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Cell biology of astrocyte-synapse interactions

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Abstract

Astrocytes, the most abundant glial cells in the mammalian brain, are critical regulators of brain development and physiology through dynamic and often bidirectional interactions with neuronal synapses. Despite the clear importance of astrocytes for the establishment and maintenance of proper synaptic connectivity, our understanding of their role in brain function is still in its infancy. We propose that this is at least in part due to large gaps in our knowledge of the cell biology of astrocytes and the mechanisms they use to interact with synapses. In this review, we summarize some of the seminal findings that yield important insight into the cellular and molecular basis of astrocyte-neuron communication, focusing on the role of astrocytes in the development and remodeling of synapses. Furthermore, we will pose some pressing questions that need to be addressed to advance our mechanistic understanding of the role of astrocytes in regulating synaptic development.

Introduction to astrocytes

Astrocytes comprise the most abundant population of glia in the mammalian brain. They are crucial for the proper health and function of the nervous system, as they provide important metabolic and trophic support to neurons (Banker, 1980). The timeline of astrocyte generation, differentiation and maturation differs between different brain regions; however, astrocyte birth and development usually happens after the main periods of neurogenesis and neuronal migration are completed in the mammalian brain (Miller and Gauthier, 2007). An important exception to this occurs within the neurogenic regions of the adult brain, such as

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the dentate gyrus of the hippocampus or the subventricular region of the cortex, in which resident astrocytes are thought to provide signals that participate in the generation of new neurons and their integration into existing circuits (Gonçalves et al., 2016; Krzisch et al., 2015; Sultan et al., 2015).

Classical neuroanatomical studies categorized rodent astrocytes into two groups on the basis of their morphology and location (Kettenmann and Verkhratsky, 2008). The first group contains the protoplasmic astrocytes of the grey matter, which are highly ramified to infiltrate the surrounding neuropil (Figure 1A). These astrocytes ensheath neuronal synapses through their fine perisynaptic processes (PAPs, Figure 1B), which emanate from the secondary and tertiary branches, giving these cells their characteristically intricate “bushy” structure (Bushong et al., 2004; Witcher et al., 2007; Witcher et al., 2010). Protoplasmic astrocytes are also in direct contact with blood vessels through a specialized cellular compartment called the end-foot (Foo et al., 2011). The second group is the fibrous astrocytes of the white matter. Fibrous astrocytes are associated with myelinated axonal tracts such as the corpus callosum and are in contact with the nodes of Ranvier. Even though all astrocytes share common properties, recent physiological and gene expression profiling studies revealed that astrocytes are a far more diverse cell population than previously appreciated. For example, astrocytes have distinct properties in different brain regions and at different periods of development (Cahoy et al., 2008; Chai et al., 2017; John Lin et al., 2017; Morel et al., 2017). In fact the study of astrocyte diversity and heterogeneity now constitutes a flourishing subject in neuroscience and we will discuss the recent discoveries on this topic in more detail (Bayraktar et al., 2015; Zhang and Barres, 2010).

Astrocyte-like cells perform important and analogous functions in species as distant as flies and humans. In the fruit fly, like in mammals, astrocytes surround neuronal cell bodies and proximal neurites, couple to the vasculature, and closely associate with synapses (Freeman, 2015). Across species the number and size of astrocytes increase with increasing brain size and cognitive capabilities (Allen, 2014; Stogsdill and Eroglu, 2017). For example, the human brain contains several more populations of astrocytes than the rodent brain, evident by their unique structures (Oberheim et al., 2009; Sosunov et al., 2014). Furthermore, human astrocytes are up to threefold larger and more than 10 fold more ramified than their rodent counterparts. Recent gene expression studies found interesting differences between human and rodent astrocytes, which indicate that the unique features of human astrocytes are in large part cell-autonomous (Zhang et al., 2016). In agreement with this possibility injection of human glial progenitors, which give rise to astrocytes and oligodendrocyte-lineage cells, into the mouse brain significantly altered mouse brain connectivity and enhanced behavior (Han et al., 2013). The ability to generate human astrocytes from fibroblast-derived induced pluripotent stem cells (iPSCs) now provides new opportunities to decipher the unique functions of human astrocytes and interrogate the importance of astrocytes in human cognition (Krencik et al., 2017; Krencik and Zhang, 2011; Sloan et al., 2017).

Astrocytes are integral components of synapses

In the mature brain the processes of protoplasmic astrocytes extensively infiltrate into the neuropil and wrap around synapses. In this way astrocytes completely parcel out the gray

matter in a non-overlapping manner, forming separate anatomical domains (Bushong et al., 2002). A single mouse cortical astrocyte is estimated to contact over 100,000 synapses; whereas a human astrocyte can contact up to 2,000,000 synapses (Bushong et al., 2002; Oberheim et al., 2009). A similar tile-like organization of other astrocyte-like radial glia cells have also been observed for cerebellar Bergmann glia, which ensheath Purkinje cell synapses, and retinal Muller Glia, which ensheath synapses within the inner and outer plexiform layers (Lippman Bell et al., 2010; Lippman et al., 2008; Wang et al., 2017). These observations indicate that astrocytes, through their fine processes, have the ability to sense and adhere to synapses and coordinate with the neighboring astrocytes to tile and completely cover the neuropil. Astrocytes do not ensheath all synapses however, and synapse association of astrocytes is a dynamic process that can be altered by neuronal activity (Bernardinelli et al., 2014; Genoud et al., 2006; Xu-Friedman et al., 2001). In agreement with this possibility, astrocyte coverage of synaptic contacts is altered during development, in response to injury and in various physiological conditions such as partition, starvation and satiety (Hirrlinger et al., 2004; Procko et al., 2011; Theodosis et al., 2008). Most of these changes occur over a slow timescale of hours to days, and it is unclear whether the cue for altered astrocyte-synapse interaction is a direct sensing of alterations in neuronal activity, or an additional signal released by neurons.

Importantly, the astrocyte processes that ensheath synapses form a critical functional compartment of the synapse, that is required for the maintenance of brain homeostasis and neuronal health (Araque et al., 1999). These perisynaptic astrocyte processes rapidly remove synaptically released neurotransmitters from the interstitial space avoiding their extrasynaptic accumulation and limiting their “spill over” to nearby synapses (Figure 1C) (Murphy-Royal et al., 2017). For example, astrocytic processes are laden with glutamate transporters, which ensure that glutamate does not accumulate extrasynaptically, which could otherwise cause excitotoxicity (Tanaka et al., 1997; Yang et al., 2009). Astrocytes then break down excess glutamate into glutamine and shuttle it back to neurons to provide the building blocks for ongoing synaptic transmission (Tani et al., 2014). Similarly, through numerous channels, astrocytes control the ionic balance at the synapse including potassium ions, which is crucial for sustainability of proper synaptic transmission (Djukic et al., 2007; Kuffler, 1967; Sibille et al., 2014).

Besides these critical functions of astrocytes at the synapse, perisynaptic astrocyte processes are also decorated with metabotropic and ionotropic neurotransmitter receptors (Agulhon et al., 2008). Thus, astrocytes possess the ability to monitor ongoing local activity of synaptic circuits (Figure 1C). The activation of these receptors are known to generate dynamic Ca^{2+} transients in astrocytes, which are observed both at the cell somas and distal astrocyte processes (Shigetomi et al., 2013). It has been proposed that the synaptic activity-driven astrocytic Ca^{2+} rise triggers secretion of neuroactive molecules, commonly known as gliotransmitters, from astrocytes that directly signal to synapses to control proper basal synaptic transmission and modulate neural plasticity (Araque et al., 2014). However, research in this area also yielded conflicting results and controversy as to the Ca^{2+} dependence of gliotransmitter release, the identity of gliotransmitters and the astrocyte specificity of the manipulations used to study these pathways (Hamilton and Attwell, 2010; Nedergaard and Verkhratsky, 2012; Sloan and Barres, 2014). It is important to note that the

Ca^{2+}-transients that occur in astrocytes are of a much slower time scale than what occurs in neurons during neurotransmission (Shigetomi et al., 2016). Thus, both the nature and the function of astrocytic Ca^{2+}-transients are likely to be distinct from that of neuronal Ca^{2+}-signaling, which underlie fast synaptic transmission. In fact recent studies in Drosophila and mice have demonstrated that astrocytes respond to slow-acting neuromodulators such as norepinephrine, tyramine and octopamine with increases in intracellular calcium (Ding et al., 2013; Ma et al., 2016; Paukert et al., 2014). In Drosophila, astrocytic activation by octopamine/tyramine leads to inhibition of dopaminergic neuron firing and alterations in behavior, demonstrating an in vivo role for astrocyte calcium signaling in regulating neuronal circuit function (Ma et al., 2016).

In summary, the studies on astrocyte-synapse interactions have revealed that astrocytes form an integral component of the nervous system architecture. These brain cells are critical regulators of brain function and plasticity, through dynamic and bidirectional interactions with synapses. In the rest of this Review, we will focus on the contributions of astrocytes to the development and remodeling of neuronal synapses, and discuss implications for defects in these pathways in neurodevelopmental disorders.

Astrocytic control of synapse development

Astrocyte-synapse interactions play a definitive role in synapse development across different regions of the central nervous system (CNS). For example, in the developing rodent cerebral cortex, neurons take their positions in their respective layers and extend their axons to their appropriate targets by birth. However, the main periods of synaptogenesis, corresponding to the second and third postnatal weeks, occur only after the birth and differentiation of astrocytes is completed (Freeman, 2010). This is not a coincidence. A number of studies, which we will discuss here, revealed that in fact astrocyte-derived signals are required for the formation, functional maturation and refinement of synapses and circuits.

The first evidence for a critical role of astrocytes in regulating synaptogenesis came from experiments carried out 20 years ago, where it was demonstrated that rodent neurons grown in isolation in vitro formed few synapses, and the addition of astrocytes to the neurons greatly increased both the number of synapses and the strength of the synapses that formed (Pfrieger and Barres, 1997; Ullian et al., 2001). These initial findings have been replicated across species, demonstrating a role for glial cells in regulating synaptogenesis in C. elegans (Colón-Ramos et al., 2007), Drosophila (Muthukumar et al., 2014), Xenopus (Cao and Ko, 2007), rodent (Nägler et al., 2001; Ullian et al., 2001) and human (Hartley et al., 1999; Johnson et al., 2007) neurons. Astrocytes regulate the formation of many types of synapses, including glutamatergic (Ullian et al., 2001), GABAergic (Elmariah et al., 2005; Hughes et al., 2010), glycinergic (Cuevas et al., 2005) and cholinergic (Cao and Ko, 2007; Reddy et al., 2003). Based on these findings an important area of research has been to identify the signals that astrocytes use to regulate neuronal synapse formation, and the pathways activated in neurons in response to these signals. We now know that astrocytes use multiple synaptogenic cues to signal to neurons, and these comprise both contact-mediated and secreted cues (Table 1).

Most work in this field has focused on identifying secreted signals that regulate formation and function of glutamatergic synapses (Figure 2A–B). The first to be identified was cholesterol (Goritz et al., 2005; Mauch et al., 2001), a lipid that is synthesized by astrocytes and secreted in complex with ApoE to be taken up by neurons. Cholesterol was shown to increase the number of vesicles present in the presynaptic terminal and increase release probability, leading to an overall increase in presynaptic function. Mice with decreased lipid synthesis specifically in astrocytes in vivo show decreased release of cholesterol from astrocytes and impaired synaptic development, with decreased numbers of presynaptic vesicles and defects in synaptic plasticity (van Deijk et al., 2017). It is unclear, however, if astrocyte-secreted ApoE itself is involved in regulating synapse function in vivo. Such a role for ApoE would have important clinical implications as mutations in ApoE are strongly linked to neurodegenerative diseases, such as Alzheimer's disease (Jaekwang et al., 2014).

Astrocytes secrete Thrombospondins (TSP1 and 2) (Christopherson et al., 2005) and SPARCL1/Hevin (Kucukdereli et al., 2011) as signals that control the formation of structural glutamatergic synapses (Figure 2A). The synapses induced by these signals contain all of the structural elements of a synapse (such as presynaptic vesicles, active release sites and postsynaptic density) and are presynaptically active because they release and reuptake synaptic vesicles. However, they are postsynaptically silent synapses, because they contain only NMDA glutamate receptors, but lack AMPA glutamate receptors (Christopherson et al., 2005; Singh et al., 2016). Interestingly, astrocytes also produce an antagonist of the prosynaptogenic Hevin, called SPARC (Kucukdereli et al., 2011). In addition to blocking Hevin-induced synapse formation, SPARC also decreases the number of AMPA glutamate receptors present in postsynaptic terminals (Jones et al., 2011). Furthermore, at cholinergic synapses SPARC prevents maturation of presynaptic terminals and triggers a cell-autonomous program of synapse elimination (Albrecht et al., 2012; López-Murcia et al., 2015). These findings collectively show that SPARC acts as an overall negative regulator of synapse number and function.

In addition to astrocyte-secreted proteins that control structural synapse formation, a number of astrocyte-derived signals that positively regulate AMPA receptor localization to postsynaptic terminals have been identified. By increasing synaptic AMPA receptor levels, these signals all act to enhance excitatory postsynaptic function. These include the heparan sulfate proteoglycans glypican 4 and 6 (Gpc4 and 6), which bring GluA1 AMPA receptors to newly forming synaptic sites and subsequent recruitment of postsynaptic density proteins and fully functional synapse formation (Figure 2B) (Allen et al., 2012). TNFα increases AMPAR levels at existing synapses and conversely decreases GABAA receptors at inhibitory synapses leading to overall increased neuronal activity, and is involved in homeostatic scaling to maintain neuronal network activity (Beattie et al., 2002; Stellwagen et al., 2005; Stellwagen and Malenka, 2006). Wingless/Wnt secreted from glia has been shown to cluster glutamate receptors at the Drosophila neuromuscular junction (Kerr et al., 2014). Studies in mammals have shown similar roles for Wnts at excitatory synapses, however the source of Wnt has not been determined (Ciani et al., 2011). Given astrocytes are enriched for mRNA for Wnt ligands (Zhang et al., 2014), it will be interesting to determine if the same pathway exists in the mammalian brain.

Contact-mediated signaling between astrocytes and neurons also regulate synaptogenesis; however, our knowledge of the nature of these signals is rather limited. Interestingly, most of the contact-mediated astrocyte-neuron signaling events that have been identified so far act in embryonic neurons suggesting that contact between astrocytes and neurons is an important permissive first step in neuronal synapse formation. For example, contact of an astrocyte on any part of an embryonic hippocampal neuron induces PKC-signaling throughout the neuron that then allows it to form synapses (Hama et al., 2004). Similarly, embryonic retinal ganglion cell neurons are unable to respond to secreted synaptogenic signals from astrocytes until they have been physically contacted by an astrocyte, again suggesting astrocyte contact changes the maturation state of the neuron (Barker et al., 2008). Gamma protocadherins, cell adhesion proteins that are found both on astrocytes and neurons, act locally as adhesion complexes between astrocyte processes and synapses that promote formation of both excitatory and inhibitory synapses (Garrett and Weiner, 2009).

An important step in synapse development is the elimination of superfluous synapses in an activity-dependent manner. With the exception of SPARC, which blocks new synapse formation in response to Hevin (Kucukdereli et al., 2011), all of the roles of astrocytes at synapses so far described are positive. To provide balance and to prevent excessive and inappropriate synaptogenesis from occurring it seems necessary that astrocytes will also regulate synapse elimination. This has been demonstrated to occur both as a direct and an indirect mechanism. Astrocytes can directly phagocytose excess synapses in the developing brain via the functions of astrocytic phagocytic receptors Mertk and Megf10 (Figure 2C) (Chung et al., 2013). Astrocytes indirectly regulate synapse elimination via the secretion of TGFβ, which upregulates C1q in neurons, leading to tagging of synapses for elimination by phagocytosis by microglia (Figure 2C) (Bialas and Stevens, 2013; Schafer et al., 2012). Both synapse elimination processes are activity-dependent; thus, are highly likely to be involved in controlling the remodeling of circuits in response to experience.

Why do astrocytes make so many different signals? This may be related to the specialized function that each signal has as described above, for example regulating receptor recruitment or presynaptic release. In addition, some of the astrocyte-secreted signals may be specifically regulating the formation and/or maturation of a subset of synapses. This has been shown to be the case for Hevin. In the developing mouse cortex, Hevin is specifically required for the formation of a subset of excitatory synapses: the thalamocortical connections between thalamic axons and cortical dendrites (Risher et al., 2014). Investigation of the ultrastructure of cortical dendrites at high resolution revealed that astrocyte-secreted Hevin is required for a developmental refinement step that occurs at the level of single dendritic spine heads. During early postnatal development dendritic spines often receive two excitatory inputs, one from a thalamic axon and another from a cortical axon. As synapses mature the spines lose one of these inputs and keep the other, achieving the characteristic “one spine = one synapse” organization. Absence of Hevin results in persistence of dendritic spines which are still doubly innervated, most likely due to the inability of thalamocortical connections to compete for spines with cortical inputs (Risher et al., 2014).

Currently, we do not know whether other astrocyte-secreted proteins such as thrombospondins and glypicans also have synapse-specific roles. Furthermore, so far each of these astrocyte synaptogenic cues has been studied in isolation. An important next step is to ask how all these diverse signals work together in vivo to sculpt developing neuronal circuits. Are all signals expressed by the same astrocytes at the same time (Figure 3)? Are they distributed equally to all the nearby neurons? A comprehensive mapping of the *in vivo* spatial and temporal expression of all currently identified astrocyte synaptogenic signals is required as a first step to answer this. There is already strong evidence from work in the developing mouse spinal cord for regional specification of astrocytes and their synaptogenic potential. It was found that spinal cord astrocytes originate from specific gliogenic domains in the dorsal or ventral spinal cord (Tsai et al., 2012), and have different gene expression (Molofsky et al., 2014). Contrary to what is often assumed, these different subgroup of astrocytes do not have redundant functions in synaptogenesis. When abundance of one class of spinal cord astrocytes was reduced by 70% by genetic ablation, the neighboring group of astrocytes were unable to compensate for their loss (Tsai et al., 2012). It is important to note that in *Drosophila* ablation of 50% of astrocytes leads to the remaining astrocytes to increase their domain size to compensate for the loss, whereas with ablation of 75% of the astrocytes the remaining cells are unable to compensate, suggesting a minimum number of astrocytes may be required for maintaining proper synaptic function (Muthukumar et al., 2014).

Astrocyte-synapse interaction regulate plasticity of neuronal circuits

Much progress has been made in understanding how astrocytes regulate the development of neuronal synapses. Synapse strength and number can change in the adult brain as a result of experience and learning, and a role for astrocyte remodeling of synapses in these processes has been proposed. Much of the evidence for this has come from studies of plasticity in mice lacking known astrocyte-secreted synapse modifying factors described above, such as Hevin, SPARC and TNFα. For example the ability to induce long term potentiation (LTP) of synapses in hippocampal slices prepared from SPARC knock out (KO) mice is impaired, and this is hypothesized to be due to the increased strength of individual synapses present in the KO before plasticity is induced which prevents the further strengthening of synapses (Jones et al., 2011). TNFα is involved in a different form of plasticity, known as homeostatic scaling, where the strength of all of the synapses on a neuron are altered in response to changes in neuronal activity patterns (Stellwagen and Malenka, 2006). Hippocampal slices prepared from TNFα KO mice show normal LTP and long term depression (LTD), but no increase in synaptic strength in response to prolonged activity deprivation. Mice lacking Hevin (Hevin KO) do not show a form of *in vivo* plasticity called ocular dominance plasticity, where synapses in the visual cortex remodel in response to changed visual experience (Singh et al., 2016). Rescue of Hevin expression specifically in developing visual cortex astrocytes of the Hevin KOs resulted in restoration of ocular dominance plasticity, showing that astrocytic expression of Hevin is sufficient to control this form of plasticity (Singh et al., 2016). Ocular dominance plasticity occurs through a combination of LTD, LTP and homeostatic scaling (Espinosa and Stryker, 2012), so it will be important to determine which of these plasticity mechanisms is defective in Hevin null mice.

Most work demonstrating a role for astrocyte factors in regulating synaptic plasticity has been performed in germline KO mice. This makes it hard to directly identify a role for these factors in synaptic plasticity when many of these mice already display defects in synaptogenesis, meaning the plasticity defect may be secondary to the synaptogenesis defect. Allowing synapses to develop normally in the presence of astrocyte synaptogenic factors, and conditionally removing them in the adult, will allow separation of a developmental from a plasticity role. In addition, the targeted removal of factors just from astrocytes, rather than globally, will help determine the requirement of an astrocytic source.

How do neurons respond to astrocyte synaptogenic cues?

Now that numerous synaptogenic signals from astrocytes have been identified, it is important to understand how they interact with neurons to regulate neuronal synapse formation and function. An attractive hypothesis is that astrocyte-secreted signals would regulate synaptogenic pathways within neurons, providing an additional level of control of these signaling cascades. This has been shown to be the case for a number of astrocyte-secreted synaptogenic proteins (Baldwin and Eroglu, 2017). Thrombospondins were found to induce synaptogenesis by binding to a neuronal receptor, the calcium channel subunit α2δ-1 (gene name: Cacna2d-1). α2δ-1 is a type 1 membrane protein with a large extracellular domain that contains a protein interaction fold known as von Willebrand Factor A (VWF-A) domain. All five members of the thrombospondin family proteins can induce synapse formation via the synaptogenic interaction that is mediated through their EGF-like domains, which bind to the VWF-A domain of α2δ-1 (Eroglu et al., 2009). This interaction is thought to cause a conformational change in α2δ-1, allowing for activation of a yet unknown synaptogenic signaling pathway in neurons (Risher and Eroglu, 2012). Interestingly, Gabapentin, a drug used to treat epilepsy and neuropathic pain, perturbs this interaction and strongly blocks thrombospondin induced synaptogenesis (Eroglu et al., 2009). This finding also suggests that astrocyte dysfunction may contribute to neuropathologies such as epilepsy and neuropathic pain (Faria et al., 2017).

On the other hand, Hevin regulates formation of thalamocortical glutamatergic synapses by bridging presynaptic neurexin-1alpha (NRX1α) with postsynaptic neuroligin-1B (NL1B) (Singh et al., 2016), two neuronal cell adhesion molecules that do not interact with each other. A region within Hevin interacts concurrently with the extracellular domains of NRX1α and NL1B. This transcellular interaction and bridging is required for Hevin's synaptogenic activity. Interestingly, thrombospondin can also interact with neuroligins (Xu et al., 2009), suggesting a potential cooperation or competition between Hevin and thrombospondins in regulating synaptogenesis. In the case of SPARC, its ability to decrease synaptic levels of AMPA receptors is mediated by inhibiting signaling through β-integrins, receptors known to stabilize AMPAR at synapses (Jones et al., 2011). How SPARC antagonizes Hevin-induced synapse formation remains to be elucidated. Astrocyte-secreted glypican 4, which induces synapse formation by first clustering GluA1 AMPA receptors, acts by signaling through the protein phosphatase receptor PTPRδ present in the presynaptic terminal. This leads to release of the AMPA receptor clustering factor neuronal pentraxin 1, which then binds to postsynaptic GluA1 AMPA receptors and stabilizes them on the surface to induce synapse formation (Farhy-Tselnicker et al., 2017).

Astrocyte-secreted factors also strongly induce inhibitory synapse formation and function, but the identity of the signals are unknown. However, it has been established that astrocytic control of inhibitory synaptogenesis requires both BDNF (Brain Derived Neurotrophic Factor) and its receptor TrkB-signaling in neurons (Elmariah et al., 2005). How astrocytes control inhibition in the CNS is a critically important question in the field, which needs to be addressed to achieve a better understanding of the role of astrocytes in synaptic connectivity and regulating neuronal excitability.

How is release of synaptogenic signals from astrocytes regulated?

Much work on understanding how astrocytes regulate neuronal synapses has focused on identifying the signals astrocytes release and the response that these signals induce in neurons. An equally important part of this mechanism is identifying whether the release of synaptogenic signals from astrocytes is regulated, and if so how. This has been investigated for a limited number of astrocyte-secreted synaptogenic signals. For example, the secretion of thrombospondins from astrocytes in vitro is stimulated by treatment with ATP acting through purinergic receptors (Tran and Neary, 2006). SPARC secretion from astrocytes is stimulated by glutamate (Jones et al., 2011), and as SPARC decreases synaptic AMPAR levels this provides a potential homeostatic feedback loop to decrease synaptic excitability in the presence of increased excitability. TNFα release is decreased by glutamate (Stellwagen and Malenka, 2006). Because TNFα increases synaptic AMPAR this feedback loop again provides a homeostatic mechanism to limit excitability.

Based on these findings it seems likely that the expression and release of synaptogenic factors from astrocytes is regulated, however evidence for this occurring in vivo is currently lacking. Experiments to address this should be conducted at two levels. First, many astrocyte synaptogenic signals show temporal differences in mRNA and protein expression levels. For example in the cortex, thrombospondins 1 and 2 are most highly expressed in the first postnatal week when synapses are first forming and downregulated after that, whereas Hevin increases in the second postnatal week when synapses are maturing (Cahoy et al., 2008; Christopherson et al., 2005; Risher et al., 2014). It is not known whether these changes in expression of synaptogenic factors are an intrinsic part of the astrocyte maturation process, or being regulated by interactions with neighboring neurons. Experiments to manipulate neuronal activity in vivo would go some way to addressing this. Interestingly, dark rearing, which changes neuronal activity patterns in the visual cortex, causes a delay in the structural maturation of astrocytes in the visual cortex (Muller, 1990), as does knocking out glutamate release from cortical neurons in vivo (Morel et al., 2014). This strongly suggests that astrocyte maturation, and therefore expression of synaptogenic factors, is influenced by the neurons they interact with.

Second, it is important to determine if the secretion of synaptogenic signals from astrocytes in vivo is constitutive or controlled by neurons, and whether it can be restricted to specific astrocyte domains (Figure 3D). It seems likely from in vitro work that the secretion of some synaptogenic signals from astrocytes will be regulated by activation of neurotransmitter receptors on astrocytes. This work has been performed on bulk cultures of astrocytes and assayed release over relatively long timeframes, with no ability to measure release in a

spatially restricted way. In the mature brain one astrocyte can contact as many as 100,000 synapses within its domain via fine processes (Bushong et al., 2002). During development there is an increase in astrocyte domain size and in the number of synapses that fall within their domain (Morel et al., 2014), again raising the possibility that astrocytes may regulate synapse formation on a local level via spatially restricted release of synaptogenic factors. Addressing this will require an understanding of where synaptogenic proteins are localized within astrocytes, how they are trafficked within the cell, and if they are present in sub-cellular compartments that make them accessible for release such as vesicles. Interestingly, recent work has shown that astrocytes have mRNA and ribosomes in their distal perisynaptic processes (Sakers et al., 2017), analogous to dendritically localized mRNA in neurons, raising the possibility that mRNA for synaptogenic factors may be localized to astrocytic regions opposed to synapses.

Do all astrocytes have the same synaptogenic potential?

There is increasing evidence that astrocytes in different brain regions are not identical, and even neighboring astrocytes within the same brain region may have differences. Much of the evidence for this comes from mRNA profiling of isolated astrocytes from different brain regions of both rodents and humans (Chai et al., 2017; Doyle et al., 2008; Malik et al., 2014; Molofsky et al., 2013; Morel et al., 2017; Zhang et al., 2014; Zhang et al., 2016). More recently single cell RNAseq has shown local differences in astrocytes and the potential for at least two populations of astrocytes to exist within the cerebral cortex (Darmanis et al., 2015; Zeisel et al., 2015). An analysis of cortical astrocytes separated based on differential expression of cell-surface markers identified five unique populations of cells (John Lin et al., 2017). Given these global differences in astrocyte populations, it is now important to use these datasets to ask whether expression of astrocytic genes that regulate synapses show regional specializations. So far most of the functional characterization of astrocyte sub-populations has been performed in vitro (see below), however determining the importance of astrocyte diversity to synaptic development and function *in vivo* is an important next step. For example, does removing a regionally-specialized astrocyte gene affect synapses just in that brain region, or does ectopic expression of that gene in astrocytes that don’t normally express it change the synapses those astrocytes interact with?

One approach to address the question of whether regional differences in astrocyte gene expression are functionally meaningful for synaptogenic potential has been to use in vitro cultures of astrocytes and neurons isolated from different brain regions. Isolating cortical and subcortical cell types demonstrated that in region-matched astrocyte-neuron cultures the astrocytes induced robust process outgrowth and active synapse formation in the neurons, whereas when astrocytes and neurons from different brain regions were cultured together there were deficiencies in process outgrowth and synaptogenesis (Morel et al., 2017). Similarly, (Buosi et al., 2017) performed in vitro experiments mixing astrocytes and neurons from different brain regions and found altered synaptogenic potency of astrocytes from different brain regions, and correlated this with different secreted levels of known synaptogenic factors such as Hevin, SPARC, Gpc4 and Gpc6. An alternative approach was to ask if subsets of astrocytes from within the cortex had different synaptogenic potential (John Lin et al., 2017). From the five identified astrocyte groups, two were tested for their

ability to induce synapses between cortical neurons. One group had higher expression of known synapse regulating genes such as Hevin and SPARC, and indeed had a stronger ability to induce synapse formation between cortical neurons compared to the astrocytes with low levels of synaptogenic genes. Given that the majority of studies identifying astrocyte synaptogenic signals have focused on cortical astrocytes, this suggests that future work should ask if unique synaptogenic factors are produced by astrocytes in other brain regions. Additionally, determining whether it is only changes in astrocytes that mediate these regional specializations, and/or changes in the receptors for these factors expressed by neurons, will give insight into the meaning of astrocyte specialization.

It is important to determine what induces regionally specialized astrocytes. For example, is it based on their developmental origins, or does the environment of the astrocytes regulate their properties (García-Marqués and López-Mascaraque, 2013)? One place this question has been explored is in the cerebellum, where two morphologically distinct types of astrocytes reside in close proximity - the radial glia-like Bergmann glia, and the protoplasmic-like velate astrocytes. In addition to obvious morphological differences, these two types of astrocytes also display many gene expression differences, such as high levels of AMPA receptors in Bergmann glia and aquaporin 4 in velate astrocytes. Sonic hedgehog (Shh) released from neighboring neurons regulates these specialized properties of Bergmann glia, demonstrating that morphogen release from neurons can regulate astrocyte function (Farmer et al., 2016). Interestingly, gene expression in *Planaria* glia is also regulated by hedgehog released from neurons, suggesting this pathway is highly conserved (Wang et al., 2016). Studies have shown that other morphogens including fibroblast growth factor (FGF) released from neurons (Stork et al., 2014) and bone morphogenetic protein (BMP) (Scholze et al., 2014) can induce structural maturation of astrocytes. It will be interesting to determine if these effects show regional specializations, and can also regulate expression of genes related to synaptogenesis in astrocytes.

Astrocyte-synapse interactions are disrupted in neurodevelopmental disorders

Alteration in synapse formation, synaptic function and synaptic remodeling has been implicated in a number of neurodevelopmental disorders, including autism spectrum disorder (ASD) (Parikshak et al., 2013). Given the important roles of astrocytes in regulating synapses in the brain, it seems likely that alterations in astrocyte-synapse interaction will be involved in these pathologies (Blanco-Suárez et al., 2017). ASD has been linked to mutations in hundreds of genes (Jeste and Geschwind, 2014), so an important question is to ask whether any of these genes are enriched in astrocytes and known to have roles in synaptic regulation. An analysis of the SFARI database of 881 genes implicated in ASD (https://gene.sfari.org/) identified cases linked to mutations in Gpc4, Gpc6, Hevin, TSP1 and FABP7, all astrocyte-enriched factors that regulate synaptogenesis (Allen et al., 2012; Christopherson et al., 2005; Ebrahimi et al., 2016; Kucukdereli et al., 2011). Conversely, this list also includes known neuronal receptors for astrocyte-secreted synaptogenic factors, including multiple neuroligins which mediate both Hevin and TSP1 synaptogenesis, and

neurexins which mediate Hevin effects (Singh et al., 2016; Xu et al., 2009). These data suggest in some cases of ASD, alterations in astrocyte function may be causative.

An alternative way of addressing this question has been to ask whether astrocytes that contain ASD mutations have adverse effects on neuronal synaptic development. This has been investigated for multiple genetic forms of ASD, using both rodent models and human patient iPSC derived astrocytes. Rett's syndrome is caused by a mutation in Mecp2, a transcriptional repressor. Astrocytes that are mutant for Mecp2 have negative effects on the development of WT neurons, including decreasing neuronal process outgrowth and decreasing synaptic function (Ballas et al., 2009; Williams et al., 2014). Rescuing the genetic deficit specifically in astrocytes in vivo in a mouse model of Rett's syndrome is sufficient to rescue some of the disease features, including increasing dendritic outgrowth and synapse number (Lioy et al., 2011). In vitro studies showed that addition of IGF-1, which is currently in clinical trials for Rett's syndrome, was able to block some of the negative effects of Rett's astrocytes on neuronal development (Williams et al., 2014), though the mechanisms underlying this rescue are not known. In Fragile X syndrome there are mutations in the RNA binding protein Fmr1. Like in Rett's syndrome, astrocytes with Fmr1 mutations inhibit WT neuron development, with neurons showing delayed dendritic outgrowth and synapse formation (Jacobs et al., 2010). At least two alterations in astrocyte protein secretion from Fragile X mutant astrocytes have been linked to these effects. The first is an increased secretion of neurotrophin 3 (NT3), with NT3 inhibiting dendritic outgrowth and synaptogenesis (Yang et al., 2012). The second is a decreased release of TSP1 from Fragile X astrocytes, with addition of TSP1 protein to Fragile X astrocyte rescuing the synaptogenesis defects (Cheng et al., 2016). In Down's syndrome, a related neurodevelopmental disorder caused by trisomy of chromosome 21, astrocytes also do not support neuronal development or synapse formation, with WT neurons showing immature spines when cultured with Down's syndrome astrocytes. Interestingly, Down's syndrome astrocytes show decreased expression of multiple synaptogenic proteins including TSP1 and Gpc6, and like with Fragile X syndrome, the addition of TSP1 to Down's syndrome astrocytes is sufficient to rescue some of the synaptogenic deficits (Chen et al., 2014; Garcia et al., 2010). Finally in Costello syndrome, caused by mutations in HRAS, an opposite alteration in astrocyte function is observed. Astrocytes appear to mature early and produce excess levels of secreted factors that regulate synapses, including TSP1 and Gpc6, and treating WT neurons with Costello syndrome astrocytes causes increased synapse formation (Krencik et al., 2015).

There is compelling evidence that astrocytes are contributing to the neuronal and synaptic deficits reported in ASDs. By examining already known factors that astrocytes use to regulate synapses, such as thrombospondins and glypicans, some of the underlying molecular mechanisms have been revealed. More in depth and unbiased analysis of alterations in protein secretion across ASDs should yield additional targets for therapy. What is striking is that each of these neurodevelopmental disorders is caused by diverse genetic mutations, yet the changes in expression and release of synaptogenic factors from astrocytes is overlapping. This points to a shared defect in astrocyte development across these disorders. Importantly, altered synaptogenic potential of astrocytes can either be a deficit, as seen in Fragile X and Down's syndrome, or an excess, like in Costello Syndrome. This

suggests that the levels of astrocyte synaptogenic proteins, not just their presence or absence, are important regulators of neuronal synaptic development.

Conclusion

Astrocytes are an integral part of neuronal circuits, and essential regulators of synaptic development. The work described above has laid the ground work for beginning to understand on a cell biological level how astrocytes are interacting with and regulating neurons. Future work to address the questions we have raised will improve our understanding of this process and reveal the importance that astrocytes have in development. Moreover, a better understanding of astrocyte biology will take us one big step closer to revealing how our brains work.

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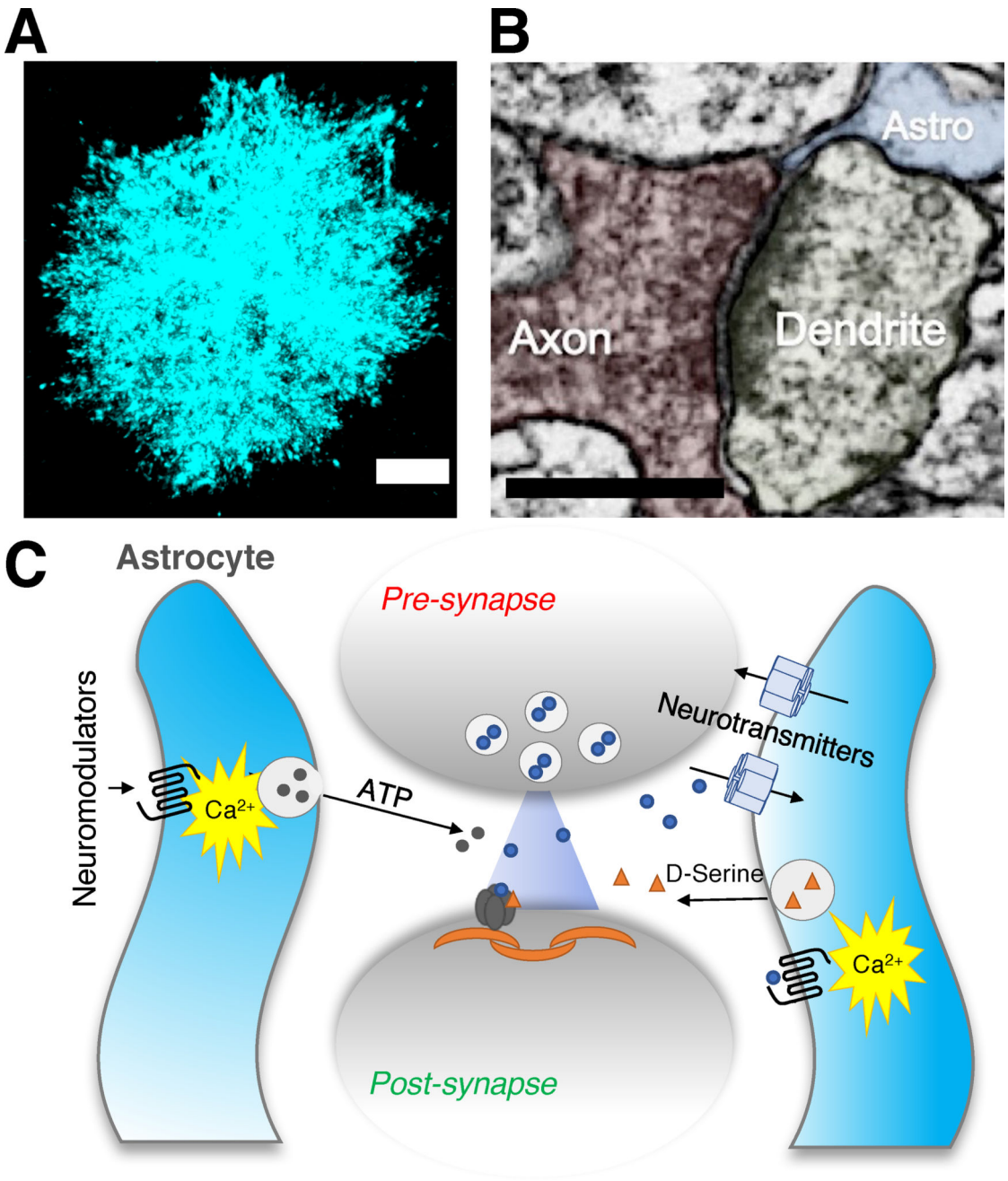

Figure 1. Perisynaptic astrocyte processes are structural and functional components of synapses

A) A membrane-associated fluorescent protein expressing cortical protoplasmic astrocyte from postnatal day 21 mouse cortex, showing the morphological complexity of astrocytes in vivo. Scale bar, 20 μm. Image courtesy of Dr. J.A. Stogsdill (Eroglu Lab).

B) An Electron Micrograph of the tripartite synapse in the mouse visual cortex (V1). The pre-(Axon) and postsynaptic (Dendrite) structures are highlighted in red and green, respectively. An astrocyte process, which makes contacts with pre and postsynaptic boutons, is labeled in blue (Astro). Scale bar, 250nm.

C) Astrocytes are also functionally linked to synapses as they possess the ability to sense synaptic activity and respond to it through intracellular Ca^{2+} transients, and by releasing neuroactive molecules that can signal back to synapses.

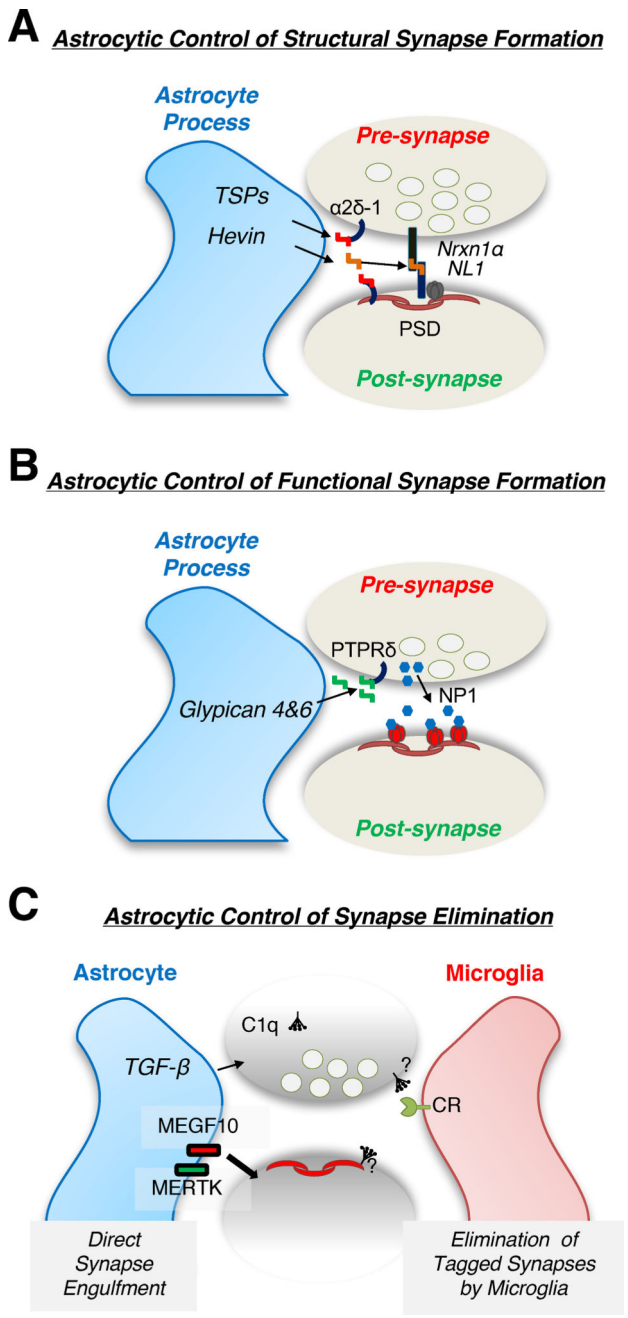

Figure 2. Astrocytes control synapse formation, maturation and elimination

A) Astrocytes secrete synaptogenic Thrombospondins (TSP) and hevin, to induce structural synapses, which are presynaptically active but postsynaptically silent due to lack of AMPARs. However, these structural synapses have postsynaptic NMDARs (grey). TSPs induce synapse formation by interacting with their neuronal receptor calcium channel subunit α2δ-1. Hevin induces formation of a subset of excitatory synapses by bridging two interaction-incompatible synaptic receptors, neurexin1-a and neuroligin 1.

B) Astrocyte-secreted glypican 4 induces functional synapse formation by signaling through presynaptic RPTPδ, leading to release of the AMPA receptor clustering factor NP1 from the presynaptic terminal, and binding of NP1 to GluA1 AMPARs (red) on the dendrite.
C) Astrocytes control synapse elimination in two different ways. First astrocytes eliminate unwanted synapses by phagocytosis through the functions of MERTK and MEGF10 receptors. Second, astrocytes release TGFβ, which induces complement protein C1q expression by neurons. C1q is localized to weak/unwanted synapses through an unknown mechanism and recruits microglia, which express complement receptors (CRs) for elimination of these unwanted synapses by phagocytosis.

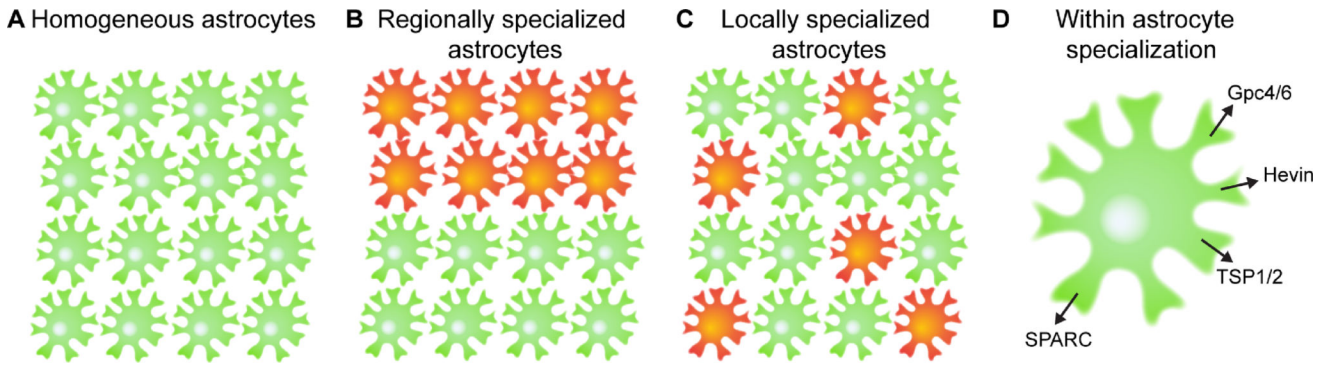

Figure 3. Models of differential astrocyte expression of synaptogenic factors

A) Homogenous astrocytes – all astrocytes express the same synaptogenic factors.

B) Regionally restricted astrocytes – astrocytes in different brain regions express different synaptogenic factors.

C) Locally specialized astrocytes – neighboring astrocytes within brain regions express different synaptogenic factors.

D) Within astrocyte specialization – one astrocyte expresses multiple synaptogenic factors, and targets them to different synaptic domains.

Table 1

Astrocyte-secreted synapse modifying signals

Molecule	Class	Function	Citations
Thrombospondin	Matricellular	Silent synapse formation. Decrease synaptic AMPAR, increase glycine receptor.	(Christopherson et al., 2005) (Hennekinne et al., 2013)
Hevin/Sparcl1	Matricellular	Silent synapse formation.	(Kucukdereli et al., 2011)
Glypican	Heparan sulfate proteoglycan	Active synaptogenesis, GluA1 AMPAR.	(Allen et al., 2012)
TNFα	Cytokine	Increase AMPAR, decrease GABAR. Homeostatic scaling.	(Stellwagen et al., 2005) (Stellwagen & Malenka, 2006)
Wingless/Wnt	Morphogen	Increase synaptic glutamate receptor.	(Kerr et al., 2014)
TGFβ	Cytokine	Schwann cell, NMJ synaptogenesis. Excitatory synapse formation. Inhibitory synapse formation.	(Feng & Ko, 2008) (Diniz et al., 2012) (Diniz et al., 2014)
Maverick/TGFβ	Cytokine	Coordinates pre- and post-synaptic maturation.	(Fuentes-Medel et al., 2012)
SPARC	Glycoprotein	Inhibit Hevin-induced synapse formation. Decrease synaptic AMPAR.	(Kucukdereli et al., 2011) (Jones et al., 2011)
Cholesterol	Lipid	Enhances presynaptic function.	(Mauch et al., 2001)
CSPGs	Chondroitin sulfate proteoglycan	Stabilize synaptic AMPARs.	(Pyka et al., 2011)