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The interplay between neurons and glia in synapse development and plasticity

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Abstract

In the brain, the formation of complex neuronal networks amenable to experience-dependent remodeling is complicated by the diversity of neurons and synapse types. The establishment of a functional brain depends not only on neurons, but also non-neuronal glial cells. Glia are in continuous bi-directional communication with neurons to direct the formation and refinement of synaptic connectivity. This article reviews important findings, which uncovered cellular and molecular aspects of the neuron–glia cross-talk that govern the formation and remodeling of synapses and circuits. *In vivo* evidence demonstrating the critical interplay between neurons and glia will be the major focus. Additional attention will be given to how aberrant communication between neurons and glia may contribute to neural pathologies.

Overview

The mammalian brain is a complex organ comprised of numerous cell types and greater than 1×10^{14} synapses. In broad classifications, two main cell types encompass the neural parenchyma: neurons and glia. Neurons are a heterogeneous group of electrically active cells, which form the framework of the complex circuitry of the brain. Glia comprise a class of non-neuronal cells including astrocytes, microglia, oligodendrocytes, and oligodendrocyte progenitor cells (OPCs) of the mammalian central nervous system (CNS). Schwann cells and satellite cells of the peripheral nervous system (PNS) also play analogous roles to CNS glia. Each glial cell type occupies a discrete role in the development and function of the CNS. Astrocytes and microglia, in particular, arise from separate cell lineages (neural and immune, respectively) and regulate distinct aspects of synaptic development and circuit connectivity.

The intricate communication between neurons and glia and their cooperative roles in synapse formation are now coming to light due in large part to advances in genetic and imaging tools. This article will examine the progress made in our understanding of the role of mammalian perisynaptic glia (astrocytes and microglia) in synapse development, maturation, and plasticity since the previous Current Opinion article [1]. An integration of

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Conflict of interest

Nothing declared.

past and new findings of glial control of synapse development and plasticity is tabulated in Box 1.

Glia control the formation of synaptic circuits

In the CNS, glial cells are in tight association with synapses in all brain regions [2]. In particular, astrocytes and microglia are ramified cells that extend numerous small processes that associate with synapses (Figure 1). These perisynaptic glial processes are proposed to actively participate in regulation of synaptic transmission [3]. This recognition gave rise to the ‘tripartite’ and ‘quad-partite’ synapse models, which include perisynaptic glial processes as integral parts of the synapse in addition to the neuronal pre and postsynaptic compartments [4,5]. Thus, glial cells are in prime position to monitor and influence local synaptic activity in response to synaptic signals and/or physiological states.

Studies using purified neuron and astroglial cultures revealed that neurons form few and weak synapses in the absence of glia [6] and mice in which gliogenesis is inhibited genetically display rampant neuron loss, diminished motor output [7], and altered synaptogenesis [8]. Nearly three decades of research has provided a framework whereby glia-derived secreted factors promote the formation and maturation of excitatory synapses [1,9]. Among the first identified proteins were the astrocyte-secreted thrombospondins 1–5 (TSP1–5), which induce the formation of structurally intact, but postsynaptically silent, excitatory synapses *in vitro* and *in vivo* [10]. TSPs induce excitatory synapse formation by their interaction with the neuronal Gabapentin receptor $\alpha 2\delta$ -1 [11]. Other glial secreted factors have since been identified to regulate various aspects of excitatory synapse formation including: cholesterol with apolipoprotein E (APOE) [12], glypicans 4 and 6 [13], TGF- β [14,15], chondroitin sulfate proteoglycans (CSPGs) [16,17] and TNF- α [18] (see Box 1: Tables 1 and 2 for more details). *In vitro* studies also showed that inhibitory synapses are induced when astroglial cells are present; however the identities of these factors remain to be elucidated [19,20].

Another astrocyte-secreted synaptogenic protein is hevin (a.k.a. SPARCL1), which induces postsynaptically silent excitatory synapses similar to the thrombospondins *in vitro* [21]. In the developing mouse cortex hevin specifically controls the formation of thalamocortical (thalamic neuron-to-cortical neuron) glutamatergic synapses. Hevin knockout mice display a significant loss of these thalamocortical synapses with a concordant increase in the number of intracortical (cortical neuron-to-cortical neuron) synapses [22]. Hevin functions by bridging two neuronal cell adhesion molecules, neurexin 1 α (Nrxn1 α) and neuroligin 1B (Nlgn1B), across the synapse and promotes the formation of both pre and postsynaptic specializations [23••]. There are numerous isoforms of Nrxns and Nlgns, which are thought to be the basis for the diversity of neuronal synaptic connections [24]. However, these findings show that astrocytes are capable of promoting the formation of select subclasses of excitatory connections in the brain by modifying this molecular code.

Microglia have also been shown to regulate synapse development in the brain. Compared to controls, mice with genetically ablated microglia show reduced dendritic spine formation, reduced motor-learning dependent synapse formation, and reduced excitatory postsynaptic

currents. Many of these anomalies are recapitulated with *Cremediated* microglia specific elimination of Brain Derived Neurotrophic Factor (BDNF), indicating a specific role for microglia-secreted BDNF in the formation and/or maintenance of proper synaptic connectivity [25•]. However, not all microglia ablation phenotypes are recapitulated with microglial BDNF loss, indicating the presence of other molecular mechanisms whereby microglia regulate synapse formation. BDNF is also released from astrocytes and neurons to regulate synaptic functions; future studies are needed to determine the significance of the precise source and timing of BDNF-signaling coming from each of these CNS cell types.

A looming question in the glial field is whether glial cells control where synapses are formed and whether glia can discriminate between synapses to provide specific machinery fitting to their needs. Studies using invertebrate genetic models, such as *Caenorhabditis elegans*, hint that glial cells indeed can dictate where synapses form [26] and can preferentially traffic and localize glial proteins to specific synapses to regulate neural receptive endings [27]. A single rodent astrocyte can ensheath more than 100 000 synapses from a number of different circuits; how it discerns one synapse from another and serves these synapses individually is a fascinating question and is only beginning to be explored [28]. Future studies are necessary to elucidate how the diverse glia-derived secreted synaptogenic factors are released from glia in a regulated fashion for temporal and spatial control of specific synaptic circuits.

Glia refine and remodel synapses and circuits

Glia are active participants in synaptic plasticity and are known to modulate individual synapses and circuits [3]. The function of astrocytic glutamate transporters GLT-1 and GLAST are classic examples of how astrocytes regulate glutamatergic synaptic transmission by controlling the neurotransmitter levels at the synapse [29,30]. Glutamate uptake normally occurs perisynaptically due to the localization of astrocyte processes [31]. New evidence shows that astrocyte process ensheathment is restricted to perisynaptic regions by the hemichannel protein connexin 30 (Cx30). Genetic deletion of Cx30 permits astrocyte process invasion into synaptic clefts, which prevents glutamate activation of the postsynapse and alters excitatory synaptic strength. These effects of Cx30 are independent of its channel function, suggesting that Cx30 in this context acts as a cell adhesion protein. Functionally, astrocytic Cx30 regulates long-term synaptic plasticity and hippocampal-based contextual memory [32••].

It has also been postulated that astrocytes regulate synaptic strength through the vesicular release of factors [33] including astrocyte-specific neurotransmitter D-serine, which is a co-agonist for NMDA receptors (NMDARs) [34]. However, the conclusions of some of these studies have been subject to scrutiny [35]. A new study that investigated the role of hippocampal astrocytes in regulating adult-born neuron circuit integration addressed some of these concerns. In two independent transgenic mouse lines used to inhibit vesicular exocytosis of astrocytes, adult-born neurons fail to generate mature dendritic spines only when passing through the affected astrocytes. These data argue that local vesicular release of astrocyte factors is required for synaptic integration [36••]. These phenotypes could be

mostly, but not fully, restored through exogenous addition of D-serine, suggesting that other vesicle-released astrocyte factors are involved in different steps of synaptic integration.

In addition to influencing the integration of new-born neurons, glia modulate the plasticity of existing synaptic circuits. A useful model for the study of developmental synaptic plasticity is in the mammalian visual cortex with a phenomenon known as ocular dominance plasticity (ODP). ODP is observed when monocular deprivation during a critical developmental period causes the rearrangement of neuronal wiring properties in the binocular zone of the visual cortex, such that inputs from the open eye strengthen as inputs from the closed eye weaken [37].

Numerous studies identified neuronal signaling pathways that are required for the ODP [38]; however, new studies now shed light on the critical glial contribution to ODP in mice. In response to monocular deprivation during the critical period, microglia rapidly alter their morphology and synaptic interactions in the binocular zone of the visual cortex [39]. Interestingly, elimination of the purinergic P2Y₁₂ receptor prevented these microglial ODP-dependent responses and abolished ODP [40]. How P2Y₁₂ regulates ODP remains to be elucidated.

Astrocytes also regulate ODP through the release of the synaptogenic protein hevin. Hevin knockout mice fail to make the ODP shift unlike their wild type siblings. Significantly, postnatal rescue of hevin expression, specifically in astrocytes of the visual cortex, completely restores ODP [23••]. The underlying mechanism of how hevin regulates ODP is unclear, though it may stem from the crucial role of this astrocyte-secreted protein in the formation and refinement of thalamocortical contacts [22] and/or its enhancement of NMDAR-dependent glutamatergic signaling [23••].

In addition to their role in regulating synaptic strength and plasticity, glial cells also actively refine circuits through pruning and phagocytosis of unnecessary and weak synapses. Microglia and astrocytes eliminate synapses in a developmentally regulated and activity-dependent manner. The early postnatal pruning process by microglia is dependent upon the CX3CR1 receptor and complement receptor, CR3 [41,42]. New evidence also shows that microglia engulf synaptic material in the adult brain through a CR3-dependent process by binding to soluble β -amyloid oligomers [43]. Inhibitory synapses are also pruned by microglia and are preferentially eliminated upon genetic deletion of progranulin, a regulator of complement production [44]. Astrocytes also express phagocytosis machinery and eliminate synapses through MEGF10 and MERTK pathways in an activity-dependent manner [45].

Glia sense and respond to synaptic activity

Signaling at the synapse between glia and neurons is not a one-way street, but is instead a delicate bi-directional communication required for the proper formation and maintenance of synapses and circuits. It was proposed that astrocyte vesicular release is regulated by neuronal activity [46]. Since circuit formation and integration of neurons may require the vesicular release of astrocytic factors [36••], it would appear that neurons and glia utilize an

intricate positive feedback system, whereby neurons signal through astrocytes to regulate synapse formation and plasticity ‘on-demand.’

The middle-man function of astrocytes has been detected in other hippocampal circuits. Hilar astrocytes act as the relay center between cholinergic inputs and the hippocampal granule cells. Acetylcholine activates hilar astrocyte intracellular calcium signaling presumably through the nicotinic and muscarinic receptors, where then astrocytes excite the hilar interneurons through glutamate release and cause downstream granule cell depolarization [47•]. It is still unclear how calcium signaling initiates glutamate release in this context.

Astrocytes undergo global and local calcium transients and the calcium signaling properties of these glial cells have been investigated for some time. Still our understanding of their roles in synaptic transmission and overall brain function is very limited. Organic calcium indicator dyes and now powerful genetically encoded calcium indicators (such as GCaMPs or GECIs) have been utilized to extensively study astrocyte calcium fluctuations in the brain under pharmacologic and sensory stimuli [48,49]. Focal calcium elevations in astrocytes near synapses are detected following synaptic neurotransmitter release [50] even at the level of single-synaptic stimulations [3]. Assorted calcium fluctuations have been live-imaged within single *in vivo* astrocytes including calcium oscillations in the soma, main branches, and within microdomains in the neuropil during the startle response [51]. Some calcium spikes are lost upon knockout of the major astrocyte inositol triphosphate receptor, IP3R2. However, calcium spikes still occur in IP3R2 knockout mice, which indicates that other mechanisms can regulate intracellular calcium in astrocytes [51]. What these calcium fluctuations mean and how they regulate glial cell behavior and output is still to be determined, and should be a major focus of future studies.

Microglia also sense neuronal communication and synaptic activity. Synaptic pruning by microglia is activity-dependent, as less active presynaptic inputs are preferentially eliminated over more active terminals [52]. Microglia regulate elimination of these synapses through the neuronal release of C1q, the initiator of the classical complement cascade and the complement ligands, C3 and C4 [53]. The precise mechanism of how synapses are tagged for elimination by C1q and C3 is still unclear. Do microglia directly or indirectly sense synaptic activity based on neuronal release of C1q, C3, the fractalkine ligand CX3CL1, or other proteins? Interestingly, altered synaptic pruning by microglia is tightly linked to dysfunctional brain connectivity and deficits in social behavior [54]; therefore alterations in microglia–neuron communication are likely to underlie neurodevelopmental and neuropsychiatric pathologies.

Aberrant interplay between glia and synapses in neurological disorders and diseases

A critical hallmark of most neurodevelopmental disorders is aberrant synapse formation and/or function. Since glia play crucial roles in synapse development and plasticity, it is not surprising that misregulated astrocytes and microglia underlie the pathological mechanisms at work in many neural disorders. Inclusively, glial cells may regulate the pathology of Rett

syndrome [55], Down syndrome [56], Spinal Muscular Atrophy [57], Fragile X syndrome [58] and others [59]. Surprisingly, abnormal phenotypes can be rescued or alleviated by wild type glial cells, even when the neurons still harbor the detrimental mutations.

More recent studies have identified that the communication between neurons and glial cells is affected in a number of neuropsychiatric and neurodegenerative disorders. Schizophrenia is a heritable psychiatric disorder that manifests late in adolescence to early adulthood and features a pronounced loss of grey matter and reduced synaptic structures. In humans, neuronal C4 levels strongly correlate with schizophrenia susceptibility. In mice, C4 mediates microglial synaptic elimination during postnatal development, suggesting that schizophrenia may be regulated by anomalous microglia-synapse communication [60].

The complement pathway and microglia have also recently been shown to regulate mouse models of the neurodegenerative diseases. Microglial-mediated neuroinflammation has been attributed to late stage Alzheimer's disease (AD) progression, yet a role for microglia in early stage AD had been determined. The new findings show that in mouse models of AD, microglia trigger excessive synapse loss through C1q and C3 signaling that precedes β -amyloid plaque deposition [43]. Huntington's disease (HD) is a neurodegenerative disorder of the basal ganglia where glial deficits contribute to neuronal dysfunction. In mouse models of HD, striatal astrocytes display altered membrane properties and lower Kir4.1 potassium ion channel levels compared to controls, which lead to increased neuronal excitability and dysfunction [61]. New evidence suggests that aberrant synaptic development may drive the neural pathologies measured in mouse models of HD [62], opening the door for glial misregulation in the establishment of synaptic connectivity in HD. The roles of glial cells in these and other neurological disorders will become more apparent with the advent of new tools and a dedicated glial focus.

Conclusions and perspectives

As reviewed here, glial cells, specifically astrocytes and microglia, have are active regulators of synaptic development and plasticity (Figure 2). However, there are still a number of important unanswered questions that need to be addressed in the upcoming years:

1. How does a single astrocyte coordinate the production and release of multiple synaptogenic proteins to organize regulated synapse formation and plasticity?
2. Do astrocytes serve each synapse individually or in groups, such as within the territory of a single astrocyte?
3. What is the role of astrocyte-synapse adhesions in synapse development and plasticity?
4. Do glial cells communicate with each other to harmonize synapse formation, maturation, and plasticity with synapse pruning?
5. Do other glial cells, such as OPCs, regulate neuronal and synaptic function?
6. What unknown factors do neurons utilize to communicate with glial cells?
7. How do glial cells convert these neuronal signals into functional outputs?

8. Recent studies revealed molecular and functional heterogeneity of perisynaptic glia [63,64]. These findings bring forward the question: Do different classes of astrocytes and microglia have specific functions in controlling synaptic connectivity?

In conclusion, here we summarized a number of recent high impact studies, which revealed important aspects of the communication between glial cells and synapses. These critical studies were made possible due to the advanced tools for studying glial cell biology in connection with synaptic functions, including mouse models and functional assays. However, to further our understanding on glia/synapse interactions there is now an even greater demand for more sophisticated tools to study glial cells *in vivo* and their associations with their neuronal partners in real time.

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Box 1**Summary of past and recent findings**

Summary of findings related to glial control of synapse formation and synaptic plasticity.

Table 1

Glial molecules that control synapse formation.

Molecule	Cell type	Finding	Reference
Thrombospondin	Astrocyte	Induces the formation of post-synaptically silent excitatory synapses; Functions through neuronal Gabapentin receptor $\alpha 2\delta$ -1.	[10,11]
Hevin	Astrocyte	Controls retinocollicular and thalamocortical excitatory synapse formation via bridging synaptic Nr1 α and NL1.	[21,22,23••]
SPARC	Astrocyte & microglia	Inhibits the synaptogenic function of hevin; Inhibits synaptic recruitment of GluA1 and GluA2 AMPARs.	[21,65]
Glypicans	Astrocyte	Increases synaptic levels of GluA1 AMPARs and induces excitatory synapse formation.	[13]
TGF-beta	Astrocyte	Controls excitatory synapse formation in the CNS; Regulates NMJ formation in the PNS.	[14,15]
BDNF	Astrocyte & microglia	Controls excitatory synapse formation.	[25•,68]
Gamma-protocadherins	Astrocyte	Regulates excitatory and inhibitory synapse formation through direct contact with neurons.	[20]

Table 2

Glial molecules that regulate synaptic plasticity.

Molecule	Cell type	Finding	Reference
CSPGs	Astrocyte	Determines surface AMPAR mobility and synaptic strength.	[16,17]
TNF-alpha	Astrocyte and microglia	Regulates AMPARs-dependent synaptic plasticity; Suppresses synaptic plasticity during chronic substance abuse.	[18,66,67]
Hevin	Astrocyte	Required for Ocular Dominance Plasticity (ODP) in the visual cortex.	[23••]
P2Y12	Microglia	Required for ODP in the visual cortex.	[40]
CX30	Astrocyte	Regulates synaptic contact of astrocyte processes thus controls glutamate uptake.	[32••]
D-serine	Astrocyte	Controls NMDAR-dependent synaptic integration of adult-born neurons.	[33,34,36••]

Molecule	Cell type	Finding	Reference
CX3CR1	Microglia	Controls synaptic pruning; activity-dependent.	[42]
CR3 and CR4	Microglia	Controls synaptic pruning; activity-dependent.	[53]
MEGF10 & MERTK	Astrocyte	Regulates engulfment of unwanted synapses by astrocytes; activity-dependent.	[45]

Figure 1.

Perisynaptic glial cells of the CNS: astrocytes and microglia. **(a)** A single layer 4 EGFP-filled cerebral cortex protoplasmic astrocyte from a 21-day old mouse. Astrocytes are morphologically complex cells with a single soma and thousands of tiny branches that ensheath 6–8 neuronal soma and over 100 000 synapses. **(b)** A zoomed-in micrograph of a cortical microglial cell from the CX3CR1-EGFP knock-in mouse. Microglia have a single soma with many branches that survey synaptic tissue to regulate synapse formation, elimination, and plasticity. Scale = $5\ \mu\text{m}^3$. *Source:* Image in B courtesy of Sagar Patel and Juan Ramirez.

Figure 2.

Model for regulation of synaptic connectivity and development by perisynaptic glia. **(a)** Synapse formation is controlled by several astrocyte and microglia-derived soluble factors. Astrocyte secreted factors regulate synapse formation (i.e. TSPs and hevin) and maturation (i.e. glypicans) by binding to neuronal receptors (such as Gabapentin Receptor, calcium channel subunit $\alpha 2\delta$ -1 for TSPs and Nrnx1 α /NL1B for hevin). BDNF released from microglia also control excitatory synapse formation presumably by binding to the TrkB receptor in neurons. The recruitment of pre and postsynaptic specializations is a key step in synapse development regulated by glia. **(b)** Synaptic plasticity is controlled by several glial mechanisms. Astrocytes regulate synaptic integration through vesicular release of D-serine and potentially via other factors. Synaptic plasticity is regulated in the visual cortex by astrocytic hevin and microglial P2Y12. **(c)** Elimination of weak synapses is controlled by microglia through the complement proteins C1q, C3 and C4 and their microglial receptors. Astrocytes engulf and remove unwanted synapses via MEGF10 and MERTK pathways.