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# Cell adhesion molecules regulating astrocyte-neuron interactions

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## Abstract

A tripartite synapse is comprised of a neuronal presynaptic axon and a postsynaptic dendrite, which are closely ensheathed by a perisynaptic astrocyte process. Through their structural and functional association with thousands of neuronal synapses, astrocytes regulate synapse formation and function. Recent work revealed a diverse range of cell adhesion-based mechanisms that mediate astrocyte-synapse interactions at tripartite synapses. Here we will review some of these findings unveiling a highly dynamic bidirectional signaling between astrocytes and synapses, which orchestrates astrocyte morphological maturation and synapse development. Moreover, we will discuss the roles of these newly discovered molecular pathways in brain physiology and function both in health and disease.

## Introduction

At its most fundamental, the brain is a highly complex web of synaptic connections. Classically, a synapse is viewed as a cell-cell adhesion between the pre-synaptic axon of a neuron and the post-synaptic dendrite of another, allowing for information to be transmitted between them. However, the intricacy, specificity and complexity of synapse formation and function is not only governed by neurons. In recent years, astrocytes, the dominant perisynaptic glial cell type in the brain, has been shown to play critical roles in the genesis, maturation, elimination, plasticity and function of synapses[1–3].

The necessity of astrocytes in synapse development were first demonstrated using purified retina ganglion cell neuron-only cultures. These neurons, despite their ability to survive and thrive under serum-free conditions, formed very few synapses when cultured alone.

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However, when media containing astrocyte-secreted factors were applied to these neurons, a profound increase in synapse numbers and synaptic activity were detected [4,5]. Since then, many astrocyte-released synaptogenic factors were discovered and a number of them were characterized for their roles in controlling synapse development *in vivo,* clearly revealing the dynamic regulation of excitatory and inhibitory synapse formation and function by astrocytes[6–8].

Another mode of signaling between astrocytes and neuronal synapses is mediated by cell adhesion molecules which will be the main focus of this review. In particular, we will focus on four recently discovered adhesion-based mechanisms (Figure 1) that control the physical and functional engagement between synapses and protoplasmic astrocytes of the grey matter. We will highlight the latest research on how these cell adhesion-based mechanisms regulate astrocyte-neuron interactions and how these findings shape our understanding of synapse function in health and disease.

## Protoplasmic astrocyte morphology reflects the complexity of astrocyte-synapse interactions

Protoplasmic astrocytes of the grey matter have highly ramified morphologies. 6-8 primary processes extend out from the astrocyte soma, branching out into secondary and tertiary processes, and finally elaborating into fine perisynaptic astrocytic processes (PAPs) that come into direct contact with the synapse[9]. Electron micrograph 3D reconstructions of rat hippocampi revealed that synapses adjacent to (<1μm) astrocytes tend to be mature (large perforated mushroom spines)[10]. This observation, coupled with evidence of activity-dependent, bidirectional signaling between astrocytes and synapses led to the concept of the tripartite synapse[11]. The tripartite synapses are abundant in the brain, accounting for 57-90% of all synapses in the hippocampus[10,12] and all excitatory synapses in the cerebellum[13,14]. In the cortex, a single astrocyte contacts up to 100 000 synapses in mice[15] and upwards of 2 million synapses in humans[16]. Indeed, these intimate astrocyte-synapse interactions are conserved through evolution[17–19] and are vital for synapse formation[18], stability and maturation[20], as well as conferring circuit specificity[21] across species. What are the molecular mechanisms that mediate astrocyte-neuron contacts at synapses? Evidently, cell adhesion emerged as major regulators of astrocyte-neuron contacts at tripartite synapses[22,23]. Here we will focus on cell surface adhesion proteins that have been identified in the past 5 years to play critical roles in the mammalian brain.

## Negative regulation of synaptogenesis by ephrin/Eph signaling at the tripartite synapse

Several Eph receptors and their ephrin ligands have been identified at the astrocyte-neuron interface. Astrocytic ephrinA3 binds to EphA4 receptors on dendritic spines, resulting in spine retraction to ensure proper dendritic morphology and synapse formation during development[24]. Reverse signaling of astrocytic ephrinA3 by neuronal EphA4 limits cell surface expression of glutamate transporter in astrocytes, ensuring optimal synaptic

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glutamate levels that are critical for synaptic plasticity[25]. Bidirectional signaling at the tripartite synapse could be dependent on sensory experience driven synaptic activity, because ephrinA3/EphA4 signaling between suprachiasmatic nucleus (SCN) astrocytes and neurons regulates circadian plasticity in response to light[26].

Recent work identified another class of ephrins, ephrinB, at the astrocyte-synapse interface (Figure 1). Different from ephrinAs, which are bound to the cell membrane with a glycosylphophatidylinositol (GPI) anchor, ephrinBs are single-pass transmembrane proteins with a PDZ-binding domain at their cytoplasmic tails[27]. This structural difference means that in addition to tyrosine phosphorylation-dependent signaling, ephrinB can also initiate reverse signaling (outside-in) via interaction with PDZ domain proteins. This finding greatly augments the possibility of ephrinB/EphB interaction for bidirectional signaling at the astrocyte-neuron interface.

Astrocytic ephrinB1 was first shown to be a critical negative regulator of adult synaptogenesis, inhibiting exuberant excitatory synaptogenesis in the hippocampal stratum radiatum by pruning away immature spines[28,29]. Overexpression of ephrinB1 in hippocampal astrocytes results in synapse loss and reduced contextual fear memory[28]. Astrocytic ephrinB1 binds to EphB receptors on presynaptic boutons to promote the engulfment of synaptosomes *in vitro,* suggesting a potential role for this molecule in astrocyte-mediated synapse elimination. Loss of function mutations in the intracellular domain of ephrinB1 decrease synaptosome engulfment by 50%, indicating that astrocytic ephrinB1: neuronal EphB reverse signaling might be involved [28]. *In vivo,* astrocytic *ephrinB1* conditional knockout mice (cKO) have significantly increased spine density and spine clustering compared to wildtype littermates, particularly on CA1 neurons[29]. Training these mice in a fear-conditioning paradigm increases AMPA receptor insertion on the surfaces of these immature spines, resulting in an increase in the number of functional excitatory synapses [28], therefore enhancing long-term contextual recall in astrocytic *ephrinB1* cKO mice[28,29].

Apart from its importance in regulating excitatory synaptogenesis in adulthood, astrocytic ephrinB1 is also critical in maintaining excitation/inhibition (E/I) balance in the developing brain[30]. *EphrinB1* astrocytic cKO mice have exuberant excitatory synapses, increased clustering of dendritic spines as well as a higher proportion of immature synapses during early development[30]. These observations are similar in the adult hippocampus, suggesting that astrocytic ephrinB1 also negatively regulates excitatory synaptogenesis both in development and adulthood. Interestingly, elimination of ephrinB1 from developing astrocytes during (postnatal days) P14-P19 results in a significant increase in both presynaptic and postsynaptic excitability of P28 hippocampal CA1 pyramidal neurons[30]. Also, there were significant decreases in the amplitudes of both evoked and miniature inhibitory postsynaptic currents (IPSCs). These changes were attributed to a marked decrease in parvalbumin (PV) neurons as well as a significant decrease in excitatory input onto PV neurons[30]. While the mechanism has yet to be elucidated, there is striking evidence that astrocytic ephrin B1 plays a critical role in the proper wiring of both excitatory and inhibitory circuits in the developing hippocampus[30].

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Astrocytic ephrinB1 function is also relevant to our understanding on the molecular basis of neurological diseases. Knocking out *ephrinB1* from astrocytes during development is sufficient to cause impairments in social memory, one aspect of social cognitive impairment observed in autism[30]. Furthermore, ephrinB1 expression increases in hippocampal astrocytes after traumatic brain injury. This suggests that astrocytic ephrinB1 can participate in synapse remodeling following injury, thus could be a therapeutic target for functional recovery after serious brain injuries[31].

Astrocytes also express ephrinB2, ephrinB3 and a full suite of Eph receptors[32], which have also been implicated in defects in astrocyte-neuron interactions. For example, elevated levels of astrocytic ephrinB3 and neuronal EphB3 (and no other Eph/ephrins) were observed in both patient and rat model of intractable temporal lobe epilepsy. This observation suggests that EphB3/ephrinB3 contact is critical in disease pathogenesis, likely through regulating synaptogenesis and synaptic plasticity[33]. Finally, astrocytes from a common mouse model of spinal muscular atrophy (*SMNΔ7* mice) have a 73% reduction in *ephrinB2* mRNA expression[34]. When *SMNΔ7* astrocytes are co-cultured with wildtype or *SMNΔ7* neurons, both synaptogenesis and synaptic function were significantly reduced. However, no synaptic changes were observed when the same experiment was repeated using a trans-well astrocyte-neuron co-culture, in which astrocytes are not in contact with neurons[34]. It is therefore hypothesized that Eph/ephrinB2-mediated astrocyte-neuron signaling is critical to the pathogenesis of early-stage spinal muscular atrophy.

## Control of astrocyte morphogenesis and synaptogenesis by neurexin/neuroligin interactions

Neurexin and neuroligin families of cell adhesion molecules have been studied extensively for their heterophilic trans-synaptic interactions[35]. However, neither neuroligins nor neurexins are solely expressed by neurons. In fact, astrocytes express three neuroligins (Nlgn1, Nlgn2 and Nlgn3) as well as two neurexins (Nrxn1 and Nrxn2) at similar or higher levels compared to neurons[36].

Recent work revealed that astrocytic neuroligins bind to neuronal neurexins (Figure 1), molecularly linking astrocyte morphogenesis and synaptogenesis during the first three postnatal weeks of cortical development[37]. Indeed, astrocytes gain their morphological and functional maturity concurrently with the peak period of synaptogenesis in the cortex [38] and bidirectional signaling between astrocytes and experience-dependent synaptic activity is critical for both biological processes[39]. However, how astrocyte morphogenesis and synaptogenesis occurred in concert was unclear. Recent work revealed astrocytic Nlgn1, 2 and 3 as key players in orchestration of astrocyte and synapse development. Using purified neuron and astrocyte co-cultures, it was shown that silencing Nlgn1, Nlgn2, and Nlgn3 individually or all together in astrocytes result in significant decreases in astrocyte morphological complexity[37]. Concordantly, silencing or sequestering neuronal Nrxn1 and 2 diminished the complexity of wildtype astrocytes[37], verifying that it is neuronal neurexin/astrocytic neuroligin transcellular interactions which mediate contact-induced astrocyte morphogenesis. When individual neuroligins were silenced in astrocytes *in vivo,*

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all significantly perturbed astrocyte morphogenesis. In particular, knockdown of astrocytic Nlgn2 resulted in continued diminished neuropil infiltration volume (i.e., a quantitative measure of PAP complexity). Importantly, loss of Nlgn2 only in astrocytes was sufficient to diminish excitatory synapse numbers and function. Whereas, loss of Nlgn2 in astrocytes increased the frequency of inhibitory synaptic events without affecting the number of inhibitory synapses[37]. Taken together these findings showed that astrocytic neuroligins, such as Nlgn2, interact with neuronal neurexins to synchronize synapse formation and astrocyte morphogenesis.

Apart from neuroligins, transcriptomic analyses have long suggested that astrocytes also express neurexins, amongst which *Nrxn1* expression is profoundly higher in mouse and human astrocytes than neurons[36,40]. Profiling of neurexin isoforms in the brain by in-situ hybridization confirmed that *Nrxn1α* and *Nrx1β* mRNA expression is abundant in hippocampal and cortical astrocytes[41]. A recent preprint suggests a role for astrocytic Nrxn1α in synaptic plasticity[42]. In the proposed model, astrocytic Nrxn1α is spliced differently from the neuronal variant and is more heavily modified by heparan sulfate and hence has different binding partners[42]. Comparison of neuronal and astrocytic *Nrxn1α* cKO suggests distinct transcriptional profiles, defects in synaptic function and behavioral impairments[42]. However, whether astrocytic neurexins can bind to neuronal neuroligins to mediate astrocyte-neuron contact dependent adhesion is not known.

These studies yield exciting new questions to be explored. How are cell adhesion molecules compartmentalized on astrocyte surfaces? In neurons, compartmentalization of neurexins to axons and neuroligins to dendrites is documented, though several studies also showed their presence in the opposing compartments[43–45]. Is there a connection between astrocytic neuroligins and neurexins and the proper polarity of these molecules in neurons? Are these molecules targeted to specific astrocyte domains? Answering these basic cell biological questions would have important disease implications, as neurexins and neuroligins have been strongly implicated in neurodevelopmental diseases such as autism[46,47] and schizophrenia[48,49].

## Promotion of dendrite arborization by γ-protocadherin homophilic interactions

The cadherin superfamily is a large group of cell adhesion proteins characterized by calcium-dependent intercellular adhesions. Protocadherins, the largest subfamily, are predominantly expressed in the central nervous system. These proteins play critical roles in neurodevelopment, particularly in generating functional diversity within neurons and promoting synaptogenesis[50]. Interestingly, homophilic γ-protocadherin interactions between astrocytes and neurons are required for proper excitatory and inhibitory synaptogenesis[51]. The initial experiments were conducted in the spinal cord[51], and now homophilic γ-protocadherin astrocyte-neuron interactions have also been shown to be critical in the developing mouse cortex[52]. Elimination of astrocytic γ-protocadherins in the cortex reduces dendritic complexity, non-cell autonomously. Specifically, astrocytic manipulation results in a decrease in the number of branchpoints and total dendrite length

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of layer V pyramidal neurons[52]. Interestingly, loss of γ-protocadherin in astrocytic processes does not appear to adversely impact astrocyte survival or complexity[52]. Notably, alternative splicing of γ-protocadherin gives rise to 22 different isoforms. A mismatch of isoforms disrupts homophilic interactions between the astrocyte and the growing dendrite[52]. Taken together, these studies show that transcellular γ-protocadherin interactions can confer specificity and significant diversity in astrocyte-dendrite interactions.

Apart from homophilic *trans*-interactions, γ-protocadherins can make *cis*-interactions with Nlgn1[53], functioning as a competitive inhibitor of transcellular neurexin-neuroligin signaling[53]. In a heterologous cell culture system, *cis*-interactions between γ-protocadherin and Nlgn1 in non-neuronal cells, such as *Cos7* cells, prevented both presynaptic differentiation and dendritic spines formation[53]. Similarly, inhibitory *cis*-interactions between γ-protocadherins and Nlgn2 were also identified recently[54]. Whether γ-protocadherins co-localize with Nlgn1 or Nlgn2 on PAPs is yet to be determined. However, it is highly plausible that astrocytic γ-protocadherins through their *trans* and *cis* binding partners control important aspects of astrocyte-neuron interactions and provide a dynamic specificity and diversity to the contacts between cells.

## Regulation of inhibitory synaptogenesis by NrCAM

In the past decade, several astrocyte-neuron signaling mechanisms regulating excitatory synaptogenesis have been elucidated. However, our understanding of how cell adhesion between astrocytes and neurons impact inhibitory synapse formation and function has been severely lacking. A recent study characterized the proteome of the astrocyte-synapse junctions as they exist *in vivo* using a targeted biotinylation-based approach. Among the 118 proteins which were identified as high confidence tripartite synaptic proteins, Neuronal Cell Adhesion Molecule (NrCAM) was shown to be a critical regulator of transcellular interactions between astrocytes and inhibitory post-synapses[55].

NrCAM is enriched at astrocyte processes. Super resolution imaging of PAPs and synaptic markers revealed that NrCAM is localized to astrocyte-inhibitory synapse contacts in the cortex. *Nrcam* deletion in cortical astrocytes results in increased astrocyte territory and neuropil infiltration volume[55], opposite of the phenotype observed with the loss of astrocytic neuroligins. These phenotypes are rescued by wildtype NcCAM but not by NrCAM lacking the extracellular immunoglobulin domains. These results indicate that astrocytic NrCAM mediates a transcellular interaction through its extracellular region[55]. In agreement, a homophilic interaction mediated by NrCAM between astrocytes and neurons were found to be critical, because knockout of *Nrcam* in neurons phenocopied the loss of astrocytic *Nrcam*[55]. These results indicate that NrCAM from both neurons and astrocytes interact transcellularly to negatively regulate astrocyte morphogenesis[55].

Importantly, homophilic NrCAM interaction between astrocytes and neurons also regulates inhibitory synapse formation, which was revealed by the comparison of inhibitory synapse numbers within the domains of NrCAM-depleted astrocytes and their wildtype neighbors[55]. Loss of NrCAM in astrocytes or neurons both result in a significant decrease in inhibitory synapses[55]. Interestingly, in neurons NrCAM is localized to the

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inhibitory postsynaptic specializations, because NrCAM directly binds to the postsynaptic scaffold protein, Gephyrin. In summary, through a homophilic transcellular interaction, NrCAM bridges growing astrocyte processes to inhibitory synapse development. When this interaction is impaired, astrocytes overgrow into the neuropil and disrupt inhibitory synapse formation[55].

*NRCAM* gene in humans has been implicated in polysubstance abuse[56]. A recent study showed that NrCAM regulates addiction-related neural circuits both during development and in the mature brain[57]. The decline in inhibitory synapses due to NrCAM loss of function is likely to profoundly impact E/I balance, which drive congenital preference for addictive substances. The exact pathogenic mechanisms are yet unknown; however, loss-of-function mutations in *NRCAM* impact both astrocytes and neurons by disrupting transcellular interactions. There are many other neurological diseases in which inhibitory circuits and E/I balance are disrupted. Therefore, a deeper knowledge of the molecular players at the inhibitory tripartite synapses would provide a better understanding of the pathophysiology of these diseases.

## Modes of contact-mediated astrocyte-synapse interactions

Initial characterization studies in the early 2000s about protoplasmic astrocyte morphological complexity and functional proximity to axons and dendrites[10,11,15] highlighted the potential importance of cell adhesion molecules at the tripartite synapse. New knowledge about these contact-mediated mechanisms have demonstrated the diversity and complexity of bidirectional signaling between astrocytes and neurons in development, plasticity, and disease. Importantly, there is newfound appreciation for how astrocyte morphological maturation directly regulates both excitatory and inhibitory synaptogenesis during development[37,55].

How do astrocyte-neuron interactions coordinate astrocyte process arborization and synaptic circuit development? As we mentioned above, astrocytic adhesions could boost[37] or inhibit[55] synapse formation and function. Thus, there could be at least two distinct modes of cell-cell interactions at the tripartite synapse: cooperation and competition (Figure 2)[58].

First, astrocytes and synapses may depend on each other to develop and mature cooperatively. Astrocyte morphological complexity brings the fine PAPs into close contact with the developing synapse. Transcellular adhesions between astrocytes and neurons help anchor axons and dendrites close to each other and stabilize interactions between the pre-and post-synaptic compartments, resulting in a functional tripartite synapse. When astrocytes fail to mature, for example in the case of *Nlgn2* knockout astrocytes[37], they are no longer in close proximity to the developing axon/dendrite and are unable to mediate excitatory synapse formation and maturation. Secondly, astrocyte-neuron interactions might be competitive, limiting astrocyte growth into neuronal synapses. For instance, NrCAM homophillic interactions between astrocytes and neurons negatively regulate astrocyte complexity and when this interaction is lost, PAPs overgrow. This overgrowth occurs concurrently with loss of inhibitory synapse formation suggesting that astrocytic PAP

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overgrowth may compete off forming inhibitory synapses by encroaching into the nascent synaptic adhesions[55].

## Conclusions

In this review, we highlight some of the latest advances in our understanding of how adhesion-based mechanisms control astrocyte-neuron interactions at the tripartite synapse. Furthermore, we discuss how these molecular interactions regulate concordant development of astrocytes and synapses. The findings emerging from these studies clearly demonstrate that synaptic cell adhesions cannot be solely viewed as made between neurons. Astrocytes use similar cell adhesion molecules to infiltrate into the developing neuropil and interact with synapses.

In the future, synaptic functions of cell adhesion molecules should be evaluated not just in neurons but also in astrocytes, because both cell types are integral to the structure and function of excitatory and inhibitory circuits. Another critical question that is yet to be resolved is how do cell adhesion molecules cross-talk at the interface of astrocytes and neurons? Do different molecular players, such as protocadherins and neuroligins, dynamically regulate each other's functions at the tripartite synapse? Do astrocyte contacts specialize in tune with the needs of the neighboring synapse? It would be important to answer these questions not only because astrocytes have been implicated in synaptic pathologies of a wide range of neurological diseases, but mutations in the cell adhesion molecules discussed in this review are also strongly associated with neuropathology. This suggests that aberrant bidirectional communication between astrocytes and neurons may be an underlying mechanism in the pathogenesis of many neurological diseases and could be an excellent target for therapeutics.

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## Highlights

1. Astrocytes directly contact axons and dendrites via cell adhesion molecules (CAMs)
2. Transcellular contact control synaptogenesis and astrocyte maturation concurrently
3. Dysfunction in astrocytic CAMs contribute to brain pathology and disease

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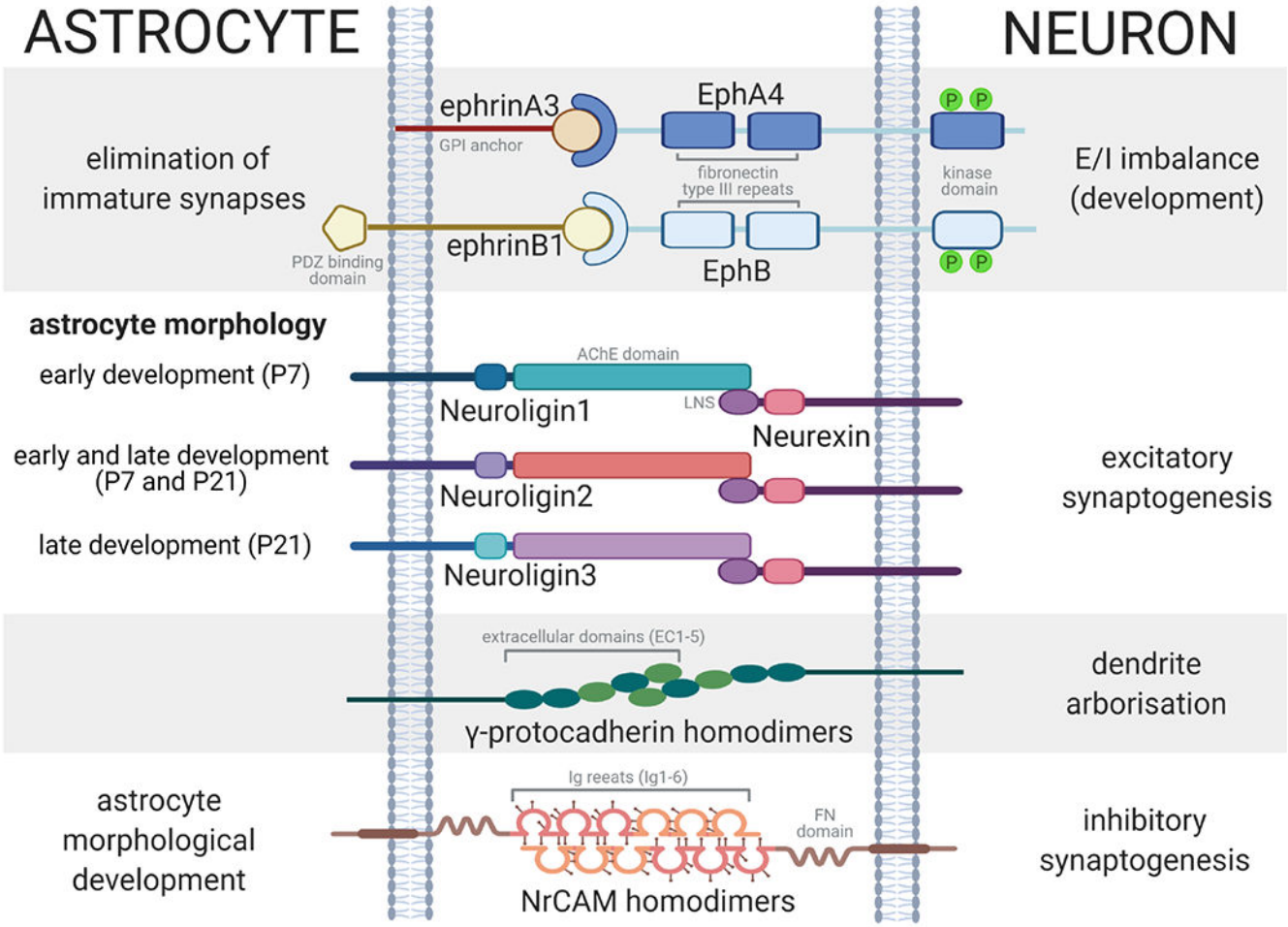


**Figure 1. Overview of cell adhesion mechanisms mediating astrocyte-neuron interactions.**

4 classes of adhesion molecules are discussed in this review. (1) Astrocytic ephrins bind to neuronal Eph receptors to negatively regulate synaptogenesis by eliminating immature synapses. ephrinAs are anchored to the astrocyte cell surface by a GPI anchor; whereas, ephrinBs have a C-terminal PDZ-binding domain that greatly augments the possiblity of astrocyte-neuron bidirectional signaling. In constrast, EphA and EphB have similar structures, comprising of extracellular fibronectin type II repeats and an intracellular kinase domain that is known to be critical for E/I balance. (2) Neuroligin-neurexin interactions are mediated by the binding of neuroligin's acetylcholinesterase (AChE) domain and neurexin's LNS (laminin, neurexin, sexhormone binding globulin) domain. Different neuroligins regulate astrocyte morphological complexity at different developmental timepoints. Collectively astrocytic neuroligin/neuronal neurexin interactions molecularly link astrocyte complexity with synaptogenesis during a critical window of postnatal cortical development. (3) γ-protocadherins trans-dimerize via binding of its extracellular domains at the astrocyte-neuron interface to regulate dendrite arborization. (4) The extracellular domain of NrCAM is composed of 6 immunoglobulin-like (Ig) repeats and several fibronectin (FN) domains. Astrocytic NrCAM forms homodimers with

neuronal NrCAM via its Ig repeats to regulate both astrocyte morphology and inhibitory synaptogenesis.

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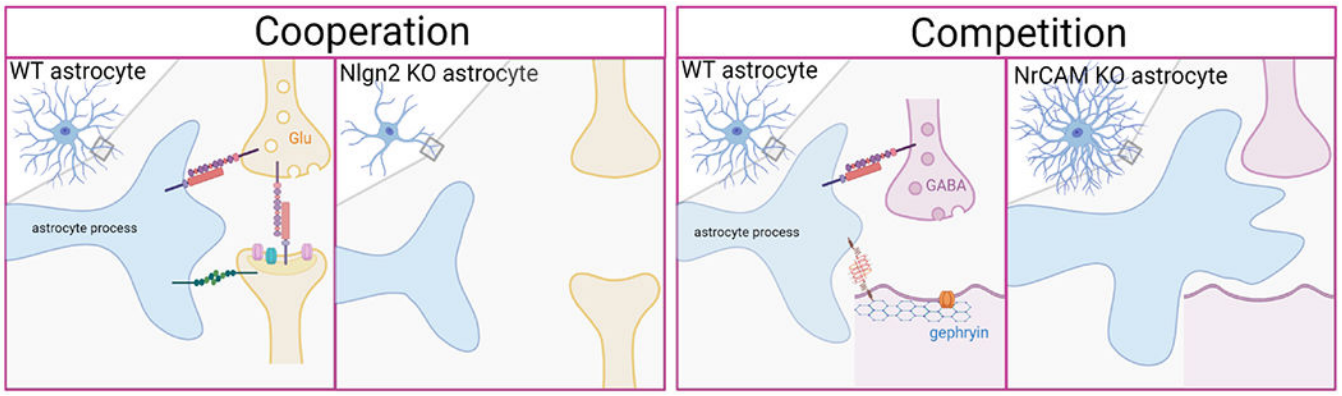


**Figure 2. Modes of astrocyte-neuron contact-contact mediated interactions.**

(Left) Astrocytes mature morphologically concurrently with synapse development. Cooperation between developing astrocytes and neurites are critical in stabilizing trans-synaptic connections, resulting in a functional tripartite synapse. (Right) On the other hand, astrocyte-neuron contacts may act as a negative regulator of astrocyte overgrowth that would otherwise encroach into the forming synapse and prevent synaptogenesis.