate is rapidly removed from the extracellular space by action of excitatory amino acid transporters (EAATs). Two glial EAAT subtypes (GLAST/EAAT1 and GLT1/EAAT2) distribute on astrocytic membranes in the vicinity of excitatory synapses. GLAST and GLT1 prevent accumulation of extracellular glutamate and overstimulation of glutamate receptors, thus preventing excitotoxic neuronal death [27–29]. Glutamate transporters also promote synapse independence by limiting glutamate spillover to neighboring synapses [30,31], limit glutamate spillout to extrasynaptic receptors [32], and modulate the intensity and duration of postsynaptic activation [33] (reviewed in [34]). Despite the importance of glial glutamate transporters for synaptic function, its molecular regulation is still unclear (Figure 2). In vitro studies suggest that neuronal secreted factors can induce GLAST and GLT1 expression [35], while in vivo studies demonstrate that alteration of sensory activity by whisker stimulation induces changes in astroglial synaptic coverage and expression levels of glial transporters [36]. Additionally, induction of LTP in the hippocampus leads to an increase in glial transporter levels [37].

A recent study identified a mechanism by which axons induce the transcriptional activation of GLT1 [38]. In a microfluidic culture platform used to co-culture neurons and astrocytes, axons entering the astrocyte compartment induced local GLT1 expression by regulating GLT1 transcription. Characterization of the GLT1 promoter in vitro and in vivo identified a small motif responsible for neuron-dependent activation. Through biochemical and mass spectrometry analysis, the kappa-B motif binding phosphoprotein (KBBP), the mouse homolog of human heterogeneous nuclear ribonucleoprotein K (hnRNP K), was found to bind to this motif. The authors could show that neuron-stimulated GLT1 transcriptional activation requires KBBP and that KBBP mediates changes in astroglial synaptic functions upon neural injury (Figure 2). Loss of presynaptic signaling to astrocytes as a result of axon transection, neuronal death induced by administration of the neurotoxin ricin, or neurodegeneration in an ALS model, resulted in reduced astrocytic KBBP and loss of GLT1 expression.

Evidence has recently accumulated for a role of glial glutamate uptake in the modulation of synaptic plasticity. In the hypothalamus, reduced glutamate clearance associated with a decrease in astrocyte coverage during lactation, was shown to affect neurotransmitter release through the modulation of metabotropic glutamate receptors [39]. Glial glutamate transporters in the barrel cortex facilitate activity-dependent remodeling of somatosensory maps during the critical period [40]. In the hippocampus, GLAST and GLT1 are regulated by a member of the Eph receptor tyrosine kinase family and one of its cognate ephrin ligands [41,42]. A unique feature of this signaling system is its bidirectionality, in the sense that an Eph receptor can also act as a ligand in the same manner that an ephrin, can act as a receptor [43]. Lack of EphA4 in the postsynaptic CA1 cell and absence of ephrinA3, mainly expressed in astrocytes, increases the abundance of astrocytic glutamate transporters [41,42]. EphrinA3 modulates transporter currents in astrocytes, possibly mediated by ephrinA3 reverse signaling in the astrocyte (Figure 2). In addition, mutant mice display impairment in theta burst-induced SC–CA1 LTP in hippocampal slices. Pharmacological inhibition of glutamate transporters rescues the LTP defects in EphA4 and ephrinA3 mutant mice.
mice, suggesting that the altered astrocytic glutamate transporter expression is responsible for the LTP deficiency. Transgenic overexpression of ephrinA3 in astrocytes reduces glutamate transporter levels and produces dystrophic dendrites with focal swellings probably owing to elevated extracellular glutamate concentrations [41]. Consistent with a role of glial glutamate uptake in modulating synaptic plasticity, ephrinA3 null mice show impairments in certain learning and memory tasks that require the hippocampus [42].

Further evidence for a role of glial glutamate uptake in the modulation of synaptic plasticity comes from studies on GLT1 at mossy fiber (MF)–CA3 synapses [44]. The authors used a β-lactam antibiotic, ceftriaxone (CEF), previously shown to up-regulate the transcription of GLT1 [45]. CEF-treated rats have a strong impairment in mGluR-dependent long-term depression (LTD) at MF–CA3 synapses and pharmacological block of GLT1 in CEF-treated slices restored LTD. Since GLT1 expression was elevated in astrocytic processes and MF presynaptic terminals, the authors concluded that GLT1 may regulate synaptic plasticity by preventing activation of presynaptic receptors.

In Drosophila melanogaster, courtship behavior is controlled by the glial amino acid transporter genderblind (gb) [46]. Gb protein regulates ambient extracellular glutamate concentration and thereby influences synaptic transmission [47]. Gb mutant males also court other males, because they have defects in chemosensory processing, causing a misinterpretation of sex-specific sensory cues. The male–male courtship phenotype is the result of increased glutamatergic synapse strength. Overexpression of the Drosophila vesicular glutamate transporter (DVGlut1) caused an increase in male–male courtship. Moreover, the Gb phenotype could be rescued by pharmacological suppression of glutamatergic neurotransmission [46]. Gb has high homology to mammalian xCT proteins that are subunits of cystine/glutamate transporters that secrete glutamate in exchange for extracellular cystine. Taken together, these results suggest that Gb normally suppresses glutamatergic synapse strength to prevent homosexual behavior.

**Regulation of inhibition by glia cells**

While the integration of astrocytes in excitatory neuronal networks is well documented, their roles in shaping inhibitory neurotransmission via GABA and glycine are poorly understood. Glial cells mainly express the GABA transporters GAT1 (also expressed by neurons) and GAT3. Genetic ablation of GAT1 and GAT3 demonstrated that glial GABA uptake plays an essential role in vivo in controlling extracellular levels of GABA in the...
Consistent with such a model, GAT3 heterozygous mice have an increased resistance to pharmacologically induced seizures [49]. In olfactory bulb astrocytes, GABA uptake was shown to evoke Ca²⁺ transients in astrocytes [50]. Glycine transport is regulated by both neuronal and glial transporters. The glycine transporter GlyT1 is predominantly expressed by glial cells of the brain stem and spinal cord. Genetic ablation of GlyT1 resulted in strong motor and respiratory deficits, pointing to the relevance of this transporter in the removal of glycine from the extrasynaptic cleft in neonatal animals [51].

Astrocytes can function as an interconnected network and as individual cells

Astrocytes are organized in networks and communicate through gap junctions. Gap junctions are composed of connexins that form channels in the plasma membrane thereby allowing direct intercellular communication [3]. Astrocyte networks could be actively associated with groups of synapses and with the vascular network (Figure 3). Although not directly demonstrated, some evidence suggests that Ca²⁺ waves in astroglial networks regulate neuronal network activity [52]. For example, mice lacking astrocytic gap junctions displayed changes in the dynamics of stimulus-induced potassium transients and a lowered threshold for the generation of epileptiform events [53]. Ca²⁺ waves in astroglial networks may also couple neuronal activity to the use of glucose in the brain [1]. For example, glutamate released during synaptic transmission evoked metabolic waves in cultured astrocytes, resulting in coordinated uptake of glucose by interconnected astrocytes [54]. Recently, neuronal activity in awake, behaving mice was shown to correlate with Ca²⁺ transients in astrocytic populations [55,56]. Two-photon microscopy through a cranial window of an awake, head-restraint mouse allowed to run on a styrofoam ball was used to visualize Ca²⁺ transients in neocortical neurons and astrocytes [55]. Similar studies were performed in the cerebellum, where radial Bergmann glia signals were found to correlate with locomotor behavior and were sensitive to blockade of neuronal activity [56]. In the cerebellum, three forms of Ca²⁺ transients were described. ‘Flares’ were triggered during locomotion and extended over macroscopic domains of several hundreds of microns. They involved large networks of astrocytes. ‘Sparkles’ and ‘bursts’ were present in resting animals; sparkles were restricted to individual fibers and dependent on neuronal activity, while bursts were radial waves of tens of cells and were dependent on purinergic signaling. Similar radially expanding bursts or waves have been reported in anesthetized rodents [57]. These observations raise the possibility that flares are part of a dynamic interplay between neurons, astrocytes, and blood flow dynamics associated with motor behavior. They also reveal differences between radially shaped Bergmann glia and star-shaped neocortical astrocytes and highlight the need to compare astrocytic physiology between different subtypes and across brain regions.

By imaging Ca²⁺ signals in the primary visual cortex of ferrets, it was shown that astrocytes, like neurons, exhibit Ca²⁺ responses to visual stimuli that were dependent on stimulus features and have receptive-field characteristics of neurons. Moreover, astrocytes were even more sharply tuned for orientation and frequency than neurons at single-cell resolution. Surprisingly, astrocytic Ca²⁺ responses generally did not propagate to other astrocytes, indicating that each astrocyte interacts independently with a small number of neurons surrounding it, rather
than functioning as an interconnected network [58**]. Application of \(\text{D}-\text{threo-}\beta\)-benzoyloxyaspartate (TBOA), which blocks astrocytic glutamate transporters, reduced the response of astrocytes to visual stimuli while increasing the neuronal response. TBOA also reduced the intrinsic optical signals, which reflect changes in blood vessel volume, suggesting that evoked neuronal activity is transferred to the adjacent astrocytes, which subsequently modulates local blood flow (Figure 3).

**Conclusions**

Astrocytes actively participate in synaptic transmission and plasticity by secreting neuroactive substances and by actively removing synaptically released neurotransmitters. Although much progress has been made in recent months, the signaling mechanisms of secretion and uptake of active substances in perisynaptic astrocytes are still largely unexplored. In addition, glial cells are quite diverse throughout the brain, and different types of glia may modulate different types of plasticity. In the future, it will be essential to investigate the bidirectional signaling between neurons and glia in awake, behaving animals and to link neuron–glia crosstalk to specific types of behavior. Moreover, neuron–glia interactions including gliovascular interactions, should be studied at the single cell and network level in order to fully understand the role of glia in brain dynamics and adaptation.

**Acknowledgements**

We thank R. Schorner for excellent technical support in preparing the figures. Our own work on neuron–glia crosstalk was funded by the Deutsche Forschungsgemeinschaft (SPP1172).

**References and recommended reading**

Papers of particular interest, published within the annual period of the review, have been highlighted as:

- of special interest
- of outstanding interest

19. By generating inducible transgenic mice that express a dominant-negative SNARE domain selectively in astrocytes, the vesicular release of neurotransmitters was selectively blocked in astrocytes. These dnSNARE animals it was demonstrated for the first time that adenine-mediated tonic suppression of synaptic transmission was dependent on astrocytic vesicular release of ATP.
21. Using the same transgenic animals as in [19] this study shows that adenine metabolism from ATP released by astrocytes participate in the homeostatic drive for sleep. dnSNARE mice did not show cognitive deficits associated with sleep loss compared to wild-type mice.


34. Tzingounis AV, Wadiche JI: Astroglial excitatory neurotransmitter transporters GLT1. Neuron 2008, 61:880-894. This study confirmed previous reports that neuronal activity might regulate astroglial GLT1 expression. The authors identified a neuronal-dependent transcription factor in astrocytes, kappa-B motif binding phosphoprotein (KBPP), that regulates GLT1 transcription. In vivo mouse models of denervation and neuronal degeneration resulted in loss of KBPP expression and concomitant loss of GLT1, indicating that the integrity of presynaptic terminals helps to maintain astrocyte function.


56. Nimmerjahn A, Mukamel EA, Schnitzer MJ: Motor behavior activates Bergmann glial networks. Neuron 2009, 62:400–412. A study on awake, head-restraint mice showing that large networks of radial glia can be activated by specific behaviors. Three different forms of Ca$^{2+}$ transients were described. One form was initiated during locomotor behavior and correlated with changes in blood perfusion, suggesting that these glia networks modulate macroscopic changes in brain dynamics and blood flow.


58. Schummers J, Yu H, Sur M: Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science 2008, 320:1638–1643. This study used two-photon imaging in the adult ferret visual cortex in vivo to demonstrate that visual stimulation triggers astrocytic Ca$^{2+}$ responses that are sharply tuned for stimulus orientation and spatial frequency. Visual stimulation did not induce intercellular Ca$^{2+}$ waves that propagate through large astrocytic networks, demonstrating that astrocytes behave relatively independently of each other. This study further suggests that astrocytic Ca$^{2+}$ signaling is important for the regulation of the hemodynamic response that generates the intrinsic optical signal.