

Astrocytes Control Synapse Formation, Function, and Elimination

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Astrocytes, through their close associations with synapses, can monitor and alter synaptic function, thus actively controlling synaptic transmission in the adult brain. Besides their important role at adult synapses, in the last three decades a number of critical findings have highlighted the importance of astrocytes in the establishment of synaptic connectivity in the developing brain. In this article, we will review the key findings on astrocytic control of synapse formation, function, and elimination. First, we will summarize our current structural and functional understanding of astrocytes at the synapse. Then, we will discuss the cellular and molecular mechanisms through which developing and mature astrocytes instruct the formation, maturation, and refinement of synapses. Our aim is to provide an overview of astrocytes as important players in the establishment of a functional nervous system.

In the central nervous system (CNS), astrocytes are closely associated with synapses. Through this association, astrocytes can monitor and alter synaptic function, thus actively controlling synaptic transmission. This close structural and functional partnership of the perisynaptic astrocytic process with the neuronal pre- and postsynaptic structures led to the “tripartite synapse” concept (Araque et al. 1999). Besides their important role at adult synapses, in the last three decades a number of critical findings highlighted the importance of astrocytes in the establishment of synaptic connectivity in the CNS. These discoveries fundamentally changed the way we view astrocytes and led to

the birth of a now thriving area of cellular neuroscience.

Our aim here is to provide the current understanding of astrocytes as active participants in the construction of synaptic circuits. In this article, we will review the key findings on astrocytic control of synapse formation, function, and elimination. We will start by evaluating our structural and functional understanding of astrocytes at the synapse. We will then discuss, in detail, the molecular mechanisms through which developing and mature astrocytes instruct the formation, maturation, and refinement of synapses. Along the way, we will also highlight the important gaps in our knowl-

Editors: Ben A. Barres, Marc R. Freeman, and Beth Stevens
Additional Perspectives on Glia available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2015;7:a020370

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edge that remain to be addressed by future research.

PROPERTIES OF ASTROCYTES AT SYNAPSES

Astrocyte Processes Ensheath Synapses and Define Functional Domains

Astrocytes closely interact with surrounding structures in the nervous system and contribute to the regulation of their functions. For example, astrocyte processes contribute to the glia limitans of the neural tube, and astrocyte end-feet contact blood vessels and control blood flow. Astrocytes also tightly ensheath neuronal somas, axons, dendrites, and synapses (Fig. 1).

Astrocytes occupy nonoverlapping territories, and these domains are established through a developmental process (Bushong et al. 2002; Ogata and Kosaka 2002; Halassa et al. 2007). During the first postnatal week, astrocyte processes are initially intermingled and then, during the second postnatal week, gradually establish independent domains. This process of segregation, also known as astrocyte tiling, is thought to be regulated by “contact inhibition” between neighboring astrocytes (Distler et al. 1991). However, the functional significance of this phenomenon and the molecular mechanisms that control this process are largely unknown. Astrocyte tiling may be crucial for normal functions of the nervous system because, in

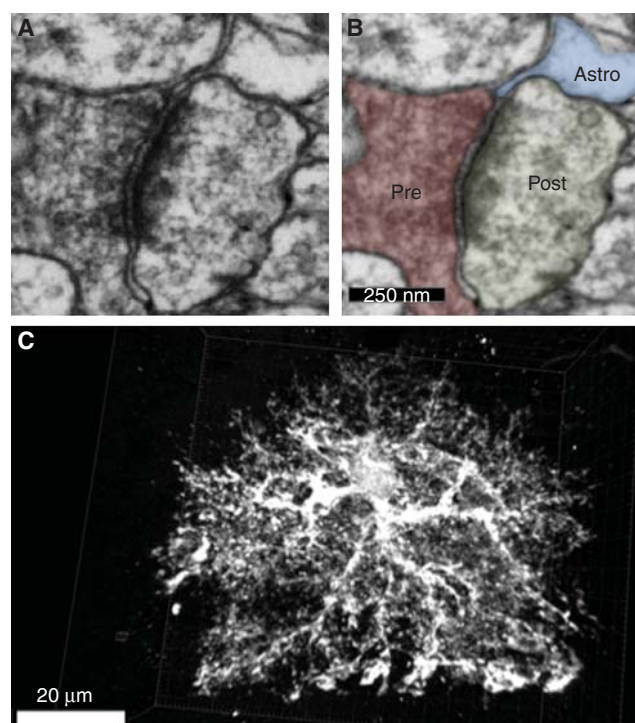


Figure 1. Astrocytic processes contact synapses. (A) Electron micrograph of a tripartite synapse in the mouse visual cortex. (B) The micrograph in A is highlighted to show an astrocyte process (blue) contacting both pre- and postsynaptic neural structures (red and green, respectively). Scale bar, 250 nm. (C) Three-dimensional reconstruction of an enhanced green fluorescent protein (EGFP)-labeled astrocyte from the 18-d-old mouse cortex reveals the complexity of astrocyte morphology. The astrocyte is comprised of several projections emanating from a central soma, which ramify to create hundreds of fine branches. These branches terminate at neuronal synapses to regulate synaptic formation and/or function or envelop blood vessels to help maintain the blood–brain barrier. Scale bar, 20 μ m. (Images are the courtesy of Jeff A. Stogsdill, Eroglu Laboratory, Duke University Medical Center.)

disease and postinjury conditions, astrocytes lose their tiling and display intermingled process morphology (Oberheim et al. 2009).

Detailed quantification of astrocyte domains in mice revealed that one cortical astrocyte enwraps multiple neuronal cell bodies and up to 600 dendrites and, through the finer processes, one astrocyte contacts $\sim 100,000$ synapses (Halassa et al. 2007). The complex of astrocyte processes with presynaptic and postsynaptic structures form the tripartite synapse (Araque et al. 1999). The tight structural interactions between synapses and astrocytes have important functional consequences. Astrocyte processes contain neurotransmitter receptors, transporters, and cell-adhesion molecules that mediate synapse–astrocyte communication. Because of this structural arrangement, astrocytes can monitor synaptic activity and, in turn, control synaptic transmission within a functional island of synapses, that is, a group of synapses confined within the boundaries of an individual astrocyte (Halassa et al. 2007). The functional consequences of the synaptic islands defined by astrocytes remain to be determined, but it is possible that an astrocyte provides an additional level of connectivity among synapses that are within its domain via signaling through the astrocyte.

Astrocyte Processes Segregate Neighboring Synapses

One of the most important functions of astrocytes at the synapse is the clearance of neurotransmitters. For example, the astrocytic processes that are associated with excitatory synapses are covered with glutamate transporters, which maintain a low ambient level of glutamate in the CNS and shape the activation of glutamate receptors at synapses. Astrocytic processes may have a specific attraction toward postsynaptic sites. Occurrence of astrocyte processes was found to be three- to fourfold higher at the postsynapse compared with the presynapse (Lehre and Rusakov 2002). Caused by this asymmetric localization of astrocytes at excitatory synapses, the glutamate escaping the synaptic cleft is 2–4 times more likely to activate

glutamate receptors that are at the periphery of the presynaptic side compared with the extrasynaptic receptors at the spines. This asymmetry is even more exaggerated in the cerebellum in which Bergman glia ensheath a vast majority of Purkinje cell spines (Grosche et al. 1999). These observations suggest that astrocyte–synapse interactions favor fast presynaptic feedback because of glutamate overflow, while preserving the specificity of postsynaptic transmission (Rusakov and Lehre 2002). Interestingly, the extent of astrocyte ensheathment differs among brain regions (e.g., 74% of the cerebellar Purkinje cell synapses are ensheathed by astrocytes as opposed to 29% of the dendritic spines in the mouse visual cortex) (Spacek 1985; Ventura and Harris 1999). Moreover, astrocyte–synapse interactions are also regulated by physiological states of animals (e.g., hydrated vs. dehydrated rats or oxygen/glucose deprivation) and can be manipulated by experience (e.g., environmental enrichment or whisker stimulation) (Wittkowski and Brinkmann 1974; Jones and Greenough 1996; Genoud et al. 2006). These observations suggest that astrocyte–synapse interactions are dynamically regulated, and the extent of interactions between astrocytes and synapses may determine synaptic characteristics.

Astrocyte–Synapse Interactions Are Dynamic

Time-lapse imaging of astrocytes and dendrites in organotypic slices from different brain regions show the dynamic nature of fine astrocytic processes, as they rapidly extend and retract to engage and disengage from postsynaptic dendritic spines. In the brain stem, astrocyte processes interact with neuronal dendrites and spines through at least two distinct microstructures: flat lamellipodia-like astrocyte processes, and the more transient filopodia-like astrocytic protrusions (Grass et al. 2004). Similarly, astrocytic processes actively interact with neuronal dendrites and spines in the mouse hippocampus (Murai et al. 2003; Haber et al. 2006). In the hippocampus, dendritic spines are well known to rapidly undergo structural changes, and these changes are linked to synaptic events, such as

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long-term potentiation (LTP) and long-term depression (LTD) (Murakoshi and Yasuda 2012). Akin to spines, astrocytic processes also undergo significant and rapid changes in volume and motility. Importantly, the changes in astrocytic microstructures are independently regulated when neighboring processes from the same astrocyte are compared, indicating a local regulation of astrocytic process dynamics at the tripartite synapse (Haber et al. 2006). Astrocyte–spine interactions are stabilized at larger spines, suggesting that stronger synapses recruit stable astrocytic contacts (Haber et al. 2006).

Our current molecular knowledge on how astrocyte–synapse interactions are mediated is limited; however, a contact-mediated mechanism involving bidirectional ephrin/EphA signaling was previously described (Murai et al. 2003). In the hippocampus, astrocytes and their processes express ephrin A3, whereas neurons express the ephrin receptor EphA4. Perturbing ephrin/EphA signaling either by delivering soluble ephrin A3 in hippocampal slice cultures or by transfecting neurons with a kinase inactive EphA4 results in defects in spine formation and maturation. Similarly, mice lacking ephA4 or ephrin-3A have aberrant spine morphology (Carmona et al. 2009; Filosa et al. 2009). It is possible that activity-dependent mechanisms that regulate ephrin/EphA signaling can modulate astrocyte–synapse interactions, thus controlling synaptic stability and potentially also synapse elimination and refinement.

In summary, astrocyte processes closely interact with neuronal synapses throughout life, and this interaction is highly dynamic, enabling ongoing modulation of synaptic function by astrocytes.

REQUIREMENT OF GLIA/ASTROCYTES FOR SYNAPSE FORMATION AND FUNCTION

In the rodent cerebral cortex, the majority of neuronal cells mature and project axons to their targets within a few days after birth; however, by the end of the first postnatal week, few synapses have been formed. The majority of excitatory synaptic structures in the rodent brain appear

during the second and third postnatal weeks. This period of extensive synapse formation coincides with the differentiation and maturation of astrocytes. These observations indicated that astrocytes may contribute to the timing and extent of synapse formation in the CNS. However, this was difficult to test because astrocytes are crucial for the survival and health of neurons, both in culture and in vivo (Banker 1980). In the mouse cortex, deletion of Mek1 and Mek2 in radial glia eliminates astrocyte and oligodendrocyte precursors, thus obliterating gliogenesis (Li et al. 2012b). These glia-free mice are born, but the majority of them cannot survive past the first 2 postnatal weeks as a result of extensive neurodegeneration, which occurs in the absence of glia. Similarly, in the peripheral nervous system (PNS), ablation of perisynaptic Schwann cells, which have analogous roles in the PNS to astrocytes in the CNS, leads to loss of normal synaptic transmission and causes degeneration and loss of neuromuscular junctions (NMJs) (Reddy et al. 2003).

Traditional neuronal culture systems, such as those that use embryonic hippocampal and cortical neurons, require astrocytes for correct neuronal differentiation and survival (Banker 1980). This major bottleneck for studying the role of astrocytes in synapse formation was overcome by the use of purified cultures of retinal ganglion cell (RGC) neurons (Meyer-Franke et al. 1995). These neurons can be isolated from postnatal rodent retinas by using antibodies specific for surface antigens on RGCs and can be cultured in the presence of known survival factors in a serum-free media, which supports their long-term survival and health. Interestingly, under these conditions, RGCs grow out neurites and contact each other, but they make very few synapses. On the contrary, if these RGCs are cultured in the presence of astrocyte feeder layers or fed by culture media that is previously conditioned by astrocytes (i.e., astrocyte conditioned media [ACM]), then RGCs establish many synapses (Fig. 2). Using this system, it was discovered that astrocytes control different stages of excitatory synapse formation (Fig. 3) via different secreted factors: (1) astrocytes increase the number of synaptic structures, (2)

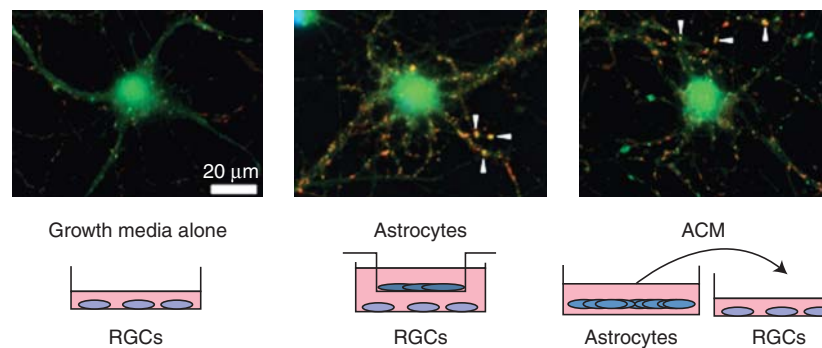


Figure 2. Retinal ganglion cells (RGCs) can be purified by sequential immunopanning to >99.5% purity from P7 Sprague–Dawley rats and cultured in a neurobasal medium-based growth media that contains several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF). RGCs are cultured for 3–4 d to allow robust process outgrowth and then cultured for 6 additional d with growth media or astrocyte feeder inserts or with astrocyte-conditioned media (ACM). Change in synapse number in response to treatments is assayed by staining these neurons with antibodies against a pre- and a postsynaptic protein (bassoon, red; homer-1, green). Pre- and postsynaptic proteins appear colocalized (arrowheads) at the synapse because of their close proximity. Astrocytes and ACM strongly increase the number of colocalized synaptic puncta. (Images are courtesy of Sehwon Koh, Eroglu Laboratory, Duke University Medical Center.)

astrocytes increase postsynaptic activity by inducing AMPA receptor localization to the postsynaptic density, and (3) astrocytes enhance presynaptic function by increasing release probabilities.

The RGC culture system provided a platform to identify astrocyte-secreted factors that control these different aspects of synapse formation and functional maturation, which will be discussed in the following sections.

ASTROCYTE-SECRETED FACTORS CONTROL STRUCTURAL SYNAPSE FORMATION AND MATURATION

Thrombospondins Are Major Contributors to Astrocyte-Regulated Synapse Formation

As outlined in the previous section, astrocytes secrete factors that greatly increase the number of structural synapses that neurons form. The first family of proteins that was identified to be a major synaptogenic factor secreted by astrocytes and present in ACM was the extracellular matrix (ECM) proteins, thrombospondins (TSPs) (Christopherson et al. 2005). There are five TSPs in mammals. Protoplasmic gray-

matter astrocytes express TSP1 and -2, whereas astrocytes originating from the subventricular zone and fibrous white matter astrocytes express TSP4 (Eroglu 2009; Benner et al. 2013). Addition of purified TSP protein to cultured neurons increased the synapse number to levels comparable to ACM. Removal of TSPs from ACM eliminated the majority of the synaptogenic activity of ACM. In agreement with these *in vitro* findings, TSP1/2 double knockout (KO) mice displayed fewer cortical excitatory synapses, indicating that TSPs are important for synapse development *in vivo*. Interestingly, in the rodent cortex, TSPs are expressed by immature astrocytes only during the first week of postnatal development, a time period that corresponds to the initiation of excitatory synapse formation in this region (Christopherson et al. 2005). In adult mice, the levels of TSPs are low, but TSP levels are greatly increased after injury. Lack of TSPs leads to defects in injury-induced structural plasticity of the developing barrel cortex (Eroglu et al. 2009) and hampers synaptic recovery after stroke, showing an important role for astrocyte-derived TSPs in regulating the formation of new synapses after injury (Liauw et al. 2008).

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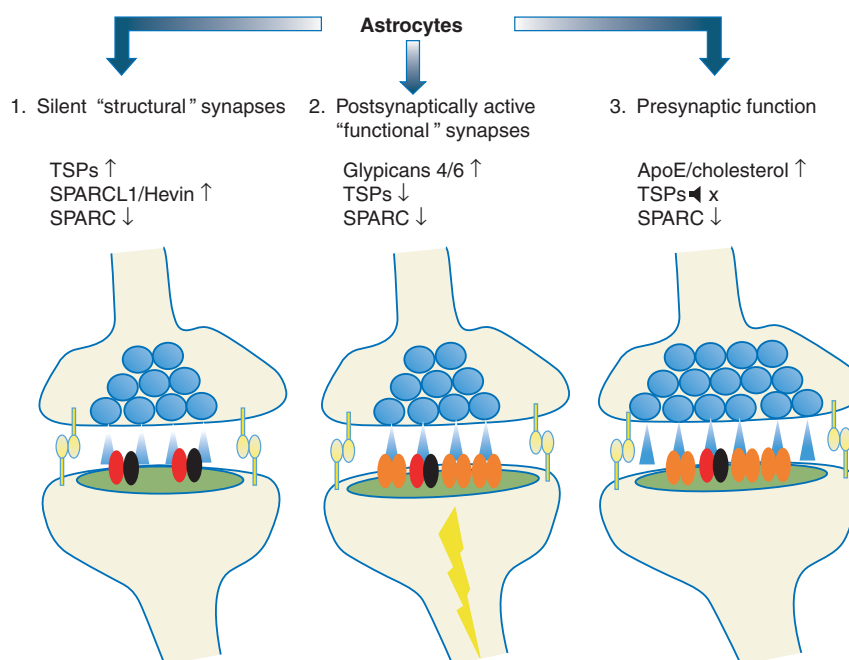


Figure 3. Astrocyte-secreted factors control different aspects of excitatory synaptic development. (1) Astrocytes increase the number of structural synapses. These synapses have normal morphology and contain *N*-methyl-D-aspartate (NMDA) receptors (red and black). However, they lack AMPA-type glutamate receptors (orange). (2) Astrocytes increase postsynaptic activity by inducing AMPA receptor localization to the postsynaptic density. (3) Astrocytes enhance presynaptic release by increasing release probabilities.

The calcium channel subunit $\alpha 2\delta 1$ (Cacna2d1) was identified as the major synaptogenic neuronal receptor for TSPs (Eroglu et al. 2009). All five mammalian TSPs share the ability to induce synapse formation by binding via their type 2 EGF-like repeats to the von Willebrand factor A (VWFA) domain of neuronal Cacna2d1. Besides Cacna2d-1, TSPs also interact with a number of other cell-surface receptors and mediate other functions in the CNS (Risher and Eroglu 2012). TSP1 was also found to interact with the postsynaptic adhesion protein neuroligin 1 and, in this way, accelerate excitatory synapse formation in cultured hippocampal neurons (Xu et al. 2009a).

Interestingly, TSP receptor Cacna2d-1 is also the receptor for two commonly used drugs called Gabapentin (Neurontin) and Pregabalin (Lyrica). These drugs are used to treat neuropathic pain and epilepsy through an unknown mechanism of action. Treatment of RGCs with

Gabapentin and TSP completely abolished TSP-induced excitatory synaptogenesis in vitro. Gabapentin also profoundly inhibited excitatory synapse formation between neurons in the developing brain. Gabapentin prevents excitatory synapse formation by blocking the ability of TSP to bind its receptor Cacna2d1, thus inhibiting the synaptogenic signaling initiated by TSP–Cacna2d-1 interaction without affecting previously formed synapses (Eroglu et al. 2009). These findings provide an additional line of evidence highlighting the important ability of astrocytes to powerfully promote synapse formation in vivo. They also indicate that TSP–Cacna2d-1 signaling and astrocyte-induced synapse formation might be involved in the pathophysiology of diseases, such as neuropathic pain and epilepsy, for which gabapentin is a common treatment. In fact, under injury or seizure conditions that lead to establishment of neuropathic pain or epilepsy, expression of

TSPs and *Cacna2d1* are highly up-regulated. Inhibition of TSP or *Cacna2d1* up-regulation, or blocking of their interaction by gabapentin, blocked establishment of pain states and prevented epileptogenic activity (Boroujerdi et al. 2011; Kim et al. 2012; Li et al. 2012a).

Role of SPARC Family Proteins in Synapse Formation

A secreted protein acidic, rich in cysteine (SPARC) family protein, hevin (also known as SPARC-like 1), was recently identified as another synaptogenic protein secreted by astrocytes (Kucukdereli et al. 2011). Hevin is highly expressed by developing and mature astrocytes in the brain and has been shown to localize to synaptic clefts (Johnston et al. 1990; Lively and Brown 2008). Hevin alone induces an increase in structural synapses among cultured RGCs, similar to the levels induced by TSPs. However, hevin was not able to exert its full synaptogenic activity in vitro when it was present within the ACM. This observation led to the discovery of an astrocyte-secreted protein that inhibits hevin-induced structural synapse formation. Astrocytes express SPARC, a secreted protein highly homologous to hevin. Unlike hevin, SPARC is not synaptogenic. On the contrary, when RGCs were treated concurrently with hevin and SPARC, the synaptogenic function of hevin was antagonized by SPARC. SPARC's antagonism is specific to hevin because SPARC does not prevent TSP-induced synapse formation (Kucukdereli et al. 2011). These findings showed that astrocytes not only provide positive signals that stimulate synapse formation, but also provide negative cues to inhibit synaptogenesis. These opposing actions of hevin and SPARC can thereby control the rate and extent of synapse formation and maturation in the CNS. In agreement with this possibility, analyses of hevin null and SPARC null mice showed that lack of hevin significantly impaired the formation and maturation of synaptic connections in the superior colliculus, the in vivo targets of RGCs, whereas lack of SPARC led to accelerated formation of synapses in this region (Kucukdereli et al. 2011).

Astrocytic Synaptogenic Cues Alter during Development

Neurons also undergo a developmental switch to be able to respond to soluble synaptogenic signals from astrocytes, and this switch is induced by direct contact with astrocytes. Interestingly, when RGCs from 17-d-old embryos (E17 RGCs) are cultured together with postnatal RGCs in the presence of ACM, E17 RGCs fail to receive synapses from postnatal RGCs, whereas their axons could establish synapses onto postnatal RGCs. By E19, RGCs started responding to ACM (Barker et al. 2008). This switch in receptivity between E17 and E19 correlates with the appearance of astrocytes in the retina. Indeed, physical contact with astrocytes but not amacrine cells in culture was sufficient for E17 RGCs to become receptive to synaptogenic signals secreted from astrocytes. Contact by astrocytes causes the synaptic adhesion molecule neurexin to partition out of the dendrites where it is inhibitory to synapse formation (Barker et al. 2008). Similarly, in cultured hippocampal neurons, contact with astrocytes was found to be critical for the neuron's ability to form synapses (Hama et al. 2004). The full extent of the molecular interactions that mediate contact-dependent synaptogenic signaling between astrocytes and neurons is not yet clear. Integrin-mediated protein kinase C signaling was proposed to play a critical role in hippocampal neurons (Hama et al. 2004). In addition, homophilic astrocyte–neuron adhesions established by a family of cell-adhesion molecules, γ -protocadherins, were shown to be important for both excitatory and inhibitory synaptogenesis in the spinal cord (Garrett and Weiner 2009).

Taken together, these findings suggest that astrocytes play an important role in determining the correct timing of structural synapse formation, as neurons are unable to form synapses until they are physically contacted by an astrocyte. Why would astrocytes require multiple signals to control excitatory synapse formation? The answer to this question may lie in the developmental expression patterns of these proteins. TSPs are expressed by immature astrocytes during a narrow developmental window

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(circa P5), coinciding with the initiation of nascent synaptic contacts between dendrites and axons (Christopherson et al. 2005). Hevin and SPARC expression in vivo peaks during the second and third weeks of development (Kucukdereli et al. 2011), a period in which experience-dependent synaptic activity drives the maturation of some synapses and the elimination of weaker synapses. Interestingly, astrocytes continue to express hevin into adulthood; however, SPARC expression is greatly reduced in the adult CNS. Thus, it is possible that in vivo TSP initiates structural synapse formation, and hevin acts to stabilize synaptic connections and facilitate their maturation.

ASTROCYTES REGULATE SYNAPTIC FUNCTION

Some of the earliest evidence that astrocytes regulate synapse development and function came from electrophysiological studies of cultured rodent neurons, showing that the presence of astrocytes greatly enhance synaptic activity and the amplitude of responses to applied neurotransmitters, including glutamate and γ -aminobutyric acid (GABA) (Liu et al. 1996; Pfrieger and Barres 1997; Li et al. 1999; Ullian et al. 2001). These initial findings on retinal (RGC) and hippocampal neurons have been repeated in neurons from multiple different brain regions and classes, including glycinergic spinal cord neurons, spinal motor neurons, cerebellar Purkinje neurons, cortical subplate neurons, neurons derived from adult hippocampal stem cells, and at the neuromuscular junction (NMJ) (reviewed in Ko and Robitaille 2015) (Song et al. 2002; Ullian et al. 2004; Cuevas et al. 2005; Cao and Ko 2007; Feng and Ko 2008; McKellar and Shatz 2009; Buard et al. 2010). In addition to rodent neurons, astrocytes enhance synaptic function between cultured human neurons, and in vivo in *Caenorhabditis elegans* sensory neuron function (Hartley et al. 1999; Johnson et al. 2007; Bacaj et al. 2008; *C. elegans* reviewed in Shaham 2014). These findings from multiple neuron types and species provide compelling evidence that astrocytes provide essential signals to neurons that regulate synaptic function.

This section will review the progress that has been made in identifying the astrocyte signals that regulate synaptic function, and will focus on factors that regulate developmental synapse maturation and receptor trafficking (for a review of the effects of astrocyte gliotransmitters on synaptic plasticity in mature neural circuits, see Haydon and Nedergaard 2015).

Astrocytes use many different signals to control specific aspects of synapse function, including presynaptic function and postsynaptic receptor recruitment, using both positive and negative signals to finely tune synaptic strength (Fig. 3; Table 1). The strength of a presynaptic connection is controlled by a number of factors. These include release probability, which is the likelihood of neurotransmitter vesicles being released from the presynaptic terminal in response to an action potential, and quantal content, which reflects the number of vesicles that are released in response to an action potential. Postsynaptic strength is determined by the number of functional neurotransmitter receptors that are clustered at the postsynaptic density. Response size can vary depending on receptor number, receptor subunit composition, phosphorylation status of the receptor, and stability of receptors in the postsynaptic density. Analysis of individual synaptic events (miniature excitatory postsynaptic currents [mEPSCs]) is commonly used to determine postsynaptic strength, as mEPSCs reflect the response of one synapse to the release of one vesicle of saturating levels of neurotransmitter, so the size of the mEPSC is a reflection of the strength of the postsynaptic side.

Presynaptic Function

Astrocyte-secreted cholesterol, complexed with apolipoprotein E lipoparticles, was identified as a positive regulator of glutamatergic presynaptic function (Mauch et al. 2001). Cholesterol enhances both presynaptic release probability and quantal content, thus increasing the efficacy of synaptic transmission by making it more likely that neurotransmitter will be released in response to an action potential. The ability of cholesterol to enhance postsynaptic function

Table 1. Astrocytic factors that control different aspects of synaptic development

Molecule	Action	Findings	References
Structural synapse formation			
Thrombospondin	Positive	Induces glutamatergic synapse formation; secreted by astrocytes	Christopherson et al. 2005; Eroglu et al. 2009; Xu et al. 2009a; Garcia et al. 2010; Hughes et al. 2010
Hevin (SPARC-like 1)	Positive	Induces glutamatergic synapse formation; secreted by astrocytes	Kucukdereli et al. 2011
γ -Protocadherins	Positive	Induces glutamatergic and GABAergic synapse formation; contact mediated	Garrett and Weiner 2009
BDNF	Positive	Induces glutamatergic synapse formation; secreted by vestibular support cells	Gómez-Casati et al. 2010
TGF- β	Positive	Induces glutamatergic synapse formation; regulates synapse maturation	Diniz et al. 2012; Fuentes-Medel et al. 2012
Estrogen	Positive	Induces glutamatergic synapse formation	Hu et al. 2007
SPARC	Negative	Inhibits glutamatergic synapse formation; antagonist of hevin	Kucukdereli et al. 2011
Presynaptic function			
Cholesterol	Positive	Increases presynaptic strength and release probability	Mauch et al. 2001; Goritz et al. 2005
Thrombospondin	Negative	Inhibits presynaptic release at glutamatergic synapses	Crawford et al. 2012
SPARC	Negative	Inhibits presynaptic maturation at cholinergic nerve terminals	Albrecht et al. 2012
Postsynaptic function			
Glypican 4,6	Positive	Increases synaptic AMPA receptors; induces glutamatergic synapse formation	Allen et al. 2012
ECM	Positive	Stabilizes AMPA receptors at synapses	Frischknecht et al. 2009; Pyka et al. 2011
ADNF	Positive	Increases synaptic NMDA receptors	Blondel et al. 2000
TNF- α	Positive and negative	Increases synaptic AMPA receptors; decreases synaptic GABA _A receptors	Beattie et al. 2002; Stellwagen et al. 2005; Stellwagen and Malenka 2006; Steinmetz and Turrigiano 2010; Hennekinne et al. 2013
Thrombospondin	Positive and negative	Increases synaptic glycine receptors; decreases synaptic AMPA receptors	Jones et al. 2011
SPARC	Negative	Decreases synaptic AMPA receptors	

ADNF, activity-dependent neurotrophic factor; BDNF, brain-derived neurotrophic factor; GABA_A, γ -aminobutyric acid; NMDA, *N*-methyl-D-aspartate; SPARC, secreted protein acidic, rich in cysteine; TNF- α , tumor necrosis factor α .

was examined in two different culture systems using RGC neurons: autaptic cultures, in which individual neurons are grown in isolation and synapse onto themselves, and dense cultures, in which neurons form synapses with neighboring cells. Neurons in both autaptic and dense cultures showed an increase in mEPSC frequency

in response to cholesterol, but only autaptic neurons had an increase in mEPSC amplitude (Mauch et al. 2001; Christopherson et al. 2005; Goritz et al. 2005), showing the predominant way that cholesterol enhances synaptic transmission is by strengthening presynaptic function.



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As described in the section on structural synapse formation, TSPs are astrocyte-secreted factors that strongly promote structural synapse formation, but these synapses are postsynaptically silent as they do not contain AMPA glutamate receptors (AMPA). It has now been shown that TSPs can also regulate presynaptic function by inducing a state called presynaptic muting (Crawford et al. 2012). Muting results in a reduction in the number of presynaptic terminals that release neurotransmitter, and is thought to be an adaption to changes in neuronal firing, and a protective mechanism against excitotoxicity by limiting the release of glutamate. TSP regulation of presynaptic muting is not caused by release of TSP in response to a muting challenge, as it has to be present before the challenge occurs. Rather, TSP is proposed to be a factor that is necessary during synapse development to make presynaptic terminals muting competent using a protein kinase A (PKA)-dependent mechanism. SPARC, which inhibits synapse formation in response to astrocytic hevin, also has effects on presynaptic maturation (Kucukdereli et al. 2011). Treatment of autaptic cholinergic neurons with SPARC results in an enhanced presynaptic release probability and a decrease in the number of vesicles available for release, features indicative of an immature presynaptic terminal (Albrecht et al. 2012). This shows that SPARC has multiple mechanisms to decrease synaptic communication in the developing brain.

Postsynaptic Function

Glutamatergic synapses are the predominant class of excitatory synapse in the CNS, and a number of astrocyte factors have been identified that regulate glutamatergic postsynaptic strength. These include factors that increase synaptic AMPARs (glypican 4 and -6, Gpc4/6; tumor necrosis factor [TNF]- α), decrease synaptic AMPARs (SPARC; TSP), stabilize surface AMPARs (chondroitin sulfate proteoglycans [CSPGs]), and increase synaptic NMDA glutamate receptors (NMDARs) (activity-dependent neurotrophic factor [ADNF]) (Blondel et al. 2000; Beattie et al. 2002; Jones et al. 2011; Pyka

et al. 2011; Allen et al. 2012; Hennekinne et al. 2013).

The finding that astrocyte-secreted factors are able to up-regulate the surface level of AMPARs on RGC neurons by threefold led to a biochemical study to identify the factor responsible (Allen et al. 2012). This identified Gpc4 and -6 as astrocyte-derived proteins that are necessary and sufficient to increase GluA1-containing AMPARs on the surface of neurons and at synapses, but surprisingly Gpc4/6 had no effect on surface levels of GluA2/3 or GluA4 AMPARs. Recording mEPSCs showed that Gpc4 is sufficient to increase synaptic activity in neurons, but the synaptic events have a more immature phenotype than those induced by astrocytes, suggesting that additional factors are released from astrocytes that recruit GluA2/3 to synapses and induce synaptic maturation. Possible maturation factors are ECM molecules, including CSPGs. Enzymatic digestion of the ECM causes increased mobility of AMPARs on dendritic surfaces, and a faster rate of exchange between synaptic and extrasynaptic receptors, suggesting that the ECM normally stabilizes AMPARs at synaptic sites (Frischknecht et al. 2009). Astrocytes are a source of a number of ECM molecules, and the ability of astrocytes to increase synapse formation is increased when cells are cultured in the presence of enzymes to digest the ECM, but the mEPSC amplitude is decreased, suggesting that the synapses are in an immature state and astrocyte ECM molecules contribute to stabilizing mature synapses (Pyka et al. 2011). It will be exciting to determine the relative contribution of astrocytic and neuronal ECM molecules to synapse maturation.

Astrocytes potently stimulate the formation and functional maturation of inhibitory GABAergic synapses via secreted factors, but the factor that regulates γ -aminobutyric acid receptor (GABA_AR) synaptic recruitment is not known. The ability of astrocytes to increase postsynaptic GABA_AR levels requires the presence of BDNF and tyrosine kinase receptor B (TrkB) in neurons, thus identifying the signaling pathway that astrocytes regulate to increase synaptic GABA_ARs (Elmariah et al. 2005). Interestingly, astrocyte factors that induce excita-

tory synapse formation, such as TSP, do not induce inhibitory GABAergic synapse formation, showing that astrocytes can dictate the class of synapse that forms via the release of specific signals (Hughes et al. 2010).

Two astrocyte factors that regulate structural synapse formation and presynaptic plasticity, SPARC and TSP, also regulate postsynaptic receptor levels, showing diverse functions for these molecules in regulating synapse development. SPARC decreases synaptic accumulation of AMPARs, fitting with its function of decreasing synapse formation and presynaptic function, suggesting it is a generally inhibitory factor (Jones et al. 2011). Secretion of SPARC from astrocytes is regulated by neuronal activity, being increased when neuronal activity increases, suggesting that SPARC acts to limit neuronal overexcitation from occurring by reducing synaptic AMPAR levels. TSP has differential effects over AMPARs and glycine receptors in mature cultures of spinal cord neurons in which synapses have already formed (Hennekinne et al. 2013). TSP increases synaptic glycine receptors and decreases synaptic AMPARs, so decreasing neuronal excitability. Again, this decrease in neuronal excitability correlates with the other known functions of TSP in inducing silent synapse formation and decreasing presynaptic release, suggesting that TSP has a role in limiting neuronal excitability in mature neuronal networks.

Synaptic Plasticity

As well as having effects on developmental synapse function, astrocyte factors have been shown to contribute to synaptic plasticity in postnatal animals. Paradigms in which a role for astrocytes has been investigated include LTP and LTD, forms of plasticity that rapidly regulate synaptic strength in response to alterations in neuronal activity, and homeostatic synaptic scaling, a compensatory up-regulation of synaptic activity in response to prolonged periods of activity deprivation. SPARC KO mice show a failure to maintain LTP in hippocampal slices (although synaptic potentiation is induced), and KO neurons in culture do not display homeostatic scaling in response to activ-

ity blockade, presumably because the KO already has a saturating level of AMPARs at the synapse so is no longer in a range to increase AMPARs to undergo plasticity (Jones et al. 2011). Astrocyte-derived TNF- α rapidly induces the surface insertion of GluA1-containing AMPARs (within 15 min of treatment), causing an increase in the frequency but not amplitude of mEPSCs in cultured hippocampal neurons (Beattie et al. 2002). Interestingly, TNF- α has the opposite effect on GABA_ARs, causing a decrease in the surface levels of these inhibitory receptors (Stellwagen et al. 2005). To investigate whether this differential effect of TNF- α on AMPA and GABA_A receptors causes changes in excitatory/inhibitory balance in an intact neural circuit, recordings were made from neurons in hippocampal slices treated with TNF- α . These cells had larger excitatory synaptic events and smaller inhibitory events, confirming that TNF- α is able to shift neurons toward a more excitable state. Does this ability of TNF- α have any implications for synaptic plasticity in the hippocampus? TNF- α KO mice show normal hippocampal LTP and LTD (Stellwagen and Malenka 2006). However, homeostatic synaptic scaling is absent in TNF- α KO hippocampus, showing TNF- α to be necessary for the up-regulation of AMPARs, which occurs during scaling. It has been suggested that the role of TNF- α in up-regulation of synaptic strength during activity blockade is not an instructive signal, but rather a permissive signal that must be present to maintain synapses in a scalable state (Steinmetz and Turrigiano 2010). The exact mechanism of how TNF- α induces homeostatic scaling awaits clarification, but, regardless of this, these studies all show that astrocytes have a powerful ability to regulate synaptic strength in response to alterations in neuronal activity.

ASTROCYTES CONTROL SYNAPSE ELIMINATION

Synapses are dynamic structures that can undergo rapid formation and elimination. During development of the nervous system, neurons initially generate excessive projections that make redundant synaptic connections with tar-

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get cells. Within critical time periods, these excessive synapses are eliminated, whereas the remaining inputs are further strengthened to form the mature neural circuit (Shatz 1983; Sanes and Lichtman 1999). Synapse-elimination processes have also been observed during synaptic plasticity in the adult brain (Xu et al. 2009b; Yang et al. 2009; Roberts et al. 2010). Sensory experience induces the formation of new spines along with the elimination of preexisting spines during learning and memory formation. Thus, synapse elimination is likely to be a central feature of remodeling and reorganization of our nervous system during learning.

How is synapse elimination achieved? A number of studies suggest that neuronal activity-dependent competition initiates the elimination of unwanted synapses, likely through the Hebbian rule; that is, correlated activities strengthen synapses, whereas uncorrelated activities weaken synapses (Hebb 1949; Balice-Gordon and Lichtman 1994; Katz and Shatz 1996; Buffelli et al. 2003). However, the detailed mechanisms of how changes in neuronal activity transduce into the structural elimination of certain synapses are not well understood. Recently, glial cells, especially microglia and astrocytes, have been shown to mediate synapse elimination, providing a new type of neuron–glia interaction and a mechanism for synapse elimination. Here, we will focus on the role of astrocytes in mediating synapse elimination in the normal developing and adult brain.

Indirect Role of Astrocytes in Mediating Synapse Elimination

In the developing mammalian visual system, RGCs from both eyes send axonal projections to their major target neurons in the dorsal lateral geniculate (dLGN) and form synaptic connections. Electrophysiology experiments have shown that initially there are more than 20 axonal connections from RGCs to dLGN neurons, which subsequently undergo substantial elimination during early postnatal stages leaving only one or two axonal connections per neuron (Chen and Regehr 2000). Microglia cells have been shown to phagocytose many of these extra

synapses and mediate eye-specific segregation in the retinogeniculate system (Schafer et al. 2012). In this model, the initiating complement protein, C1q, can opsonize unwanted synapses in the dLGN, along with C3 (Stevens et al. 2007), and these C1q/C3-coated synapses are recognized and engulfed by microglia through C3R-mediated phagocytosis (Schafer et al. 2012). Importantly, the expression of C1q messenger RNA (mRNA) in RGCs is dependent on secreted factors from astrocytes (Stevens et al. 2007), whose identity has been recently identified as transforming growth factor- β (TGF- β) (Bialas and Stevens 2013). TGF- β is expressed by astrocytes and induces C1q expression in purified RGCs through the corresponding receptor TGFBR2. TGFBR2 is expressed by RGCs, with the highest level at P5, and its expression sharply decreases by P15, thus generating a critical time window for astrocytes to induce C1q expression. Genetic and pharmacological manipulations to block TGFBR2 show that C1q expression in RGCs induced by astrocytic TGF- β is critical for phagocytic functions of microglia (Bialas and Stevens 2013). Interestingly, C1q mRNA is also highly expressed by microglial cells, but its expression is not regulated by TGF- β . In contrast to the retina, in which C1q expression in RGCs decline sharply by P15, C1q is continuously being expressed by microglial cells in the brain and its protein level dramatically accumulates in the normal aging brain (Stephan et al. 2013). Thus, these data reveal the indirect role of astrocytes in initiating C1q expression in RGCs and mediating microglial-dependent synapse elimination (Fig. 4A).

Direct Role of Astrocytes in Mediating Synapse Elimination

Through gene expression analysis, astrocytes were found to express a plethora of genes that have been implicated in engulfment and phagocytosis (Cahoy et al. 2008). These phagocytic genes can be categorized into three main pathways, which begin in serial, but then converge into common downstream pathways in ways that are still poorly understood. The first pathway includes the proteins CrkII, DOCK180, ELMO, and Rac1, which control rearrangement

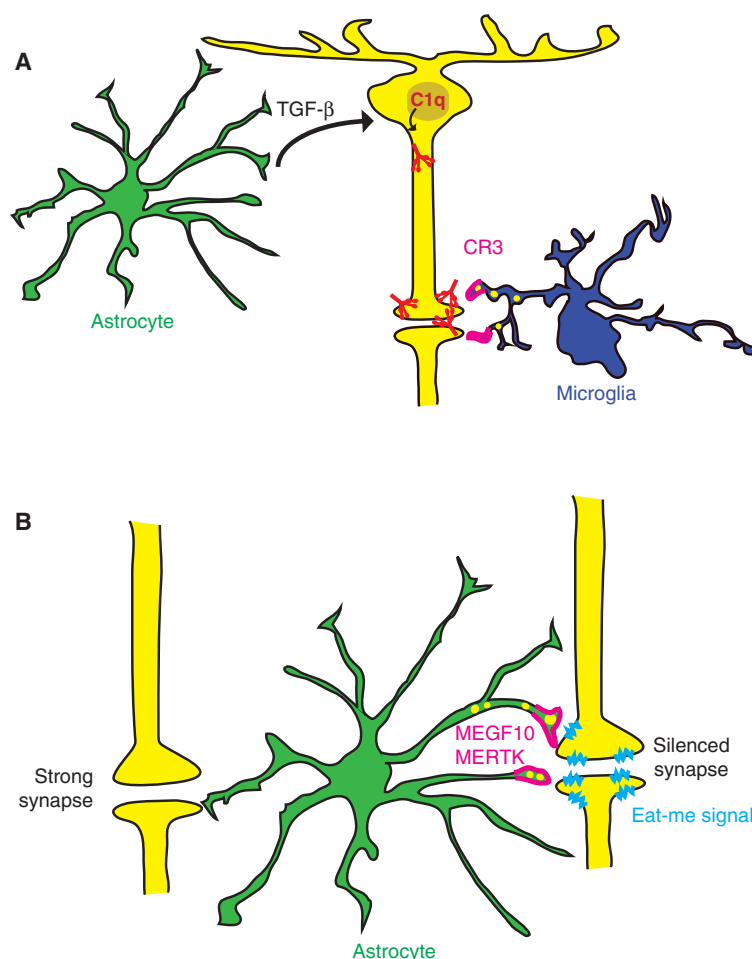


Figure 4. Astrocytes mediate synapse elimination through indirect (A), and direct (B) mechanisms. (A) Astrocytes (green) induce C1q expression (red) in RGCs (yellow) through TGF-β signaling. C1q-labeled synapses can be recognized by complement component-3 receptors (C3R, magenta) in microglia (dark blue) and eliminated through complement-dependent phagocytosis. (B) Astrocytes (green) directly eliminate synapses by recognizing “eat-me signals” (light blue) presented in the silent synapses and phagocytosing them through MEGF10 and MERTK phagocytic pathways (magenta).

of the actin cytoskeleton which is required for membrane dynamics of phagocytes when surrounding cellular debris (Kinchin et al. 2005). Bai1 has been identified as a phagocytic receptor that is expressed by astrocytes, and acts upstream of these components (Park et al. 2007). The second pathway includes the TAM receptors, TYRO3, AXL, and MERTK (Lemke 2013), which in part work with the integrin pathway to regulate CrkII/DOCK180/ELMO/Rac1 modules (Wu et al. 2005). The last pathway consists of

MEGF10 (an ortholog of *Drosophila* Draper and *C. elegans* CED-1), GULP (an ortholog of *Drosophila* dCed-6 and *C. elegans* CED-6), and ABCA1 (ATP-binding cassette, subfamily A member 1), and participates in cellular debris recognition and engulfment (Zhou et al. 2001; MacDonald et al. 2006; Yu et al. 2008).

The phagocytic capacity of astrocytes has been shown previously in in vitro culture (Park et al. 2007; Loov et al. 2012) and in vivo models of injury (al-Ali and al-Hussain 1996;

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Bechmann and Nitsch 1997), glioma (Lantos 1974), and developmental neuronal/axonal death (Berbel and Innocenti 1988; Mazlo et al. 2004). More recently, evidence of a phagocytic capacity of in vivo astrocytes came from a study showing axonal organelle engulfment by adult astrocytes in the optic nerve head (Nguyen et al. 2011). Astrocytes in the optic nerve head express Mac-2 (Lgals3), a bridging molecule that mediates the binding of target debris with phagocytic receptors (Caberoy et al. 2012), and astrocytes continuously phagocytose axonal organelles in the noninjured optic nerve (Nguyen et al. 2011). A recent follow-up study has revealed that the axonal evulsions contain axoplasmic components including mitochondria, suggesting large amounts of neuronal mitochondria are degraded by astrocytes within the optic nerve head (Davis et al. 2014). Although further studies are required to determine the functional requirement of Mac-2 for axonal phagocytosis by astrocytes, and its physiological role in the optic nerve head, this study shows that astrocytes are continuously phagocytic in normal adult stages and suggests astrocytes may play important roles in maintaining homeostasis of the nervous system through clearing damaged organelles and debris.

The role of phagocytic activity of astrocytes in directly mediating synapse elimination has been recently discovered (Fig. 4B) (Chung et al. 2013). Using an in vitro engulfment assay, as well as in vivo analysis in the developing retinogeniculate system, astrocytes were found to eliminate synapses and neural debris efficiently. Astrocyte-mediated synapse elimination is dependent on two phagocytic receptors, MEGF10 and MERTK. Astrocytes deficient in either one of these phagocytic receptors show a 50% reduction in their relative phagocytic capacity, and astrocytes deficient in both receptors show greater defects, suggesting that MEGF10 and MERTK work in parallel to mediate synapse-elimination processes. Importantly, in mice deficient in both MEGF10 and MERTK, developing RGCs fail to normally refine their connections and retain excess functional synapses with neurons in the dLGN, thus providing evidence that astrocytes actively participate in

synapse elimination rather than simply cleaning up already dead synapses. Previous studies have shown that eye-specific segregation in the dLGN is regulated by spontaneous retinal waves, which are bursts of action potentials that spread across large regions of the RGC layer and occur before eye opening (Penn et al. 1998; Huberman et al. 2002; Stellwagen and Shatz 2002; Feller 2009). Interestingly, synapse-elimination processes by astrocytes are strongly dependent on neural activity, because blocking spontaneous retinal waves in both eyes significantly reduces astrocyte-mediated phagocytosis of bilateral synaptic inputs, whereas selective blocking of activity in only one eye induces preferential engulfment of the silenced synapses by astrocytes (Chung et al. 2013). Therefore, astrocytes actively contribute to neural activity-dependent synapse pruning and elimination that mediates neural circuit refinement by phagocytosing unwanted synapses through MEGF10 and MERTK pathways.

The expression of phagocytic machineries including MEGF10 and MERTK in astrocytes persists into adulthood, raising the question of whether astrocytes continuously engulf synapses in the adult brain. By examining the somatosensory cortex from 1- and 4-mo-old mouse brains, astrocytes were shown to actively engulf both excitatory and inhibitory synapses. *Drosophila* glia, which resemble mammalian astrocytes, also phagocytose synapses during metamorphosis using Draper (an ortholog of Megf10) and Crk/Mbc/dCed-12 (orthologs of CrkII/DOCK1/ELMO) signaling pathways, indicating that the phagocytic function of astrocytes is evolutionarily conserved (Tasdemir-Yilmaz and Freeman 2014).

Thus, astrocytes share with microglia the ability to actively engulf and eliminate synapses in response to neural activity during development and adult stages, but synapse engulfment by astrocytes uses distinct phagocytic pathways from those used by microglia (Fig. 4B). Because astrocytes normally ensheath synapses and are in the perfect position to monitor synaptic activity, this newly discovered phagocytic function of astrocytes in eliminating synapses suggests that the synaptic architecture



may be constantly remodeled by astrocytes in response to experience.

CONCLUDING REMARKS

Here we have reviewed some of the advances in our knowledge of astrocyte–synapse interactions, which have provided new insights into the important and vast roles of astrocytes in the formation, function, and elimination of synapses. We focused particularly on astrocytes from mammalian systems. Interestingly, many of the cellular and molecular mechanisms used by mammalian astrocytes to achieve the correct synaptic connectivity are evolutionarily conserved, further indicating the importance of astrocyte–synapse interactions for a functional nervous system. Many important questions still remain to be answered. How do astrocyte-derived factors facilitate synapse formation, alter synaptic function, and accomplish synaptic pruning with high precision? Do astrocytes regulate critical periods of plasticity? Do astrocytes participate in experience-dependent modulation of neuronal circuit function? Importantly, the pathogenesis of many neurological disorders, such as amyotrophic lateral sclerosis (ALS), Rett syndrome, fragile-X syndrome, and psychiatric disorders, have been tightly linked to astrocyte dysfunction (Sloan and Barres 2014). Therefore, answering these questions will not only provide the necessary in-depth understanding of the nervous system but also may present important clues for overcoming neurological pathologies.

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Cold Spring Harb Perspect Biol 2015; doi: 10.1101/cshperspect.a020370 originally published online February 6, 2015

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