Mini-Review

New roles for astrocytes in developing synaptic circuits

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Key words: glia, retinal ganglion cells, synaptogenesis, spine motility, synaptic plasticity

Introduction

Glial cells comprise 90% of the human brain.1 Glia are divided into two subtypes, the microglia which function largely as scavengers to engulf apoptotic cell debris and the macroglia comprised of oligodendrocytes that myelinate axons, and astrocytes.2 Here we focus on the astrocyte which has many well-defined functions; astrocytes control ion homeostasis, uptake glutamate at the synapse and provide metabolic support for neighboring neurons.1,3 The past several decades have seen an expansion in our understanding of astrocyte roles in the brain, specifically at the synapse where they influence formation and function.3 Here, we review in vitro experiments that led to the identification of astrocyte secreted factors and astrocyte contact effects that influence synapse formation. We then shift to a discussion of in vivo data, where we focus on the developmental role of astrocytes. Recent imaging studies have expanded our understanding of how neurons and astrocytes interact during these initial contacts, when synapses are extensively forming. We highlight recent work from our lab where we demonstrate that astrocyte contact is necessary for the synaptic maturation of developing neurons. Finally, we contrast these developmental interactions with astrocyte organization in the adult brain and discuss what these differences might mean in the context of plasticity.

Astrocytes Release Soluble Factors that Influence Synapse Formation

Despite early recognition of the large astrocyte presence in the brain, reviewed by Garcia-Marin et al., 20074 understanding astrocyte-neuron interactions has been complicated by the need to isolate and maintain neurons in the absence of astrocyte-derived trophic support. By using an immunopanning procedure developed to isolate retinal ganglion cells (RGCs) to greater than 99.5% purity, and a defined serum-free media to maintain RGCs in culture for long periods (up to several months), the first questions about astrocyte effects on synapse number could be answered.5,6 These CNS neurons, when grown in isolation exhibit few ultrastructural synapses, and little to no activity as assessed by whole-cell patch clamp recordings of EPSCs.7 Direct contact with astrocytes or astrocyte conditioned media (ACM) greatly increases both synapse number and synaptic activity.8,9 That ACM alone was sufficient to induce structural and functional synapses suggested the identity of astrocyte-released soluble factors with synapse promoting capability. And indeed this proved to be the case when such factors were identified. Cholesterol was shown to act presynaptically to increase quantal content and the efficacy of transmitter release.10 More recently, astrocyte-secreted proteins, thrombospondin (TSP) 1 and 2 were shown to increase synapse number in cultured RGCs.11 TSP-induced synapses are ultrastructurally normal, but lack postsynaptic functional activity, underscoring the complexity of the astrocyte contribution to synapse formation. While this finding purports the identity of an additional, activity inducing, astrocyte factor(s), mechanisms of TSP action also merit further investigation. For instance, under what conditions do astrocytes release TSP? How do neurons respond to TSP to increase synapse number? The developmental specificity of TSP1/2 action has been proposed given its high early postnatal expression and its downregulation in adult brain.11

Astrocyte-Neuron Signaling

While the underlying mechanisms of TSP action are still unknown, we can learn about the diversity and complexity of astrocyte to neuron signaling from other examples. For example, astrocyte-mediated PKC signaling has been demonstrated as a requirement for global synapse induction in hippocampal autapses. Here, synapse formation is mediated by direct contact with astrocytes through integrin receptors.12 Interestingly, PKC signaling does not mediate synapse formation in retinal ganglion cells,13 suggesting that different signaling pathways may underlie synapse formation in different populations of neurons. Still other signaling pathways have been described in inhibitory synaptogenesis.14 Astrocyte contact and ACM also contribute to increased inhibitory synapse number presynaptically by increasing the number of VGAT+ presynaptic terminals through TrkB independent mechanisms.

In addition to the release of molecules that mediate increases in synapse number, it has become clear that astrocytes influence the synapse through both pre and postsynaptic mechanisms to modulate functional aspects of synaptic plasticity (reviewed in ref. 15) and synaptic transmission (reviewed in refs. 16 and 17). For example, neural activity induces intracellular Ca++ oscillations in astrocytes,
which in turn release neuroactive molecules termed gliotransmitters. Additionally, glial-derived TNFalpha increases synaptic strength through trafficking AMPAR to synapses in hippocampal neurons in a homeostatic response to diminished activity.18,20 Interestingly, gliotransmitters act to both increase and decrease synaptic function. ATP has been shown to dampen synaptic transmission.21 In contrast glial-derived D-serine functioning as a co-agonist for NMDA receptors, enhances postsynaptic responses and plasticity.22,23

**Astrocyte Presence Influences Synapse Formation During Development**

Striking observations have been made about the onset of synaptogenesis and astrocyte maturation throughout the developing brain. For example, after afferent inputs from retinal ganglion cells arrive at their targets in the superior colliculus synapse formation is delayed until the time of astrocyte ingrowth, nearly one week later.8,24,25 In the cerebellum maturation of Bergmann glia is also strongly associated with synapse formation and dendrite outgrowth in the Purkinje cells.26 Similarly, the appearance of mature synapses coincides with astrocyte maturation in the rodent hippocampus. In this region, studies of developing astrocyte morphology reveal the appearance of molecularly distinct astrocytes at the end of the first postnatal week (postnatal day (P)7). These immature astrocytes possess filopodia-like processes that are highly interdigitated. A week later at P14, astrocytes display a high level of immunochemical homogeneity, and well-defined domains. Refinement continues through P28 with most adult morphologies and domains intact by P21.27 As astrocytes are maturing during the first two postnatal weeks, their processes are highly motile, providing opportunities to influence simultaneously occurring neuronal plasticity.

Recently, we found that neurons isolated from the embryonic retina remain unable to receive synapses on their dendrites in the absence of direct contact with astrocytes.13 Developmentally, astrocytes migrate into the retina along the optic nerve and contact RGCs at embryonic day (E)19.28 RGCs isolated and cultured before this migration occurs from E17 retina exhibit marked reductions in synapse number even when grown under conditions that promote synapse formation in neurons isolated at older ages. We demonstrated that these neurons were able to form, but not receive synapses: i.e., they were postsynaptically unresponsive. Direct contact with astrocytes, but not other retinal cell types restored synapse formation. Additionally, astrocyte contact produced changes in the localization of dendritically expressed neurexin and the dendritic overexpression of neurexin inhibited astrocyte contact increases in synapse formation, suggesting a mechanistic role for neurexins in mediating the effect of astrocytes on synapse formation. Interestingly RGCs purified from retina after in vivo contact with astrocytes were synaptically receptive and did not require continuous astrocyte contact, but still required soluble signals from astrocytes to form synapses. These studies further demonstrate the complex role of astrocyte contact and astrocyte secreted factors in influencing neuronal expression and localization of synaptic proteins. How these synaptic proteins are directly regulated by astrocyte signals remains an open question.

One surprising aspect of our work is the necessity of astrocytes in signaling a neuron’s ability to receive synapses. Once differentiated, neurons were thought to be intrinsically capable of synaptogenesis. However, studies in hippocampal cultures revealed a developmental delay between a neuron’s ability to form and receive synapses,29 and additional work contributed to the revision of this hypothesis by demonstrating the role of neurotrophins in synapse formation.30 We expand on this previous work and show for the first time an important contribution of astrocyte contact in altering the ability of neuronal dendrites to receive synapses. We further demonstrate that astrocyte contact is required even in the presence of other hallmarks of postsynaptically receptive neurons (i.e., saturating conditions of the neurotrophin BDNF and elaborate dendritic fields). If astrocyte contact is necessary for a neuron's dendrites to become competent to receive synapses it presents the possibility that astrocytes may highly modulate both temporal and spatial aspects of synapse formation in the developing brain. Evidence from invertebrates, demonstrates a striking glial involvement in synapse specificity.31 In the retina, perhaps similar mechanisms are in play, whereby astrocytes appear when all RGCs have been generated and astrocyte contact signals the RGCs to become postsynaptically receptive. Such a scenario would allow for a measure of control during synapse formation, helping to ensure that all RGCs are present when synapse formation in the retina begins. One important caveat is that RGCs do not form synapses with one another in vivo, so careful analysis of astrocyte interactions and RGC positioning with their endogenous targets is needed; although astrocytes have been shown to increase synapse formation between RGCs and their target neurons from the superior colliculus.8

**Astrocyte-Neuron Interactions Are Highly Dynamic**

Throughout the CNS astrocytes are intimately associated with synaptic sites.32 At sites of synaptic contact, astrocyte processes are preferentially opposed to dendritic spines when compared to presynaptic sites and also make contacts at extrasynaptic sites.33 The close apposition of astrocytes and dendrites raises the question of whether astrocytes could influence synapse shape. This is an important question because dendritic spine morphology adjusts as synapses are strengthened or weakened, in dynamic response to activity. For example, increases in spine size have been correlated with long-term potentiation (LTP)34 and similarly decreased spine length is associated with long-term depression (LTD).35 Additionally, filopodial spine morphology is a hallmark of disease.36

Recently several techniques for differential targeting of neurons and glia with fluorescent markers has allowed for real-time imaging of astrocyte-neuron interactions, revealing their highly dynamic nature.37-40 By selectively infecting astrocytes and neurons with farnesylated EGFP or RFP to induce membrane localization, Haber et al., simultaneously imaged the movement of astrocyte processes and opposing dendritic protrusions in a slice preparation. Surprisingly, astrocytic processes displayed on average greater motility than their dendritic neighbors. Despite this more dynamic behavior, astrocyte process motility was often correlated with movement in associated dendritic protrusions. As astrocytes extended or retracted, so did opposing astrocyte processes. The stabilization of a spine has been shown to be an important step in the synaptogenic process and while astrocytes are intimately associated with spines the question remains whether astrocytes may influence synapse formation by stabilizing spines. Recent work suggests this is true. Studies with two-photon microscopy that track the dynamics of astrocyte processes and the fate of dendritic protrusions reveal several
contributions of astrocyte contact. Dendritic protrusions making even transient contacts with astrocytic processes, ranging from 30 minutes to 5.5 hours in duration, exhibit longer lifetimes and more mature morphologies. Furthermore these transient contacts between dendrite and astrocyte correlate with enhanced spine stabilization and increased transition from immature filopodia to longer-lasting spines. In this study, astrocytic motility was reduced through the infection of a mutant Rac1, suggesting that cytoskeletal rearrangements underlie process motility. Astrocyte-spine interactions were also inhibited with the application of soluble EphAR/Fc or ephrin-A3/Fc. Both mutant Rac1 expression and Eph/ephrin treatment led to an increase in the number of spines with immature filopodia morphology. Interestingly, in additional experiments increasing glutamatergic tone through application of bicuculline resulted in a stabilization of dendritic processes, but no loss of astrocyte motility. These studies were performed during development when synapses are forming. Similar imaging studies occurring during synaptic remodeling associated with learning, memory and injury may reveal novel astrocyte contributions.

How are these astrocyte-neuron interactions mediated? Ephrin-A3 expression on astrocytes has been shown to make connections with its receptor EphA4R expressed on dendritic spines. EphA4R has been shown to interact with members of the Rho/Ras pathways, suggesting that EphAR/ephrin-A interactions may underlie aspects of actin-driven astrocyte motility. Nestor et al. examined EphR expression in astrocytes at both the transcript and protein level revealing that astrocytes also express EphA4R. Activation of this receptor via application of ephrin-A3/Fc increased the motility of astrocyte processes and inhibited intracellular Ca\(^{+2}\) oscillations. While EphA4R/ephrin-A3 interactions have been well-characterized, multiple Eph/ephrins are expressed in both neurons and astrocytes of the hippocampus. Perhaps these molecules influence synapse formation. Several interesting possibilities present themselves. For example, if a spine is not expressing an EphR that finds a match in a neighboring astrocyte, a likely consequence of losing astrocyte contact is a decrease in the spine’s chance for stabilization. A less stable spine is less likely to be available to engage with an axon and form a synapse. In this situation the astrocyte may act as a first-pass editing mechanism to ensure that spines are expressing the right surface molecules to meet their presynaptic partners. If this expression is absent, the nascent spine decreases its chances of being stabilized. Alternatively, astrocyte contact may contribute to stabilization of nascent synapses after axons are correctly aligned with appropriate targets. Giall guidance of synapse specificity is not unprecedented. For example, elegant studies in C. elegans demonstrate that glia can function as guideposts, directing migrating axons to their targets. Interestingly, Bergmann glia have also recently been shown to specify synaptic connections by guidance GABAergic stellate axons as they innervate their targets sites on the dendrites of the Purkinje neurons. These studies suggest a role for Bergmann glia in ensuring correct targets reach one another and thus minimizing opportunities for excess synapse formation. Are glia mediating a similar role in the other brain regions? These possibilities raise still other questions. Can a neuron’s expression of Eph/ephrins or other surface molecules help predict its final synaptic partner? Do astrocytes facilitate the synaptic pairing process by only recognizing and stabilizing certain spines based on their molecular surface expression? And if so, do future presynaptic partners exert any influence on this process? The relative specificity with which neurons find their synaptic partners and wire up, suggests that many mechanisms are in play helping to guide neurons to their targets. The large astrocyte presence in the brain, their intimate connections with neurons and their dynamic behavior while synapses are forming, make them well positioned to fill this guiding role. Tracking the interactions of astrocytes, axons, and their presumptive synaptic partners in these imaging studies might provide avenues for investigating some of these questions.

Implications for synaptic specificity notwithstanding, these live imaging studies provide compelling evidence to suggest a developmental role for astrocyte contact in priming the neuron for synapse formation, a process that is likely dependent on both Rac1/actin and EphA4R/ephrin-A3 signaling. While the bulk of imaging studies have been restricted to the hippocampus, astrocytes inhabiting other regions of the brain display similar dynamic behavior. Bergmann glia have well-characterized motility requiring actin polymerization, but not Rac1 or RhoG activity. Live imaging studies in the brainstem reveal high degrees of astrocyte motility at synaptic sites. The extent of astrocyte-synapse contact can be altered in various physiological states. For instance, in the magnocellular neurons of the hypothalamus changes in astrocyte ensheathment of synapses occur in response to certain physiological states, such as parturition, lactation, and prolonged dehydration, leading to profound changes in synaptic function. Recently, Holtmaat et al. also reported that changes in sensory input to the barrel cortex increased the stability of newly formed transient spines in a cell-type specific way. Could astrocytes be contributing to this stability, perhaps by increasing their contact with spines under these conditions? Expanding these studies to track astrocyte movement would be an exciting next step in understanding this process.

Astrocyte Organization in the Adult Brain

In the adult brain astrocytes occupy distinct territories where neighboring processes exhibit minimal overlap, yet extensive gap junction coupling provides an avenue for astrocyte communication. Single astrocyte domains are estimated to make contacts with between 300 and 600 dendrites and upwards of 10^5 synapses. Evidence for selective Ca\(^{+2}\) wave transmission among neighboring astrocytes suggests these domains may be organized into functional units, with the potential to coordinate responses to activity. Recent in vivo imaging suggests astrocyte responses to physiologic stimuli are both fine-tuned and highly selective. Perturbations of astrocytic territories and intercellular communication may underlie disease states. Indeed disruption of astrocyte domains has been associated with several mouse models for epilepsy and aberrant expression of the astrocyte gap junction protein connexin43 has also been reported in the autistic brain. The presence of non-overlapping astrocyte domains and their selective interactions with neighboring neurons present the intriguing possibility that astrocytes contribute to specifying not only the timing of synapse formation but also its location. The well-defined spacing of the astrocyte network with its capacity for intercellular communication has the potential to function as a boundary system for neurons, guiding their search for targets during development or ensuring the activity of mature synapses is appropriately contained.
In the mature brain neurogenesis continues throughout the lifetime of mammals, albeit in restricted regions. Several studies have implicated astrocyte involvement in this process. Contact with monolayers of astrocytes, but not ACM supports neurogenesis in vitro. Adult stem cells isolated from the hippocampus were found to selectively develop into neurons when contacting astrocytes from neonatal or adult hippocampus. Strikingly, mature astrocytes were competent to mediate these effects, although neurogenesis in the developing brain precedes astrocyte maturation. Astrocyte secreted factors are also likely to contribute to the induction of neurogenesis.

Conclusions

There can be little doubt that astrocytes are dynamic players in the neuronal world. Astrocytes ensheath synapses, contribute to neurogenesis, secrete soluble factors that enhance synaptogenesis and neuroactive molecules that mediate plasticity. Both astrocyte contact and secreted factors are important in regulating synapse formation and function. While mechanisms to distinguish between the two have been helpful in determining molecular underpinnings of astrocyte influence, it is unlikely that they are separated in vivo. Interactions between neuron-astrocyte likely underlie stabilization of developing synapses and transient astrocyte contact lends stability to dendritic spines, and thus may help to specify the synapse. This observation is especially striking as transient contacts are also sufficient to induce a neuron’s dendrites to engage in synapse formation. The motility of astrocytes in the developing brain has been characterized, and in some settings (hippocampus) outstrips the motility of neighboring dendrites. In combination, the dynamic interactions between astrocytes and neurons and our recent work in demonstrating astrocyte contact effects on the maturation of a neuron’s postsynaptic receptivity may provide a new lens to consider the seminal work of Müller and Best in which embryonic neurons injected into the adult visual cortex restore a state of plasticity. Perhaps one way in which these immature astrocytes contribute to plasticity is to turn on a neuron’s ability to receive new synapses. It is interesting to note that both immature and mature astrocytes retain their ability to induce neurogenesis in progenitor cells in vitro. Further imaging studies in the adult brain, if they prove astrocytes less dynamic may help explain a key component of the developing brain’s plasticity. Perhaps this is why we can retain memories for decades and perhaps this process goes awry in disease? The possibilities for understanding astrocyte involvement in synaptogenesis continue to expand with our technology, ensuring the next chapter of astrocyte research will certainly be an interesting one.

Acknowledgements

We thank members of the Ullian lab for their comments and discussion. This work was supported by the Sandler Family Fund, the Alfred P. Sloan Foundation, a March of Dimes Basil O’Connor Award, a Research to Prevent Blindness Young Investigator Award, the Autism Speaks Foundation, and That Man May See (Erik M. Ullian).

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