

Chapter 2

Astrocyte–Neuron Communications

Sally R. McIver, Mathilde Faideau, and Philip G. Haydon

2.1 Introduction

When astrocytes were first visualized by Virchow in 1846, he characterized them as a type of “glue” filling in the interstitial space. The term “astrocyte” first appeared in 1893 when improvements in histological techniques made it possible to distinguish individual cell morphology within this cerebral “glue” [1]. The importance of these cells, though not well understood, was appreciated by the fact that astrocytes occupy a substantial amount of space in the brain, representing up to 50% of cerebral volume [2]. Interestingly, the ratio of astrocytes to neurons varies among species and according to the relative complexity of the brain [3], increasing proportionally with the complexity of the neural network. This is perhaps one of the first pieces of evidence hinting at a role for astrocytes in the integration of neuronal activity. With the advancement of staining techniques came a greater appreciation for the unique structure of these cells which subsequently provided great insight into their diverse functions. Astrocytes have multiple primary processes and fine branching processes which are able to expand and contract, allowing them to dynamically contact both synapses and microvasculature. In addition, by forming independent microdomains, with little or no overlap with neighboring astrocytes, astrocytes are able to effectively modulate communication between neuronal networks and glial–vascular coupling. For example, the end feet of astrocytes contact blood vessels and modulate blood flow via Ca^{2+} -dependent release of vasoactive agents, effectively regulating neuronal access to nutrients required to sustain metabolic demand. Similarly, astrocyte morphology can change in response to their environment. Hormonally responsive astrocytes in the arcuate nucleus of the adult female rat respond to estradiol with dramatic changes in their morphology, including an increased coverage of neuronal perikarya, impacting synaptic communication [4]. These changes in

S.R. McIver • M. Faideau • P.G. Haydon, Ph.D. (✉)
Department of Neuroscience, Tufts University School of Medicine,
136 Harrison Avenue, Boston, MA 02111, USA
e-mail: philip.haydon@tufts.edu

morphology have been recently correlated with changes in glutamate–glutamine cycling, indicating functional plasticity of neuronal–glial communication in the normal adult brain [5]. Another important function of astrocytes is their role in the “tripartite synapse,” or the communication between the astrocytic process and the pre- and postsynaptic terminals [6]. Despite the complex morphology and numerous ramifications of astrocytes, it is still rather surprising to consider that a single astrocyte residing in area CA1 of the rat hippocampus can contact up to 140,000 synapses [7].

Much of what is widely known about the function of astrocytes has been discovered using *in vitro* and *in situ* preparations, providing valuable information on how these glial cells help maintain extracellular homeostasis by buffering potassium and reuptaking glutamate, for example. However, the dynamic nature of astrocyte physiology is undoubtedly best appreciated *in vivo*. The recent progress in molecular genetic techniques has allowed for astrocyte-specific expression of reporter proteins, such as GFP and various transgenes of interest, and contributed to the surge in research investigating the multifaceted role of these cells as key players in brain function. It is now widely accepted that astrocytes are in direct communication with neurons, modulating neuronal function at the synaptic and network levels, ultimately providing a significant impact on physiological and pathological conditions. This chapter begins by describing different types of glia–neuron communication at the synaptic and network levels. It then aims to link these glia–neuron signaling mechanisms to what is currently known regarding how glia–neuron communication, or perturbations thereof, contribute to healthy brain physiology as well as neuroinflammation and other related neurological conditions such as epilepsy, Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), and addiction.

2.2 Gliotransmission

According to the definition of “classical neurotransmitter,” the signaling molecule must be released from the presynaptic terminal. This definition reflects the historically neuron-centric considerations of brain function. As neuroscience has progressed toward a greater encompassment of glial biology, it is now widely accepted that astrocytes are also capable of transmitter release through a process called “gliotransmission.” Similar to neurons, astrocytes contain the cellular mechanics necessary for transmitter packaging and release, which can occur either via lysosomal or small vesicle fusion [8–11]. Vesicle fusion and exocytosis of gliotransmitters is calcium-dependent and involves formation of the SNARE complex, similar to neurons. Early demonstrations of gliotransmission, particularly those using calcium imaging, were primarily performed in cell culture, and though invaluable to the progress in current understanding of glial biology, these studies posed limitations that would later impact interpretations of astrocyte function [12–16]. Thanks to recent advances in technology and innovation, many of these early studies are being confirmed *in situ* and *in vivo*. This section summarizes the discoveries and current understanding of gliotransmission as a key player in glia–neuron communication.

2.2.1 *Glutamate*

Glutamate is the major excitatory neurotransmitter in the nervous system and is critically involved in many functions, including cerebral development and motor and cognitive functions. It has been recognized as a neurotransmitter since the 1950s [17], but its role as a gliotransmitter, which was first demonstrated in the 1990s in vitro [18–21], is currently under debate [22]. Using astrocyte–neuron cocultures and photostimulation of astrocytes, Parpura et al. [20] was among the first to demonstrate that an increase in intracellular calcium in astrocytes causes an increase in intracellular calcium in neighboring neurons in vitro. This effect was blocked with administration of a broad-spectrum glutamate receptor antagonist (D-glutamylglycine), suggesting a calcium-dependent mechanism of glutamate release by astrocytes [20]. Subsequent studies using rat and mouse hippocampal and cortical slices employed either pharmacological or cell-specific photolytic elevation of Ca^{2+} and substantiated the relevance of Ca^{2+} -dependent glutamate release from astrocytes as a mechanism of gliotransmission that could modulate either neuronal excitability or synaptic transmission and plasticity [23–25].

Further evidence supporting the capacity of astrocytes to release glutamate was later provided by biochemical and structural evidence demonstrating that VGLUT transporters and SNARE complex proteins are expressed in astrocyte processes in vitro and in acutely isolated brain slices, showing that these cells are potentially capable of vesicular glutamate release [10, 11, 26, 27]. Indeed, in purified astrocyte cultures, calcium-induced glutamate release depends on proteins involved in vesicular function, including the SNARE element synaptobrevin 2 and the calcium-binding protein synaptotagmin IV [11, 28].

Astrocytic glutamate can be synthesized de novo in astrocytes through conversion of alpha-ketoglutarate by glutamate dehydrogenase (GDH), which is mainly expressed in astrocytes [29–34]. Astrocytic glutamate also comes from the high-capacity uptake of synaptic glutamate by the astrocytic transporters GLAST and GLT-1 and the Cl-dependent glutamate/cysteine exchanger [35, 36]. Following uptake, glutamate is quickly metabolized into glutamine by the glutamine–glutamate cycle via a reaction mediated by glutamine synthetase [36].

Multiple factors have been shown to stimulate glutamate release from astrocytes in vitro, from the classic neurotransmitters, including glutamate itself, to inflammatory molecules such as TNF-alpha [37], ATP [38, 39], prostaglandins, and CXCL12 [40, 41]. In some cases, multiple convergent signaling cascades are required to produce elevated calcium-induced release, suggesting that astrocytes are capable of integrating incoming signals to produce a response. For example, basal concentrations of TNF-alpha are required for P2Y1R-evoked and Ca^{2+} -dependent glutamate release from astrocytes [41, 42], whereas higher concentrations of TNF-alpha can directly increase the glutamate release, independently of P2Y1R in granule cells of the mouse hippocampus [41].

The release of glutamate from astrocytes has been shown to modulate synaptic activity in multiple brain regions in situ including the hippocampus, thalamus, nucleus accumbens, and olfactory bulb. In many cases, it produces these effects by

activating neuronal extrasynaptic NMDA and metabotropic glutamate receptors to modulate neuronal activity [23, 43–49].

These early studies were critical in demonstrating that astrocytes not only communicate with neurons but are capable of modulating neuronal activity via calcium-dependent release of transmitters. However, further studies using slice preparations and *in vivo* models have become increasingly necessary for understanding the functional impact of astrocytic glutamate release.

Although numerous studies have shown the potential for glutamate to be released from astrocytes and signal directly to neurons, efforts to directly monitor glutamate-mediated gliotransmission by many methods, including microdialysis and biosensor detection, have been challenged by the fact that astrocytes are endowed with high-affinity glutamate transporters that rapidly and avidly remove glutamate from the extracellular space. Of course, the inability to detect does not mean that signaling does not occur. Because of the difficulties inherent in direct measurements, more indirect approaches have been used to infer the functional relevance of glutamate release.

Recently, studies from one laboratory used molecular-genetic approaches to alter intracellular calcium signaling in astrocytes and concluded that calcium-dependent glutamate-mediated gliotransmission does not occur in the hippocampus, contrary to other reports [13]. Specifically, the authors used two lines of transgenic mice, one in which intracellular calcium levels could be selectively increased in astrocytes by pharmacological activation of ectopic expression of a receptor (MrgA1R+) and one in which the IP_3 signaling pathway was selectively impaired in astrocytes ($IP_3R2^{-/-}$), causing reduced intracellular calcium. Based on whole cell recordings of CA1 pyramidal neurons in hippocampal slices, the authors failed to detect a difference in evoked or spontaneous NMDA-mediated EPSCs, as well as LTP induction or maintenance, in either of the transgenic mouse lines compared to WT [13]. Results from this study directly challenged previous results from several independent laboratories, fueling a debate concerning the importance of glutamate-mediated gliotransmission. A crucial consideration, however, is that “absence of evidence is not evidence of absence.” The contradictions in results are likely to reflect variations in experimental approaches, all of which pose limitations [50]. For example, cell culture studies undoubtedly are restricted by the lack of intact physiology; slice preparations maintain network connections, but the electrophysiological approaches that are generally employed in these studies do not always abide by consistent paradigms and on some occasions may depend upon a narrow range of physiological metrics (such as measurements of basal neuronal or synaptic currents) that may obscure functionally relevant responses. Finally, *in vivo* studies using astrocyte-specific manipulations in transgenic mice minimize the need for pharmacological approaches but should be regarded with careful criticism as well, since in many cases the transgenes expressed may function by introducing new cellular pathways that are not inherent in astrocytes (rather than introducing mutations in preexisting astrocyte pathways), or may be expressed constitutively, allowing for potential developmental compensations to impact function. These difficulties necessitate a combined approach employing multiple independent measurements to assess the role of glutamatergic gliotransmission in the regulation of neuronal function.

In contrast to the reports indicating a lack of impact of disrupting astrocytic calcium on some forms of activity, other studies employed a multifaceted approach and demonstrated that glutamatergic gliotransmission does play a role in modulating synaptic plasticity in the hippocampus. For example, Navarette et al. recently showed that *in vivo* calcium signals in astrocytes are required for cholinergic-induced LTP in the hippocampus [51]. Using *in vivo* calcium imaging and electrophysiology, they demonstrated that somatosensory stimulation (via tail pinch) or electrical stimulation of cholinergic activity evoked increases in intracellular calcium in hippocampal astrocytes along with subsequent cholinergic LTP, through a pathway that required activation of muscarinic receptors and, in the case of LTP, of metabotropic glutamate receptors (mGluRs). To further examine astrocytic mechanisms contributing to cholinergic LTP, the authors use a combination of calcium imaging, electrophysiology, and calcium uncaging in hippocampal slice preparations. Consistent with the *in vivo* results, these experiments showed that cholinergic LTP required calcium-dependent glutamate release from astrocytes—LTP was abolished when calcium chelators were applied to astrocytes in rat hippocampus and in hippocampal slices prepared from mice in which IP_3 -mediated calcium signaling in astrocytes is transgenically knocked out ($IP_3R2^{-/-}$). Interestingly, using simultaneous calcium uncaging in astrocytes and a minimal stimulation paradigm, they show that astrocytic calcium elevations induce release of glutamate that activates presynaptic mGluRs and that LTP requires a tight coordination of these events with postsynaptic depolarization. Thus, by using similar techniques (including the same $IP_3R2^{-/-}$ as used in the aforementioned study) but also by broadening the scope of physiologically relevant paradigms for monitoring synaptic activity, this group provided rigorous evidence showing that calcium-dependent release of glutamate from astrocytes modulates cholinergic LTP in the hippocampus [51].

Thus, the pendulum is swinging back in favor of substantial evidence supporting calcium-dependent glutamate-mediated gliotransmission. However, in the future, it will be important to use cell-specific recombination of floxed alleles of genes to understand the precise physiological and behavioral role of this form of glial–neuronal interaction.

2.2.2 D-Serine

D-serine has been generally considered to be selectively expressed in astrocytes, along with serine racemase, which converts L-serine into D-serine [52, 53]. D-serine is an endogenous co-agonist of NMDA receptors and binds to the “glycine-binding site” on the NR1 subunit, a subunit which is present on all NMDA receptor assemblies [54–56]. *In vitro* studies of astrocytes demonstrated that D-serine release is Ca^{2+} -dependent and occurs via SNARE-mediated exocytosis following AMPA receptor activation [57]. Evidence from *in vitro* cortical astrocyte cultures and *in vivo* hippocampal astrocytes studies show that D-serine and glutamate are contained in similar vesicular organelles called synaptic-like microvesicles (SLMVs) in astrocytes [58, 59].

In contrast to early studies showing astrocyte specificity of D-serine, the recent development of new antibodies suggests that neurons express more serine racemase

than astrocytes [60–64]. Serine racemase expression has been observed in pyramidal neurons of the cortex and hippocampus and GABAergic medium-spiny neurons of the striatum, but was not detected in glial cells [62, 63]. More recently, Ding et al. produced new antibodies against serine racemase and found immunoreactivity in mouse cortical neurons and to a lower extent in oligodendrocytes and astrocytes of the corpus callosum [64]. Similar to what was reported in astrocyte cultures, AMPA receptor stimulation on cortical neuronal cultures leads to D-serine release; however this seems to occur independently of exocytosis [61]. Neuronal D-serine release can be differentiated from astrocytic D-serine release using application of veratridine in cortical slices, which enhances depolarization and subsequent neuronal release of D-serine through Asc-1 transporters [61]. The precise mechanism of D-serine signaling pathways is still rather unclear, though Wolosker et al. [60] proposed the existence of a neuron–astrocyte “serine shuttle,” where neurons and astrocytes provide sources of D-serine and L-serine, respectively. In this scenario, astrocytic L-serine produced from glucose would be used by neurons to produce D-serine, which would then accumulate in astrocytes [61].

The action of D-serine on the glutamatergic synapses of the supraoptic nucleus of the hypothalamus is finely modulated by the distance between the astrocytic processes and the postsynaptic element. During lactation, the astrocytic processes retract from the synapse, which reduces the D-serine concentration at the synapse and thereby induces long-term depression (LTD) [49]. The contribution of D-serine in synaptic plasticity also has been demonstrated within the hippocampus and cortex [65, 66]. For example, impairing intracellular Ca^{2+} within a single astrocyte prevents LTP induction at Schaffer collateral/CA1 synapses, and LTP can be rescued with subsequent application of D-serine [65]. Furthermore, LTP is also suppressed following treatment with a serine racemase inhibitor, which decreases astrocytic release of D-serine. Astrocytic D-serine was also shown to be involved in LTP formation in layers V/VI of the prefrontal cortex, where inhibition of astrocytic metabolic activity with fluoroacetate reduced LTP [66].

Since several lines of evidence indicate localization of serine racemase expression in neurons as well as astrocytes, the relative contribution of astrocytic and neuronal D-serine was investigated using cell-selective serine racemase KO mice [67]. These studies showed that neuronal absence of serine racemase significantly reduced LTP in Schaffer collateral/CA1, whereas astrocytic loss of serine racemase did not. This study does not necessarily contradict original reports of D-serine as a gliotransmitter but suggests that in normal conditions neuronal D-serine predominantly impacts synaptic activity. Future studies using these mice or other cell-specific approaches may determine whether astrocytic D-serine plays a more important role in synaptic function under conditions of neuronal dysfunction or metabolic dysregulation.

2.2.3 GABA

Transient expression of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), has been observed in astrocytes during development [68, 69]. GAD67

(67 kDa form of GAD) and the GABA transaminase, GABA-T, have also been reported to be expressed in human astrocytes [70]. However, the potential role of GABA release in astrocyte–neuron signaling has been unclear until recently, although an astrocytic inhibitory modulation on neuronal activity was suggested in the past [71, 72]. Kozlov et al. demonstrated that mitral cells of the olfactory bulb present synchronous slow outward currents, which are dependent on GABA release from astrocytes [48]. Since then, studies have confirmed a role for astrocytic GABAergic signaling in tonic inhibition, modulating a continuous current which was found to be dependent on extrasynaptic GABA-A receptors that control the neuronal excitability [73] and which has been shown to be involved in sleep, memory, epilepsy, and alcohol vulnerability [74–77].

Tonic inhibition mediated by astrocytic GABA appears to be released by the anion channel Best1, as was shown recently in the cerebellum [78]. Best1 is activated by intracellular Ca^{2+} and by changes in cell volume and is also permeable to glutamate. Although Best1 is expressed in astrocytes of the CA1 layer in the hippocampus, Yoon et al. [79] observed a very low tonic inhibition correlated with low levels of GABA in astrocytes [79]. Bergmann glia of the cerebellum provide a higher tonic inhibition due to their higher concentration of intracellular GABA [79].

In addition to providing a source of tonic inhibition to neurons, astrocytic GABA signaling has anti-inflammatory properties. GABA receptors expressed on astrocytes and microglia (GABA-A and GABA-B) inhibit the NF- κ B and p38-MAP kinase pathways and the release of TNF- α and IL-6 under inflammatory stimulation [70]. In return, activation of GABA-A and GABA-B on astrocytes increases their release of GABA in a Ca^{2+} -dependent manner [80]. GABA transporters GAT1, GAT2, and GAT3 are able to release GABA from astrocytes via a Ca^{2+} -independent pathway when intracellular GABA concentration is too high [80], although these mechanisms are less clear.

2.2.4 Adenosine Triphosphate

Adenosine triphosphate (ATP) is critical for metabolic cellular processes but can also act as a transmitter. Astrocytes are equipped with machinery for ATP release, as was appreciated by visualization of ATP-containing vesicles in cultured astrocytes [39, 81]. Early studies using astrocyte cultures demonstrated that calcium wave propagation was mediated by astrocyte release of ATP [82–84]. Astrocytic ATP signaling was thought to propagate calcium waves via chemical coupling [85], providing a mechanism for rapid intercellular communication across broad domains. ATP also proved important for astrocyte cross talk with neurons. Zhang et al. elegantly demonstrated a functional impact of glia–neuron signaling by showing that ATP release from astrocytes tonically suppresses glutamatergic synapses via activation of presynaptic purinergic receptors *in vitro*, an effect which was dependent on synaptic glutamate release and subsequent activation of glutamate receptors on astrocytes [84]. Similar findings were reported in studies of CA1 synapses in hippocampal slices, where adenosine, a metabolite of

ATP, was found to modulate synaptic depression [84]. A key role for purinergic gliotransmission was further established through the development of transgenic mice in which SNARE-dependent exocytosis was selectively blocked in astrocytes [86]. Specifically, this study demonstrated that astrocytic ATP and its metabolite, adenosine, act to tonically suppress synaptic activity via activation of A1 receptors.

ATP release from astrocytes can occur through activation of purinergic P2Y and P2X receptors and impacts physiological and pathological responses [87–89]. Indeed, activation of P2Y1 and P2Y2 receptors on astrocytes has been shown to be critical for intercellular calcium signaling [90], the source of which is thought to be ATP.

Purinergic signaling from astrocytes extends beyond ATP release. Once released, ATP can be rapidly hydrolyzed into adenosine, which can act on adenosine A1 and A2a receptors. Activation of presynaptic A1 receptors tonically inhibits glutamate release, whereas activation of A2a receptors upregulates synaptic transmission, and there is evidence for dynamic astrocytic regulation of synaptic activity via both A1 and A2a receptors [86, 91, 92]. Several lines of evidence point to a critical role for this astrocytic source of adenosine in modulation of synaptic plasticity, discussed in the subsequent sections, memory consolidation and sleep homeostasis.

2.2.5 *TNF-Alpha*

TNF-alpha is a well-known proinflammatory cytokine, but the studies of Beattie and Stellwagen in 2002 and 2006 suggest that it can also act as a gliotransmitter [93, 94]. When applied to neurons in vitro, TNF-alpha taken from conditional media of astrocyte cultures increases the amount of AMPAR expression at the membrane surface, thereby increasing neuronal sensitivity to glutamate [93]. In response to prolonged blockade of activity (via TTX treatment), TNF-alpha treatment still increases AMPAR surface expression [94], suggesting that TNF-alpha is secreted by astrocytes and modulates neuronal activity by increasing surface AMPAR expression and increasing the synaptic strength.

2.3 Synaptic Plasticity

As part of the tripartite synapse, astrocytes are uniquely positioned to monitor synaptic activity. Signaling at the synapse can activate receptors on astrocytes, and astrocytes are readily equipped to modulate the uptake and release of neuroactive signaling molecules, including glutamate, D-serine, ATP, and TNF-alpha, subsequently regulating activation of pre-, post-, and extrasynaptic receptors. Communication at the tripartite synapse, therefore, can be both static and dynamic, modulating synaptic plasticity and network activity through a variety of mechanisms, many of which are calcium-dependent.

2.3.1 Intracellular Calcium Signaling

There has been conflicting evidence regarding the role of calcium-dependent gliotransmitter release on LTP. Several different methods have been used to assess this, and it is likely that a difference in techniques accounts for the discrepancies. For example, several studies have shown that calcium clamping astrocytes impairs LTP in astrocytes [95]. However, results from Agulhon et al. [13] stimulated quite a bit of controversy by demonstrating a lack of an effect on LTP in two transgenic mouse lines with an induced impairment in calcium signaling specifically in astrocytes [13]. These authors failed to detect a difference in LTP induction and maintenance in hippocampal slices isolated from transgenic mice with astrocyte-specific impairment in the IP_3 signaling pathway. However, a recent study using the same IP_3 knockout mouse showed that calcium-dependent glutamate-mediated gliotransmission does indeed contribute to cholinergic LTP in the hippocampus [51]. Despite conflicting evidence, there are several additional sophisticated approaches that demonstrate the impact of intracellular calcium signaling on synaptic transmission (for review [96]). For example, visualization of high spatial and temporal resolution using two-photon laser scanning microscopy revealed two types of local calcium dynamics in individual hippocampal astrocytes [97]. “Focal” events were characterized by small increases in calcium $[Ca^{2+}]_i$ that were highly localized to a small subregion and accounted for the majority of calcium events in astrocytic processes. “Expanded” events were detected simultaneously in contiguous segments of astrocytic processes and were marked by large calcium increases $[Ca^{2+}]_i$. The expanded events showed a higher degree of dependence on neuronal activity, whereas the focal events only slightly decreased in frequency when neuronal activity was blocked and therefore appeared to primarily reflect spontaneous synaptic release. Furthermore, these calcium events were dependent on IP_3 receptor signaling and purinergic receptor activation and participated in bidirectional communication, such that activation of the purinergic receptor, P2Y₁R, stimulated IP_3 -mediated release of intracellular calcium causing transmitter release at excitatory synapses.

Using combined techniques of whole cell recordings, calcium imaging in astrocytes and glutamate imaging in hippocampal slices, it is possible to measure simultaneous responses in neurons and astrocytes, providing solid evidence for astrocytic glutamate release. Panatier et al. [92] visualized calcium dynamics localized within “compartments” of astrocyte processes using real-time confocal imaging and found that stimulation of single synapses induced calcium events confined to a local astrocytic compartment and occurring simultaneously with postsynaptic glutamatergic transmission [92]. These events were dependent on mGluR5 activation of astrocytes, which induced calcium signaling and subsequent release of an astrocytic source of adenosine acting on presynaptic A2a receptors to modulate basal excitatory synaptic transmission.

Recent studies have also discovered that astrocytic calcium signaling can occur in microdomains of the astrocytic processes [98], which further impacts the ability of these cells to communicate discretely at the synapse.

2.3.2 *Astrocyte Signaling Molecules and Synaptic Plasticity*

Astrocyte–neuron signaling also occurs locally through interactions between signaling molecules and ligand receptors. These interactions are often bidirectional, impacting both astrocyte and neuron physiology and, in many cases, synaptic plasticity.

2.3.2.1 Ephrin

Ephrin ligands and their receptors are widely recognized for their role in axon guidance and synapse development, but recent studies show that they are also involved in synaptic plasticity in the adult brain. For example, recent [99, 100] studies indicate that ephrin-mediated signaling among astrocytes and synapses impacts LTP in the hippocampus. Astrocytic processes express ephrin-A3, whose binding partner, EphA4, is expressed neuronally. LTP is attenuated in mice lacking either ephrin-A3 or EphA4 in the CA1 region of the hippocampus. Furthermore, reduced synaptic plasticity in these knockout mice was associated with increased glutamate uptake via a selective increase in expression of the astrocytic glutamate transporters GLT1 and GLAST, subsequently reducing the availability of synaptic glutamate [99].

2.3.2.2 Endocannabinoid Signaling

Endocannabinoids and their endogenous receptor CB1 have widespread effects on neuronal activity, including synaptic plasticity, mainly by reducing presynaptic release of neurotransmitters. For example, astrocytes were recently shown to express functional CB1 receptors that, upon activation, regulate glutamate release from hippocampal astrocytes in a calcium-dependent manner [101]. Further studies revealed that activation of CB1 receptors modulates synaptic plasticity in a bidirectional manner. Specifically, activation of presynaptic CB1 receptors depresses synaptic activity, whereas activation of astrocytic CB1 receptors potentiates synaptic plasticity via calcium-dependent release of glutamate and subsequent activation of presynaptic mGluR1 receptors [102]. These results suggest that endocannabinoid signaling may induce inhibitory effects locally at the synapse but may also potentiate synaptic transmission across a broader network via CB1R activation (and subsequently calcium signaling) on astrocytes. To investigate the specific role of astrocytic CB1R, Han et al. recently generated a conditional transgenic mouse lacking CB1R selectively in astrocytes, GABAergic, or glutamatergic neurons [103]. This study showed that CB1Rs expressed by astrocytes, but not by glutamatergic or GABAergic hippocampal neurons, are necessary for endocannabinoid-induced synaptic depression in hippocampal CA3/CA1 synapses and cannabinoid-induced impairment of spatial working memory [103]. These studies provide further evidence of astrocytic modulation of synaptic plasticity.

2.3.2.3 Aquaporin

Aquaporin 4 (APQ4) is a water-permeable channel that is mainly expressed by astrocytes in the CNS. It responds to changes in extracellular milieu and has been implicated in cerebral edema following injury [104]. Since APQ4 is colocalized with K^+ channels [104], it was hypothesized that it has the potential to alter excitability. Indeed, mice null for APQ4 show attenuated theta-burst stimulation (TBS) LTP and LTD, though baseline activity and short-term plasticity are intact [105]. Interestingly, LTP impairment in APQ4 $^{-/-}$ mice was attributed to reduced BDNF, which is known to be required for TBS–LTP. How a lack of APQ4 modifies BDNF expression is unknown.

2.4 Network Modulation

While astrocytes are ideally situated to monitor synaptic activity at the tripartite synapse, they are also able to communicate long distances via the expression of gap junctions. Astrocytes have been shown to be organized in structurally nonoverlapping domains [106] and make contact with neurons and blood vessels. With its impressive volume, one astrocyte can contact tens of thousands of synapses [7, 106, 107], while one synapse is surrounded by only one astrocyte. This anatomical aspect is essential for the comprehension of their role in the plasticity and regulation of synaptic activity. Indeed, astrocytes are organized in a network, or syncytium, to communicate with one another via gap junctions, which are formed by connexins that are permeable to compounds with molecular weights lower than 1,000 Da [108]. The astrocytic syncytium can then be easily visualized via dye propagation through gap junctions, where it is confined within the network.

Though it is clear that astrocytic signaling modulates neuronal network activity, both through intercellular calcium signaling, gap junctions, and through lactate transport, the intricacies of the molecular mechanisms governing these pathways are still somewhat unclear.

2.4.1 Intercellular Calcium Signaling (“Calcium Waves”)

Unlike neurons, astrocytes do not produce action potentials. Their membrane potential is very stable and has low resistance due to the high resting permeability to K^+ ions and because of the gap junction connectivity in an astrocytic syncytium. In early work, Nedergaard used astrocyte–neuron cocultures to show that astrocytes communicate to each other and to neurons through calcium signals, or “waves” that propagate via gap junctions [109]. Since then, two forms of excitation have been characterized in astrocytes based on “calcium waves”: a spontaneous, autonomous excitation and an excitation which is dependent on chemical signals relayed within the neuronal circuit [107]. Until recently, calcium waves were mainly characterized

in vitro or in acute brain slices and were found to be independent of neuronal activity and relatively slow and localized [110, 111]. There are discrepancies in the results of these earlier studies, most of which were done in vitro or in situ, and subsequently there are two proposed mechanisms of calcium wave propagation: one poses that it occurs via cytosolic transfer of IP_3 through gap junctions [112], whereas the other proposes that it occurs via ATP release and diffusion, activation of purinergic P2YRs on astrocytes, and then subsequent activation of IP_3 signaling [85]. It is important to realize that these two pathways are not mutually exclusive, and it is likely that maintenance of calcium wave propagation varies according to brain region and the related heterogeneity of astrocyte cell types. Models of these two pathways have been described [113]. Further studies using in vivo techniques are required to determine the relevance of calcium wave propagation and the signaling mechanisms that govern it.

As an example, recent improvements in calcium imaging techniques in vivo contributed to the confirmation that calcium waves, or “glissandi,” propagate through astrocytes en masse in the hippocampus [114], occurring more frequently than sporadic calcium activity. Interestingly, glissandi were blocked by tetrodotoxin, demonstrating for the first time that calcium wave propagation is dependent on neuronal activity, relative to sporadic activity, which is TTX-insensitive, and therefore results from intrinsic intracellular calcium signaling in individual astrocytes. Calcium wave propagation was also found to be initiated by ATP application and blocked by inhibitors of gap junctions or ATP receptors, suggesting a role for both intra- and extracellular signaling molecules. Moreover, these large-scale calcium waves coincided with reduced flow of red blood cells through vessels within the same region, providing even more evidence that astrocytic signaling coordinates network activity on multiple levels.

2.4.2 *Gap Junctions*

When a large number of gap junctions were detected among astrocytes over three decades ago, many scientists were led to the conclusion that this “glial syncytium” functioned as more of a support system. However, more recent studies indicate that astrocytes communicate through gap junctions to integrate intra- and extracellular signals in response to changes in neurovasculature and neuronal metabolic demands, effectively integrating signals to modulate network activity. Contrary to early belief, astrocytes are uniquely structured to morphologically occupy discrete anatomical domains, without structural overlap, therefore necessitating an efficient mechanism for intercellular communication. This is achieved primarily by the formation of gap junctions, which are composed of connexins 30 and 43 (Cx30, Cx43) in astrocytes. Regulation of network activity by gap junction signaling in astrocytes can be easily appreciated in the somatosensory barrel cortex of rodents, where dye coupling of astrocytes shows strong coupling almost exclusively within a barrel [115]. Similarly, astrocytic networks also modulate sensory integration within olfactory glomeruli, which are highly structurally organized in functional units, mainly via

activity-dependent generation of extracellular potassium and Cx30-mediated gap junction communication [116].

Studies from Cx30 and Cx43 knockout mice also highlight the impact of astrocytic regulation of hippocampal network activity. Hippocampal network activity is altered in these mice, with increased synaptic activity at CA1 pyramidal cells and increased basal excitatory synaptic transmission, which occludes LTP and enhances LTD [117]. This aberrant synaptic plasticity was attributed to decreased/slower glutamate uptake and slower K^+ clearance. Despite the finding that Cx30 $^{-/-}$ and Cx43 $^{-/-}$ astrocytes still uptake a significant amount of glutamate and potassium, they are uncoupled and therefore unable to redistribute it, causing cell swelling in response to neuronal activity. These results show the importance of astrocytic networks in maintaining extracellular homeostasis during basal synaptic transmission.

2.4.3 Energy Metabolism (Lactate Transport)

Neuronal activity, and in particular glutamatergic activity, requires a great quantity of energy resulting from the metabolism of glucose. Cerebral consumption of glucose accounts for 20 % of the total body glucose utilization [2] and is mainly utilized in support of excitatory neuronal activity; action potentials, reestablishment of ion gradients and postsynaptic responses all rely on glucose availability [118].

Astrocytes are ideally located to interact with both blood vessels, via end-feet contact, and neurons within the tripartite synapse. Here, astrocytes are critical regulators of brain energy metabolism as they provide energy to neurons during synaptic activity [119–121]. As proposed in the astrocyte–neuron lactate shuttle (ANLS) [122], the astrocytic energy supply to neurons depends on their glutamate and glucose uptake and their high glycolytic activity [119, 120]. Astrocytes uptake glutamate via Na^+ -coupled glutamate transporters (GLAST and GLT-1) in response to glutamate release from neurons. The Na^+/K^+ pump then restores the Na^+ gradient, requiring ATP consumption, which in turn stimulates glucose uptake by astrocytes via the transporter GLUT-1 [123] and glycolysis [122, 124–127]. The glycolytic product, lactate, is then transported via the astrocytic MCT transporters (MCT1 and MCT4) to the neuronal MCT2. In addition to their direct utilization of glucose, neurons can rapidly use the lactate oxidative energy substrate [128–131].

An interesting study performed by Rouach et al. [132] showed that within the hippocampus, an entire astrocyte syncytium, supported by the connexins Cx43 and Cx30, is able to restore neuronal activity under conditions of glucose deprivation due to lactate provided by the astrocytes and its ability to diffuse through the syncytium [132]. This study showed that the fluorescent derivative of glucose 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG), injected in a perivascular astrocyte of a mouse hippocampal slice, can diffuse in a score of minutes in a network of astrocytes (60–90 astrocytes). This diffusion was shown to be dependent on the gap junction proteins, Cx43 and Cx30, and increased during neuronal activity. Moreover, it suggests that diffusion through the astrocytic network, including the perivascular astrocyte, is capable of shifting differentially

according to energy demands. Specifically, this study showed that the diffusion of 2-NBDG propagated toward the electrically stimulated area, which was deprived of glucose, and subsequently restored neuronal activity. This restoration was shown to be dependent on lactate [132]. Thus, the ANLS is more than the response of one individual astrocyte to synaptic activity but is also a dynamic response to an entire network, with the ability to adapt its size to the intensity of the energetic demand. This metabolic coupling between the astrocytes, the neurons, and of course the blood capillaries is a perfect illustration of the complex integration of signaling in the cerebral parenchyma [133].

2.5 Impact of Glia–Neuron Communication on Behavior

The previous sections were designed to introduce astrocyte–neuron signaling as it occurs at the synaptic and network levels. The recent surge in technology and interest focused on the potential contribution of astrocyte signaling to brain function has challenged traditional views of neuroscience, which are primarily neuron-centric, promoting the idea that neuronal activity is the basis of cognition and behavior. Indeed, there are currently several lines of evidence that highlight the impact of astrocyte signaling on behavior.

2.5.1 *Sleep Homeostasis*

Ramon y Cajal was among the first neuroscientists to appreciate the unique structure of astrocytes, predicting the importance of these glial cells in brain function, namely, sleep–wake states [134]. Despite the availability of only static micrographs of astrocytes, Cajal imagined a very dynamic structure and function of astrocyte processes, based on their morphology and relative proximity to neurons. Indeed, he theorized that astrocytes retract their processes during wakefulness, allowing neurons to directly communicate, and then induce sleep by extending their processes to the synapse, subsequently interrupting synaptic communication [134]. Over a century later, through the advancements in techniques applied to glial biology research, aspects of his precocious insight have been confirmed. Using a transgenic mouse line in which SNARE-mediated transmitter release is selectively attenuated in astrocytes (called “dnSNARE” mice [86]), it was recently shown that gliotransmission regulates sleep pressure by providing a tonic source of adenosine acting on adenosine A1R [91]. Specifically, 6-h sleep deprivation effectively increased sleep pressure (measured by the intensity of low-frequency SWA component of NREM sleep in the cortex) in WT, but not in dnSNARE mice. This effect on sleep pressure was demonstrated to be dependent on A1R activation, as the A1R antagonist CPT mimicked the dnSNARE phenotype when administered to WT mice. Interestingly, the cognitive impairments associated with sleep deprivation,

while present in WT mice, were markedly attenuated in dnSNARE mice, suggesting that an astrocytic source of adenosine regulates the accumulation of sleep pressure that is associated with memory impairments following sleep loss. Extending on these findings, a recent study examined the late-phase LTP (L-LTP) in hippocampal slices of dnSNARE or WT mice after sleep deprivation and found that sleep loss does not impair L-LTP in hippocampus of dnSNARE mice, as it does in WT mice, an effect which was shown to be dependent on A1R activation [135]. Similarly, spatial object memory, a hippocampal-dependent function, was not impaired following sleep deprivation in dnSNARE mice or CPT-treated mice, as it was in WT and vehicle controls. These studies consistently demonstrate the impact of an astrocytic source of adenosine acting on synaptic A1Rs on sleep homeostasis and memory impairments following sleep loss.

Studies linking genetic influences to sleep disorders also point to a role of adenosine, which may likely include astrocytic-derived adenosine. Specifically, a functional polymorphism in adenosine deaminase (ADA), the enzyme responsible for the breakdown of adenosine into inosine, was linked to high sleep pressure in otherwise healthy individuals [136, 137]. These results are consistent with animal models showing the importance of ADA as a regulator of sleep homeostasis [138, 139]. In light of aforementioned studies of dnSNARE mice, and given that ADA is thought to be more abundantly expressed in astrocytes than neurons [138, 140], adenosine signaling in astrocytes may be a critical regulator of sleep homeostasis.

2.5.2 *Breathing*

Recent evidence has shown a crucial role for astrocyte–neuron signaling in regulating respiration. In an elegant study by Gourine et al., astrocytes in the ventral medulla oblongata were genetically induced to selectively express a calcium indicator to examine whether astrocytes respond to respiratory pH changes via calcium signaling [141]. Indeed, artificially induced decreases in pH stimulated immediate increases in astrocytic calcium, particularly in astrocytes adjacent to blood vessels, independent of neuronal activation. pH-sensitive calcium signaling was found to partially involve gap junctions but was primarily dependent on activation of ionotropic ATP receptors. Using optogenetically controlled calcium channels in astrocytes, they next showed that calcium signaling in astrocytes induced release of ATP which subsequently depolarized neighboring neurons. The functional importance of this signaling was then remarkably demonstrated by optogenetic stimulation of calcium influx in astrocytes that robustly induced respiratory activity in anesthetized rats [141].

Astrocytes also couple with respiratory neurons and regulate rhythmic firing in the pre-Botzinger complex in the midbrain. The burst firing of action potentials causes an abundance of extracellular K^+ and glutamate, which initiate astrocytic responses characterized by rhythmic inward currents carried by inward-rectifying K^+ channels and electrogenic uptake of glutamate via GLT-1 transporter [142].

2.5.3 *Circadian Regulation*

Thanks to the amenability of genetic manipulations in *Drosophila*, Suh and Jackson discovered that glial cells synthesize (and secrete) biogenic amines necessary for circadian regulation of locomotor behaviors [143]. More recently, several different lines of transgenic mice were used to assay circadian release of ATP from cultured astrocytes [144] and showed that it is dependent on clock genes. Using cultures isolated from dnSNARE mice, they show that circadian release of ATP does not rely on SNARE-dependent transmitter release. Using another line of transgenic mice in which IP_3 signaling (and therefore intracellular Ca^{2+}) is selectively upregulated in astrocytes, they showed that IP_3 -dependent calcium signaling contributes to the amplitude of rhythmic ATP release. In light of evidence showing astrocytic SNARE-dependent modulation of sleep homeostasis [91], it is interesting to consider the idea that two mechanisms of ATP release from astrocytes independently regulate two components of sleep and wake: circadian rhythms and sleep pressure.

2.5.4 *Memory*

Astrocytic modulation of network activity via lactate transport was recently demonstrated in an elegant study by Suzuki et al. [145], where it was shown to have an impact on plasticity and related memory impairments [145]. Specifically, they showed that inhibition of glycolysis in the hippocampus impaired long-term memory but had no effect on acquisition (“training”) or short-term memory. Administration of lactate restored long-term memory, suggesting that there is rapid glycogenesis during training and, subsequently, lactate release that is important for long-term memory consolidation. Impairment of lactate release via the knockdown of the astrocytic lactate transporters MCT1 or MCT4 significantly impaired long-term memory (but not acquisition or short-term memory), an effect which could be rescued by injection of lactate, but not glucose. Furthermore, long-term memory was also impaired when the neuronal lactate transporter MCT2 was knocked down, but neither lactate nor glucose rescued it, suggesting lactate release by MCT1 and MCT4 from astrocytes and import by MCT2 into neurons is necessary for long-term memory consolidation.

Mice lacking expression of the receptor for the cytokine IL1 (which is highly, but not exclusively, expressed in astrocytes) also exhibit impairments in hippocampal-dependent memory and LTP [146–148]. However, when neural stem cells (NSC) from wild-type mice were implanted into the hippocampus of IL1 $^{-/-}$ mice and allowed to differentiate into astrocytes, spatial memory was recovered [149], suggesting that astrocytic IL1 signaling plays an important role in hippocampal-dependent memory. Similarly, LTP is impaired and learning and memory deficits manifest in transgenic mice with astrocyte-targeted deletion of nuclear factor-kappa B (NF- κ B), a transcription factor originally recognized for its involvement in inflammatory and immune responses, but shown to play a role at the synapse [150–153]. Interestingly, these deficits were associated with reduced expression of

the metabotropic glutamate receptor mGluR5 and PSD-95 in the hippocampus and cortex and importantly were exclusively pronounced in female mice [154], implicating a role for sexually dimorphic pathways in astrocytes.

2.6 Glia–Neuron Dysfunction in Disease and Neuroinflammation

Astrocytes have long been recognized as key players in neuroinflammatory conditions. Notably, along with microglia, astrocytes respond to injury by becoming “reactive,” a somewhat controversial term used to describe the morphological and histochemical profile of these cells under injury conditions. Indisputable changes occurring in “reactive” astrocytes include hypertrophic morphology, upregulation of the cytoskeletal protein GFAP, proliferation, loss of nonoverlapping domains, and ultimately glial scar formation. The functional roles of reactive astrocytes appear to be both neuroprotective and neurotoxic. For example, selective ablation of reactive astrocytes increases neuronal death under several injury conditions (e.g., [155]), and inducible, transgenic blockade of STAT3, a signaling molecule for several cytokines, impairs astrogliosis, increases inflammation, and impedes functional recovery after spinal cord injury [156, 157]. On the other hand, blockade of other inflammatory pathways in astrocytes, such as NF-kappa B, has been shown to be neuroprotective in models of spinal cord injury, ischemia, and experimental autoimmune encephalopathy [158–160]. To date, there are several reviews describing the role of astrocytes in neuroinflammation associated with neurodegenerative diseases (e.g., see [160–164]).

Collectively, these and other studies suggest that numerous signaling pathways are likely to be involved in astrocytic response to inflammation.

2.6.1 Epilepsy

Multiple osmotic functions of astrocytes are impaired in epilepsy, including ionic balance (K^+ buffering), energy metabolism, vascular coupling, and glutamate uptake (for review, see [165]). In general, these astrocytic changes were thought to occur secondary to neuronal dysfunction. However, recent evidence suggests that astrocyte signaling contributes to epileptogenesis and seizure activity through changes in glutamate transport and release, energy metabolism, calcium signaling, and adenosine.

Regions affected by epilepsy in human brains show high extracellular levels of glutamate and the associated excitotoxic neuronal death. Both decreases and increases in expression of astrocytic glutamate transporters have been observed, so how these transporters are involved in aberrant levels of extracellular glutamate is not clear [166–169]. Similarly, animal models usually exhibit an impairment in glutamate transport function [170–176]. Shortly after status epilepticus, an increased coupling and decreased efficiency of glutamate uptake has been observed in the hippocampus of a kainate-induced status epilepticus rat model [177], supporting a role for astrocytic glutamate transport in epileptogenesis.

The glutamine–glutamate cycle in astrocytes modulates levels of glutamate and GABA produced by neurons. This cycle has been shown to be impaired in sclerotic hippocampus [178, 179]. Epileptiform activity is dependent on neuronal glutamine uptake [180], and reactive astrocytes have a decreased expression of glutamine synthetase, ultimately resulting in a decreased GABA inhibition [181]. In this manner, it is possible that astrocytes contribute to the hyperexcitability of epileptiform activity.

Furthermore, dysfunctional energy metabolism in astrocytes was recently shown to be involved in the latent phase of epileptogenesis, which marks the transition from status epilepticus to seizure behaviors. As described recently in Alvestad et al., impairment of glutamate–glutamine cycle and astrocytic GABA production from glucose have been observed specifically in the latent phase of a kainate rat model of epilepsy [182]. Thus, changes in metabolism of both glutamate and GABA may contribute to epileptogenesis.

Astrocytic calcium signaling may also contribute to epileptiform activity. For example, seizure activity induces an upregulation of metabotropic glutamate receptor expression in astrocytes and increases intracellular Ca^{2+} [183, 184]. As described previously, Ca^{2+} signaling in astrocytes increases Ca^{2+} -dependent gliotransmission. Though glutamate release from astrocytes does not generate epileptiform activity [185], it is possible that it contributes to sustained hyperexcitability during seizures and subsequent excitotoxic neuronal death. Intracellular calcium signaling in astrocytes and extrasynaptic (potentially astrocytic) sources of glutamate have also been shown to contribute to aberrant depolarization during interictal events [186], suggesting that an astrocytic source of glutamate contributes to epileptiform activity. Indeed, inhibition of astrocytic metabotropic glutamate receptors (mGluR5) after status epilepticus delays neuronal death, as does application of a Ca^{2+} chelator [187]. To more closely examine the role of astrocytes on epileptiform activity, Gomez-Gonzalez et al. monitored astrocytic Ca^{2+} signaling in parallel with neuronal epileptiform activity in entorhinal cortical slices from rat brain. Intracellular astrocytic Ca^{2+} signaling increased during the development of a focal ictal event; selective inhibition of intracellular astrocytic Ca^{2+} near the focal ictal event reduced the intensity of this event. Thus, these results showed that astrocytes can play a role in the initiation of ictal activity during seizures [188].

Adenosine, a by-product of the gliotransmitter ATP, typically counteracts neuronal hyperexcitability through its action on presynaptic A1 receptors. Along the same line, overexpression of adenosine kinase (ADK), which metabolizes adenosine into ADP and therefore reduces adenosine levels, is sufficient to induce epileptiform activity [189]. Immunohistology from brains of temporal lobe epilepsy patients reveals enhanced ADK expression in the hippocampus [190], and inhibition of ADK expression is able to prevent seizures in mouse models of epilepsy [191].

In addition to local signaling, the astrocytic syncytium has been shown to be involved in seizure development, as deletion of the gap junction proteins, Cx30 and Cx43, increases the potency of epileptiform activity [192]. Furthermore, astrocytic domains were shown to be disrupted in three different mouse models of epilepsy. Diolistic labeling in cortical slices showed that the domains normally occupied by independent astrocytes begin to overlap following seizure events, an effect which was lessened by treatment with antiepileptic drugs [193]. Interestingly, the loss of

astrocytic domains coincided with changes in dendritic morphology, including an increase in spine density, though it is yet unknown whether the changes in astrocyte morphology precede those in dendrites, or vice versa.

2.6.2 *Neurodegenerative Diseases*

Neurodegenerative diseases are characterized by neuronal degeneration and necrotic cell death and in later stages are often associated with severe cognitive and/or motor deficits. Though initially thought to result from a primary impairment in neuronal function, accumulating evidence suggests a role for dysfunction in astrocyte–neuron signaling as a critical contributor to pathology for most neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and ALS [164, 194–199]. AD, PD, and ALS primarily occur sporadically, with the few inherited forms resulting from abnormal mutation of specific genes, while HD is inherited through acquisition of the well-known huntingtin gene mutation. Reactive astrocytes are hallmark features of pathology in these diseases and correlate with the intensity of neuronal dysfunction and death [198, 200, 201]. This section will focus on AD and ALS, for which there is substantial evidence showing the role of astrocytes in neurodegeneration.

Accumulation of amyloid plaques is a hallmark pathology of AD and is generally associated with neighboring reactive astrocytes (e.g., [202]). Though astrocytes accumulate around amyloid plaques, they are not able to degrade amyloid β ($A\beta$) as they do in culture [203]. Rather, these reactive astrocytes, along with microglia, release proinflammatory cytokines, including interleukin 1 β , interleukin 6, and TNF α [204], though whether this response is neuroprotective or toxic is not well understood. Inhibitors of proinflammatory cytokines were found to attenuate both synaptic and cognitive deficits in mice treated with $A\beta$ infusion, suggesting that these inflammatory responses of astrocytes have detrimental downstream effects [205]. However, the relevance of these findings to clinical evidence is questionable [206].

Notably, an abnormally increased level of TNF α is a characteristic feature of neuroinflammation in AD patients [207], and there is convincing evidence highlighting a role for astrocytic release of TNF α in aberrant Ca^{2+} signaling and extracellular glutamate levels in a mouse model of AD [208, 209]. Specifically, TNF α -stimulated, Ca^{2+} -dependent release of glutamate was decreased in hippocampal slices isolated from aged AD mice [209], a finding which is somewhat incongruous with studies showing increased Ca^{2+} signaling in astrocytes (which should increase glutamate gliotransmission) in AD mouse models [208, 210, 211]. The altered levels of glutamate transporter and receptor expression that is characteristically pronounced in AD models [212, 213] may partially account for these results (see for review [164]). Given the established role of astrocytes in modulating synaptic activity, as described in previous sections, it is likely that aberrations in astrocyte signaling contribute to the progression of impairments in synaptic transmission associated with cognitive decline in AD. The gliotransmitter ATP has recently been proposed as a neuroprotective agent in AD. A study on primary astrocyte cultures

observed an increased release of ATP after application of the peptide A β 42 in the medium [214]. ATP application on neuronal cultures prevented A β 42 toxicity via P2-type purinergic receptors and prevented A β 42-induced impairment of LTP on acute hippocampal slices.

ALS is characterized by a progressive paralysis due to motor neuron death. The etiology of ALS is mainly sporadic, but 10 % of the patients present a dominant negative mutation, and among them 25 % have a mutation of the antioxidant enzyme superoxide dismutase 1 gene (SOD1) [215]. Changes in microglia, macrophages, and astrocytes have been documented in transgenic mice that express mutant SOD1 ubiquitously. Immunohistochemical examination of the CNS from these mice revealed that microglia activation was pronounced in the motor cortex, motor nuclei of the brainstem, corticospinal tract, and ventral horn of the spinal cord, and preceded the onset of motor neuron degeneration, which suggests the potential to serve as a pre-clinical indicator of ALS [216, 217]. In fact, positron emission tomography (PET) has been used to examine microglia activation in patients with ALS, revealing an association between microgliosis and upper (but not lower) motor neuron damage, again suggesting that microglia responses may occur prior to motor neuron degeneration in humans [218]. Macrophage activation is prominent in the sciatic nerve in SOD1 mutant mice, occurring throughout the disease onset and progression [219]. Astrogliosis appears in the dorsal and ventral horn of the spinal cord and is not restricted to motor areas of the brain, extending through both cortical gray and subcortical white matter in SOD1 mutant mice [220–222]. The activation of astrocytes occurs later than that of microglia, in time with the onset of motor neuron degeneration.

Given the changes that occur in microglia and astrocytes in SOD1 model of ALS, transgenic mice with a specific manipulation of mutant SOD1 expression in Cd11b-expressing microglia or GFAP-expressing astrocytes were created to examine more closely how these cells contribute to the initiation and progression of ALS [194, 223]. Transgenic mice expressing mutant SOD1 do not display features of neurodegeneration when transgene expression is restricted to motoneurons [224]. This observation suggests that neurodegeneration in ALS is not cell-autonomous, but rather it also results from dysfunction in nonneuronal cells. Specific deletion of mutant SOD1 in Cd11b-expressing cells, including microglia and macrophages, or in GFAP-expressing astrocytes does not change the disease onset but slows the disease progression and increases the survival of mutant SOD1 mice [194, 223, 225]. When mutant SOD1 expression is absent in microglia only, astrogliosis and microgliosis persist [223]. When mutant SOD1 is absent in astrocytes only, astrogliosis, but not microgliosis, is delayed compared to mice with ubiquitous expression of mutant SOD1 [225]. This suggests that the microglia response depends in part on factors released by astrocytes expressing mutant SOD1 [225]. In conclusion, neuronal mutant SOD1 expression is necessary for the onset of SOD1-related ALS but expression in nonneuronal cells is involved in the progression of the disease. More particularly, both astrocytes and microglia are important in the late progression phase of the disease and in survival [223, 225].

Several lines of evidence indicate that astrocytes are actively involved in ALS pathogenesis, particularly due to an impairment in glutamate uptake. For example, brain regions affected by the sporadic form of the ALS show a 95 % loss of the

astrocytic glutamate transporter EAAT2 and an increased level of glutamate in CSF [226, 227].

The impairment in energy metabolism observed in ALS also appears to involve astrocytes. For example, mutant SOD1 expression in astrocytes decreases lactate efflux to motor neurons *in vitro*, and this loss of metabolic support leads to a decrease in neuronal survival [228].

Similar to AD pathology, inflammation is also a characteristic feature of ALS pathogenesis [229]. Depletion of SOD1 in microglia or astrocytes leads to secretion of cytokines [229], and treatment with anti-inflammatory drugs slows down the progression of the disease in transgenic mice [230, 231].

Collectively, this evidence suggests that neuron–astrocyte interactions have a strong impact on the progression of neurodegenerative diseases, and therefore, astrocytes may be a novel therapeutic target for treatment of these diseases.

2.6.3 *Drugs of Abuse*

To date, there are limited studies investigating the putative contribution of glial cells to drug and alcohol abuse and addiction. However, there is some evidence from both human and mouse models that suggest astrocytes may play a role in drug and alcohol behavior. For example, there are marked changes in the immunohistochemical profile and morphology of astrocytes during chronic morphine treatment, such as increased GFAP expression and reduced expression of GLT-1 and GLAST [232, 233], suggesting an alteration in glutamate clearance. Similar changes occur following alcohol exposure, with the degree (and direction) of GFAP expression dependent on the duration of alcohol exposure [234, 235]. Interestingly, the density of GFAP-positive astrocytes is decreased in the prefrontal cortex of alcohol-preferring rats compared to non-preferring rats [236, 237], which may be either coincidental or meaningful to the same finding in prefrontal cortex of depressed patients, who also show decreased GFAP protein and mRNA expression in prefrontal cortex, and have a high comorbidity with alcoholism [238–242]. The link between astrocyte dysfunction and neuronal signaling is not yet clear in models of addiction; however, there is evidence from genetic screens and manipulations to suggest that it involves glutamate signaling. Notably, a single nucleotide mutation in the EAAT2 (GLT-1) gene has been linked to impulsive behaviors in alcoholics [243]. Furthermore, mutations in the circadian clock gene, *Per2*, lead to increased alcohol consumption in mice, coincident with decreased expression of another glial glutamate transporter, EAAT1 [244]. This phenotype is consistent with reports of associations between *Per2* mutations and increased alcohol intake in humans.

Both astrocytes and microglia have been recognized as key players in neuroinflammation and, as stated above, are often characterized as “reactive” based on changes in morphology and immunohistochemical profile [245]. However, dysfunction in glia–neuron signaling during neuroinflammation has only recently received attention as a key contributor to neuropathological conditions. Toll-like receptor (TLR) type 4, which is responsible for immediate detection and response to

insults to the immune system, is mainly expressed in glial cells [246] and is receiving increasing attention for its role in pathology associated with neurodegenerative diseases and chronic alcohol exposure [247–250]. For example, astrocyte and microglia activation and associated impairments in memory and anxiety behaviors that typically occur following chronic alcohol exposure were attenuated in mice lacking expression of TLR4 [248]. Though TLR4 is expressed on microglia and astrocytes, and upregulated in response to a neuroimmune challenge (including chronic alcohol exposure), a recent study using mixed glial cultures with or without microglia depletion showed that the astrocytic response to lipopolysaccharide, an activator of TLR4, was completely dependent on the presence of microglia [251]. This study suggests that activation of TLR4 on microglia precedes and induces subsequent activation of astrocytes *in vitro*. Further investigation is required to examine the extent to which microglia TLR4 signaling impacts astrocyte response *in vivo*.

2.7 Summary

Our understanding of astrocyte–neuron signaling mechanisms is ever-increasing. It is now widely appreciated that astrocytes' contribution to brain function extends beyond the basic extracellular “housekeeping” tasks to include modulation of neuronal communication at the synaptic, network, and behavioral levels. As a component of the tripartite synapse, astrocytes are capable of fine-tuning synaptic transmission via calcium-dependent gliotransmitter release, dynamically regulating the physiological range required for synaptic plasticity. Intercellular calcium signaling, or calcium waves, among astrocytes helps to coordinate neuronal activity and neurovascular resources to satisfy the metabolic demands of network activity. Recent advances in molecular genetic techniques, including transgenic mice with astrocyte-specific knockout, overexpression or mutation of genes, and *in vivo* application of optogenetics, especially when used in combination with rigorous electrophysiological, real-time imaging, and behavioral assays, are allowing glial biologists to more directly probe the functions of astrocytes. From studies published so far, there is now mounting evidence showing direct involvement of astrocytes in several behaviors, including sleep, circadian rhythms, memory, and respiration. It is also becoming increasingly clear, both in experimental models and clinical cases, that astrocytes play a role in neuropathological conditions, including neurodegeneration, neuroinflammation, and addiction. Any remaining debate in regard to the significance of astrocyte–neuron communication should serve as an impetus to set higher and more specified experimental standards, incorporating *in vivo* techniques with acute slice preparations and pharmacology, and importantly sampling a range of electrophysiological parameters which may be more physiologically relevant to conditions in which astrocytes modulate synaptic activity. In summary, the understanding that astrocytes contribute to overall brain function, but do so by continuously monitoring and tuning network activity rather than directly relaying it (as neurons do), is becoming an essential component to fundamental neuroscience and may be a key to the progression toward therapeutics for neurological conditions.

2.8 Conclusion

Over the past decade, discoveries in glial biology have brought forth a new era in brain research, moving beyond a neuron-centric approach to encompass the increasingly defined impact of glial signaling. Glia not only play an active role in modulating synaptic activity but, through intercellular communication, serve to integrate information at the network level. In this manner, glia–neuron signaling has a much more significant impact on both physiological and pathological conditions than what was initially thought. For example, glial cell involvement in the onset and/or progression of pathology in many neurodegenerative diseases is now widely accepted. Though the role of astrocytes in drug and alcohol abuse is only beginning to be understood, progress made thus far has provided a greater potential for insight into mechanisms of addiction. Astrocytes express proteins known to be impacted by drugs and alcohol, but the signaling pathways that contribute to addiction are not known. Given the ever-increasing development of techniques for probing glial function, and what is currently known regarding the role of astrocytes in neurodegenerative diseases and addiction, the future holds the possibility for identifying novel diagnostic techniques and therapeutics.

References

1. Kettenmann H, Verkhratsky A (2008) Neuroglia: the 150 years after. *Trends Neurosci* 31(12):653–659
2. Magistretti PJ, Pellerin L (1999) Astrocytes couple synaptic activity to glucose utilization in the brain. *News Physiol Sci* 14:177–182
3. Banachlocha MA (2007) Neuromagnetic dialogue between neuronal minicolumns and astroglial network: a new approach for memory and cerebral computation. *Brain Res Bull* 73(1–3):21–27
4. Garcia-Segura LM, Chowen JA, Duenas M, Torres-Aleman I, Naftolin F (1994) Gonadal steroids as promoters of neuro-glial plasticity. *Psychoneuroendocrinology* 19(5–7):445–453
5. Blutstein T, Baab PJ, Zielke HR, Mong JA (2009) Hormonal modulation of amino acid neurotransmitter metabolism in the arcuate nucleus of the adult female rat: a novel action of estradiol. *Endocrinology* 150(7):3237–3244
6. Haydon PG, Blendy J, Moss SJ, Rob JF (2009) Astrocytic control of synaptic transmission and plasticity: a target for drugs of abuse? *Neuropharmacology* 56(suppl 1):83–90
7. Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 22(1):183–192
8. Li D, Ropert N, Koulakoff A, Giaume C, Oheim M (2008) Lysosomes are the major vesicular compartment undergoing Ca²⁺-regulated exocytosis from cortical astrocytes. *J Neurosci* 28(30):7648–7658
9. Liu T, Sun L, Xiong Y, Shang S, Guo N, Teng S et al (2011) Calcium triggers exocytosis from two types of organelles in a single astrocyte. *J Neurosci* 31(29):10593–10601
10. Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V (2006) Vesicular transmitter release from astrocytes. *Glia* 54(7):700–715
11. Zhang Q, Pangrsic T, Kreft M, Krzan M, Li N, Sul JY et al (2004) Fusion-related release of glutamate from astrocytes. *J Biol Chem* 279(13):12724–12733
12. Agulhon C, Petravic J, McMullen AB, Sweger EJ, Minton SK, Taves SR et al (2008) What is the role of astrocyte calcium in neurophysiology? *Neuron* 59(6):932–946

13. Agulhon C, Fiacco TA, McCarthy KD (2010) Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling. *Science* 327(5970):1250–1254
14. Fiacco TA, Agulhon C, Taves SR, Petravic J, Casper KB, Dong X et al (2007) Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. *Neuron* 54(4):611–626
15. Fiacco TA, Agulhon C, McCarthy KD (2009) Sorting out astrocyte physiology from pharmacology. *Annu Rev Pharmacol Toxicol* 49:151–174
16. Petravic J, Fiacco TA, McCarthy KD (2008) Loss of IP3 receptor-dependent Ca^{2+} increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. *J Neurosci* 28(19):4967–4973
17. Watkins JC (2000) L-glutamate as a central neurotransmitter: looking back. *Biochem Soc Trans* 28(4):297–309
18. Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL et al (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 391(6664):281–285
19. Jętrinija SD, Jętrinija KV, Stefanovic G, Liu F (1996) Neuroligand-evoked calcium-dependent release of excitatory amino acids from cultured astrocytes. *J Neurochem* 66(2):676–684
20. Parpura V, Basarsky TA, Liu F, Jętrinija K, Jętrinija S, Haydon PG (1994) Glutamate-mediated astrocyte–neuron signalling. *Nature* 369(6483):744–747
21. Parpura V, Fang Y, Basarsky T, Jahn R, Haydon PG (1995) Expression of synaptobrevin II, cellubrevin and syntaxin but not SNAP-25 in cultured astrocytes. *FEBS Lett* 377(3):489–492
22. Hamilton NB, Attwell D (2010) Do astrocytes really exocytose neurotransmitters? *Nat Rev Neurosci* 11(4):227–238
23. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron* 43(5):729–743
24. Pasti L, Volterra A, Pozzan T, Carmignoto G (1997) Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. *J Neurosci* 17(20):7817–7830
25. Santello M, Volterra A (2009) Synaptic modulation by astrocytes via Ca^{2+} -dependent glutamate release. *Neuroscience* 158(1):253–259
26. Montana V, Ni Y, Sunjara V, Hua X, Parpura V (2004) Vesicular glutamate transporter-dependent glutamate release from astrocytes. *J Neurosci* 24(11):2633–2642
27. Ormel L, Stensrud MJ, Bergersen LH, Gundersen V (2012) VGLUT1 is localized in astrocytic processes in several brain regions. *Glia* 60(2):229–238
28. Zhang Q, Fukuda M, Van Bockstaele E, Pascual O, Haydon PG (2004) Synaptotagmin IV regulates glial glutamate release. *Proc Natl Acad Sci U S A* 101(25):9441–9446
29. Aoki C, Milner TA, Sheu KF, Blass JP, Pickel VM (1987) Regional distribution of astrocytes with intense immunoreactivity for glutamate dehydrogenase in rat brain: implications for neuron–glia interactions in glutamate transmission. *J Neurosci* 7(7):2214–2231
30. Kaneko T, Shigemoto R, Mizuno N (1988) Metabolism of glutamate and ammonia in astrocyte: an immunocytochemical study. *Brain Res* 457(1):160–164
31. Madl JE, Clements JR, Beitz AJ, Wenthold RJ, Larson AA (1988) Immunocytochemical localization of glutamate dehydrogenase in mitochondria of the cerebellum: an ultrastructural study using a monoclonal antibody. *Brain Res* 452(1–2):396–402
32. Wenthold RJ, Altschuler RA, Skaggs KK, Reeks KA (1987) Immunocytochemical characterization of glutamate dehydrogenase in the cerebellum of the rat. *J Neurochem* 48(2):636–643
33. Rothe F, Brosz M, Storm-Mathisen J (1995) Quantitative ultrastructural localization of glutamate dehydrogenase in the rat cerebellar cortex. *Neuroscience* 64(4):iii–xvi
34. Rothe F, Brosz M, Storm-Mathisen J (1994) Quantitative ultrastructural localization of glutamate dehydrogenase in the rat cerebellar cortex. *Neuroscience* 62(4):1133–1146
35. Anderson CM, Swanson RA (2000) Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 32(1):1–14

36. Danbolt NC (2001) Glutamate uptake. *Prog Neurobiol* 65(1):1–105
37. Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E et al (2001) CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci* 4(7):702–710
38. Bal-Price A, Moneer Z, Brown GC (2002) Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. *Glia* 40(3):312–323
39. Coco S, Calegari F, Pravettoni E, Pozzi D, Taverna E, Rosa P et al (2003) Storage and release of ATP from astrocytes in culture. *J Biol Chem* 278(2):1354–1362
40. Cali C, Bezzi P (2010) CXCR4-mediated glutamate exocytosis from astrocytes. *J Neuroimmunol* 224(1–2):13–21
41. Santello M, Bezzi P, Volterra A (2011) TNF α controls glutamatergic gliotransmission in the hippocampal dentate gyrus. *Neuron* 69(5):988–1001
42. Domercq M, Brambilla L, Pilati E, Marchaland J, Volterra A, Bezzi P (2006) P2Y₁ receptor-evoked glutamate exocytosis from astrocytes: control by tumor necrosis factor- α and prostaglandins. *J Biol Chem* 281(41):30684–30696
43. Angulo MC, Kozlov AS, Charkpak S, Audinat E (2004) Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. *J Neurosci* 24(31):6920–6927
44. Araque A, Sanzgiri RP, Parpura V, Haydon PG (1999) Astrocyte-induced modulation of synaptic transmission. *Can J Physiol Pharmacol* 77(9):699–706
45. Fellin T, D’Ascenzo M, Haydon PG (2007) Astrocytes control neuronal excitability in the nucleus accumbens. *Sci World J* 7:89–97
46. Haydon PG, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* 86(3):1009–1031
47. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M et al (2007) Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci* 10(3):331–339
48. Kozlov AS, Angulo MC, Audinat E, Charkpak S (2006) Target cell-specific modulation of neuronal activity by astrocytes. *Proc Natl Acad Sci USA* 103(26):10058–10063
49. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA et al (2006) Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 125(4):775–784
50. Nedergaard M, Verkhratsky A (2012) Artifact versus reality – how astrocytes contribute to synaptic events? *Glia* 60(7):1013–1023
51. Navarrete M, Perea G, de Sevilla DF, Gomez-Gonzalo M, Nunez A, Martin ED et al (2012) Astrocytes mediate in vivo cholinergic-induced synaptic plasticity. *PLoS Biol* 10(2):e1001259
52. Williams SM, Diaz CM, Macnab LT, Sullivan RKP, Pow DV (2006) Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. *Glia* 53(4):401–411
53. Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: A glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proc Natl Acad Sci USA* 96(23):13409–13414
54. Johnson JW, Ascher P (1987) Glycine potentiates the Nmda response in cultured mouse-brain neurons. *Nature* 325(6104):529–531
55. Schell MJ, Molliver ME, Snyder SH (1995) D-Serine, an endogenous synaptic modulator – localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA* 92(9):3948–3952
56. Mothet JP, Parent AT, Wolosker H, Brady RO, Linden DJ, Ferris CD et al (2000) D-Serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA* 97(9):4926–4931
57. Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G (2005) Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci USA* 102(15):5606–5611
58. Bergersen LH, Morland C, Ormel L, Rinholm JE, Larsson M, Wold JF et al (2011) Immunogold detection of L-glutamate and D-serine in small synaptic-like microvesicles in adult hippocampal astrocytes. *Cereb Cortex* 22(7):1690–1697

59. Martineau M, Galli T, Baux G, Mothet JP (2008) Confocal imaging and tracking of the exocytotic routes for D-serine-mediated gliotransmission. *Glia* 56(12):1271–1284
60. Wolosker H (2011) Serine racemase and the serine shuttle between neurons and astrocytes. *Biochim Biophys Acta-Proteins Proteomics* 1814(11):1558–1566
61. Rosenberg D, Kartvelishvili E, Shleper M, Klinker CMC, Bowser MT, Wolosker H (2010) Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. *FASEB J* 24(8):2951–2961
62. Yoshikawa M, Takayasu N, Hashimoto A, Sato Y, Tamaki R, Tsukamoto H et al (2007) The serine racemase mRNA is predominantly expressed in rat brain neurons. *Arch Histol Cytol* 70(2):127–134
63. Miya K, Inoue R, Takata Y, Abe M, Natsume R, Sakimura K et al (2008) Serine racemase is predominantly localized in neurons in mouse brain. *J Comp Neurol* 510(6):641–654
64. Ding XH, Ma N, Nagahama M, Yamada K, Semba R (2011) Localization of D-serine and serine racemase in neurons and neuroglia in mouse brain. *Neurol Sci* 32(2):263–267
65. Henneberger C, Papouin T, Oliet SHR, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463(7278):232–U120
66. Fossat P, Turpin FR, Sacchi S, Dulong J, Shi T, Rivet JM et al (2011) Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. *Cereb Cortex* 22(3):595–606
67. Benneyworth MA, Li Y, Basu AC, Bolshakov VY, Coyle JT (2012) Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. *Cell Mol Neurobiol* 32(4):613–624
68. Lake N (1992) Taurine, Gaba and Gfap immunoreactivity in the developing and adult-rat optic-nerve. *Brain Res* 596(1–2):124–132
69. Ochi S, Lim JY, Rand MN, During MJ, Sakatani K, Kocsis JD (1993) Transient presence of Gaba in astrocytes of the developing optic-nerve. *Glia* 9(3):188–198
70. Lee M, Schwab C, Mcgeer PL (2011) Astrocytes are gabaergic cells that modulate microglial activity. *Glia* 59(1):152–165
71. Sakatani K, Hassan AZ, Ching W (1991) Age-dependent extrasynaptic modulation of axonal conduction by exogenous and endogenous Gaba in the rat optic-nerve. *Exp Neurol* 114(3):307–314
72. Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat Neurosci* 1(8):683–692
73. Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* 6(3):215–229
74. Jia F, Pignatario L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic GABA(A) receptor mediates tonic inhibition in thalamic VB neurons. *J Neurophysiol* 94(6):4491–4501
75. Cope DW, Hughes SW, Crunelli V (2005) GABA(A) receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci* 25(50):11553–11563
76. Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA (2010) Alpha 5GABA(A) receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. *J Neurosci* 30(15):5269–5282
77. Fleming RL, Acheson SK, Moore SD, Wilson WA, Swartzwelder HS (2011) GABA transport modulates the ethanol sensitivity of tonic inhibition in the rat dentate gyrus. *Alcohol* 45(6):577–583
78. Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS et al (2010) Channel-mediated tonic GABA release from glia. *Science* 330(6005):790–796
79. Yoon BE, Jo S, Woo J, Lee JH, Kim T, Kim D et al (2011) The amount of astrocytic GABA positively correlates with the degree of tonic inhibition in hippocampal CA1 and cerebellum. *Mol Brain* 4(1):42
80. Lee M, Mcgeer EG, Mcgeer PL (2011) Mechanisms of GABA release from human astrocytes. *Glia* 59(11):1600–1611
81. Maienschein V, Marxen M, Volkandt W, Zimmermann H (1999) A plethora of presynaptic proteins associated with ATP-storing organelles in cultured astrocytes. *Glia* 26(3):233–244

82. Cotrina ML, Lin JH, Nedergaard M (1998) Cytoskeletal assembly and ATP release regulate astrocytic calcium signaling. *J Neurosci* 18(21):8794–8804
83. Wang Z, Haydon PG, Yeung ES (2000) Direct observation of calcium-independent intercellular ATP signaling in astrocytes. *Anal Chem* 72(9):2001–2007
84. Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL et al (2003) ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 40(5):971–982
85. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB (1999) ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 19(2):520–528
86. Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY et al (2005) Astrocytic purinergic signaling coordinates synaptic networks. *Science* 310(5745):113–116
87. Illes P, Verkhratsky A, Burnstock G, Franke H (2011) P2X receptors and their roles in astroglia in the central and peripheral nervous system. *Neuroscientist*
88. Peterson TS, Camden JM, Wang Y, Seye CI, Wood WG, Sun GY et al (2010) P2Y2 nucleotide receptor-mediated responses in brain cells. *Mol Neurobiol* 41(2–3):356–366
89. Tozaki-Saitoh H, Tsuda M, Inoue K (2011) Role of purinergic receptors in CNS function and neuroprotection. *Adv Pharmacol* 61:495–528
90. James G, Butt AM (2002) P2Y and P2X purinoceptor mediated Ca²⁺ signalling in glial cell pathology in the central nervous system. *Eur J Pharmacol* 447(2–3):247–260
91. Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T et al (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 61(2):213–219
92. Panatier A, Vallee J, Haber M, Murai KK, Lacaille JC, Robitaille R (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell* 146(5):785–798
93. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, von Zastrow M et al (2002) Control of synaptic strength by glial TNF alpha. *Science* 295(5563):2282–2285
94. Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-alpha. *Nature* 440(7087):1054–1059
95. Henneberger C, Rusakov DA (2010) Synaptic plasticity and Ca(2+) signalling in astrocytes. *Neuron Glia Biol* 6(3):141–146
96. Parpura V, Grubisic V, Verkhratsky A (2011) Ca(2+) sources for the exocytotic release of glutamate from astrocytes. *Biochim Biophys Acta* 1813(5):984–991
97. Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D et al (2011) Local Ca²⁺ detection and modulation of synaptic release by astrocytes. *Nat Neurosci* 14(10):1276–1284
98. Shigetomi E, Kracun S, Khakh BS (2010) Monitoring astrocyte calcium microdomains with improved membrane targeted GCaMP reporters. *Neuron Glia Biol* 6(3):183–191
99. Filosa A, Paixao S, Honsek SD, Carmona MA, Becker L, Feddersen B et al (2009) Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nat Neurosci* 12(10):1285–1292
100. Murai KK, Pasquale EB (2011) Eph receptors and ephrins in neuron-astrocyte communication at synapses. *Glia* 59(11):1567–1578
101. Navarrete M, Araque A (2008) Endocannabinoids mediate neuron-astrocyte communication. *Neuron* 57(6):883–893
102. Navarrete M, Araque A (2010) Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* 68(1):113–126
103. Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F et al (2012) Acute cannabinoids impair working memory through astroglial CB(1) receptor modulation of hippocampal LTD. *Cell* 148(5):1039–1050
104. Saadoun S, Papadopoulos MC (2010) Aquaporin-4 in brain and spinal cord oedema. *Neuroscience* 168(4):1036–1046
105. Skucas VA, Mathews IB, Yang J, Cheng Q, Treister A, Duffy AM et al (2011) Impairment of select forms of spatial memory and neurotrophin-dependent synaptic plasticity by deletion of glial aquaporin-4. *J Neurosci* 31(17):6392–6397
106. Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG (2007) Synaptic islands defined by the territory of a single astrocyte. *J Neurosci* 27(24):6473–6477

107. Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 6(8):626–640
108. Rose CR, Ransom BR (1997) Gap junctions equalize intracellular Na⁺ concentration in astrocytes. *Glia* 20(4):299–307
109. Nedergaard M (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263(5154):1768–1771
110. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247(4941):470–473
111. Scemes E, Giaume C (2006) Astrocyte calcium waves: what they are and what they do. *Glia* 54(7):716–725
112. Giaume C, Venance L (1998) Inter-cellular calcium signaling and gap junctional communication in astrocytes. *Glia* 24(1):50–64
113. Goldberg M, De Pitta M, Volman V, Berry H, Ben Jacob E (2010) Nonlinear gap junctions enable long-distance propagation of pulsating calcium waves in astrocyte networks. *PLoS Comput Biol* 6(8). pii:e1000909
114. Kuga N, Sasaki T, Takahara Y, Matsuki N, Ikegaya Y (2011) Large-scale calcium waves traveling through astrocytic networks in vivo. *J Neurosci* 31(7):2607–2614
115. Houades V, Koulakoff A, Ezan P, Seif I, Giaume C (2008) Gap junction-mediated astrocytic networks in the mouse barrel cortex. *J Neurosci* 28(20):5207–5217
116. Roux L, Benchenane K, Rothstein JD, Bonvento G, Giaume C (2011) Plasticity of astroglial networks in olfactory glomeruli. *Proc Natl Acad Sci USA* 108(45):18442–18446
117. Pannasch U, Vargova L, Reingruber J, Ezan P, Holcman D, Giaume C et al (2011) Astroglial networks scale synaptic activity and plasticity. *Proc Natl Acad Sci USA* 108(20):8467–8472
118. Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab* 21(10):1133–1145
119. Magistretti PJ, Sorg O, Naichen Y, Pellerin L, de Rham S, Martin JL (1994) Regulation of astrocyte energy metabolism by neurotransmitters. *Ren Physiol Biochem* 17(3–4):168–171
120. Pellerin L, Magistretti PJ (2011) Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* 32(7):1152–1166
121. Sokoloff L, Takahashi S, Gotoh J, Driscoll BF, Law MJ (1996) Contribution of astroglia to functionally activated energy metabolism. *Dev Neurosci* 18(5–6):344–352
122. Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R et al (2007) Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 55(12):1251–1262
123. Kacem K, Lacombe P, Seylaz J, Bonvento G (1998) Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 23(1):1–10
124. Herard AS, Dubois A, Escartin C, Tanaka K, Delzescaux T, Hantraye P et al (2005) Decreased metabolic response to visual stimulation in the superior colliculus of mice lacking the glial glutamate transporter GLT-1. *Eur J Neurosci* 22(7):1807–1811
125. Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* 91(22):10625–10629
126. Schousboe A, Sickmann HM, Walls AB, Bak LK, Waagepetersen HS (2010) Functional importance of the astrocytic glycogen-shunt and glycolysis for maintenance of an intact intra/extracellular glutamate gradient. *Neurotox Res* 18(1):94–99
127. Voutsinos-Porche B, Bonvento G, Tanaka K, Steiner P, Welker E, Chatton JY et al (2003) Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron* 37(2):275–286
128. Bouzier-Sore AK, Merle M, Magistretti PJ, Pellerin L (2002) Feeding active neurons: (re) emergence of a nursing role for astrocytes. *J Physiol Paris* 96(3–4):273–282
129. Bouzier-Sore AK, Serres S, Canioni P, Merle M (2003) Lactate involvement in neuron-glia metabolic interaction: (13)C-NMR spectroscopy contribution. *Biochimie* 85(9):841–848
130. Itoh Y, Esaki T, Shimoji K, Cook M, Law MJ, Kaufman E et al (2003) Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia in vitro and on glucose utilization by brain in vivo. *Proc Natl Acad Sci USA* 100(8):4879–4884

131. Ivanov A, Mukhtarov M, Bregestovski P, Zilberter Y (2011) Lactate effectively covers energy demands during neuronal network activity in neonatal hippocampal slices. *Front Neuroenerg* 3:2
132. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C (2008) Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322(5907):1551–1555
133. Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci* 11(2):87–99
134. Garcia-Marin V, Garcia-Lopez P, Freire M (2007) Cajal's contributions to glia research. *Trends Neurosci* 30(9):479–487
135. Florian C, Vecsey CG, Halassa MM, Haydon PG, Abel T (2011) Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. *J Neurosci* 31(19):6956–6962
136. Bachmann V, Klaus F, Bodenmann S, Schafer N, Brugger P, Huber S et al (2011) Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. *Cereb Cortex* 22(4):962–970
137. Retey JV, Adam M, Honegger E, Khatami R, Luhmann UF, Jung HH et al (2005) A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. *Proc Natl Acad Sci USA* 102(43):15676–15681
138. Okada T, Mochizuki T, Huang ZL, Eguchi N, Sugita Y, Urade Y et al (2003) Dominant localization of adenosine deaminase in leptomeninges and involvement of the enzyme in sleep. *Biochem Biophys Res Commun* 312(1):29–34
139. Franken P, Chollet D, Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. *J Neurosci* 21(8):2610–2621
140. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM (2005) Adenosine and brain function. *Int Rev Neurobiol* 63:191–270
141. Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S et al (2010) Astrocytes control breathing through pH-dependent release of ATP. *Science* 329(5991):571–575
142. Schnell C, Fresemann J, Hulsmann S (2011) Determinants of functional coupling between astrocytes and respiratory neurons in the pre-Botzinger complex. *PLoS One* 6(10):e26309
143. Suh J, Jackson FR (2007) *Drosophila* ebony activity is required in glia for the circadian regulation of locomotor activity. *Neuron* 55(3):435–447
144. Marpegan L, Swannstrom AE, Chung K, Simon T, Haydon PG, Khan SK et al (2011) Circadian regulation of ATP release in astrocytes. *J Neurosci* 31(23):8342–8350
145. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ et al (2011) Astrocyte–neuron lactate transport is required for long-term memory formation. *Cell* 144(5):810–823
146. Avital A, Goshen I, Kamsler A, Segal M, Iverfeldt K, Richter-Levin G et al (2003) Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. *Hippocampus* 13(7):826–834
147. Ban EM, Sarliewe LL, Haour FG (1993) Interleukin-1 binding sites on astrocytes. *Neuroscience* 52(3):725–733
148. Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben Hur T et al (2007) A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology* 32(8–10):1106–1115
149. Menachem-Zidon O, Avital A, Ben Menahem Y, Goshen I, Kreisel T, Shmueli EM et al (2011) Astrocytes support hippocampal-dependent memory and long-term potentiation via interleukin-1 signaling. *Brain Behav Immun* 25(5):1008–1016
150. Albeni BC, Mattson MP (2000) Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity. *Synapse* 35(2):151–159
151. Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D (2003) NF-kappa B functions in synaptic signaling and behavior. *Nat Neurosci* 6(10):1072–1078
152. O'Mahony A, Raber J, Montano M, Foehr E, Han V, Lu SM et al (2006) NF-kappaB/Rel regulates inhibitory and excitatory neuronal function and synaptic plasticity. *Mol Cell Biol* 26(19):7283–7298

153. Yu Z, Cheng G, Wen X, Wu GD, Lee WT, Pleasure D (2002) Tumor necrosis factor alpha increases neuronal vulnerability to excitotoxic necrosis by inducing expression of the AMPA-glutamate receptor subunit GluR1 via an acid sphingomyelinase- and NF-kappaB-dependent mechanism. *Neurobiol Dis* 11(1):199–213
154. Bracchi-Ricard V, Brambilla R, Levenson J, Hu WH, Bramwell A, Sweatt JD et al (2008) Astroglial nuclear factor-kappaB regulates learning and memory and synaptic plasticity in female mice. *J Neurochem* 104(3):611–623
155. Sofroniew MV, Bush TG, Blumauer N, Lawrence K, Mucke L, Johnson MH (1999) Genetically-targeted and conditionally-regulated ablation of astroglial cells in the central, enteric and peripheral nervous systems in adult transgenic mice. *Brain Res* 835(1):91–95
156. Herrmann JE, Imura T, Song B, Qi J, Ao Y, Nguyen TK et al (2008) STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. *J Neurosci* 28(28):7231–7243
157. Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K et al (2006) Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med* 12(7):829–834
158. Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A, Karmally S et al (2005) Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. *J Exp Med* 202(1):145–156
159. Brambilla R, Hurtado A, Persaud T, Esham K, Pearse DD, Oudega M et al (2009) Transgenic inhibition of astroglial NF-kappa B leads to increased axonal sparing and sprouting following spinal cord injury. *J Neurochem* 110(2):765–778
160. Dvoriantchikova G, Barakat D, Brambilla R, Agudelo C, Hernandez E, Bethea JR et al (2009) Inactivation of astroglial NF-kappa B promotes survival of retinal neurons following ischemic injury. *Eur J Neurosci* 30(2):175–185
161. Allaman I, Belanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships: for better and for worse. *Trends Neurosci* 34(2):76–87
162. Chung YC, Ko HW, Bok E, Park ES, Huh SH, Nam JH et al (2010) The role of neuroinflammation on the pathogenesis of Parkinson's disease. *BMB Rep* 43(4):225–232
163. Li C, Zhao R, Gao K, Wei Z, Yin MY, Lau LT et al (2011) Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 8(1):67–80
164. Rossi D, Volterra A (2009) Astrocytic dysfunction: insights on the role in neurodegeneration. *Brain Res Bull* 80(4–5):224–232
165. de Lanerolle NC, Lee TS, Spencer DD (2010) Astrocytes and epilepsy. *Neurotherapeutics* 7(4):424–438
166. Hoogland G, Spierenburg HA, van Veelen CW, van Rijen PC, van Huffelen AC, de Graan PN (2004) Synaptosomal glutamate and GABA transport in patients with temporal lobe epilepsy. *J Neurosci Res* 76(6):881–890
167. Tessler S, Danbolt NC, Faull RL, Storm-Mathisen J, Emson PC (1999) Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience* 88(4):1083–1091
168. Mathern GW, Mendoza D, Lozada A, Pretorius JK, Dehnes Y, Danbolt NC et al (1999) Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology* 52(3):453–472
169. Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MG, Schrama LH et al (2002) Distribution of glutamate transporters in the hippocampus of patients with pharmacoresistant temporal lobe epilepsy. *Brain* 125(Pt 1):32–43
170. Moreira JD, de Siqueira LV, Lague VM, Porciuncula LO, Vinade L, Souza DO (2011) Short-term alterations in hippocampal glutamate transport system caused by one-single neonatal seizure episode: implications on behavioral performance in adulthood. *Neurochem Int* 59(2):217–223
171. Binder DK, Steinhilber C (2006) Functional changes in astroglial cells in epilepsy. *Glia* 54(5):358–368
172. Zhang G, Raol YS, Hsu FC, Brooks-Kayal AR (2004) Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. *J Neurochem* 88(1):91–101

173. Ueda Y, Doi T, Tokumaru J, Yokoyama H, Nakajima A, Mitsuyama Y et al (2001) Collapse of extracellular glutamate regulation during epileptogenesis: down-regulation and functional failure of glutamate transporter function in rats with chronic seizures induced by kainic acid. *J Neurochem* 76(3):892–900
174. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K et al (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* 276(5319):1699–1702
175. Simantov R, Crispino M, Hoe W, Broutman G, Tocco G, Rothstein JD et al (1999) Changes in expression of neuronal and glial glutamate transporters in rat hippocampus following kainate-induced seizure activity. *Brain Res Mol Brain Res* 65(1):112–123
176. Miller HP, Levey AI, Rothstein JD, Tzingounis AV, Conn PJ (1997) Alterations in glutamate transporter protein levels in kindling-induced epilepsy. *J Neurochem* 68(4):1564–1570
177. Takahashi DK, Vargas JR, Wilcox KS (2010) Increased coupling and altered glutamate transport currents in astrocytes following kainic-acid-induced status epilepticus. *Neurobiol Dis* 40(3):573–585
178. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD (2002) Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia* 43(7):703–710
179. Eid T, Thomas MJ, Spencer DD, Runden-Pran E, Lai JC, Malthankar GV et al (2004) Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* 363(9402):28–37
180. Tani H, Dulla CG, Huguenard JR, Reimer RJ (2010) Glutamine is required for persistent epileptiform activity in the disinhibited neocortical brain slice. *J Neurosci* 30(4):1288–1300
181. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ et al (2010) Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci* 13(5):584–591
182. Alvestad S, Hammer J, Qu H, Haberg A, Ottersen OP, Sonnewald U (2011) Reduced astrocytic contribution to the turnover of glutamate, glutamine, and GABA characterizes the latent phase in the kainate model of temporal lobe epilepsy. *J Cereb Blood Flow Metab* 31(8):1675–1686
183. Aronica E, Gorter JA, Jansen GH, van Veelen CW, van Rijen PC, Ramkema M et al (2003) Expression and cell distribution of group I and group II metabotropic glutamate receptor subtypes in taylor-type focal cortical dysplasia. *Epilepsia* 44(6):785–795
184. Steinhauser C, Seifert G (2002) Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol* 447(2–3):227–237
185. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG (2006) Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci* 26(36):9312–9322
186. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J et al (2005) An astrocytic basis of epilepsy. *Nat Med* 11(9):973–981
187. Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF et al (2007) Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci* 27(40):10674–10684
188. Gomez-Gonzalo M, Losi G, Chiavegato A, Zonta M, Cammarota M, Brondi M et al (2010) An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biol* 8(4):e1000352
189. Li T, Lan JQ, Boison D (2008) Uncoupling of astrogliosis from epileptogenesis in adenosine kinase (ADK) transgenic mice. *Neuron Glia Biol* 4(2):91–99
190. Aronica E, Zurolo E, Iyer A, de Groot M, Anink J, Carbonell C et al (2011) Upregulation of adenosine kinase in astrocytes in experimental and human temporal lobe epilepsy. *Epilepsia* 52(9):1645–1655
191. Theofilas P, Brar S, Stewart KA, Shen HY, Sandau US, Poulsen D et al (2011) Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. *Epilepsia* 52(3):589–601
192. Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C (2006) The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci* 26(20):5438–5447

193. Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B et al (2008) Loss of astrocytic domain organization in the epileptic brain. *J Neurosci* 28(13):3264–3276
194. Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M et al (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302(5642):113–117
195. Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G et al (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum Mol Genet* 19(15):3053–3067
196. Halliday GM, Stevens CH (2011) Glia: initiators and progressors of pathology in Parkinson's disease. *Mov Disord* 26(1):6–17
197. Maragakis NJ, Rothstein JD (2006) Mechanisms of disease: astrocytes in neurodegenerative disease. *Nat Clin Pract Neurol* 2(12):679–689
198. Salmina AB (2009) Neuron-glia interactions as therapeutic targets in neurodegeneration. *J Alzheimers Dis* 16(3):485–502
199. Mallajosyula JK, Kaur D, Chinta SJ, Rajagopalan S, Rane A, Nicholls DG et al (2008) MAO-B elevation in mouse brain astrocytes results in Parkinson's pathology. *PLoS One* 3(2):e1616
200. Escartin C, Bonvento G (2008) Targeted activation of astrocytes: a potential neuroprotective strategy. *Mol Neurobiol* 38(3):231–241
201. Serrano-Pozo A, Mielke ML, Gomez-Isla T, Betensky RA, Growdon JH, Frosch MP et al (2011) Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. *Am J Pathol* 179(3):1373–1384
202. Wyss-Coray T (2006) Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med* 12(9):1005–1015
203. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F et al (2003) Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med* 9(4):453–457
204. McGeer EG, McGeer PL (2010) Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. *J Alzheimers Dis* 19(1):355–361
205. Ralay RH, Craft JM, Hu W, Guo L, Wing LK, Van Eldik LJ et al (2006) Glia as a therapeutic target: selective suppression of human amyloid-beta-induced upregulation of brain proinflammatory cytokine production attenuates neurodegeneration. *J Neurosci* 26(2):662–670
206. Leuba G, Savioz A, Vernay A, Carnal B, Kraftsik R, Tardif E et al (2008) Differential changes in synaptic proteins in the Alzheimer frontal cortex with marked increase in PSD-95 postsynaptic protein. *J Alzheimers Dis* 15(1):139–151
207. Llano DA, Li J, Waring JF, Ellis T, Devanarayan V, Witte DG et al (2011) Cerebrospinal fluid cytokine dynamics differ between alzheimer disease patients and elderly controls. *Alzheimer Dis Assoc Disord*
208. Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science* 323(5918):1211–1215
209. Rossi D, Brambilla L, Valori CF, Crugnola A, Giaccone G, Capobianco R et al (2005) Defective tumor necrosis factor-alpha-dependent control of astrocyte glutamate release in a transgenic mouse model of Alzheimer disease. *J Biol Chem* 280(51):42088–42096
210. Stix B, Reiser G (1998) Beta-amyloid peptide 25-35 regulates basal and hormone-stimulated Ca²⁺ levels in cultured rat astrocytes. *Neurosci Lett* 243(1–3):121–124
211. Haughey NJ, Mattson MP (2003) Alzheimer's amyloid beta-peptide enhances ATP/gap junction-mediated calcium-wave propagation in astrocytes. *Neuromolecular Med* 3(3):173–180
212. Li S, Mallory M, Alford M, Tanaka S, Masliah E (1997) Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol* 56(8):901–911
213. Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T, Zander N et al (2007) Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. *J Alzheimers Dis* 11(1):97–116
214. Jung ES, An K, Seok HH, Kim JH, Mook-Jung I (2012) Astrocyte-originated ATP protects abeta1-42-induced impairment of synaptic plasticity. *J Neurosci* 32(9):3081–3087

215. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362(6415):59–62
216. Hall ED, Oostveen JA, Gurney ME (1998) Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. *Glia* 23(3):249–256
217. Kawamata T, Akiyama H, Yamada T, Mcgeer PL (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 140(3):691–707
218. Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ et al (2004) Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [¹¹C](R)-PK11195 positron emission tomography study. *Neurobiol Dis* 15(3):601–609
219. Chiu IM, Phatnani H, Kuligowski M, Tapia JC, Carrasco MA, Zhang M et al (2009) Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. *Proc Natl Acad Sci USA* 106(49):20960–20965
220. Nagy D, Kato T, Kushner PD (1994) Reactive astrocytes are widespread in the cortical gray matter of amyotrophic lateral sclerosis. *J Neurosci Res* 38(3):336–347
221. Kushner PD, Stephenson DT, Wright S (1991) Reactive astrogliosis is widespread in the subcortical white matter of amyotrophic lateral sclerosis brain. *J Neuropathol Exp Neurol* 50(3):263–277
222. Schiffer D, Cordera S, Cavalla P, Migheli A (1996) Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. *J Neurol Sci* 139(suppl):27–33
223. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G et al (2006) Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312(5778):1389–1392
224. Pramatarova A, Laganieri J, Roussel J, Brisebois K, Rouleau GA (2001) Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J Neurosci* 21(10):3369–3374
225. Yamanaka K, Chun SJ, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann DH et al (2008) Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat Neurosci* 11(3):251–253
226. Bristol LA, Rothstein JD (1996) Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. *Ann Neurol* 39(5):676–679
227. Sasaki S, Komori T, Iwata M (2000) Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis. *Acta Neuropathol* 100(2):138–144
228. Ferraiuolo L, Higginbottom A, Heath PR, Barber S, Greenald D, Kirby J et al (2011) Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. *Brain* 134(Pt 9):2627–2641
229. Philips T, Robberecht W (2011) Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *Lancet Neurol* 10(3):253–263
230. West M, Mhatre M, Ceballos A, Floyd RA, Grammas P, Gabbita SP et al (2004) The arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid inhibits tumor necrosis factor alpha activation of microglia and extends survival of G93A-SOD1 transgenic mice. *J Neurochem* 91(1):133–143
231. Kiaei M, Petri S, Kipiani K, Gardian G, Choi DK, Chen J et al (2006) Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 26(9):2467–2473
232. Beitner-Johnson D, Guitart X, Nestler EJ (1993) Glial fibrillary acidic protein and the mesolimbic dopamine system: regulation by chronic morphine and Lewis-Fischer strain differences in the rat ventral tegmental area. *J Neurochem* 61(5):1766–1773
233. Bruce-Keller AJ, Turchan-Cholewo J, Smart EJ, Geurin T, Chauhan A, Reid R et al (2008) Morphine causes rapid increases in glial activation and neuronal injury in the striatum of inducible HIV-1 Tat transgenic mice. *Glia* 56(13):1414–1427
234. Evrard SG, Duhalde-Vega M, Tagliaferro P, Mirochnic S, Caltana LR, Brusco A (2006) A low chronic ethanol exposure induces morphological changes in the adolescent rat brain that are

- not fully recovered even after a long abstinence: an immunohistochemical study. *Exp Neurol* 200(2):438–459
235. Lewohl JM, Wixey J, Harper CG, Dodd PR (2005) Expression of MBP, PLP, MAG, CNP, and GFAP in the Human Alcoholic Brain. *Alcohol Clin Exp Res* 29(9):1698–1705
 236. Miguel-Hidalgo JJ, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2006) Reduced glial and neuronal packing density in the orbitofrontal cortex in alcohol dependence and its relationship with suicide and duration of alcohol dependence. *Alcohol Clin Exp Res* 30(11):1845–1855
 237. Miguel-Hidalgo JJ (2006) Withdrawal from free-choice ethanol consumption results in increased packing density of glutamine synthetase-immunoreactive astrocytes in the prelimbic cortex of alcohol-preferring rats. *Alcohol* 41(4):379–385
 238. Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *J Affect Disord* 127(1–3):230–240
 239. Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY et al (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 45(9):1085–1098
 240. Johnston-Wilson NL, Sims CD, Hofmann JP, Anderson L, Shore AD, Torrey EF et al (2000) Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium. *Mol Psychiatry* 5(2):142–149
 241. Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA et al (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. *Biol Psychiatry* 48(8):861–873
 242. Si X, Miguel-Hidalgo JJ, O'Dwyer G, Stockmeier CA, Rajkowska G (2004) Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. *Neuropsychopharmacology* 29(11):2088–2096
 243. Sander T, Ostapowicz A, Samochowiec J, Smolka M, Winterer G, Schmidt LG (2000) Genetic variation of the glutamate transporter EAAT2 gene and vulnerability to alcohol dependence. *Psychiatr Genet* 10(3):103–107
 244. Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC et al (2005) The clock gene *Per2* influences the glutamatergic system and modulates alcohol consumption. *Nat Med* 11(1):35–42
 245. Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. *Trends Immunol* 28(3):138–145
 246. Okun E, Griffioen KJ, Lathia JD, Tang SC, Mattson MP, Arumugam TV (2009) Toll-like receptors in neurodegeneration. *Brain Res Rev* 59(2):278–292
 247. Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I, Guerri C (2010) Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. *J Neurosci* 30(24):8285–8295
 248. Pascual M, Balino P, Alfonso-Loeches S, Aragon CM, Guerri C (2011) Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. *Brain Behav Immun* 25(suppl 1):S80–S91
 249. Waak J, Weber SS, Waldenmaier A, Gerner K, Alunni-Fabbroni M, Schell H et al (2009) Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ-1. *FASEB J* 23(8):2478–2489
 250. Salaria S, Badkoobehi H, Rockenstein E, Crews L, Chana G, Masliah E et al (2007) Toll-like receptor pathway gene expression is associated with human immunodeficiency virus-associated neurodegeneration. *J Neurovirol* 13(6):496–503
 251. Holm TH, Draeby D, Owens T (2012) Microglia are required for astroglial Toll-like receptor 4 response and for optimal TLR2 and TLR3 response. *Glia* 60(4):630–638

Neural-Immune Interactions in Brain Function and
Alcohol Related Disorders

Cui, C.; Grandison, L.; Noronha, A. (Eds.)

2013, VIII, 588 p., Hardcover

ISBN: 978-1-4614-4728-3