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# Molecular mechanisms of astrocyte-induced synaptogenesis

Katherine T Baldwin[1] and Cagla Eroglu[1,2,3]

Astrocytes are morphologically complex cells that perform a wide variety of critical functions in the brain. As a structurally and functionally integrated component of the synapse, astrocytes secrete proteins, lipids, and small molecules that bind neuronal receptors to promote synaptogenesis and regulate synaptic connectivity. Additionally, astrocytes are key players in circuit formation, instructing the formation of synapses between distinct classes of neurons. This review highlights recent publications on the topic of astrocyte-mediated synaptogenesis, with a focus on the molecular mechanisms through which astrocytes orchestrate the formation of synaptic circuits.

**Addresses**
[1] Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, United States
[2] Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, United States
[3] Duke Institute for Brain Sciences (DIBS), Durham, NC 27710, United States

Corresponding authors: Baldwin, Katherine T (katie.baldwin@duke.edu), Eroglu, Cagla (cagla.eroglu@duke.edu)

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## Introduction/overview

Synapses are the building blocks of neuronal networks in the brain. A synapse is traditionally defined as a specialized cell-cell adhesion between the presynaptic axon of one neuron and the postsynaptic dendrite of another neuron. During synaptogenesis, the presynaptic terminal accumulates vesicles, and the postsynaptic side recruits neurotransmitter receptors. Synaptic cell adhesion molecules facilitate this process by mechanically stabilizing synaptic contacts, organizing presynaptic release machinery and postsynaptic scaffolding proteins, and employing downstream signaling molecules that interact with the cytoskeleton [1]. In the mammalian brain, synapse formation is an intricately regulated developmental process that occurs simultaneously in numerous brain regions and between many different types of neurons. A single neuron receives thousands of synaptic inputs. Neuronal activity, sensory experience, extrinsic cues, and intrinsic signaling pathways all help to shape and define synaptic connections, thus establishing the complex circuitry of the brain.

Numerous studies over the past 20 years have revealed that astrocytes of the gray matter (aka protoplasmic astrocytes) are integral components of synapses that dynamically participate in the control of synapse formation and function [2]. Astrocytes are morphologically complex cells with extensively branched processes terminating in fine structures, called perisynaptic astrocytic processes (PAPs), that structurally and functionally interact with synapses [3]. A single astrocyte can ensheathe over 100 000 synapses in the mouse, and over one million synapses in the human brain [4]. Through their intimate association with synapses, astrocytes regulate key aspects of synapse formation, maturation, and function. In this review, we will focus on recent findings on the topic of astrocyte-induced synaptogenesis, since the previous Current Opinion article [5]. Collectively, these new studies provide a framework for how astrocytes, through a number of distinct molecular pathways, promote formation of synaptic circuits.

## Astrocyte-secreted factors induce synaptogenesis and specify circuit formation

The synaptogenic role of astrocytes was initially discovered using a purified retinal ganglion cell (RGC) culture system. RGC neurons grown in the absence of astroglia form very few synapses. However, synapse formation is greatly enhanced upon addition of astrocyte-conditioned media (ACM) [6]. This seminal finding, and the cell culture system that was used to reveal this phenomenon, led to the identification of a number of astrocyte-secreted factors, including proteins, lipids, and small molecules that control different aspects of excitatory synapse formation (Table 1). Furthermore, several recent studies have uncovered distinct molecular mechanisms employed by astrocyte-secreted factors to induce synapse formation. Interestingly, some of these studies highlight a role for astrocytes in directing the formation of specific types of synaptic connections to build different circuits. Below we will highlight some of these molecular mechanisms.

**Table 1**

**Astrocyte-expressed molecules that regulate synapse formation**

| Molecule | Neuronal receptor[a] | Synapse type | Finding | References |
|---|---|---|---|---|
| Thrombospondin | α2δ-1 | Excitatory | Induces formation of silent structural synapses | [7,8,9] |
| Hevin | NL1B, NRX1α | Excitatory thalamocortical | Bridges NL1B and NRX1α to stabilize thalamocortical excitatory synapses | [13,14,15••] |
| SPARC | Unknown | Excitatory | Antagonizes Hevin-induced synaptogenesis | [13] |
| TGF-β1 | TGFβRs? (not tested) | Excitatory and Inhibitory | Induces excitatory and inhibitory synapse formation | [22,23] |
| D-Serine | NMDAR | Excitatory | Promotes synapse formation in adult born neurons | [25] |
| Glypican 4 and 6 | Unknown | Excitatory | Induces excitatory synapse formation; increases synaptic levels of GluA1 AMPARs | [26] |
| Sema3A | Npn1/Plexin A | Excitatory and inhibitory motor neuron inputs | Positional cue required for proper establishment of motor neuron and sensory neuron circuit formation | [36••] |
| γ-Protocadherin | γ-protocadherin | Excitatory and Inhibitory | Promotes excitatory and inhibitory synaptogenesis via direct contact with neurons | [29] |
| EphrinA3 | EphA4 | Excitatory | Promotes normal dendritic spine morphology via direct contact with neurons | [30,31] |
| Cholesterol/ApoE | Unknown | Excitatory | Promotes excitatory synapse formation | [28] |
| BDNF | TrkB | Excitatory | Promotes excitatory synapse formation | [27] |

[a] Here we list the neuronal receptor(s) required for the described synaptogenic function. While receptors for some of these factors are known, whether they participate in astrocyte-induced synaptogenesis is not known.

## Control of excitatory synapse formation by thrombospondins

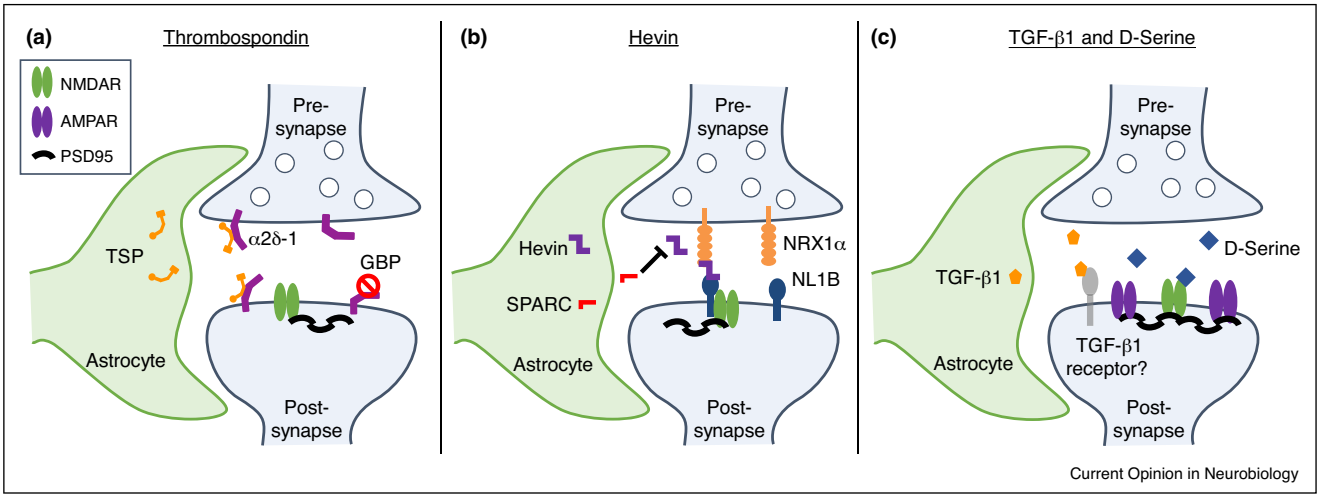
The thrombospondins (TSP), a family of 5 extracellular matrix proteins, were among the first astrocyte-secreted synaptogenic factors to be identified [7,8]. Addition of purified TSPs to cultured RGCs induces the formation of structurally mature, but functionally silent synapses. These silent structural synapses contain NMDA receptors, but lack AMPA receptors [7]. In the developing mouse brain, immature protoplasmic gray-matter astrocytes express TSP1 and TSP2 for the first two postnatal weeks. In agreement for an important role in triggering excitatory synapse formation *in vivo*, TSP1/2 knockout (KO) mice, display fewer excitatory synapses in the cortex [7]. TSPs induce synaptogenesis by binding to their neuronal receptor, the calcium channel subunit α2δ-1 (Cacna2d-1) [8]. The synaptogenic activity of TSP is mediated through its EGF-like domains, which bind to the von Willebrand Factor A (VWF-A) domain of α2δ-1 [8]. This interaction is thought to cause a conformational change in α2δ-1, allowing for the recruitment and assembly of a putative synaptogenic signaling complex [9] (Figure 1a). Interestingly, α2δ-1 is also a receptor for Gabapentin, a drug used to treat epilepsy and neuropathic pain. Gabapentin strongly inhibits excitatory synapse formation *in vitro* and *in vivo* by preventing TSP from binding α2δ-1, thus blocking TSP-induced synaptogenesis [8]. This finding highlights not only the important role of astrocyte-secreted TSP in synapse formation, but also suggests that astrocyte dysfunction may contribute to neuropathologies such as epilepsy and neuropathic pain [10–12].

## Circuit specific control of excitatory synapse maturation and plasticity by astrocytic Hevin/SPARCL1

Besides TSPs, two members of the secreted protein acidic, enriched in cysteine (SPARC) family proteins, Hevin (also known as SPARC-like 1/SPARCL1) and SPARC were identified as astrocyte-secreted factors that control synapse formation between cultured RGCs. Similar to TSPs, treatment of RGCs with purified Hevin is sufficient to induce formation of ultrastructurally mature, but postsynaptically silent synapses. Conversely, SPARC is not synaptogenic, and antagonizes Hevin-induced synaptogenesis [13]. In the developing mouse brain, Hevin and SPARC are expressed at high levels in astrocytes during the second and third weeks, coinciding with periods of heightened synapse stabilization and synaptic refinement. In adulthood, Hevin expression remains high, while SPARC expression is significantly reduced [13]. Analyses of developing visual cortices of Hevin-null mice revealed that Hevin is required for proper thalamocortical synaptic connectivity [14]. Hevin KO mice have significantly fewer thalamocortical synapses, while the number of intracortical synapses is significantly increased. Furthermore, ultrastructural analysis revealed that Hevin KO spines and dendrites are structurally immature [14].

Recently, significant progress was made in identifying the molecular mechanism of Hevin-induced synapse formation. Hevin regulates formation of thalamocortical glutamatergic synapses by bridging presynaptic neurexin-1alpha (NRX1α) with postsynaptic neuroligin–1B (NL1B) [15••], two neuronal cell adhesion molecules that do not interact with each other (Figure 1b). The region of Hevin between amino acids 351–440 interacts with the extracellular domains of NRX1α and NL1B, and is required for Hevin's synaptogenic activity. Hevin recruits NL1 and NL1-associated proteins, including PSD95 and NMDA receptor subunits, to synapses. Neurons express several isoforms of presynaptic neurexins and postsynaptic neuroligins. The interaction code of these different isoforms is thought to instruct the formation of a diverse

**Figure 1**




Astrocyte-secreted factors induce excitatory synapse formation. **(a)** Astrocytes secrete thrombospondins (TSP) which bind to neuronal a2d-1 to induce the formation of silent, structural synapses. The anti-epileptic drug Gabapentin (GBP) binds to a2d-1, preventing TSP-induced synaptogenesis. **(b)** Astrocyte-secreted Hevin/SPARCL1 promotes synapse formation through its interactions with presynaptic NRX1a and postsynaptic NL1B, two proteins that do not normally interact. Astrocyte-secreted SPARC antagonizes Hevin-induced synapse formation through an unknown mechanism. **(c)** Astrocyte-secreted TGF-β1 promotes the formation of excitatory synapses through a mechanisms that requires NMDA receptor activity, along with the NMDA receptor agonist D-serine.

array of synaptic connections [16]. By bridging the connection between two incompatible isoforms, Hevin acts as a synaptic linker protein, modifying the neurexin/neuroligin isoform code to induce thalamocortical synapse formation. Interestingly, mutations in Hevin, neurexins, and neuroligins are strongly associated with Autism Spectrum Disorder, suggesting that these molecules play critical roles in proper brain development [17,18].

Hevin and SPARC have similar domain structures, with an N-terminal acidic domain and C-terminal follistatin-like and SPARC-like domains. They share 60% homology in their C-terminal domains, but differ substantially in their N-terminal domains. The mechanism through which SPARC antagonizes Hevin-induced synaptogenesis is unknown. Hevin and SPARC do not interact with one another [13], but given their C-terminal homology, SPARC may antagonize Hevin function by binding and sequestering common interacting partners. Interestingly, SPARC expression is negatively regulated by enhanced excitatory synaptic activity in astrocyte-neuron co-cultures *in vitro*. In addition, SPARC negatively regulates AMPAR recruitment to synapses, potentially via interactions with integrins [19]. Furthermore, SPARC was shown to inhibit maturation of cholinergic presynaptic terminals [20] and induce a cell autonomous program of synapse elimination via collapse of presynaptic terminals [21]. The molecular mechanisms through which SPARC exerts these inhibitory actions in synapse formation remains to be elucidated.

## TGF-beta promotes formation of excitatory and inhibitory synapse formation

Transforming growth factor beta-1 (TGF-β1) is a secreted protein belonging to the TGFβ superfamily of cytokines, which participate in a plethora of developmental signaling pathways. While studies of astrocyte-secreted synaptogenic factors have mainly identified factors that promote excitatory synapse formation, TGF-β1 application promotes formation of both excitatory and inhibitory synapses. In cultured mouse cortical neurons, addition of TGF-β1 significantly enhances the formation of structural and functional excitatory and inhibitory synapses [22,23]. *In vivo*, TGF-β1 overexpressing mice display increased levels of AMPA and NMDA receptor subunits in the hippocampus [24]. TGF-β1-induced excitatory synaptogenesis requires NMDA receptor activity and the NMDA co-activator D-serine (Figure 1c). D-serine treatment is sufficient to mimic TGF-β1-induced synaptogenesis, and requires the function of serine racemase, the enzyme which converts L-serine to D-serine [22]. Interestingly, D-serine administration also induces synapse formation in adult-born neurons *in vivo* [25].

TGF-β1 requires NMDA receptor activity to induce inhibitory synapse formation, but employs distinct signaling mechanisms from excitatory synaptogenesis. TGF-β1 increases phosphorylation of CAMKII, a major downstream signaling component of NMDA receptors, at Thr286, a key site for inhibitory synapse regulation. Accordingly, blocking CAMKII activity prevents

TGF-β1-induced inhibitory synapse formation, but has no effect on formation of excitatory synapses. TGF-β1 also increases neuroligin 2 (NL2) expression and gephyrin/NL2 clustering [23], two important components of inhibitory postsynapses. Injection of TGF-β1 into mice increases inhibitory synapse formation *in vivo*. Collectively, these studies demonstrate that TGF-β1 is capable of inducing both excitatory and inhibitory synapse formation, yet whether TGF-β1 is required for synapse formation *in vivo* under physiological conditions remains to be determined.

In addition to the factors we highlighted above, several other astrocyte-secreted synaptogenic factors have been identified, including glypican (Gpc) family members Gpc4 and Gpc6 [26], Brain-Derived Neurotrophic Factor (BDNF) [27], and cholesterol with apolipoprotein-E [28]. Furthermore, astrocyte-neuron contact may also play roles in synaptogenesis. Along these lines homotypic interactions between gamma-protocadherins and heterotypic interactions between Ephrin A3/EphA were shown to be important for synapse formation [29–31] (See Table 1 for more details).

## Astrocyte heterogeneity underlies neural circuit formation

Thus far, we have discussed astrocytes as a homogenous population. However, increasing evidence demonstrates that astrocytes comprise a heterogeneous population of cells with distinct molecular properties and functions [32–34]. Astrocyte heterogeneity occurs not only between brain regions, but may also occur within the same region. While our understanding of astrocyte heterogeneity is in its infancy, a number of recent studies describe how different types of astrocytes differentially instruct synapse formation and neural circuit formation.

### Developmentally-encoded astrocyte domains regulate circuit formation in the spinal cord

During development of the mouse spinal cord, astrocytes are allocated to specific domains based on their site of origin in the ventricular zone. Depletion of astrocytes from one domain causes neuronal and synaptic defects that cannot be rescued by astrocytes from neighboring domains [35]. This phenomenon is accomplished via different positional cues expressed by regionally-distinct astrocytes. Gene expression analysis of mouse spinal cord astrocytes identified 38 genes that are differentially expressed between dorsal and ventral astrocytes [36••]. Interestingly, many of these genes encode proteins with previously defined roles in positional guidance during brain development.

Semaphorin 3a (Sema3a) expression is highly enriched in ventral spinal cord astrocytes. Sema3a is an extracellular matrix protein that signals through a PlexinA/neuropilin 1 receptor (Nrp1) complex [37]. In the spinal cord, α-Motor neurons (α-MNs) and TrkA+ sensory neurons express high levels of Nrp1. A combination of *in vivo* and *in vitro* experiments revealed that astrocyte-secreted Sema3a is required for α-MN survival, circuit integration, and function. Knockdown of Sema3A in astrocytes decreases excitatory inputs and increases inhibitory inputs onto α-MNs [36••]. Together, these findings identify a role for astrocytes in positional regulation of circuit formation. Furthermore, they suggest that proper astrocyte development and regional allocation is a prerequisite for establishing and maintaining the complex circuitry of the central nervous system.

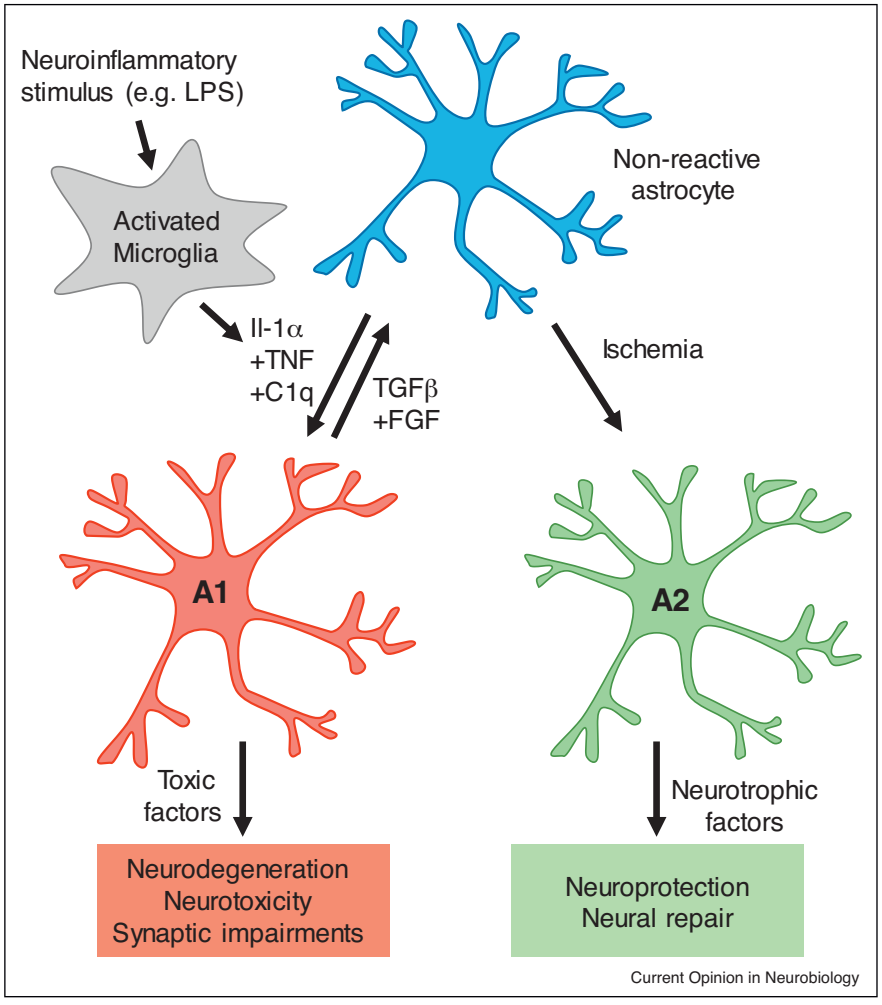
In addition to the spinal cord, a recent study examined astrocyte heterogeneity in different brain regions, including the cortex, thalamus, cerebellum, olfactory bulb, and brain stem [38•]. This study used a cell sorting approach to identify five different populations of astrocytes in the brain based on their expression of different cell surface antigens. These different populations of astrocytes (named population A, B, C, D, and E) have unique gene signatures and different functional properties. For example, population C astrocytes, which account nearly 50% of all astrocytes in the cortex, cerebellum, and brainstem, are strongly enriched for genes associated with synaptogenesis and synaptic function. Accordingly, population C astrocytes appear more synaptogenic than other populations of astrocytes when cultured with neurons *in vitro* [38]. Additional studies are needed to determine how these different populations of astrocytes control the formation of specific neural circuits *in vivo*.

### Altered synaptogenic potential in different types of reactive astrocytes

Astrocyte heterogeneity is present not only under normal physiological conditions, but also amongst reactive astrocytes [39]. Following injury or disease, astrocytes enter a reactive state characterized by changes in astrocyte morphology and gene expression. Numerous studies over the past three decades have found that reactive astrocytes are neurotoxic in some instances [40,41], yet neuroprotective in others [42–44], suggesting that different types of reactive astrocytes may serve different functions. Indeed, a recent study found that different types of stimuli induce distinct types of reactive astrocytes. For example, systemic injection of lipopolysaccharide (LPS) induces harmful A1 reactive astrocytes in mice, whereas ischemic insult induces neuroprotective A2 reactive astrocytes [45] (Figure 2).

How does astrocyte reactivity impact synaptogenesis? In the mouse cortex, activated microglia secrete several cytokines to induce A1 reactive astrocytes, including interleukin 1α (Il1-α), tumor necrosis factor (TNF), and complement component 1, subcomponent q (C1q) [46••]. Adding these three microglia-secreted cytokines to cultures of purified primary cortical astrocytes is sufficient

**Figure 2**




Heterogeneity amongst reactive astrocytes. Following insult or injury, astrocytes enter a reactive state, characterized by changes in astrocyte morphology and gene expression. Depending on the stimulus, astrocytes can become neurotoxic A1 type reactive astrocytes, or neuroprotective A2 type reactive astrocytes. Neuroinflammatory stimuli, such as LPS, yield A1 reactive astrocytes, by activating microglia to secrete the inflammatory cytokines Il-1a, TNF, and C1q. A1 reactive astrocytes promote neurodegeneration and neurotoxicity, and are not synaptogenic. Interestingly, application of TGFβ and FGF can revert type A1 reactive astrocytes to a non-reactive state in vitro. Ischemia induces the formation of A2 reactive astrocytes, through an unknown mechanism. These astrocytes have a different gene signature from A1 reactive astrocytes, and secrete neurotrophic factors to promote neuroprotection and neural repair.

to produce A1 reactive astrocytes. Conditioned media from A1 reactive astrocyte cultures (A1 ACM) fails to induce synapse formation in cultured RGC neurons [46••]. Interestingly, many astrocyte-secreted synaptogenic factors are expressed by A1 reactive astrocytes. While Hevin and Gpc6 mRNA levels are substantially decreased, and SPARC levels remain unchanged, levels of Gpc4 are increased nearly two-fold, and levels of TSP1 and TSP2 increase 7–8 fold [46••]. That A1 astrocytes fail to induce synaptogenesis despite expressing such high levels of TSP1, TSP2, and Gpc4, implies that A1 reactive astrocytes secrete a toxic signal that adversely affects synapse formation.

In addition to having distinct responses to different types of stimuli, different groups of astrocytes may have distinct reactions to the same stimuli [45]. In cortical astrocytes, LPS induces an A1 reactive phenotype via microglia, but has no direct effect on astrocytes [46••]. Conversely, a recent study found that hippocampal astrocytes in juvenile mice respond to LPS treatment via a TLR4/MyD88-dependent signaling pathway to activate Erk1/2 signaling and enhance excitatory synapse development [47]. These LPS-treated mice display an increased susceptibility to early-life seizures, which can be rescued by blocking Erk1/2 activation, suggesting a role for reactive gliosis in the pathogenesis of neurological disorders such as

epilepsy. In addition to epilepsy, reactive astrocytes are observed in many other neurological disorders, including Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and Multiple Sclerosis [46••], where they may adversely affect synapse and neuronal health. For a more in-depth discussion of astrocyte-synapse interactions in brain disorders, we refer to recent review articles [48,49].

## Conclusions and future directions

Here we have summarized a number of recent, high-impact studies detailing the molecular mechanisms of astrocyte-mediated synaptogenesis. Together, these studies demonstrate the critical role that astrocytes play in promoting the formation of synaptic circuits. However, there are still a number of important outstanding questions:

1) What mechanisms control astrocytic expression and secretion of synaptogenic factors?
2) How do astrocyte-synapse adhesions form, and what is their role in synaptogenesis?
3) Do astrocytes communicate with each other to coordinate synaptogenesis?
4) What astrocyte-intrinsic signaling mechanisms are required for astrocyte-induced synaptogenesis?
5) How does astrocyte-neuron cross-talk shape synapse formation during development

Going forward, new tools for studying astrocytes will be essential for answering these questions. Recently developed tools and resources, including RNA sequencing databases of mouse [50] and human [51] astrocytes, new astrocyte-specific Cre mouse lines [52•], and methods for studying human astrocytes [53•,54,55] are paving the way towards furthering our understanding of astrocyte biology. Given the implications for astrocyte dysfunction in human brain disorders, answering these questions will be of fundamental importance not only for deepening our understanding of the mechanisms guiding astrocyte-induced synaptogenesis, but also for developing therapeutic strategies to combat neurological disease.

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- •• of outstanding interest

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