REVIEW

ASTROCYTES IN MEMORY FUNCTION: PIONEERING FINDINGS AND FUTURE DIRECTIONS

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Abstract—Astrocytes have been generally believed to perform mainly homeostatic and supportive functions for neurons in the central nervous system. Recently, a growing body of evidence suggests previously unrecognized and surprising functions for astrocytes, including regulation of synaptic formation, transmission and plasticity, all of which are considered as the infrastructure for information processing and memory formation and stabilization. This review discusses the involvement of astrocytes in memory functions and the possible mechanisms that may underlie it. We review the important breakthroughs obtained in this field, as well as some of the controversies that arose from the past difficulty to manipulate these cells in a cell type-specific and non-invasive manner. Finally, we present new research avenues based on the advanced tools becoming available in recent years: optogenetics and chemogenetics, and the potential ways in which these tools may further illuminate the role of astrocytes in memory processes.

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Contents

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INTRODUCTION

Many if not all CNS diseases are characterized, among other symptoms, by cognitive impairments and astrocytic abnormalities (for review see Barres, 2008; De Keyser et al., 2008; Dallérac and Rouach, 2016). Neurodegenerative diseases, such as Alzheimer's disease, as well as numerous neuropsychiatric disorders, including major depression, schizophrenia and autism, are all associated with changes in the number, morphology and function of astrocytes (Rajkowska et al., 1999; Johnston-Wilson et al., 2000; Miguel-Hidalgo et al., 2000; Czeh et al., 2006; Bolivar et al., 2007; Lioy et al., 2011; Furman et al., 2012). These observations suggest a crucial role for these cells in normal cognitive and emotional functions. This review specifically discusses the involvement of astrocytes in memory functions and the possible mechanisms that may underlie it (Fig. 1). We review the important breakthroughs that were obtained in this field, as well as new research avenues based on the advanced tools becoming available, and the ways in which they may further illuminate the role of astrocytes in memory processes.

WHY ASTROCYTES?

Astrocytes are star-shaped glial cells, with diversified processes that establish close contacts with neurons and blood vessels (Oberheim et al., 2009), and envelope thousands of synapses (Bushong et al., 2002; Genoud
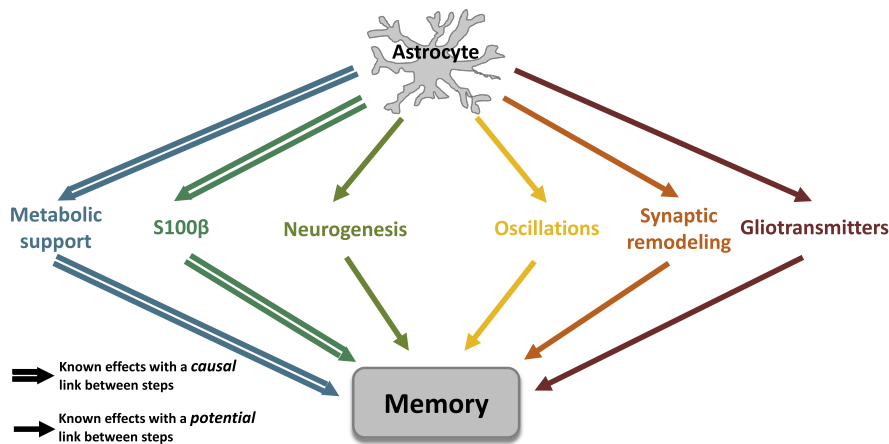
Fig. 1. Potential mechanisms for the involvement of astrocytes in memory processes. This figure presents all the known causal effects of astrocytes on different neuronal functions, which may consequently affect memory. The single-line arrows denote known proven effects of astrocytes on the different processes marked in the middle line, and the known involvement of these processes in memory. For instance, astrocytes affect neurogenesis, and neurogenesis affects memory performance, but astrocytes were not yet shown to affect memory via neurogenesis. The double-line arrows mark the cases in which astrocytes where causally implicated in memory performance via these processes. For example, astrocytes were shown to affect memory via their involvement in metabolic support of neuronal activity. *Note that all arrows represent established causal effects. The different arrow designs represent only the proven causality *between* two consecutive arrows.

et al., 2006). They can be interconnected via gap-junctions (Houades et al., 2008), and form highly organized anatomical domains with very little overlap between adjacent cells (Bushong et al., 2002; Wilhelmsson et al., 2006; Oberheim et al., 2009). Thus, their unique morphological and phenotypic features ideally position them to sense their surroundings and dynamically respond to changes in their environment. Indeed, astrocytes have many well-established homeostatic and supportive functions, including glucose metabolism maintenance, glutamate- and potassium-level monitoring, regulation of cerebral blood flow and neurotrophic factors secretion (reviewed in Sofroniew and Vinters, 2010; Allaman et al., 2011). Clearly, all of these established supportive functions are crucial for normal neuronal function, and thus for normal cognition, as will be discussed in the following sections.

In the past two decades, surprising new functions have been revealed for astrocytes, showing direct effects on neuronal activity, including regulation of synaptic formation, synaptic transmission, neuromodulation and plasticity (Haydon, 2001; Volterra and Meldolesi, 2005; Eroglu and Barres, 2010; Araque et al., 2014), all of which are considered as the infrastructure for information processing and memory formation. The following sections present these novel effects, and discuss their potential involvement in memory processes.

BILATERAL NEURON–ASTROCYTE INTERACTIONS AS POTENTIAL MECHANISMS FOR MEMORY MODULATION

For memories to be formed, recently acquired information is gradually transformed from an initially liable state, in which it is more vulnerable to disruptions, into enduring stable memories, a process termed 'memory consolidation' (Glickman, 1961; Lechner et al., 1999; McGaugh, 2000; Dudai, 2004; Dudai et al., 2015). The mechanisms of this transformation span the entire space from molecular changes at the synaptic level to neuronal network reorganization. The stabilization of changes in synaptic connectivity (e.g., the growth of new synaptic connections as well as the restructuring of existing ones) is believed to be a crucial mechanism for the acquisition and stabilization of new memories (Frankland and Bontempi, 2005). Here, we will discuss multiple evidence suggesting bilateral neuron–astrocyte interactions regulating synaptic connectivity and activity.

Regulation of synaptic formation

In vitro studies using purified neurons and astrocytes paved the way for understanding the extent to which astrocytes shape synapse formation. Astrocytes increase the number of mature, functional synapses and are required for synaptic maintenance in vitro (Pfrieger and Barres, 1997; Ullian et al., 2001). This is the result of the release of different soluble molecules from astrocytes, such as thrombospondin (Christopherson et al., 2005) and cholesterol (Mauch et al., 2001). When the communication between astrocytes and neurons is blocked, synaptogenesis is impaired both in vitro and in vivo (Christopherson et al., 2005; Kucukdereli et al., 2011), demonstrating the necessity of astrocytes to synapse formation in vivo. Furthermore, astrocytes regulation of synaptic formation is not restricted to the developing brain, since they were found to regulate synapse elimination and spine morphology in the adult brain as well (Carmona et al., 2009; Chung et al., 2013). Taken together, these results suggest that astrocytes have an important role in remodeling the synaptic architecture of our brains. Recent studies suggest that synaptic structural plasticity has a crucial role in memory formation (Fu and Zuo, 2011). These studies demonstrate that the formation and elimination of synaptic structures happen rapidly in a subpopulation of cortical neurons during various sensorimotor learning tasks, and that stabilized synaptic structures are associated with long-lasting memory of the tasks (Xu et al., 2009; Yang et al., 2009). Therefore, astrocyte regulation of synaptic formation and elimination provides a possible underlying mechanism for the modulation of learning and memory (Fig. 1).

Synaptic modulation

Responding to synaptic activity. Astrocytes are not electrically excitable, i.e. they do not generate action

potentials, and hence were considered as silent and unable to communicate. Nevertheless, astrocytic excitability is manifested as elevation of cytosolic Ca^{2+} concentration, mainly as a result of mobilization of Ca^{2+} stored in the endoplasmic reticulum (Cornell-bell et al., 1990; Dani et al., 1992; Porter and McCarthy, 1996). Astrocytes express a wide variety of functional neurotransmitter receptors, many of them are G-protein-coupled receptors (GPCRs). Stimulation of these GPCRs evokes a variety of glial cell responses, the most studied of which is elevation of intracellular calcium concentration (Porter and McCarthy, 1997). Astrocyte Ca^{2+} elevations can be triggered in vivo by neurotransmitter release and physiological sensory stimuli (Wang et al., 2006; Dombeck et al., 2007; Winship et al., 2007; Bekar et al., 2008; Petzold et al., 2008; Schummers et al., 2008; Di Castro et al., 2011; Kanemaru et al., 2014). These results suggest that astrocytes are capable of sensing and possibly integrating synaptic transmission, and therefore can be involved in information processing in the brain.

Shaping synaptic activity. Pioneering studies demonstrate that astrocytes directly affect neuronal activity, and support the presence of a reciprocal communication between astrocytes and neurons: astrocytic calcium signal can be elicited by a wide variety of neurotransmitters released from synaptic terminals. Consequently, astrocytes can release neuroactive substances, called gliotransmitters, which include glutamate, adenosine triphosphate (ATP) and D-serine, in a Ca^{2+}-dependent manner. These gliotransmitters can bind to presynaptic and/or postsynaptic receptors, resulting in the regulation of neuronal excitability and synaptic transmission (Fellin et al., 2004; Perea and Araque, 2007; Di Castro et al., 2011; Panatier et al., 2011; Santello et al., 2011; Sasaki et al., 2011). These evidences have led to the establishment of the concept "Tripartite Synapse", by which astrocytes are integral elements of the synapse, i.e., they do not merely encapsulate and insulate synapses, but actively exchange information with the pre- and postsynaptic neurons and modify synaptic activity (Araque et al., 1999; Perea et al., 2009).

It should be noted that the concept of gliotransmission is highly debated, and some contradictory observations exist (Fiacco et al., 2007; Petravicz et al., 2008, 2014; Agulhon et al., 2010), which question the ability of astrocytes to release neuroactive compounds in a Ca^{2+}-dependent manner and subsequently modulate neuronal synaptic activity. This contradiction might be partially explained by the difficulty in selectively and physiologically perturbing astrocytes without simultaneously directly influencing the nearby neurons (Agulhon et al., 2008; Araque et al., 2014). Moreover, the debate mainly revolves around the mechanism by which astrocytes release transmitters in response to Ca^{2+} elevations. In particular, there is skepticism regarding the vesicular release of gliosignaling molecules from astrocytes. Yet, disregarding the specific release mechanism, bidirectional astrocyte–neuron signaling is well accepted (Bazargani and Attwell, 2016).

The function and efficacy of synaptic transmission are determined not only by the composition and activity of pre- and postsynaptic components, but also by the environment in which a synapse is embedded. Thus, the direct effects of astrocytes on synaptic function described above come in addition to their supportive and homeostatic effects. Indeed, studies have shown the importance of these supportive roles, like lactate transport from astrocytes to neurons and potassium clearance, to synaptic activity (Suzuki et al., 2011; Sibille et al., 2014).

Regulating synaptic envelopment. Astrocytes do not wrap around all synapses and the presence and extent of astrocyte coverage might be regulated (Eroglu and Barres, 2010). They can rapidly extend and retract their processes to engage and disengage from dendritic spines and are more motile than their dendritic counterparts (Haber et al., 2006; Nishida and Okabe, 2007). Astrocytic processes motility is regulated by synaptically released glutamate, activating metabotropic glutamate receptors (mGluRs) on astrocytes and generating astrocytic Ca^{2+} transients. This motility and spine coverage correlate with spine stability (Bernardinelli et al., 2014b). Thus, perisynaptic astrocytic processes possess the machinery to both sense neuronal activity and remodel their actin filaments in an activity-dependent manner, possibly with the purpose of regulating the stability of new spines (Bernardinelli et al., 2014a).

Neuromodulation

Astrocytes express a diverse array of cholinergic, adrenergic, dopaminergic and serotonergic receptors (Porter and McCarthy, 1997), putting them in a position to contribute to the global effects of neuromodulation. For example, hippocampal astrocytes are a target of cholinergic pathway to the hippocampus. Thus, direct acetylcholine application and stimulation of cholinergic afferents in hippocampal slices increase intracellular Ca^{2+} levels in astrocytes through activation of muscarinic cholinergic receptors (Araque et al., 2002). In-vivo, cholinergic activity evoked by sensory stimulation or electrical stimulation of the septal nucleus increases Ca^{2+} in hippocampal astrocytes, and this Ca^{2+} increase is necessary for cholinergic-induced synaptic plasticity (Navarrete et al., 2012). Ca^{2+} elevation in astrocytes was also shown to mediate the effect of electrical stimulation of the nucleus basalis, the principal source of cholinergic innervation to the cortex, on local field potential elevation during whisker stimulation (Takata et al., 2011) and on the visual responses in V1 during visual stimulation (Chen et al., 2012).

Astrocytes also respond to neurotransmitters released by the post-synaptic neuron, such as endocannabinoids, which were shown to diffuse in a retrograde manner across the synapse, and subsequently activate presynaptic type 1 cannabinoid receptors (CB1Rs) to inhibit neurotransmitter release (Kano et al., 2009). Concomitantly, endocannabinoids activate CB1Rs in nearby astrocytes, which lead to Ca^{2+}-dependent release of glutamate and subsequent activation of pre-synaptic

mGluRs, and to a transient potentiation of synaptic transmission in relatively more distant neurons (Navarrete and Araque, 2008, 2010). Therefore, endocannabinoid stimulation of astrocyte signaling can have far-reaching neuromodulatory effects by releasing glutamate in distal regions, leading to potentiation of relatively distant synapses (Navarrete et al., 2014).

Astrocytes and adult neurogenesis

Adult neurogenesis, the process that gives rise to new neurons in the adult brain, occurs in the dentate gyrus of the hippocampus and the subventricular zone (Altman, 1963, 1969; Altman and Das, 1965; Gould, 2007). Adult hippocampal neurogenesis, mainly in the dorsal hippocampus, has been shown to contribute to learning and memory (Zhao et al., 2008; Cameron and Glover, 2015). Furthermore, there is extensive evidence for altered neurogenesis in neurodegenerative diseases, such as Alzheimer's disease (Mu and Gage, 2011), in which memory loss was found to be related to disturbed neurogenesis in the hippocampus of patients. Astrocytes release molecules that control different steps of adult neurogenesis, including the proliferation of neural stem cells, their differentiation into neurons, their synapse formation and integration and their survival (Song et al., 2002; Platel et al., 2010; Cao et al., 2013; Sultan et al., 2015). Several studies reported that impaired neurogenesis, reduced cognitive performance and astrocytic abnormalities go hand in hand, and that treatments that correct cognitive function are accompanied by increased neurogenesis and a normalization of astrocytic morphology (Hattiangady and Shetty, 2012; Hagemann et al., 2013; Parihar et al., 2013; Pardo et al., 2016).

MODULATION OF SYNAPTIC PLASTICITY

Long-term potentiation (LTP) and depression (LTD) of synaptic transmission are considered experimental models for studying mechanisms of memory. Hippocampal LTP can be triggered by the coincidence of postsynaptic activity and astrocyte Ca^{2+} elevation, which stimulates glutamate release. This form of LTP requires presynaptic mGluR activation (Perea and Araque, 2007). Also in the hippocampus, astrocytic release of glutamate increases the probability of transmitter release, an effect which is mediated by presynaptic N-methyl-D-aspartate receptors (NMDARs) (Jourdain et al., 2007). On the other hand, in the neocortex, astrocytic release of glutamate following stimulation activates presynaptic NMDARs and induces spike-timing-dependent long-term depression (t-LTD) of excitatory transmission (Min and Nevian, 2012). Astrocytic release of ATP, which accumulates as adenosine, technically suppresses synaptic transmission and as a result regulates the dynamic range for LTP generation (i.e., enhances the capability of synapses to express synaptic plasticity; Pascual et al., 2005). Another gliotransmitter suggested to be involved in synaptic plasticity is D-serine, the endogenous coagonist of postsynaptic NMDARs. Henneberger et al. (2010) elegantly demonstrated that astrocytic release of D-serine is necessary for the induction of NMDAR-mediated LTP (Henneberger et al., 2010). However, the methodology employed to inhibit astrocytes (and D-serine production) is nowadays criticized (Wenker, 2010) with regard to its specificity. In fact, recent studies demonstrate that the enzyme serine racemase (bio synthesizer of D-serine) is expressed almost entirely by neurons, and it is the neuronal rather than astrocytic D-serine that modulates synaptic plasticity (Wolosker et al., 2016). Nevertheless, there is no dispute that astrocytes are intricately involved in the regulation of synaptic strength and plasticity, even if the mechanisms allowing them to do so are not yet entirely deciphered.

Synaptic plasticity is typically homosynaptic, meaning that the synapses that receive a stimulation are the ones who will later show altered efficiency (Bear and Malenka, 1994). However, plasticity may also take place heterosynaptically whenever the stimulation of a modulatory interneuron leads to a change in the strength of a synaptic connection between input pathways that have not been stimulated (Bailey et al., 2000). Heterosynaptic plasticity that counterbalances input strengths requires astrocyte NMDARs and Ca^{2+} signaling (Letellier et al., 2016), and astrocyte-derived adenosine can mediate activity-dependent heterosynaptic depression (Pascual et al., 2005).

Astrocytic modulation of synaptic plasticity has been recently demonstrated in vivo as well, whereby cholinergic activation combined with somatosensory or visual stimulation induce potentiation of the synaptic response, which is mediated by direct cholinergic activation of astrocytes via muscarinic acetylcholine receptors, astrocyte Ca^{2+} elevations and gliotransmitter release (Takata et al., 2011; Chen et al., 2012; Navarrete et al., 2012). The elevation of astrocytic Ca^{2+} is crucial in this type of synaptic plasticity, as the plasticity could not be induced in inositol1,4,5-trisphosphate receptor type 2 knockout (IP3R2-KO) mice, which lack GPCR-mediated Ca^{2+} signaling in astrocytes. Conversely, the IP3R2 knockout mice demonstrate NMDAR-dependent LTP that is identical in amplitude to those of wild-type mice (Agulhon et al., 2010), suggesting that different forms of synaptic plasticity involve different astrocytic signaling pathways, and some forms may rely purely on neuronal mechanisms. Taken together, the results described in this chapter indicate that astrocytes are heavily involved in the regulation of synaptic strength and plasticity, suggesting their importance for memory formation as well (Fig. 1).

ASTROCYTES IN LEARNING AND MEMORY

The classic studies that established the field of astrocyte-mediated neuronal modulation, were performed mainly in cultured astrocytes and recently also in slice preparations. However, little is known regarding their direct contribution to cognitive functions and behavior. Nevertheless, the spatial relationship of astrocytes with synapses, their homeostatic functions and the metabolic support they provide, as well as the diversity of neurotransmitter receptors they express, intrigued scientists, and motivated them to study the role of astrocytes in higher brain functions in vertebrates.

One of the first indications that astrocytes are at play in learning and memory came from the study of Halassa et al. (2009) demonstrating that the conditional astrocyte-selective expression of the SNARE domain of the protein synaptobrevin II (dnSNARE), preventing astrocyte vesicular release, modulates memory performance following sleep deprivation, but not normal memory function, through a pathway involving adenosine A1 receptors (Halassa et al., 2009). It is important to note that the use of dnSNARE mice as a specific tool to modulate astrocytic gliotransmission is currently under debate since it was recently demonstrated that these mice also express the dnSNARE transgene in their neurons (Fujita et al., 2014). When using a different mouse model, vesicular release in astrocytes was found to contribute to the maintenance of oscillations in the gamma frequency band (25–80 Hz), which have been correlated with learning, memory storage and retrieval (Başar-Eroglu et al., 1996), and to recognition memory, suggesting that vesicular release in astrocytes may be an essential contributor to memory formation and consolidation (Lee et al., 2014).

In addition to vesicular release, other mechanisms were suggested by which astrocytes can modulate synaptic function (Bazargani and Attwell, 2016), for example connexin hemichannels (Chever et al., 2014). When connexin43 hemichannels are blocked during memory consolidation, long-term memory is found to be impaired. This memory loss can be recovered after co-infusion of a mixture of putative gliotransmitters known to be released from astrocytes, including glutamate, glutamine, lactate, D-serine, glycine, and ATP (Stehberg et al., 2012). Altogether, these findings suggest that memory formation and consolidation require uptake and supply of neuroactive molecules by astrocytes, possibly by multiple pathways.

One of the candidates suggested to be involved in astrocyte-to-neuron signaling is the calcium-binding protein S100β. S100β is considered to be mainly synthesized in astrocytes, and thus is used as an astrocytic marker. Transgenic mice overexpressing human S100β exhibit impaired hippocampal LTP and spatial learning (Gerlai et al., 1995), while mutant mice devoid of S100β exhibit enhanced hippocampal LTP and enhanced hippocampus-dependent learning and memory (Nishiyama et al., 2002). However, the specificity of the methodology used in these studies is again under debate, since S100β was found to be localized also in neural cell types (Steiner et al., 2007).

Recently, several studies have shown that metabolic support from astrocytes plays a role in memory formation in chicks (Gibbs et al., 2006; Hertz and Gibbs, 2009) and rodents (Newman et al., 2011; Suzuki et al., 2011; Tadi et al., 2015). Suzuki et al. (2011) demonstrated that pharmacological inhibition of glycogenolysis (the breakdown of glycogen into glucose) during the initial phase of memory storage, but not at later stages, prevents recall of the learned task. Moreover, extracellular lactate levels increase in the hippocampus immediately after learning. When glycogenolysis is blocked, administration of exogenous lactate rescues the memory impairment. Disrupting the expression of the astrocytic and neuronal lactate transporters blocks memory retention, but only the former is rescued by lactate administration, suggesting that astrocyte–neuron lactate transport is required for long-term memory consolidation (Suzuki et al., 2011). The importance of metabolic support provided by astrocytes to neurons was also demonstrated for drug-related memory formation and retention (Boury-Jamot et al., 2016a,b; Zhang et al., 2016).

Structural plasticity of synapses is thought to be a prominent mechanism of memory storage. Following fear learning, it was demonstrated that astrocytic processes are less prevalent in proximity to large synapses in the lateral amygdala. This finding suggests that astrocytic contacts are not randomly distributed, but are dynamically retracted in order to allow active synapses to enlarge following learning (Ostroff et al., 2014).

Astrocytic functioning is also found to be impaired in memory-related neurological diseases, such as Alzheimer's disease (e.g., Nagele et al., 2003; Kuchibhotla et al., 2009; Mei et al., 2010; Olabarria et al., 2010; Simpson et al., 2010; Furman et al., 2012; Wu et al., 2014). A recent study by Orr et al. (2015) demonstrated that humans with Alzheimer's disease, as well as aging mice expressing human amyloid precursor protein (hAPP), show increased levels of Gs-coupled adenosine receptor in astrocytes. Genetic deletion of this receptor from astrocytes enhances long-term memory in young and aging wild-type mice and in hAPP mice, and chemogenetic activation of the Gs pathway specifically in astrocytes reduces long-term memory without affecting acquisition (Orr et al., 2015). Interestingly, transplanting neural precursor cells that give rise to astrocytes can rescue impaired memory in another model of impaired cognitive function (Ben Menachem-Zidon et al., 2011). Finally, astrocytes were found to have an important role in spatial working memory impairment induced by exogenous cannabinoids. Transgenic mice lacking CB1Rs selectively in astrocytes demonstrate no spatial working memory impairment and in vivo LTD of hippocampal synaptic transmission following acute exposure of exogenous cannabinoids. Blockade of NMDARs and of synaptic trafficking of AMPARs also abolishes the effects of cannabinoids on spatial working memory and LTD, suggesting that the impairment of working memory may be due to activation of astrocytic CB1R and release of glutamate from these cells, which induces LTD by internalization of AMPA receptors (Han et al., 2012).

Almost all of our knowledge of astrocytes and their involvement in synaptic and memory functions is based entirely on studies of astrocytes' physiology in rodent models. Compared to rodents, human astrocytes are larger and exhibit greater architectural complexity and cellular diversity, as well as faster propagation of calcium signals (Oberheim et al., 2009). In a recent study by Han et al. (2013), an innovative approach was used to explore the properties and function of human astrocytes, by engrafting human glial progenitor cells into the forebrain of neonatal immunodeficient mice, resulting in a widespread integration of human astrocytes in the mouse brain. The engrafted human astrocytes retained the

structural and physiological phenotype of hominid astrocytes and the human-glial chimeric mice demonstrated enhanced LTP and memory (Han et al., 2013), identifying astrocytes as potential contributors to the development of cognitive abilities throughout evolution (Zhang and Barres, 2013).

CHALLENGES AND FUTURE DIRECTIONS

The pioneering studies described in the previous sections show the vast promise in researching the role of astrocytes in learning and memory. The reason that there are so few of them, and that the field is riddled with controversies, stems at least in part from the
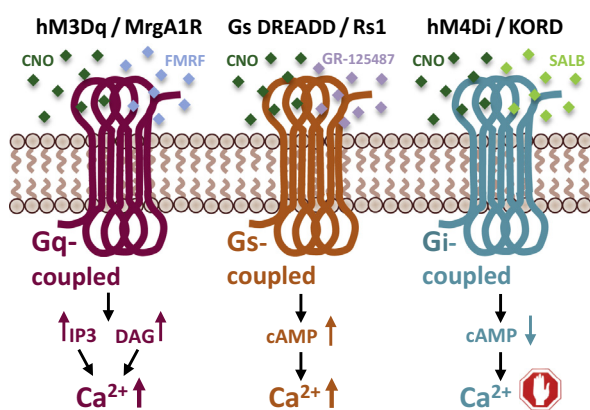
A. Optogenetic modulation of membrane potential
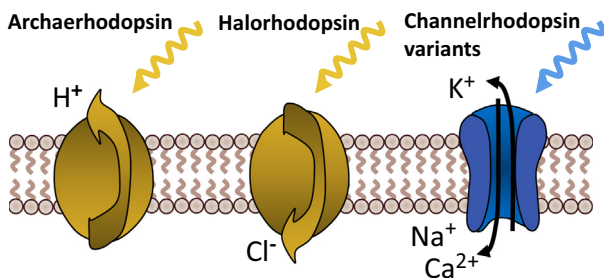
B. Optogenetic modulation of intracellular signaling
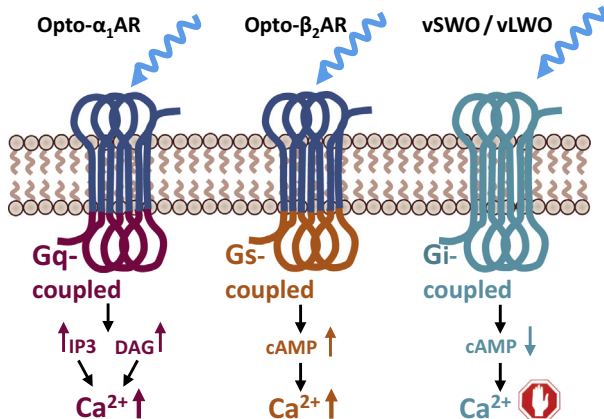
C. Chemogenetic modulation of intracellular signaling

technical difficulty to specifically modulate astrocytic activity in a physiologically relevant manner without also directly affecting the neighboring neurons. Specifically, given the fact that astrocytes share many of their receptors with neurons, it is indeed difficult to specifically alter their activity without, at the same time, directly influencing neuronal functions (Fiacco et al., 2007; Araque et al., 2014; Bazargani and Attwell, 2016). Population electrical stimulations is also impossible for the same reason. It should be noted that these arguments against what may seem to be a "specific" activation but in fact lacks specificity are just as true for neurons. Namely, every pharmacological or electrical manipulation of neuronal activity will, by definition, involve direct astrocytic modulation, which many people choose to ignore. The solution is to use genetic markers of astrocytes to gain specific access to these cells. This approach had been widely used to generate mouse lines with permanent deletion or overexpression of various genes in astrocytes. However, chronic expression (or deletion) has multiple drawbacks, primarily the recruitment of various compensation mechanisms. Specific astrocytic promoters can also be used to target this cell population using viral
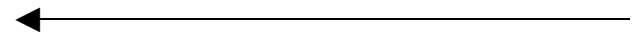

Fig. 2. Modern tools for astrocyte modulation. Optogenetic and chemogenetic tools are gradually being integrated into the field of astrocyte research. This figure presents a partial list of the major families of tools, and their effects on the cells. (A). *Tools for optogenetic modulation of membrane potential:* These are the major tools used for neuronal modulation, and are now being employed for astrocyte research too. Channelrhodopsin (ChR) variants include: *ChR2*, a cation channel opening in response to light and depolarizing the cell by allowing the inwards influx of Na+ and Ca^{2+} ions. This is currently the most commonly used tool in astrocyte research. *CatCh*, (Ca^{2+} translocating ChR) with enhanced Ca^{2+} permeability, was shown to evoke more reliable Ca^{2+} elevations than ChR2 in cultured astrocytes. Two tools inducing hyperpolarization are *Halorhodopsin* (NpHR), a Cl^- pump pushing negatively charged ions into the cell upon illumination and *Archaerhodopsin* (Arch), a proton pump pushing H^+ ions out of the cell upon illumination. The problem with the latter is its effect on local pH levels. (B) *Tools for optogenetic modulation of intracellular signaling:* The Opto-α1AR and Opto-β2AR are chimeric proteins, with the intracellular part of GPCRs that can recruit the Gq and Gs pathways (respectively, originally from the α1 and β2 adrenergic receptors), and the transmembrane and extracellular part of rhodopsin. Thus, their downstream intracellular cascades are now activated in response to light instead of ligand binding. Specifically, Optoα1AR (Gq-coupled) activates phospholipase C, thus increasing IP3 and DAG levels and consequently causing an increase in intracellular Ca^{2+}, released from ER stores, similar to the increase naturally observed in activated astrocytes. Opto-β2AR (Gs-coupled) activates adenylyl cyclase, thus increasing cAMP levels and consequently elevating Ca^{2+} via cAMP-gated Ca^{2+} channels. The vSWO and vLWO are Gi-coupled vertebrate cone opsins, both reducing cAMP levels, and thus can prevent an increase in intracellular Ca^{2+}. In neurons they also induce an inward K^+ current and inhibit firing. This opsin may serve to interfere with normal astrocytic activity by disrupting the important astrocytic function of K^+ buffering, necessary for synaptic plasticity. (C) *Tools for chemogenetic modulation of intracellular signaling:* These tools are genetically engineered GPCRs that were mutated to respond to a designer drug instead of their natural ligand. Most are inert until this drug is applied, and transiently activated by its administration, resulting in the recruitment or their respective G protein and the activation of their downstream intracellular cascades described above.

vectors, allowing the expression of various indicators and actuators. Indeed, genetically encoded Ca^{2+} indicators are already successfully used to explore astrocytic activity (Bazargani and Attwell, 2016), and early studies using optogenetic and chemogenetic tools are starting to be published as well. This section will discuss the use of such specific tools, followed by their caveats.

Optogenetics and chemogenetics

Optogenetics is a ground-breaking versatile technique allowing membrane potential or intracellular signaling in genetically defined cell populations to be controlled by expressing opsins (light-sensitive proteins) in the cell membrane, and stimulating them with light (Deisseroth and Schnitzer, 2013). A versatile optogenetic toolkit is available, allowing the depolarization or hyperpolarization of cells in response to light, with various kinetics, ionic selectivity, and wavelength sensitivity (Mattis et al., 2011). Furthermore, engineering of novel chimeric proteins now allows light-control of different GPCRs (Fig. 2; Airan et al., 2009). Optogenetics combines the advantages of other methods (high temporal resolution as in electrical stimulation, cell type specificity as in genetic manipulations) while avoiding their drawbacks (no side effects on neighboring cells as in electrical stimulation and some pharmacological agents, no time for compensatory activity to take place as in chronic genetic manipulations and pharmacology). Optogenetics requires illumination, which usually entails tethering the animal to an optical fiber right before the experiment. This procedure is somewhat stressful, but with the appropriate control groups employed had been widely used for memory studies (Goshen, 2014).

Chemogenetics (also known as Pharmacogenetics) allows bidirectional control of cellular activity by employing a different strategy – designer receptors exclusively activated by designer drugs (DREADDs). Specifically, intracellular signaling in genetically-defined cell populations is controlled by expressing a designer receptor engineered to respond to no innate ligands in the cell membrane, and activating it by the application of an otherwise inert designer drug (Roth, 2016). Several DREADDs activating different intracellular pathways and responding to different ligands are available (Fig. 2), potentially allowing combinatorial experiments (Vardy et al., 2015; Roth, 2016). Chemogenetic excitation in neurons is typically achieved by expressing Gq-coupled receptors, which depolarize the expressing neurons and increase their spiking activity in response to ligand application. Inhibition is achieved by Gi-coupled receptors, which result in reduced spiking when the designer drug is applied. Like optogenetics, chemogenetics allows inducible and reversible modulation of pre-defined cell types, while avoiding side effects on neighboring cells. The designer drug is administered in most cases by intraperitoneal injection (though some choose to administer it directly into the brain, or give the drug in the drinking water), but despite the mild stress induced by this procedure, the technique was already used for memory research (e.g., Garner et al., 2012; Robinson et al., 2014; Zhu et al., 2014).

While optogenetics allows much better temporal resolution, chemogenetics is slower, thus exposing the experiment to possible compensatory effects. Nevertheless, such slow kinetics are advantageous when longer duration modulation is required, especially for chronic modulations over multiple days. Each tool has its own advantage for use in astrocytes, and importantly, both optogenetics and chemogenetics allow the use of the same manipulation in all the relevant levels of investigation, from the single cell and the network in slice, to in vivo physiology and freely moving behaving animals, providing an opportunity to map the causal effects of a specific cell type activity in all the relevant levels of investigation. Indeed, these techniques had already revolutionized the field of neuroscience.

Optogenetic modulation of astrocytes

As astrocytes can be easily targeted genetically, they can also be optogenetically manipulated, providing an opportunity for a major leap forward in this field. Until now, optogenetic astrocyte stimulation was employed in several studies usually using channelrhodopsin (ChR) variants, and resulted, for example, in decreased firing rate in the sub thalamic nucleus, activation of chemoreceptor neurons, inspiratory neurons, and respiratory responses upon brainstem astrocytic stimulation (Gradinaru et al., 2009; Gourine et al., 2010; Okada et al., 2012). Additionally, astrocytic stimulation in the cerebellum triggered pupil dilation, in V1 it modified orientation selectivity in neurons, in the anterior cingulate cortex it induced sleep disturbance, and in the hypothalamus it increased sleep and suppressed feeding (Sasaki et al., 2012; Perea et al., 2014; Yamashita et al., 2014; Pelluru et al., 2016; Sweeney et al., 2016). Maybe most relevant to memory processes, a recent study showed that increasing astrocytic intracellular Ca^{2+} using the outward proton pump archaerhodopsin (Arch) shifts the oscillatory state of the surrounding cortex to slow oscillations (Poskanzer and Yuste, 2016).

These studies show the vast potential of the field of optogenetic astrocyte manipulation, and on the other hand demonstrate the urgent need to expand the existing toolbox to enable other astrocytic modulations that may better mimic their physiological activity. For example, the effects of ChR variants in astrocytes on Ca^{2+} levels, and consequently on neuronal activity and physiological behavior, were very slow, from tens of seconds up to several minutes, and using Arch 5 seconds (Gradinaru et al., 2009; Gourine et al., 2010; Okada et al., 2012; Poskanzer and Yuste, 2016). Because of these long timeframes, together with the fact that robust changes in membrane potential are less physiologically relevant to astrocytes compared to neurons, it would be interesting to test other optogenetic tools in astrocytes, like the cell-signaling modifying opsins. To date, these chimeric opsins (the Gq-coupled optoα1AR and the Gs-coupled optoβAR) were shown to induce Ca^{2+} increases in cultured astrocytes as (if not more) efficiently as ChR2 (Figueiredo et al., 2014). They are yet to be used in slice and behavioral experiments.

Chemogenetic modulation of astrocytes

Transgenic mice expressing DREADDs in their astrocytes have been in use for several years, and more recent studies employ viruses to achieve DREADDs expression in astrocytes. The advantage of the first approach is that it does not require intracranial viral injections, but its clear disadvantage is that the widespread expression of the DREADD can lead to systemic effects upon designer drug administration, for example the reported activation of the autonomic nervous system and global changes in motor activity (Agulhon et al., 2013). The advantage of the viral-based approach is that it allows region-specific (not just population specific) expression.

Viral-delivered DREADDs were already used in several studies in astrocytes, yielding interesting insights regarding their role in neuronal activity and behavior. Several studies had shown that activation of the Gq-coupled hM3Dq DREADD by its specific ligand CNO resulted in an increase in intracellular Ca^{2+} levels and induced glutamate release (Agulhon et al., 2013; Bonder and McCarthy, 2014; Bull et al., 2014; Scofield et al., 2015; Chen et al., 2016). These changes in astrocyte activity consequently affected neuronal activity. In the arcuate nucleus, for example, it was shown in one study to reduce firing in agouti-related peptide (AgRP) neurons (Yang et al., 2015) and in another to increase neuronal activity in the same neuronal population (Chen et al., 2016).

Astrocytic activation with hM3Dq also induced behavioral changes. For example, when expressed in the Nucleus Accumbens it inhibited both cue-induced cocaine reinstatement and ethanol self-administration after abstinence (Bull et al., 2014; Scofield et al., 2015). Two other studies had employed hM3Dq in the arcuate nucleus to investigate feeding behavior, and reported opposing results, probably stemming from CNO dose differences (Yang et al., 2015; Chen et al., 2016).

The use of chemogenetics for memory research is not just a future possibility, but was employed already in one study: Orr et al. (2015) had used transgenic mice with conditional adult expression of the Gs-coupled DREADD Rs1 (Chang et al., 2007) to activate astrocytes during memory acquisition and consolidation. They found that activating the Gs pathway during 2 days of water maze training did not impair acquisition, but resulted in impaired recall 2 days later. Furthermore, when astrocytes were activated 1 day after acquisition (but not during training) recall was also impaired due to disturbed consolidation (Orr et al., 2015). This study has two caveats: First, the brain-wide Rs1 expression of the transgene – not restricted to the hippocampus, but also in thalamus and mildly in cortex. Second, Rs1 was found to have constitutive ligand-independent Gs-coupled activity, and thus its chronic expression by itself impaired memory (Orr et al., 2015). The strong, impressive results of this study, even in light of these caveats, show the vast potential of chemogenetics in illuminating the role of astrocytes in cognitive function.

Challenges in modulating astrocytic activity

While the integration of optogenetics and chemogenetics into astrocyte research has multiple advantages and is sure to boost this field significantly (Bang et al., 2016), their drawbacks should always be kept in mind. Some of the challenges are general, i.e. relevant to the use of these tools in neurons as well, for example:

(a) *Invasiveness:* both optogenetics and chemogenetics usually require intracranial virus injection resulting in a strong expression of a foreign protein, often combined with fiber insertion or ligand injection. These procedures are likely to induce at least minimal immune activation in the brain, and due to the role of astrocytes in immune activity, that may modify their activity even before the light or the ligand are introduced. Thus, one should always control for these effects, for example by expressing a fluorophore alone under the same promoter in the control group, and make sure this control group does not show a different phenotype from non-treated mice.

(b) *Using Forced activation patterns:* Since we do not yet understand the spatiotemporal pattern of astrocytic activity in response to different stimuli, we cannot mimic it in an exact way. Rather, we are using optogenetics or chemogenetics to force an external pattern of activity, which may or may not be relevant to the way these cells actually work. In neurons, changing the optogenetic stimulation pattern from tonic to phasic yielded interesting insights (e.g., Tsai et al., 2009), thus, in the future, it may be interesting to test the effects of different activation patterns on astrocytes as well. Another synthetic feature of forced activation is the simultaneous recruitment of the entire transfected population, whereas in reality the cells do not necessarily behave so. This can be addressed, at least for optogenetic manipulation, by the integration of patterned illumination of single astrocytes. However, this application is not trivial in vivo (Deisseroth and Schnitzer, 2013; Packer et al., 2013).

(c) *Can brief modulation lead to permanent effect?* It is not unlikely that an optogenetic or a chemogenetic modulation, that efficiently recruits a population of cells, will result in plastic changes. This is especially true when using tools that lead to changes in intracellular Ca^{2+} levels, which may directly influence long-term processes in the cell. A few studies have already shown that optogenetic activation and even inhibition can independently induce plastic changes in neurons (Raimondo et al., 2012; Chun et al., 2013). Thus, even brief optogenetic or chemogenetic modulation may remodel the perturbed circuit during the experiment. Indeed, optogenetic activation of astrocytes was shown to induce long-term depression in purkinje neurons (Sasaki et al., 2012). To conclude, it is important not to assume a return to baseline activity once the light or designer drug are no longer present without verifying that it is so.

Other challenges in the use of optogenetics and pharmacogenetics tools are specific for astrocytic manipulations, as discussed in the following sections:

How is astrocytic excitation or inhibition defined?. When manipulating neuronal activity, one has several ways to measure the effectiveness of the manipulation, for example by quantifying the number of spikes induced (or reduced) in the target cell. But, how is astrocytic activation or inhibition defined? The current standard in the field is to measure astrocytic Ca^{2+} levels as a proxy for activity, but the interpretation of this readout is under debate. It is becoming clear that Ca^{2+} dynamics in different compartments of the astrocyte (soma vs. proximal processes) have different speed, duration and spatial spread, and are mediated by different intracellular mechanisms (Bazargani and Attwell, 2016). These different Ca^{2+} events may represent different kinds of information processing within the astrocyte. Astrocytic inhibition is even harder to define, so indirect measurements like cFos expression levels in astrocytes are used (Chen et al., 2016). As there is no clear and commonly agreed definition of what comprises astrocytic excitation or inhibition, each group using new tools should strive to demonstrate, using as many approaches as possible, the physiological effects of the employed tools on astrocytic activity. Hopefully this will lead to insight that may be used in the future to guide a better quantification of the level of astrocytic activity.

Genetic specificity in a heterogeneous population. Several pan-astrocytic promoters, like GFAP and Aldh1L1, allow highly specific genetic access to astrocytes, and thus are widely used, either packaged directly as the driving promoter in viral vectors, or to drive Cre in transgenic lines in combination with Cre-dependent viral constructs. However, astrocytes show significant heterogeneity in their morphological and functional characteristics, both between and within different brain regions (Haim and Rowitch, 2016). Importantly, these differences directly affect the interactions of astrocytes with each other and with their neighboring neurons (e.g., Martin et al., 2015). This fact further stresses the need for subpopulation identification and modulation. Modern molecular studies will hopefully isolate unique markers for astrocytic sub-types, and thus enable genetic access to these distinct populations, allowing their specific modulation, in order to causally determine their roles in the brain.

SUMMARY AND FUTURE DIRECTIONS

The present is an exciting time for studying the role astrocytes in memory and in behavior in general, as this field is still in its infancy and many new discoveries are waiting to be revealed. The integration of modern tools to astrocyte research now allows better temporal and spatial resolution in astrocytic modulation, and better resemblance to the physiological activity of these cells. Indeed, in an extremely short period of time an impressive variety of new findings regarding the

involvement of astrocytes in neuronal activity and behavior were collected.

As more astrocyte researchers will adopt new optogenetic and chemogenetic tools and employ them in their studies, we may be able to better characterize the roles of astrocytes in neuronal activity and plasticity, and finally in memory processes.

REFERENCES

Agulhon C, Boyt KM, Xie AX, Friocourt F, Roth BL, Mccarthy KD, Mccarthy KD (2013) Modulation of the autonomic nervous system and behaviour by acute glial cell Gq protein-coupled receptor activation in vivo. J Physiol 591:5599–5609.

Agulhon C, Fiacco TA, McCarthy KD (2010) Hippocampal short-and long-term plasticity are not modulated by astrocyte Ca2+ signaling. Science 327:1250–1254.

Agulhon C, Petravicz J, McMullen AB, Sweger EJ, Minton SK, Taves SR, Casper KB, Fiacco TA, McCarthy KD (2008) What is the role of astrocyte calcium in neurophysiology? Neuron 59:932–946.

Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K (2009) Temporally precise in vivo control of intracellular signalling. Nature 458:1025–1029.

Allaman I, Bélanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships: For better and for worse. Trends Neurosci 34:76–87.

Altman J (1963) Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat Rec 145:573–591.

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457.

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335.

Araque A, Carmignoto G, Haydon PG, Oliet SHR, Robitaille R, Volterra A (2014) Gliotransmitters travel in time and space. Neuron 81:728–739.

Araque A, Martin ED, Perea G, Arellano JI, Buno W (2002) Synaptically released acetylcholine evokes Ca^{2+} elevations in astrocytes in hippocampal slices. J Neurosci 22:2443–2450.

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: Glia, the unacknowledged partner. Trends Neurosci 22:208–215.

Bailey CH, Giustetto M, Huang YY, Hawkins RD, Kandel ER (2000) Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? Nat Rev Neurosci 1:11–20.

Bang J, Kim HY, Lee H (2016) Optogenetic and chemogenetic approaches for studying astrocytes and gliotransmitters. Exp Neurobiol 25:205.

Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430–440.

Başar-Eroglu C, Strüber D, Schürmann M, Stadler M, Başar E (1996) Gamma-band responses in the brain: a short review of psychophysiological correlates and functional significance. Int J Psychophysiol 24:101–112.

Bazargani N, Attwell D (2016) Astrocyte calcium signaling: the third wave. Nat Neurosci 19:182–189.

Bear MF, Malenka RC (1994) Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4:389–399.

Bekar LK, He W, Nedergaard M (2008) Locus coeruleus alpha-adrenergic-mediated activation of cortical astrocytes in vivo. Cereb Cortex 18:2789–2795.

Ben Menachem-Zidon O, Avital A, Ben-Menahem Y, Goshen I, Kreisel T, Shmueli EM, Segal M, Ben Hur T, Yirmiya R (2011) Astrocytes support hippocampal-dependent memory and long-term potentiation via interleukin-1 signaling. Brain Behav Immun 25:1008–1016.

Bernardinelli Y, Muller D, Nikonenko I (2014a) Astrocyte-synapse structural plasticity. Neural Plast 2014:1–13.

Bernardinelli Y, Randall J, Janett E, Nikonenko I, König S, Jones EV, Flores CE, Murai KK, Bochet CG, Holtmaat A, Muller D (2014b) Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability. Curr Biol 24:1679–1688.

Bolivar VJ, Walters SR, Phoenix JL (2007) Assessing autism-like behavior in mice: Variations in social interactions among inbred strains. Behav Brain Res 176:21–26.

Bonder DE, McCarthy KD (2014) Astrocytic Gq-GPCR-linked IP3R-dependent Ca^{2+} signaling does not mediate neurovascular coupling in mouse visual cortex in vivo. J Neurosci 34:13139–13150.

Boury-Jamot B, Carrard A, Martin JL, Halfon O, Magistretti PJ, Boutrel B (2016a) Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine. Molecular psychiatry 21:1070–1076.

Boury-Jamot B, Halfon O, Magistretti PJ, Boutrel B (2016b) Lactate release from astrocytes to neurons contributes to cocaine memory formation. BioEssays 38:1266–1273.

Bull C, Freitas KC, Zou S, Poland RS, Syed WA, Urban DJ, Minter SC, Shelton KL, Hauser KF, Negus SS, Knapp PE, Bowers MS (2014) Rat nucleus Accumbens core astrocytes modulate reward and the motivation to self-administer ethanol after abstinence. Neuropsychopharmacology 39:2835–2845.

Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22:183–192.

Cameron HA, Glover LR (2015) Adult neurogenesis: beyond learning and memory. Annu Rev Psychol 66:53–81.

Cao X, Li LP, Qin XH, Li SJ, Zhang M, Wang Q, Hu HH, Fang YY, Gao YB, Li XW, Sun LR, Xiong WC, Gao TM, Zhu XH (2013) Astrocytic adenosine 5'-triphosphate release regulates the proliferation of neural stem cells in the adult hippocampus. Stem cells 31:1633–1643.

Carmona MA, Murai KK, Wang L, Roberts AJ, Pasquale EB (2009) Glial ephrin-A3 regulates hippocampal dendritic spine morphology and glutamate transport. Proc Natl Acad Sci U S A 106:12524–12529.

Chang WC, Ng JK, Nguyen T, Pellissier L, Claeysen S, Hsiao EC, Conklin BR (2007) Modifying ligand-induced and constitutive signaling of the human 5-HT4 receptor. PLoS One 2:e1317.

Chen N, Sugihara H, Kim J, Fu Z, Barak B, Sur M, Feng G, Han W (2016) Direct modulation of GFAP-expressing glia in the arcuate nucleus bi-directionally regulates feeding. Elife 5:5599–5609.

Chen N, Sugihara H, Sharma J, Perea G, Petravicz J, Le C, Sur M (2012) Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes. Proc Natl Acad Sci U S A 109:E2832–E2841.

Chever O, Lee C-Y, Rouach N (2014) Astroglial Connexin43 hemichannels tune basal excitatory synaptic transmission. J Neurosci 34:11228–11232.

Christopherson KS, Ullian EM, Stokes CCA, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–433.

Chun S, Bayazitov IT, Blundon JA, Zakharenko SS (2013) Thalamocortical long-term potentiation becomes gated after the early critical period in the auditory cortex. J Neurosci 33:7345–7357.

Chung W-S, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, Joung J, Foo LC, Thompson A, Chen C, Smith SJ, Barres BA (2013) Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature 504:394–400.

Cornell-Bell A, Finkbeiner S, Cooper M, Smith S (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science 247:470–473.

Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31:1616–1626.

Dallérac G, Rouach N (2016) Astrocytes as new targets to improve cognitive functions. Prog Neurobiol 144:48–67.

Dani JW, Chernjavsky A, Smith SJ (1992) Neuronal activity triggers calcium waves in hippocampal astrocyte networks. Neuron 8:429–440.

De Keyser J, Mostert JP, Koch MW (2008) Dysfunctional astrocytes as key players in the pathogenesis of central nervous system disorders. J Neurol Sci 267:3–16.

Deisseroth K, Schnitzer MJ (2013) Engineering approaches to illuminating brain structure and dynamics. Neuron 80:568–577.

Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, Tiret P, Volterra A (2011) Local Ca2+ detection and modulation of synaptic release by astrocytes. Nat Neurosci 14:1276–1284.

Dombeck DA, Khabbaz AN, Collman F, Adelman TL, Tank DW (2007) Imaging large-scale neural activity with cellular resolution in awake, mobile mice. Neuron 56:43–57.

Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol 55:51–86.

Dudai Y, Karni A, Born J (2015) The consolidation and transformation of memory. Neuron 88:20–32.

Eroglu C, Barres BA (2010) Regulation of synaptic connectivity by glia. Nature 468:223–231.

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 43:729–743.

Fiacco TA, Agulhon C, Taves SR, Petravicz J, Casper KB, Dong X, Chen J, McCarthy KD (2007) Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. Neuron 54:611–626.

Figueiredo M, Lane S, Stout RF, Liu B, Parpura V, Teschemacher AG, Kasparov S (2014) Comparative analysis of optogenetic actuators in cultured astrocytes. Cell Calcium 56:208–214.

Frankland PW, Bontempi B (2005) The organization of recent and remote memories. Nat Rev Neurosci 6:119–130.

Fu M, Zuo Y (2011) Experience-dependent structural plasticity in the cortex. Trends Neurosci 34:177–187.

Fujita T, Chen MJ, Li B, Smith NA, Peng W, Sun W, Toner MJ, Kress BT, Wang L, Benraiss A, Takano T, Wang S, Nedergaard M (2014) Neuronal transgene expression in dominant-negative SNARE mice. J Neurosci 34:16594–16604.

Furman JL, Sama DM, Gant JC, Beckett TL, Murphy MP, Bachstetter AD, Van Eldik LJ, Norris CM (2012) Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease. J Neurosci 32:16129–16140.

Garner AR, Rowland DC, Hwang SY, Baumgaertel K, Roth BL, Kentros C, Mayford M (2012) Generation of a synthetic memory trace. Science 335:1513–1516.

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. PLoS Biol 4:2057–2064.

Gerlai R, Wojtowicz JM, Marks A, Roder J (1995) Overexpression of a calcium-binding protein, S100 beta, in astrocytes alters synaptic plasticity and impairs spatial learning in transgenic mice. Learn Mem 2:26–39.

Gibbs ME, Anderson DG, Hertz L (2006) Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. Glia 54:214–222.

Glickman SE (1961) Perseverative neural processes and consolidation of the memory trace. Psychol Bull 58:218–233.

Goshen I (2014) The optogenetic revolution in memory research. Trends Neurosci 37:511–522.

Gould E (2007) How widespread is adult neurogenesis in mammals? Nat Rev Neurosci 8:481–488.

Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S (2010) Astrocytes control breathing through pH-dependent release of ATP. Science 329:571–575.

Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K (2009) Optical deconstruction of parkinsonian neural circuitry. Science 324:354–359.

Haber M, Zhou L, Murai KK (2006) Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. J Neurosci 26:8881–8891.

Hagemann TL, Paylor R, Messing A (2013) Deficits in adult neurogenesis, contextual fear conditioning, and spatial learning in a Gfap mutant mouse model of Alexander disease. J Neurosci 33:18698–18706.

Haim LB, Rowitch D (2016) Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci 18:31–41.

Halassa MM, Florian C, Fellin T, Munoz JR, Lee S-Y, Abel T, Haydon PG, Frank MG (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61:213–219.

Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, Koehl M, Abrous DN, Mendizabal-Zubiaga J, Grandes P, Liu Q, Bai G, Wang W, Xiong L, Ren W, Marsicano G, Zhang X (2012) Acute cannabinoids impair working memory through astroglial CB 1 receptor modulation of hippocampal LTD. Cell 148:1039–1050.

Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar L, Betstadt S, Silva AJ, Takano T, Goldman SA, Nedergaard M (2013) Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell 12:342–353.

Hattiangady B, Shetty AK (2012) Neural stem cell grafting counteracts hippocampal injury-mediated impairments in mood, memory, and neurogenesis. Stem cells translational medicine 1:696–708.

Haydon PG (2001) GLIA: listening and talking to the synapse. Nat Rev Neurosci 2:185–193.

Henneberger C, Papouin T, Oliet SHR, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. Nature 463:232–236.

Hertz L, Gibbs ME (2009) What learning in day-old chickens can teach a neurochemist: focus on astrocyte metabolism. J Neurochem 109:10–16.

Houades V, Koulakoff A, Ezan P, Seif I, Giaume C (2008) Gap junction-mediated astrocytic networks in the mouse barrel cortex. J Neurosci 28:5207–5217.

Johnston-Wilson NL, Sims CD, Hofmann J-P, Anderson L, Shore AD, Torrey EF, Yolken RH (2000) Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. Mol Psychiatry 5:142–149.

Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A (2007) Glutamate exocytosis from astrocytes controls synaptic strength. Nat Neurosci 10:331–339.

Kanemaru K, Sekiya H, Xu M, Satoh K, Kitajima N, Yoshida K, Okubo Y, Sasaki T, Moritoh S, Hasuwa H, Mimura M, Horikawa K, Matsui K, Nagai T, Iino M, Tanaka KF (2014) In Vivo visualization of subtle, transient, and local activity of astrocytes using an ultrasensitive Ca2 + indicator. Cell Rep 8:311–318.

Kano M, Ohno-shosaku T, Hashimotodani Y, Uchigashima M (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89:309–380.

Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323:1211–1215.

Kucukdereli H, Allen NJ, Lee AT, Feng A, Ozlu MI, Conatser LM, Chakraborty C, Workman G, Weaver M, Sage EH, Barres BA, Eroglu C (2011) Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. Proc Natl Acad Sci U S A 108:E440–E449.

Lechner HA, Squire LR, Byrne JH (1999) 100 years of consolidation—remembering Müller and Pilzecker. Learn Mem 6:77–87.

Lee HS, Ghetti A, Pinto-Duarte A, Wang X, Dziewczapolski G, Galimi F, Huitron-Resendiz S, Pina-Crespo JC, Roberts AJ, Verma IM, Sejnowski TJ, Heinemann SF (2014) Astrocytes contribute to gamma oscillations and recognition memory. Proc Natl Acad Sci U S A 111:E3343–E3352.

Letellier M, Park YK, Chater TE, Chipman PH, Gautam SG, Oshima-Takago T, Goda Y (2016) Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci U S A 113:E2685–E2694.

Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, Hirrlinger PG, Kirchhoff F, Bissonnette JM, Ballas N, Mandel G (2011) A role for glia in the progression of Rett's syndrome. Nature 475:497–500.

Martin R, Bajo-Graneras R, Moratalla R, Perea G, Araque A (2015) Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. Science 349:730–734.

Mattis J, Tye KM, Ferenczi EA, Ramakrishnan C, O'Shea DJ, Prakash R, Gunaydin LA, Hyun M, Fenno LE, Gradinaru V, Yizhar O, Deisseroth K (2011) Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. Nat Methods 9:159–172.

Mauch DH, Nägler K, Schumacher S, Göritz C, Müller EC, Otto A, Pfrieger FW (2001) CNS synaptogenesis promoted by glia-derived cholesterol. Science 294:1354–1357.

McGaugh JL (2000) Memory–a Century of Consolidation. Science 287:248–251.

Mei X, Ezan P, Giaume C, Koulakoff A (2010) Astroglial connexin immunoreactivity is specifically altered at β-amyloid plaques in β-amyloid precursor protein/presenilin1 mice. Neuroscience 171:92–105.

Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48:861–873.

Min R, Nevian T (2012) Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat Neurosci 15:746–753.

Mu Y, Gage FH (2011) Adult hippocampal neurogenesis and its role in Alzheimer's disease. Mol Neurodegener 6:85–94.

Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang H-Y (2003) Astrocytes accumulate Aβ42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res 971:197–209.

Navarrete M, Araque A (2008) Endocannabinoids mediate neuron-astrocyte communication. Neuron 57:883–893.

Navarrete M, Araque A (2010) Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron 68:113–126.

Navarrete M, Diez A, Araque A (2014) Astrocytes in endocannabinoid signalling. Philos Trans R Soc B Biol Sci 369:20130599.

Navarrete M, Perea G, de Sevilla DF, Gómez-Gonzalo M, Núñez A, Martín ED, Araque A (2012) Astrocytes mediate in vivo cholinergic-induced synaptic plasticity. PLoS Biol 10:e1001259.

Newman LA, Korol DL, Gold PE (2011) Lactate produced by glycogenolysis in astrocytes regulates memory processing. PLoS One 6:e28427.

Nishida H, Okabe S (2007) Direct astrocytic contacts regulate local maturation of dendritic spines. J Neurosci 27:331–340.

Nishiyama H, Knöpfel T, Endo S, Itohara S (2002) Glial protein S100B modulates long-term neuronal synaptic plasticity. Proc Natl Acad Sci U S A 99:4037–4042.

Oberheim NA, Takano T, Han X, He W, Lin JHC, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely hominid features of adult human astrocytes. J Neurosci 29:3276–3287.

Okada Y, Sasaki T, Oku Y, Takahashi N, Seki M, Ujita S, Tanaka KF, Matsuki N, Ikegaya Y (2012) Preinspiratory calcium rise in

putative pre-Bötzinger complex astrocytes. J Physiol 590:4933–4944.

Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58:831–838.

Orr AG, Hsiao EC, Wang MM, Ho K, Kim DH, Wang X, Guo W, Kang J, Yu G-Q, Adame A, Devidze N, Dubal DB, Masliah E, Conklin BR, Mucke L (2015) Astrocytic adenosine receptor A2A and Gs-coupled signaling regulate memory. Nat Neurosci 18:423–434.

Ostroff LE, Manzur MK, Cain CK, Ledoux JE (2014) Synapses lacking astrocyte appear in the amygdala during consolidation of Pavlovian threat conditioning. J Comp Neurol 522:2152–2163.

Packer AM, Roska B, Häusser M (2013) Targeting neurons and photons for optogenetics. Nat Neurosci 16:805–815.

Panatier A, Vallée J, Haber M, Murai KK, Lacaille J-C, Robitaille R (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. Cell 146:785–798.

Pardo J, Uriarte M, Console GM, Reggiani PC, Outeiro TF, Morel GR, Goya RG (2016) Insulin-like growth factor-I gene therapy increases hippocampal neurogenesis, astrocyte branching and improves spatial memory in female aging rats. Eur J Neurosci 44:2120–2128.

Parihar VK, Hattiangady B, Shuai B, Shetty AK (2013) Mood and memory deficits in a model of Gulf War illness are linked with reduced neurogenesis, partial neuron loss, and mild inflammation in the hippocampus. Neuropsychopharmacology 38:2348–2362.

Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul J-Y, Takano H, Moss SJ, McCarthy K, Haydon PG (2005) Astrocytic purinergic signaling coordinates synaptic networks. Science 310:113–116.

Pelluru D, Konadhode RR, Bhat NR, Shiromani PJ (2016) Optogenetic stimulation of astrocytes in the posterior hypothalamus increases sleep at night in C57BL/6J mice. Eur J Neurosci 43:1298–1306.

Perea G, Araque A (2007) Astrocytes potentiate transmitter release at single hippocampal synapses. Science 317:1083–1086.

Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32:421–431.

Perea G, Yang A, Boyden ES, Sur M (2014) Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. Nat Commun 5:3262.

Petravicz J, Boyt KM, McCarthy KD (2014) Astrocyte IP3R2-dependent Ca2 + signaling is not a major modulator of neuronal pathways governing behavior. Front Behav Neurosci 8:384.

Petravicz J, Fiacco TA, McCarthy KD (2008) Loss of IP3 receptor-dependent Ca2 + increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. J Neurosci 28:4967–4973.

Petzold GC, Albeanu DF, Sato TF, Murthy VN (2008) Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. Neuron 58:897–910.

Pfrieger FW, Barres BA (1997) Synaptic efficacy enhanced by glial cells in vitro. Science 277:1684–1687.

Platel JC, Dave KA, Gordon V, Lacar B, Rubio ME, Bordey A (2010) NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. Neuron 65:859–872.

Porter JT, McCarthy KD (1996) Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J Neurosci 16:5073–5081.

Porter JT, McCarthy KD (1997) Astrocytic neurotransmitter receptors in situ and in vivo. Prog Neurobiol 51:439–455.

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci U S A 113:E2675–E2684.

Raimondo JV, Kay L, Ellender TJ, Akerman CJ (2012) Optogenetic silencing strategies differ in their effects on inhibitory synaptic transmission. Nat Neurosci 15:1102–1104.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45:1085–1098.

Robinson S, Todd TP, Pasternak AR, Luikart BW, Skelton PD, Urban DJ, Bucci DJ (2014) Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory preconditioning. J Neurosci 34:10982–10988.

Roth BL (2016) DREADDs for neuroscientists. Neuron 89:683–694.

Santello M, Bezzi P, Volterra A (2011) TNFαControls glutamatergic gliotransmission in the hippocampal dentate gyrus. Neuron 69:988–1001.

Sasaki T, Beppu K, Tanaka KF, Fukazawa Y, Shigemoto R, Matsui K (2012) Application of an optogenetic byway for perturbing neuronal activity via glial photostimulation. Proc Natl Acad Sci U S A 109:20720–20725.

Sasaki T, Matsuki N, Ikegaya Y (2011) Action-potential modulation during axonal conduction. Science 331:599–601.

Schummers J, Yu H, Sur M (2008) Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science 320:1638–1643.

Scofield MD, Boger HA, Smith RJ, Li H, Haydon PG, Kalivas PW (2015) Gq-DREADD selectively initiates glial glutamate release and inhibits cue-induced cocaine seeking. Biol Psychiatry 78:441–451.

Sibille J, Pannasch U, Rouach N (2014) Astroglial potassium clearance contributes to short-term plasticity of synaptically evoked currents at the tripartite synapse. J Physiol 592:87–102.

Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F, Savva G, Brayne C, Wharton SB (2010) Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging 31:578–590.

Sofroniew MV, Vinters HV (2010) Astrocytes: Biology and pathology. Acta Neuropathol 119:7–35.

Song H, Stevens CF, Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417:39–44.

Stehberg J, Moraga-Amaro R, Salazar C, Becerra A, Echeverria C, Orellana JA, Bultynck G, Ponsaerts R, Leybaert L, Simon F, Saez JC, Retamal MA (2012) Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. FASEB J 26:3649–3657.

Steiner J, Bernstein H-G, Bielau H, Berndt A, Brisch R, Mawrin C, Keilhoff G, Bogerts B (2007) Evidence for a wide extra-astrocytic distribution of S100B in human brain. BMC Neurosci 8:202–210.

Sultan S, Li L, Moss J, Petrelli F, Cassé F, Gebara E, Lopatar J, Pfrieger FW, Bezzi P, Bischofberger J, Toni N (2015) Synaptic integration of adult-born hippocampal neurons is locally controlled by astrocytes. Neuron 88:957–972.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144:810–823.

Sweeney P, Qi Y, Xu Z, Yang Y (2016) Activation of hypothalamic astrocytes suppresses feeding without altering emotional states. Glia 64:2263–2273.

Tadi M, Allaman I, Lengacher S, Grenningloh G, Magistretti PJ (2015) Learning-induced gene expression in the hippocampus reveals a role of neuron-astrocyte metabolic coupling in long term memory. PLoS One 10:e0141568.

Takata N, Mishima T, Hisatsune C, Nagai T, Ebisui E, Mikoshiba K, Hirase H (2011) Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo norio. J Neurosci 31:18155–18165.

Tsai H-C, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic firing in dopaminergic neurons. Science 324:1080–1084.

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291:657–661.

Vardy E et al (2015) A new DREADD facilitates the multiplexed chemogenetic interrogation of behavior. Neuron 86:936–946.

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6:626–640.

Wang X, Lou N, Xu Q, Tian G-F, Peng WG, Han X, Kang J, Takano T, Nedergaard M (2006) Astrocytic Ca2+ signaling evoked by sensory stimulation in vivo. Nat Neurosci 9:816–823.

Wenker I (2010) An active role for astrocytes in synaptic plasticity? J Neurophysiol 104:1216–1218.

Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M, Ellisman MH, Pekny M (2006) Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc Natl Acad Sci U S A 103:17513–17518.

Winship IR, Plaa N, Murphy TH (2007) Rapid astrocyte calcium signals correlate with neuronal activity and onset of the hemodynamic response in vivo. J Neurosci 27:6268–6272.

Wolosker H, Balu DT, Coyle JT (2016) The rise and fall of the d-serine-mediated gliotransmission hypothesis. Trends Neurosci 39:712–721.

Wu Z, Guo Z, Gearing M, Chen G (2014) Tonic inhibition in dentate gyrus impairs long-term potentiation and memory in an Alzhiemer's disease model. Nat Commun 5:4159.

Xu T, Yu X, Perlik AJ, Tobin WF, Zweig JA, Tennant K, Jones T, Zuo Y (2009) Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462:915–919.

Yamashita A, Hamada A, Suhara Y, Kawabe R, Yanase M, Kuzumaki N, Narita M, Matsui R, Okano H, Narita M (2014) Astrocytic activation in the anterior cingulate cortex is critical for sleep disorder under neuropathic pain. Synapse 68:235–247.

Yang G, Pan F, Gan W-B (2009) Stably maintained dendritic spines are associated with lifelong memories. Nature 462:920–924.

Yang L, Qi Y, Yang Y (2015) Astrocytes control food intake by inhibiting AGRP neuron activity via Adenosine A1 receptors. Cell Rep 11:798–807.

Zhang Y, Barres BA (2013) A smarter mouse with human astrocytes. BioEssays 35:876.

Zhang Y, Xue Y, Meng S, Luo Y, Liang J, Li J, Ai S, Sun C, Shen H, Zhu W, Wu P, Lu L, Shi J (2016) Inhibition of lactate transport erases drug memory and prevents drug relapse. Biol Psychiatry 79:928–939.

Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132:645–660.

Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL (2014) Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39:1880–1892.