Astrocyte Regulation of Synaptic Behavior

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Abstract

Astrocytes regulate multiple aspects of neuronal and synaptic function from development through to adulthood. Instead of addressing each function independently, this review provides a comprehensive overview of the different ways astrocytes modulate neuronal synaptic function throughout life, with a particular focus on recent findings in each area. It includes the emerging functions of astrocytes, such as a role in synapse formation, as well as more established roles, including the uptake and recycling of neurotransmitters. This broad approach covers the many ways astrocytes and neurons constantly interact to maintain the correct functioning of the brain. It is important to consider all of these diverse functions of astrocytes when investigating how astrocyte-neuron interactions regulate synaptic behavior to appreciate the complexity of these ongoing interactions.

Contents

INTRODUCTION

Neurons are the electrically excitable cells of the central nervous system (CNS) and communicate with other neurons at synapses, which are specialized chemical junctions (schematized in **Figure 3**) (Waites et al. 2005). Neurons are surrounded by glial cells, which is a term that encompasses the majority of cells in the brain that are not neurons, including astrocytes, oligodendrocytes, oligodendrocyte precursor cells, and microglia. Glial cells were first described

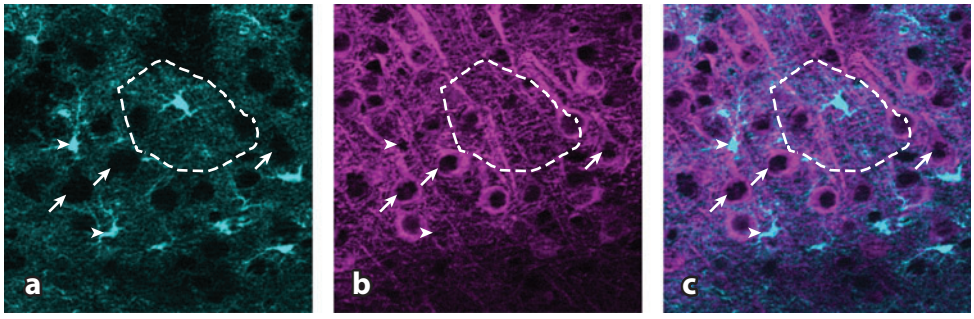

Figure 1

Astrocytes and neurons are intermingled in the mouse cerebral cortex. (*a*) Astrocytes are labeled with the fluorescent dye tdTomato (*pseudocoloured in cyan*). Astrocyte cell bodies are strongly labeled, examples marked by arrowheads. A single astrocyte profile is outlined with a dotted line to illustrate the extent of the range of one cell. Arrows mark holes where astrocyte processes are absent—this is where neuronal cell bodies are located. (*b*) MAP2 staining of the same tissue section to label neuronal cell bodies and dendritic processes. The same markings are applied to the image. (*c*) Merged image, illustrating the strong overlap between astrocyte processes and neuronal dendrites. Note that one astrocyte is extending its processes over dendrites from multiple neurons. Image provided by M. Boisvert and N. Allen.

by Virchow and colleagues in the 1860s and were considered as part of the connective tissue that holds neurons together (for a review of the history of glial discovery, see Kettenmann & Verkhratsky 2008). Since then, our understanding of glial cells has grown to appreciate that these cells do more than just provide structural support for neurons; they are actively involved in regulating many aspects of neuronal function. This review focuses on the interaction of one class of glial cell, the astrocyte, with neuronal synapses.

Properties of Astrocytes

Astrocytes are interspersed with neurons throughout synaptic layers of the brain (**Figure 1**). One astrocyte extends many fine processes, which can be visualized by labeling the cytoplasm of an individual cell (**Figure 2**) (Shigetomi et al. 2013). Traditional astrocyte markers, such as glial fibrillary acidic protein (GFAP), label only the major cytoskeleton of an astrocyte, making it hard to appreciate how closely astrocytes and neurons interact. With new methods that label astrocyte membranes, the true volume and bushy structure of the cells can be appreciated (**Figure 2**). One astrocyte can contact many thousands of synapses via its processes, and in the adult hippocampus it has been estimated that a single astrocyte can contact up to 140,000 synapses (Bushong et al. 2002). These synapses can be from different neurons, putting astrocytes in a position to sense and integrate information from neighboring neurons. During development, the processes of astrocytes are overlapping, but in the adult brain processes no longer overlap, and each astrocyte encompasses a unique, nonoverlapping domain, meaning that one synapse will be regulated by only one astrocyte (Bushong et al. 2002, 2004; Halassa et al. 2007). Astrocytes express receptors for many different neurotransmitters and show calcium signaling in response to their activation as means of nonelectrical excitability (Agulhon et al. 2008). This provides a mechanism by which astrocytes can monitor and respond to ongoing synaptic transmission. In addition to fine processes that interact with synapses, every astrocyte sends out a process that touches a nearby blood vessel, providing a link to the periphery and enabling astrocytes to take up nutrients, which are delivered to neurons (Foo et al. 2011).

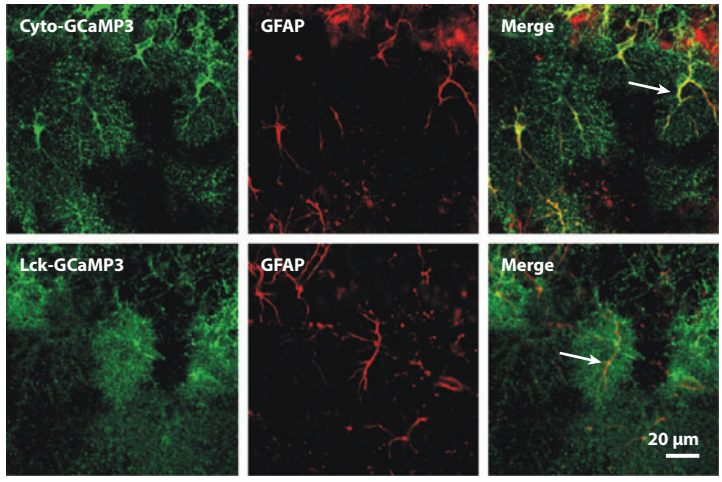

Figure 2

Astrocytes are complex highly process-bearing cells. (*top row*, *left*) Astrocytes are labeled with the cytosolic calcium indicator GCaMP3, which labels the fine processes of the cells (*green*). (*middle*) The same tissue section is immunostained for the astrocyte cytoskeletal marker GFAP (*red*). (*right*) Merge of the two images demonstrates that GFAP labels only the primary processes of astrocytes and fails to show the full size and complexity of the cell. Example marked with arrow. (*bottom row*) Same as the top, except astrocytes are labeled with a membrane-localized variant of GCaMP3, which reveals the bushy nature of the cells. Images adapted with permission from Shigetomi et al. (2013).

Evolution of Glia-Neuron Interactions

As a general rule, the ratio of glial cells (including astrocytes) to neurons has increased as animals have developed larger brains (Friede 1963). In the nematode *Caenorhabditis elegans*, we have a precise count of the cell types: 302 neurons and 56 glial cells are present, so 15% of cells are glia (Oikonomou & Shaham 2011). In the rat somatosensory cortex, 30% of the cells are glia (Bass et al. 1971); in the human brain on average 50% of the cells are glia (Azevedo et al. 2009); and in larger mammals, including elephants and whales, 80% or more of the cells are glia (Goodman et al. 2009, Hawkins & Olszewski 1957). Note that the ratio of glia to neurons varies between brain regions and even between different layers of the cortex. For example, the human brain has an average of 50% glial cells, but in the cerebral cortex, glia account for nearly 80% of the cells in the whole cortex and 60% of the cells in the gray matter, whereas in the cerebellum glia make up just 20% of the cells (Azevedo et al. 2009). Increased cortical thickness was found to be due to a higher number of astrocytes being present in synaptic layers of neuropil, whereas the density of astrocytes and neurons stayed constant (Carlo & Stevens 2013). Thus, the increase in the ratio of glia to neurons with increasing brain size is likely due to an increase in the number of synaptic connections and a requirement to regulate these synapses.

Astrocytes Are a Heterogeneous Population of Cells

It is important to consider astrocytes as a heterogeneous population of cells, much like neurons. This is obvious on a structural level when comparing the radial glia–like Bergmann glia (BG) of the cerebellum to protoplasmic astrocytes of the cortex, but it is equally possible that protoplasmic astrocytes within different cortical layers have specialized functional properties (Zhang & Barres 2010). Gene-profiling studies comparing the expression of genes in astrocytes from different brain

regions have begun to address this, and it will be interesting to extend these studies to determine if astrocytes have region-specific properties that match differences in gene expression (Doyle et al. 2008, Yeh et al. 2009). In humans, distinct classes of astrocytes have been identified that appear to be unique to humans and higher primates. These include astrocytes that project long processes across many cortical layers, as well as protoplasmic astrocytes that are twofold larger than rodent astrocytes (Oberheim et al. 2006, 2009; Sosunov et al. 2014). The function of human astrocytes has been studied by transplanting mice with human glial progenitor cells, which became astrocytes and engrafted into the mouse brain (Han et al. 2013). The human astrocytes integrated with mouse astrocytes; were larger than mouse astrocytes, suggesting size is an intrinsic feature; and enhanced the ability of the mice to perform behavioral tasks. An intriguing explanation offered for this enhanced behavioral performance is that, owing to their larger size, human astrocytes can interact with more synapses than rodent astrocytes can, and they are thus able to sense and modulate information from a greater number of synaptic inputs (Han et al. 2013, Oberheim et al. 2009).

Astrocytes Are Essential for Neuronal Survival

Teasing out the precise function of astrocytes in regulating synaptic function is made challenging by the fact that in most organisms the presence of astrocytes is essential for neuronal survival, and hence survival of the whole organism. This has been demonstrated both in cell culture and in the whole animal (Banker 1980, Müller et al. 1995, Pyka et al. 2011a). Ablation of astrocytes in the mouse brain in vivo leads to neuronal degeneration and death as a result of excitotoxicity caused by a failure to remove the excitatory neurotransmitter glutamate from the extracellular space and by excessive activation of glutamate receptors (Cui et al. 2001, Delaney et al. 1996, Sofroniew et al. 1999, Wagner et al. 2006). Therefore, the majority of studies of astrocyte-synapse interaction focus on identifying and manipulating specific molecules and pathways, such that the influence of astrocytes on specific aspects of synaptic function can be revealed. This review provides an overview of how astrocytes interact with and regulate neuronal synapses from development through adulthood. New findings in each area are highlighted and discussed. Specifically, I focus on

1. how astrocytes regulate synapse formation and remodeling during nervous system development,
2. how astrocytes interact with synapses in the mature brain to modulate synaptic plasticity,
3. traditional support functions of astrocytes that impact on synaptic function and plasticity, and
4. structural constraints on neurotransmission caused by astrocyte-synapse physical interactions.

ASTROCYTE REGULATION OF SYNAPSE FORMATION AND REMODELING IN THE DEVELOPING BRAIN

Astrocytes have important roles in regulating neuronal synapse number during development, both by promoting the formation of new synapses and by eliminating excess synapses. This section summarizes the status of the field and highlights some recent findings and outstanding questions.

Astrocytes Induce Synapse Formation Between Neurons

In the developing brain, astrocytes are generated after neurons and are derived from radial glia in the ventricular zone and through local division of newly generated astrocytes within cortical layers (Ge et al. 2012, Miller & Gauthier 2007). The observation that the beginning of neuronal

synapse formation correlates with the appearance of astrocytes (Correa-Gillieron & Cavalcante 1999) led to the hypothesis that astrocytes may be actively involved in regulating synaptogenesis. Numerous studies have demonstrated that the presence of astrocytes does lead to the formation of more synapses between developing neurons in culture, as well as an increase in synaptic activity (Blondel et al. 2000, Li et al. 1999, Liu et al. 1996, Nägler et al. 2001, Pfrieger & Barres 1997, Ullian et al. 2001). More recent studies have addressed this question in vivo. As discussed in the introduction, the ablation of all astrocytes leads to neuronal degeneration and death, so is not a useful approach to identify the role of astrocytes specifically in synapse formation. In mice, this was overcome by selectively eliminating a small region of astrocytes in the developing spinal cord (Tsai et al. 2012). Analysis of synapse formation in the region lacking astrocytes demonstrated a selective effect on particular synapse classes that formed. The formation of cholinergic and VGlut2 glutamatergic synapses onto motor neurons was unaffected, whereas the number of VGlut1 glutamatergic synapses was decreased and the number of GABAergic synapses was increased. These synapse classes derive from different neuronal input sources, e.g., sensory inputs or local interneurons, demonstrating that astrocytes can selectively control the type of synapse that forms onto one neuron during neuronal circuit formation in vivo.

Identification of Astrocyte Synaptogenic Factors Using In Vitro Models

A productive approach to determine the role of astrocytes in synapse formation has been to identify the individual astrocyte-derived factors that are responsible, so that these can be manipulated individually in vivo to determine their role in synapse formation. In rodents, the majority of studies have used cell culture systems that have been optimized to enable the survival of neurons in the absence of astrocytes, such that individual astrocyte factors can be added back to the cultures and their effect on synapse formation assayed. The first studies of this type used retinal ganglion cell neurons (RGCs), and more recently hippocampal and cortical neuron cultures have been used (Elmariah et al. 2005, Hu et al. 2007, Meyer-Franke et al. 1995, Pfrieger & Barres 1997, Ullian et al. 2001). One hypothesis is that the effect of astrocytes on synapse formation is secondary to the provision of prosurvival factors and energy substrates to neurons that increase neuronal health, thus enabling neurons to form more synapses. This hypothesis was disproved with the discovery that many different factors from astrocytes regulate specific steps of synapse formation via interaction with specific receptors on neurons. The signals that astrocytes use to interact with neurons also change with development. At embryonic stages, neurons require physical contact with an astrocyte to induce synapses, whereas postnatal neurons are able to respond to secreted signals from astrocytes, showing that the signaling interactions between neurons and astrocytes change with maturation (Barker et al. 2008, Hama et al. 2004, Ullian et al. 2001).

Astrocytes Use Multiple Signals to Regulate Glutamatergic Synapse Formation

The formation and maturation of excitatory glutamatergic synapses has been studied extensively. Secreted astrocyte molecules identified include thrombospondins and hevin, which induce structural synapse formation (Christopherson et al. 2005, Kucukdereli et al. 2011); cholesterol, which enhances presynaptic release probability (Mauch et al. 2001); glypicans, Wnts, CSPGs, and tumor necrosis factor α (TNFα), which recruit AMPA glutamate receptors (AMPARs) to the synapse (Allen et al. 2012, Beattie et al. 2002, Frischknecht et al. 2009, Kerr et al. 2014, Pyka et al. 2011b); SPARC, which inhibits synapse formation and decreases synaptic AMPARs (Jones et al. 2011, Kucukdereli et al. 2011); and activity-dependent neurotrophic factor, which increases levels of NMDA glutamate receptors (Blondel et al. 2000). Additionally γ-protocadherins mediate

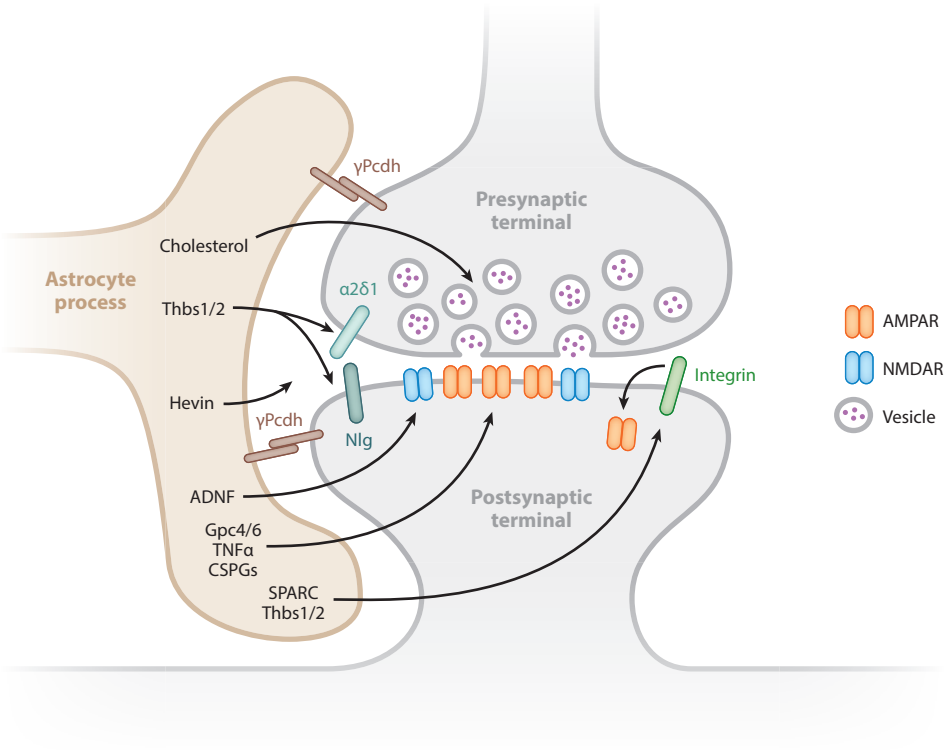

Figure 3

Astrocytes use multiple pathways to regulate glutamatergic synapse formation. Schematic representation of a synapse, consisting of a presynaptic axon terminal, a postsynaptic terminal attached to a dendrite, and an associated astrocyte process. Astrocytes release thrombospondin (Thbs1/2) and hevin, which induce structural synapse formation, Thbs1/2 via α2δ1 and neuroligin (Nlg) synaptic receptors; cholesterol, which induces presynaptic maturation; glypican 4/6 (Gpc4/6), TNFα, and CSPGs, which recruit AMPA receptors to the postsynaptic density; ADNF, which recruits NMDA receptors to the postsynaptic density; and SPARC and Thbs1/2, which decrease synaptic levels of AMPA receptors via integrins. Transmembrane γ-protocadherins (γPcdh) form a homophilic adhesion with receptors in neurons.

contact-induced synaptogenesis (Garrett & Weiner 2009). The detailed mechanism by which each of these factors works has been reviewed extensively in other articles (**Figure 3**) (Allen 2012, 2013). It can be appreciated that each of these factors acts on a subpart of synapse formation, and no factor has been identified that can induce the formation of a fully functional synapse by itself. An important next step will be to work out how all of these different factors work together in the intact brain to induce a mature glutamatergic synapse. For example, are all of these factors expressed at the same time or in the same brain region? Does one astrocyte express all these factors, and if so, what determines their release?

Astrocyte Synaptogenic Factors Act Through Known Neuronal Synaptogenic Receptors

As stated above, an important step forward in determining a specific role for astrocyte factors in regulating synapse formation was the discovery of receptors on neurons that mediate the

synaptogenic effect. Two receptors have been identified that induce glutamatergic synapse formation in response to thrombospondin: α2δ1, which was originally identified as an accessory subunit of the voltage-gated calcium channel, and neuroligin, which is best known as the synaptogenic partner of neurexin (Eroglu et al. 2009, Xu et al. 2009). Both of these receptors are located at synaptic sites, placing them in the correct location to mediate a local synaptogenic effect of thrombospondin and suggesting that astrocytes may be able to regulate precisely when and where individual synapses form by the localized release of thrombospondin. The action of SPARC in regulating synaptic AMPARs is through β3-integrins, which are known to stabilize AMPARs at synapses (Jones et al. 2011). The finding that astrocyte factors are acting through known neuronal synaptogenic pathways strengthens the hypothesis that astrocytes are actively regulating neuronal synapse formation and circuit maturation via modulation of these pathways.

Astrocyte Regulation of Inhibitory Synapse Formation Uses Factors Distinct from Glutamatergic Synapses

Multiple signals have been identified that regulate stages of excitatory glutamatergic synapse formation, but even though the presence of astrocytes enhances the formation of GABAergic and glycinergic inhibitory synapses, the molecular mechanisms responsible are only just beginning to be identified (Cuevas et al. 2005, Elmariah et al. 2005, Hughes et al. 2010, Korn et al. 2012). What is known is that different signals are required to regulate different classes of synapse formation. For example, treatment of hippocampal neurons with the astrocyte synaptogenic factor thrombospondin increases the number of glutamatergic synapses that form but has no effect on the number of GABAergic synapses (Hughes et al. 2010). This demonstrates that astrocytes have the ability to regulate the class of synapse that forms via the release of specific signals. Identifying what these signals are will enable studies of whether and how astrocytes are involved in establishing the correct balance of excitatory and inhibitory synapses in the developing brain, which has important implications for developmental disorders, including autism and epilepsy, where this balance is thought to be disrupted.

Astrocyte Factors Regulate Synaptic Balance

In mature synapses, the same astrocyte factor can have opposing effects on synaptic levels of excitatory and inhibitory receptors. For example, TNFα increases synaptic AMPAR levels and decreases synaptic GABAAR levels, leading to an overall increase in neuronal excitability (Stellwagen et al. 2005). This effect of astrocyte TNFα is important in homeostatic synaptic scaling, a type of plasticity that occurs when the activity of a whole neuronal network is altered, ensuring that the relative strengths of synapses are maintained (Stellwagen & Malenka 2006). In addition to inducing structural synapse formation, thrombospondin regulates neurotransmitter receptor levels in mature synapses. Thrombospondin decreases synaptic AMPAR levels and increases synaptic glycine receptors, leading to an overall decrease in neuronal excitability, the opposite effect to that of TNFα (Hennekinne et al. 2013). This action of thrombospondin in decreasing synaptic AMPAR levels in mature neurons correlates with its described role in the developing brain, where it induces the formation of structurally mature but postsynaptically silent synapses that lack AMPARs (Christopherson et al. 2005). These combined data suggest that the role of thrombospondin is to induce silent synapse formation, and that other factors coming from astrocytes, including glypicans and CSPGs, are responsible for increasing synaptic AMPAR levels and positively regulating synaptic strength (Allen et al. 2012, Frischknecht et al. 2009, Pyka et al. 2011b).

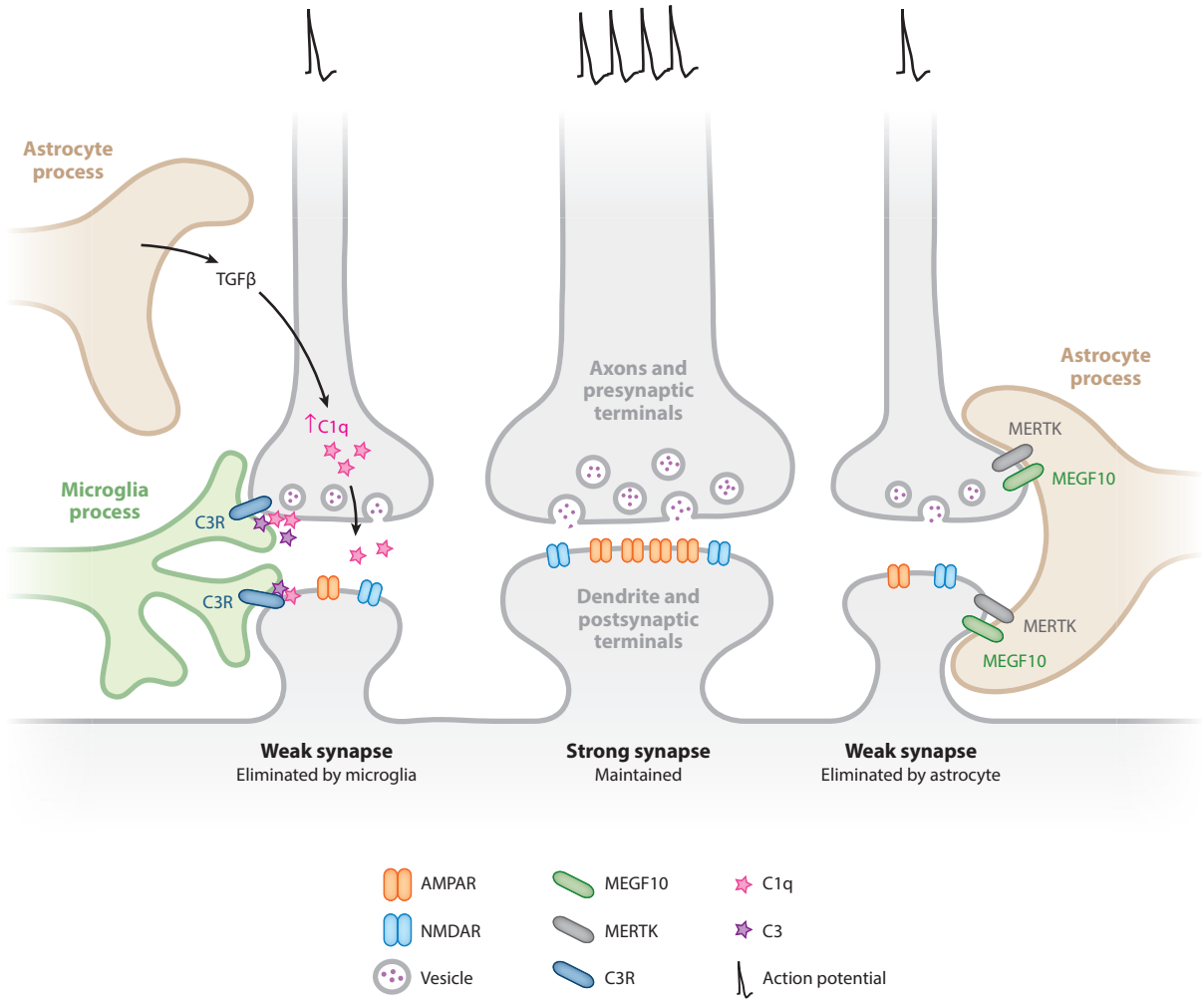

Figure 4

Astrocytes eliminate neuronal synapses by using both direct and indirect mechanisms. During development, strong, electrically active synapses are maintained and strengthened via recruitment of presynaptic neurotransmitter vesicles and postsynaptic receptors (*middle synapse*). Weaker, less electrically active synapses can be eliminated via two distinct glial pathways, terminating in phagocytosis of synapses by microglia (*left*) or astrocytes (*right*). Microglia pathway: Astrocytes release the cytokine TGFβ, which induces expression of the initiating protein of the complement cascade, C1q, in neurons. Neuronal-released C1q localizes to weak synapses and recruits the downstream component of the cascade C3, and nearby microglia recognize C3-coated synapses via the C3 receptor (C3R) and eliminate these synapses by phagocytosis. Astrocyte pathway: Astrocytes express the phagocytic receptors MEGF10 and MERTK, which each form a distinct phagocytic pathway. Astrocyte processes interact with weak synapses via these receptors and eliminate synapses by phagocytosis.

Role of Astrocytes in Synapse Elimination

Astrocytes do not just induce synapse formation, they are also actively involved in the elimination of excess synapses (**Figure 4**). In the developing brain, many more synapses form than are needed in adulthood, and these excess, inappropriate synapses must be eliminated for correct neuronal function. Astrocytes regulate this process both directly and indirectly. Gene expression profiling

of purified CNS cell populations revealed that astrocytes are highly enriched for genes involved in phagocytosis, including the phagocytic receptors *Megf10* and *Mertk*, which had been previously shown to mediate the removal of axonal debris in *Drosophila* by glia using the ortholog of *Megf10*, *draper* (Cahoy et al. 2008, Doherty et al. 2009, MacDonald et al. 2006). In the developing mouse visual system, there is a stereotyped removal of excess synapses from relay neurons in the thalamus over the first three postnatal weeks, such that relay cells go from being innervated by 10–20 different retinal ganglion neurons to just 1–2 in the adult (Chen & Regehr 2000). In mice lacking both *Megf10* and *Mertk*, thalamic relay cells remain innervated by multiple axons at three weeks of age, after axon removal and synaptic remodeling have ceased in a wild type (Chung et al. 2013). This is due to a decrease in the ability of astrocytes lacking either *Megf10* or *Mertk* to phagocytose synapses, and this defect is enhanced when both receptors are removed, showing that these are two parallel pathways through which astrocytes phagocytose synapses. Astrocytes employ an indirect pathway to further regulate synapse elimination in the developing visual system. Retinal astrocytes release transforming growth factor β, which induces the expression of the complement protein C1q in retinal ganglion neurons (Bialas & Stevens 2013). Mice lacking *C1q* display a similar phenotype to *Megf10;Mertk* knockout mice, that is, a failure to eliminate excess synaptic inputs in the thalamus (Stevens et al. 2007). This is through activation of the classical complement cascade and involves the downstream component C3 and phagocytosis of synapses by microglia (Schafer et al. 2012). In *Drosophila*, distinct pathways have been identified by which glia phagocytose either apoptotic neuronal cell bodies or degenerating axons and synapses from the same neuron, demonstrating that the context in which degeneration occurs is important for determining the phagocytic pathway that is activated (Tasdemir-Yilmaz & Freeman 2014). So, just as with synapse formation, multiple molecules and pathways are being identified through which astrocytes regulate synapse elimination.

ASTROCYTE MODULATION OF ONGOING SYNAPTIC ACTIVITY AND PLASTICITY IN THE ADULT BRAIN

Since the initial discovery that cultured astrocytes respond to the neurotransmitter glutamate with a rise in intracellular calcium, and the calcium rise can propagate to neighboring astrocytes as a wave, the hypothesis that astrocytes sense and respond to neuronal activity via long-range calcium signaling has been investigated intensively (Cornell-Bell et al. 1990). How astrocyte calcium signaling affects synaptic function, and recent findings in this area, is presented here.

Astrocytes Express Neurotransmitter Receptors and Release Neuromodulators

The discovery of astrocyte calcium signaling led to the model that astrocytes express receptors that sense neurotransmitter release from neighboring active synapses and release their own substances to signal back to neurons and modulate ongoing neurotransmission. This is the tripartite hypothesis, where astrocytes form a third active element of the synaptic apparatus (Araque et al. 1999). Various aspects of this model are the subject of ongoing debate, which is thoroughly reviewed from all sides in several review articles (Araque et al. 2014, Nedergaard & Verkhratsky 2012, Rusakov et al. 2014). Astrocytes express receptors for a multitude of different neurotransmitters and neuromodulators, including but not limited to glutamate, GABA, adenosine, norepinephrine, acetylcholine, and endocannabinoids (Agulhon et al. 2008). The majority of these transmitters signal through G protein–coupled receptors, which leads to release of calcium from intracellular stores, which in astrocytes occurs via IP_3R2. G protein–coupled receptors are well suited as receptors for neurotransmitters in astrocytes owing to their high affinity for

ligands, which is important as only low levels of neurotransmitter will diffuse to the extra synaptic sites where astrocyte processes are located. Following an increase in intracellular calcium, astrocytes can release many different neurotransmitters or neuromodulators, which are collectively called gliotransmitters, including ATP, adenosine, glutamate, and D-serine. The proposed modes of transmitter release from astrocytes include vesicular, lysosomal, volume regulated anion channel, transporter reversal, and gap junction hemichannel mechanisms. The exact mechanisms of release, and which factors are released, are hotly debated topics that are not covered in detail here but have been extensively reviewed by others (Hamilton & Attwell 2010).

Astrocytes Respond to Neurotransmitters with Calcium Rises

The study of astrocyte calcium signaling has progressed from studies of cultured astrocytes and neurons, to interactions between astrocytes and neurons in brain slices, to in vivo imaging of astrocyte calcium responses in anesthetized animals, and most recently to in vivo imaging of astrocyte calcium responses in awake behaving animals. Although most studies of astrocyte-neuron interactions use calcium responses in astrocytes as a measure of excitability, it should be noted that this is just one way by which astrocytes can respond to neuronal activity. A recent study in the adult mouse cortex found that astrocytes upregulate the expression of the metabotropic glutamate receptor mGluR3 with age, which couples to the adenylate cyclase pathway, and downregulate mGluR5 in the astrocyte cell body, which couples to calcium signaling via the release of calcium from intracellular stores (Sun et al. 2013). This means that many potential astrocyte responses are currently not being monitored owing to the lack of the appropriate tools to detect them. In addition, not all calcium responses are via store-regulated release of calcium. Spotty localized calcium responses have been detected using membrane bound calcium indicators, and these are due to calcium influx through membrane TRPA1 channels (Shigetomi et al. 2012).

Astrocytes Regulate Synaptic Plasticity

Astrocytes have been shown to participate in three major forms of synaptic plasticity in the brain. First, astrocytes can regulate short-term plasticity, which is a transient alteration in the strength of a synaptic connection. Second, astrocytes participate in long-term plasticity (LTP), which is a long-lasting increase in the strength of specific synaptic connections. Third, astrocytes regulate homeostatic synaptic scaling, which is a long-term change in the strength of all synapses on a given neuron (reviewed in the synaptogenesis section). Astrocytes regulate short-term synaptic plasticity by affecting the release of neurotransmitter from the presynaptic terminal in response to an action potential. This is via the release of ATP, which is converted into adenosine in the extracellular space. At some synapses, adenosine acts through presynaptic A1 receptors and decreases presynaptic release probability and so weakens the connection, whereas at other synapses adenosine acts through A2A receptors and increases presynaptic release probability, so strengthening the connection (Araque et al. 2014, Panatier et al. 2011, Pascual et al. 2005). Astrocytes can control the ability to induce LTP by regulated release of the NMDA receptor coagonist D-serine, which is required to activate the NMDA receptor in the presence of glutamate. Surprisingly NMDA receptors are not saturated with D-serine under basal conditions, and calcium-dependent release of D-serine from astrocytes regulates the induction of postsynaptic LTP (Henneberger et al. 2010). However, in experiments where the release of calcium from intracellular stores was abolished in astrocytes, this had no effect on the ability to induce LTP in the hippocampus (Agulhon et al. 2010). Therefore, when examining the contribution of astrocytes to synaptic plasticity, it is

important to consider the context, as differing results may be observed depending on the brain region being studied and the type of plasticity being induced (discussed in detail in Araque et al. 2014).

Astrocytes Can Detect and Modulate Activation of Individual Synapses

Many studies that have demonstrated astrocyte responses to neuronal activation have used strong stimuli to activate many synapses simultaneously, and responses were recorded from relatively large regions of astrocytes; therefore, it was unclear whether astrocytes were able to detect and modulate the activity of one synapse. This is an important question in determining whether astrocytes can independently regulate the activity of neighboring synapses, including those formed by different neurons within the domain of the same astrocyte. This was addressed by two studies in hippocampal slices, which used minimal stimulation of axons to induce neurotransmitter release at just one synapse, while simultaneously recording the calcium response of a neighboring astrocyte process, and showed a correlation between the timing and appearance of a neuronal postsynaptic response and an astrocyte calcium response (Di Castro et al. 2011, Panatier et al. 2011). In addition, the synaptically evoked local calcium responses in astrocyte processes were much faster than those observed in astrocyte cell bodies, being on the order of hundreds of milliseconds as opposed to seconds. Further, if a calcium chelator was introduced to the astrocyte during the experiment, it decreased the probability of axonal stimulation being able to induce a synaptic event, showing that ongoing communication between the astrocyte process and the synapse was setting the basal tone of presynaptic excitability, via adenosine receptor activation modulating presynaptic release (Panatier et al. 2011).

Astrocytes Show Calcium Responses In Vivo During Behavior

Astrocytes in vivo respond with calcium increases when specific sensory or motor pathways are activated. This was first demonstrated in anesthetized mice (Schummers et al. 2008, Takata & Hirase 2008, Wang et al. 2006) and then in awake behaving mice (Dombeck et al. 2007, Nimmerjahn et al. 2009). It should be noted that the presence of anesthetic has a strong effect on the astrocyte calcium response and that astrocytes are affected by levels of anesthesia that do not block neuronal transmission. Anesthesia decreases both spontaneous calcium responses and sensory-evoked calcium responses and prevents the widespread synchronous activation of groups of astrocytes (Thrane et al. 2012). Therefore, to truly understand the role of astrocytes in modulating synaptic transmission in vivo, it is necessary to conduct these experiments in awake animals.

Although the slice studies described above have shown astrocytes are capable of sensing and modulating activity at one synapse, several in vivo studies are pointing toward a role for astrocytes in setting the overall tone of neurotransmission, and perhaps regulating attention, via the cholinergic and adrenergic systems. Activation of muscarinic acetylcholine receptors on astrocytes, via stimulation of inputs from cholinergic nuclei, enhances the astrocyte calcium response to sensory input, e.g., visual inputs to V1 cortex or whisker stimulation to the barrel cortex, and also hippocampal plasticity (Chen et al. 2012, Navarrete et al. 2012, Takata et al. 2011). The increase in astrocyte calcium is dependent on calcium release from intracellular stores, as it is abolished in IP_3R2 knockout mice. Interestingly, the enhanced neuronal response that is also observed when the sensory and cholinergic stimuli are paired is abolished in IP_3R2 knockout mice, demonstrating that the effect of cholinergic innervation on plasticity and attention is mediated by astrocytes. In the barrel cortex, this is via astrocyte release of D-serine, which potentiates the postsynaptic NMDA receptor response (Takata et al. 2011). Adrenergic activation has been shown to be necessary for the widespread coordinated astrocyte calcium responses that are observed in awake

behaving mice and has been suggested to be the mechanism by which norepinephrine modulates attention (Bekar et al. 2008, Ding et al. 2013).

In brain slices, the direct stimulation of astrocyte calcium responses is sufficient to modulate synaptic transmission in neighboring neurons. This has been achieved by applying metabotropic receptor agonists and uncaging IP_3 or calcium inside astrocytes (Agulhon et al. 2008). To date, the only in vivo experiments that have addressed sufficiency of astrocyte calcium increases have used optogenetic approaches, where the light-sensitive cation channel channelrhodopsin 2 (ChR2) is expressed in astrocytes. Astrocytes are not electrically excitable cells, so modulation is presumably working by allowing calcium entry through ChR2 into astrocytes (Gourine et al. 2010, Perea et al. 2014, Sasaki et al. 2012). When ChR2-expressing astrocytes are activated in vivo, there is an effect on neuronal activity and animal behavior, showing that the in vivo modulation of astrocyte calcium signaling is sufficient to regulate neuronal activity. The mechanism of this effect is unclear—it is likely through calcium-dependent regulation of gliotransmitter release, as discussed above. Alternatively, neurotransmitter transporter activity may have been affected, or potassium buffering in the extracellular space may have been changed, as discussed in the next section.

TRADITIONAL ROLES OF ASTROCYTES IN REGULATING NEURONAL SYNAPTIC TRANSMISSION

Owing to the relatively recent discovery that astrocytes can regulate synaptic and neuronal function through the release of gliotransmitters, more established roles of astrocytes in regulating transmission through functions including maintenance of the extracellular ion balance, removal of excess neurotransmitter from the extracellular space, and supply of energy substrates to neurons have been somewhat overlooked. Some researchers have questioned whether in fact these established functions of astrocytes are contributing to the calcium-dependent modulation of neurotransmission by astrocytes, and recent evidence for this is presented here (Nedergaard & Verkhratsky 2012; Wang et al. 2012a, 2012b).

Potassium Buffering by Astrocytes

During active neurotransmission, potassium ions build up in the extracellular space, as the exit of potassium ions from depolarized neurons is responsible for returning neurons to a resting state. If neuron-released potassium were not removed from the extracellular space, then it would enhance the basal excitability of neighboring neurons by depolarizing their membrane and eventually lead to seizure generation. Kuffler (1967) and colleagues first suggested that astrocytes were responsible for removing excess potassium from the extracellular space; they demonstrated that astrocytes express potassium-permeable channels that conduct potassium into astrocytes and proposed that potassium is then spread to neighboring coupled astrocytes through gap junctions to be released at a distant site. Recent studies have sought to identify the molecular basis of astrocyte potassium uptake, as well as the channels responsible. Inwardly rectifying potassium channels (K_{ir}), in particular $K_{ir}4.1$, were prime candidates. Mice lacking *$K_{ir}4.1$* specifically in astrocytes develop seizures and die within three weeks of birth (Djukic et al. 2007). The *$K_{ir}4.1$* knockout astrocytes have a greatly reduced ability to take up potassium and also show impaired glutamate uptake (probably owing to the requirement for potassium for correct operation of the glutamate transporter; see below). Basal synaptic transmission is normal, but short-term synaptic plasticity in response to a train of action potentials is enhanced in the *$K_{ir}4.1$* knockout, suggesting astrocytes are responsible for removing and buffering potassium during periods of intense activity (Djukic et al. 2007, Sibille

et al. 2014). In addition to $K_{ir}4.1$, the astrocyte sodium potassium ATPase is responsible for uptake of some potassium during periods of intense neurotransmission (Larsen et al. 2014).

Uptake of Neurotransmitter by Astrocyte Transporters

High concentrations of neurotransmitter are released into the synaptic cleft during neurotransmission, and the only mechanisms of removal to terminate signaling are via diffusion or transporter-mediated uptake into cells. The rapid removal of neurotransmitter is crucial to maintain the spatial and temporal encoding of synaptic transmission. In the developing hippocampus, in the first postnatal week, the major route of glutamate clearance is via diffusion, but after this time the size of the extracellular space decreases, glutamate transporters are upregulated in astrocytes, and transporter uptake becomes the dominant mechanism of glutamate removal (Thomas et al. 2011). Astrocytes express transporters for the major neurotransmitters (glutamate, GABA, and glycine), and these are located in processes next to the synapse. Glutamate is taken up by GLT-1 and GLAST transporters on astrocytes, which are estimated to remove 80% of synaptically released glutamate (with the rest being taken back up into neurons) (López-Bayghen & Ortega 2011). Glutamate transporters operate by taking up one glutamate molecule along with three Na^+ and one H^+ in exchange for one K^+; therefore, the ability of astrocytes to remove glutamate from the extracellular space is affected by the sodium and potassium concentration (see above), as well as the membrane potential of the astrocyte. Blocking glutamate transporters has no effect on the kinetics of spontaneous synaptic events but prolongs synaptic events induced by sustained activation of synapses, showing an important role for transporters in segregating synaptic events (Marcaggi & Attwell 2004). Genetic removal of the major astrocyte glutamate transporter *GLT-1* in mice leads to spontaneous seizures and death owing to increased levels of extracellular glutamate, demonstrating the importance of maintaining low levels of extracellular glutamate (Tanaka et al. 1997).

Levels of glutamate transporters in astrocytes are dynamically regulated by interactions with neurons and the level of ongoing synaptic transmission (Yang et al. 2009). Decreased synaptic activity downregulates transporter level, and increased synaptic activity increases transporter level (Benediktsson et al. 2012). This regulation occurs in vivo—24 h of whisker stimulation is sufficient to cause a twofold increase in the level of GLT-1 and GLAST glutamate transporters in the barrel cortex, specifically within the cortical column that receives inputs from the stimulated whisker, and transporter levels return to baseline levels four days after stimulation ceases, demonstrating an ongoing dynamic regulation of transporters (Genoud et al. 2006). Increased glutamate levels likely stimulate the upregulation of transporters in astrocytes to ensure they are still able to take up all of the glutamate that is being released and maintain independent operation of neighboring synapses. This same paradigm also induces an increased wrapping of synapses by astrocyte processes (see below), showing a second mechanism by which astrocytes can constrain activity to individual synapses.

Physical interactions between astrocyte processes and dendritic spines, via Eph-ephrin interactions (neuronal EphA4 and astrocyte ephrin-A3), are sufficient to regulate the levels of glutamate transporters on astrocyte processes (Klein 2009, Murai & Pasquale 2011). The removal of *EphA4* from hippocampal neurons causes an increase in the level of glutamate transporters in astrocyte processes (Filosa et al. 2009). The converse manipulation, removal of *ephrin-A3* from astrocytes, increases the action of glutamate transporters in astrocyte processes, with both manipulations leading to a decreased extracellular glutamate concentration (Carmona et al. 2009). This suggests that Eph-ephrin interactions between neuronal spines and astrocyte processes decrease levels of glutamate transporters and hence glutamate uptake, potentially increasing the strength of transmission at that synapse by increasing local glutamate levels. Both of these manipulations alter the ability to

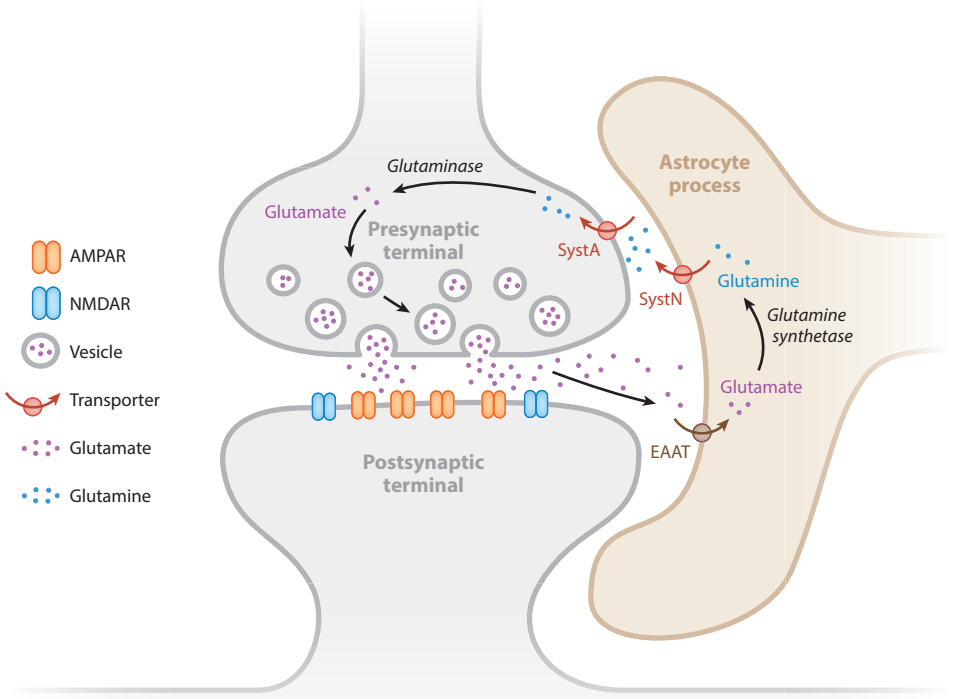

Figure 5

Astrocytes recycle the neurotransmitter glutamate via the glutamate-glutamine cycle. The neurotransmitter glutamate is stored in vesicles ready for release from presynaptic terminals. After glutamate is released, its action is terminated by uptake into neighboring astrocyte processes via glutamate transporters (EAATs). Within the astrocyte, glutamate is converted to glutamine by glutamine synthetase. Glutamine is exported from astrocytes by system N (SystN) glutamine transporters and taken up into neighboring neuronal presynaptic terminals by system A (SystA) glutamine transporters. Within the neuron, glutamine is converted back to glutamate by phosphate-activated glutaminase and repackaged into vesicles by vesicular glutamate transporters, ready to be used again.

induce synaptic plasticity in the hippocampus, showing an important role for glutamate transporter levels in regulating neuronal function by keeping synapses in a plastic state (Filosa et al. 2009).

Recycling of Neurotransmitters by Astrocytes

Besides the termination of synaptic transmission, another major role of astrocyte neurotransmitter transporters is to enable recycling of neurotransmitter back to presynaptic terminals, and hence reduce the demand for synthesis of new neurotransmitters from precursors taken up from the periphery (Marcaggi & Attwell 2004). This is achieved by the glutamate-glutamine cycle (**Figure 5**). Glutamate taken up into astrocytes is converted to glutamine by glutamine synthetase, and glutamine is released back to the extracellular space and taken up by neighboring neurons (glutamine does not activate signaling receptors itself) (Uwechue et al. 2012). This occurs at both

glutamatergic and GABAergic synapses (Tani et al. 2014), as glutamate is the precursor of GABA (Liang et al. 2006).

Astrocytes Supply Neurons with Energy Substrates

Astrocytes directly interact both with blood vessels via end feet and with neurons via fine processes, so they are uniquely positioned to take up nutrients from the blood supply and deliver them to neurons. Astrocytes supply neurons with substrates to make ATP for energy use, which is largely used to power mechanisms that maintain synaptic transmission and the neuronal membrane potential (Harris et al. 2012). The astrocyte-neuron lactate shuttle, whereby astrocytes produce lactate by glycolysis and shuttle it to neighboring neurons for use in oxidative phosphorylation, was first proposed 20 years ago (Pellerin & Magistretti 1994, 2012). This model states that during synaptic activity, the uptake of glutamate into astrocytes via transporters and subsequent conversion into glutamine would be powered by glycolysis, and the pyruvate produced by glycolysis would be converted to lactate and shuttled to neurons to produce ATP by oxidative phosphorylation to maintain synaptic transmission. Experimental evidence has been provided that both agrees with and refutes this model. For example, experiments in hippocampal slices have demonstrated that glucose and oxidative phosphorylation are sufficient to sustain synaptic transmission, and that the production of lactate is not required (Hall et al. 2012, Ivanov et al. 2014). Conversely, extracellular lactate levels have been shown to increase in the rat hippocampus during a learning task, and blocking lactate production had no effect on short-term memory formation but prevented long-term memories from being formed, as did disruption of lactate transporters (Suzuki et al. 2011). Taken together, these data suggest that the astrocyte-neuron lactate shuttle is not operational during basal synaptic transmission, but can be used during periods of intense neuronal activity to replenish the energy needed to sustain high activity levels (Magistretti 2006).

Astrocytes Synthesize Lipids that Enhance Neuronal Synaptic Function

The brain is shielded from the peripheral circulation by the blood-brain barrier, which limits the entry of molecules from the blood into the brain. In mammals, the blood-brain barrier prevents circulating lipoproteins from crossing into the brain, so all lipoproteins and cholesterol within the brain are synthesized in situ (Hayashi 2011). Astrocytes are the major source of lipid synthesis in the adult brain (Camargo et al. 2009), and gene expression analysis demonstrated that astrocytes are highly enriched for lipid synthesis enzymes compared with neurons (Cahoy et al. 2008). Astrocytes synthesize and secrete cholesterol complexed to apolipoprotein E, which is taken up by neurons via binding to low-density lipoprotein receptors followed by endocytosis (Hayashi 2011). This process has been shown to be particularly important during synaptic development, when astrocyte-derived cholesterol contributes to presynaptic maturation and enhances the probability of release of neurotransmitter from presynaptic terminals, strengthening the synaptic connection (Mauch et al. 2001).

ASTROCYTES PLACE PHYSICAL CONSTRAINTS ON NEUROTRANSMISSION

Astrocyte processes are closely associated with synapses and provide a physical barrier to limit the diffusion of neurotransmitter away from an active release site, thus limiting spillover and the activation of neighboring synapses. Interesting questions about astrocyte-synapse interactions arise: (*a*) Does every synapse have an astrocyte process associated with it? (*b*) How much of

the synaptic perimeter is ensheathed by an astrocyte? (*c*) Does the degree of astrocyte-synapse interaction vary across brain regions and synapse type? (*d*) Is the degree of astrocyte-synapse interaction altered by changes in neuronal activity? These questions have been addressed using serial section electron microscopy and 3D reconstruction. It is necessary to use this technique to visualize fine astrocyte processes that are difficult to detect using light microscopy (Bourne & Harris 2008). To date, the majority of analysis has been of astrocyte interactions with glutamatergic synapses, and these data are discussed here.

Astrocyte Ensheathment of Synapses Limits Neurotransmitter Spillover

The hippocampus has been the most extensively studied region, particularly the synapses on CA1 pyramidal neuron dendrites. In both rat and human hippocampus, approximately 50% of CA1 synapses have an astrocyte process associated with them (Ventura & Harris 1999; Witcher et al. 2007, 2010). For synapses that are contacted by an astrocyte process, the process covers less than half of the synaptic cleft, meaning that the synapse is still open to the extracellular space. The larger a synapse is, the more likely it is to have an astrocyte process associated with it, suggesting that mature synapses are contacted by astrocytes, and immature synapses are free of astrocyte contact.

An interesting question is whether synapses that form onto the same neuron and that have different origins and functional properties have the same level of astrocyte ensheathment. In the cerebellum, Purkinje neurons (PC) receive synaptic inputs from two different sources, and these synapses are anatomically segregated on the dendritic tree (Xu-Friedman et al. 2001). Climbing fibers (CF) originate in the inferior olive, and one axon makes multiple synaptic contacts onto the proximal dendrites of a PC—these synapses have a high probability of release of neurotransmitter and are depressed by high-frequency stimulation; i.e., they are highly likely to release neurotransmitter in response to one action potential but less likely to release neurotransmitter in response to a second action potential. Parallel fibers (PFs) are the axons of granule cells in the cerebellum, and each axon forms only a few synapses onto the distal dendrites of a PC—these synapses have a low release probability and are facilitated by high-frequency stimulation, the opposite properties of a CF. In the mature cerebellum, one PC receives innervation from one CF and thousands of PFs. The percentage of synapses ensheathed by an astrocyte process along more than 90% of the synaptic perimeter is strikingly different between the two types of synapse—58% of CF synapses are highly ensheathed, compared with only 29% of PF synapses (Xu-Friedman et al. 2001). This suggests that CF synapses are highly segregated from each other, whereas PF synapses are relatively open and more likely to be able to sense spillover of neurotransmitter from neighboring synapses, which may be linked to the different physiological properties of the synapses.

Increased neuronal activity is capable of changing the level of astrocyte ensheathment of synapses. This was demonstrated in vivo in the barrel cortex, where 24 h of whisker stimulation led to an increase in the number of synapses that were completely ensheathed by an astrocyte, suggesting that more active synapses have a closer association with astrocytes (Genoud et al. 2006). There was no difference in the number of synapses that were contacted by an astrocyte, which was 90% before and after stimulation, showing that many more synapses in the barrel cortex than the hippocampus are contacted by astrocytes. In the amygdala, two different learning paradigms have opposing effects on astrocyte-synapse interactions (Ostroff et al. 2013). Before learning, 50% of synapses are contacted by an astrocyte, and these synapses are ensheathed along only 20% of the perimeter, similar to astrocyte-synapse interactions observed in the hippocampus. After fear conditioning, which increases synaptic strength in the amygdala, there is a transient increase in the number of synapses that are not associated with an astrocyte. In

contrast, after conditioned inhibition, which decreases the strength of synapses in the amygdala, there is an increase in the number of small synapses that are associated with an astrocyte. This could be interpreted as meaning that new synapses form in the absence of physical contact with an astrocyte during fear conditioning, and that the presence of an astrocyte process enables synapses to decrease in size during conditioned inhibition (Ostroff et al. 2013). The ability to follow synaptic rearrangement in real time would address this point and is discussed in the next section.

Even though the percentage of synapses contacted by astrocytes varies by brain region, a general set of principles has emerged. (*a*) Many synapses are not contacted directly by an astrocyte. (*b*) Astrocytes contact and cover only part of the synaptic cleft, and rarely fully ensheath the whole synapse, meaning that neighboring synapses are not completely segregated from each other and will potentially be able to communicate through spillover of neurotransmitter. (*c*) Larger, presumably more mature synapses are more likely to be contacted by an astrocyte than small filipodia-like synapses, which may have implications for the stability of the synapse (see next section).

Physical Interactions Between Astrocytes and Synapses

The morphological studies of astrocyte-synapse interaction described in the previous section all demonstrated that larger synapses are more likely to be contacted by an astrocyte and suggested that this contact caused synapses to be more stable. Several studies investigated this question using time-lapse imaging of dendritic spines and astrocyte processes in brain slices. These studies revealed that astrocyte processes are very dynamic and extend and retract away from synapses during the imaging period (Haber et al. 2006, Hirrlinger et al. 2004, Nishida & Okabe 2007). In general, the larger the dendritic spine, the more stable the interaction with an astrocyte process, in agreement with the static electron microscopy studies. This hypothesis was tested by disrupting the movement of astrocyte processes, which led to the appearance of more filipodia on the dendrite and less mature spines, suggesting that astrocytes normally contribute to synapse maturation (Nishida & Okabe 2007). At the *Drosophila* neuromuscular junction, the growth of glial cell processes matches synapse formation and maturation and is correlated with synaptic activity (Brink et al. 2012). In mice, by imaging the interaction of BG processes with PC dendrites across the period of developmental synapse formation, Lippman et al. (2008) demonstrated that BG are more motile while synapses are forming and become more stable once synaptogenesis has ceased. Over the same period, the level of synapse ensheathment also increases. Interestingly, if the interaction of BG with synapses is decreased by causing the retraction of BG processes, then the number of synapses on the PC increases, showing that the presence of BG processes normally limits the number of synapses that form and stabilizes those that do form (Iino et al. 2001, Lippman Bell et al. 2010). The retraction of astrocyte processes away from neurons occurs naturally in the hypothalamus during physiological processes including lactation and dehydration, and this allows new synapses to form onto regions of the neuron that were previously blocked. This is a rapid (within hours) and reversible process and shows the plasticity of astrocyte-synapse interactions that continues into adulthood (reviewed extensively in Theodosis et al. 2008). In *C. elegans*, the amphid sheath glial cells remodel when the animal enters a dauer state, which is a response to environmental stress, showing that glial morphological remodeling can regulate glia-synapse interaction in many organisms (Procko et al. 2011).

The question of whether glial cells determine the location of specific synapses in the adult nervous system has been addressed in *C. elegans*. Genetic removal of *cima-1* from epidermal cells allowed synapses to form normally during embryonic development, but then excess ectopic

presynaptic sites appeared in mature animals, which did not form synapses onto the correct target neuron (Shao et al. 2013). This was due to a defective interaction between epidermal cells and glial cells, which led to an increase in glial cell size and an increase in contact between the glial cell and axon at ectopic sites. The ectopic contact led to formation of excess presynaptic sites, which is different from the effect observed in rodents, where the contact of glial cells with neurons in the hypothalamus and cerebellum decreases the number of synapses that form. It will be interesting to determine the mechanistic reasons for these different responses.

Molecular Mechanisms of Astrocyte-Synapse Physical Interactions

The strongest candidates for the molecular mechanisms that astrocytes and synaptic spines use to physically interact are members of the Eph-ephrin family of membrane receptors (reviewed in Klein 2009, Murai & Pasquale 2011). Dendritic spines express the receptor tyrosine kinase EphA4, and astrocyte processes express the ligand ephrin-A3. The removal of *EphA4* from developing hippocampal neurons leads to formation of long, disorganized spines, showing this physical interaction normally suppresses spine growth and is suggested to be necessary for spine maturation (Murai et al. 2003). The converse manipulation, removal of *ephrin-A3* from astrocytes, induces the same spine phenotype (Carmona et al. 2009). This suggests a model whereby Eph-ephrin interactions between neuronal spines and astrocyte processes cause a retraction and stabilization of the spine and aid in spine maturation. These manipulations also affect glutamate transporter function, as discussed in the transporter section. Another manipulation that affects astrocyte glutamate transporter function, but probably indirectly by changing the physical association between astrocyte processes and the synaptic cleft, is genetic removal of the astrocyte gap junction protein connexin 30 (Pannasch et al. 2014). Astrocytes from *connexin 30* knockout mice have a larger volume than astrocytes from wild-type mice, and importantly, astrocyte processes now protrude into the synaptic cleft rather than just surrounding it. This places astrocyte glutamate transporters closer to the synaptic release site, leading to enhanced uptake of glutamate, which decreases the amplitude of synaptic transmission and inhibits synaptic plasticity.

SUMMARY

The interaction of astrocytes with neuronal synapses varies across the lifetime of an animal. When investigating how specific manipulations can change these interactions, it is important to consider every way in which astrocytes and synapses interact. Alteration of one factor in an astrocyte may lead to multiple functional changes in how astrocytes interact with synapses, and many different functional disruptions within astrocytes may cause the same physiological outcome. For example, an alteration in the amplitude of an action-potential-evoked glutamatergic synaptic event could be due to changes in (*a*) the number of synapses, which can be affected by astrocyte synaptogenic factors including thrombospondin and hevin; (*b*) presynaptic release of glutamate, which can be affected by astrocyte factors including cholesterol and adenosine; (*c*) the number of glutamate receptors at the synapse, which can be affected by astrocyte factors including glypican, TNFα, and CSPGs; (*d*) the corelease of the NMDA receptor agonist D-serine from astrocytes; (*e*) the number of glutamate transporters in the astrocyte, which can affect the synaptic concentration of glutamate; or (*f*) the physical proximity of the astrocyte process to the synapse, which can also affect the synaptic concentration of glutamate.

Therefore, when studying how astrocytes and synapses interact, it is important to consider the many diverse contributions that astrocytes make to ensuring neuronal circuits function correctly throughout life.

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