Role of glia in synapse development

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Recent studies suggest that glial cells regulate certain aspects of synapse development. Neurons can form synapses without glia, but may require glia-derived cholesterol to form numerous and efficient synapses. During synapse maturation, soluble and contact-dependent factors from glia may influence the composition of the postsynaptic density. Finally, synaptic connections appear to require glia to support their structural stability. Given the new evidence, it may be time now to acknowledge glia as a source for synaptogenesis-promoting signals. Scrutinizing the molecular mechanisms underlying this new function of glia and testing its relevance *in vivo* may help to understand how synapses develop and why they degenerate under pathological conditions.

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Abbreviations

ADNF	activity-dependent neurotrophic factor
CNS	central nervous system
GCM	glia-conditioned medium
NMDA	*N*-methyl-D-aspartate
NMJs	neuromuscular junctions
PSD	postsynaptic density
RGCs	retinal ganglion cells
TNFα	tumor necrosis factor α
TSCs	terminal Schwann cells

Introduction

Views on the liaison between synapses and glial cells have changed within the last few years, with once avantgardistic opinions on glial function [1] gaining a foothold in mainstream neuroscience [2–6]. Until recently, synaptogenesis has been regarded as a purely neuronal affair. Here, I summarize new evidence that glia-derived signals control the extent of synapse formation, induce postsynaptic maturation processes and help to maintain synaptic stability. Updates on aspects of neuron–glia interactions beyond the scope of this article can be found elsewhere [7–16].

Synaptic birth control by glia

Within the last years, our understanding of how neurons establish synaptic connections has greatly expanded. Genetic, biochemical and cell culture screens as well as advanced imaging techniques revealed new cellular components and mechanisms that are involved in this fundamental process (reviewed in [17–24]). A still unresolved question is, however, whether neurons form synapses autonomously or whether they require external signals. A possible source of such signals is glial cells, which support different aspects of neuronal differentiation (reviewed in [25–31]).

A series of recent papers suggests that glia control the extent of synapse formation (reviewed in [32,33]). They were prompted by the observation that soluble factors released by macroglial cells strengthen synaptic transmission in cultures of highly purified retinal ganglion cells (RGCs) without affecting neuronal excitability, survival or neurite outgrowth [34]. This effect was examined in more detail by two follow-up studies [35••,36••], which showed, in remarkable agreement, that the glial factor increased the number of synapses by about seven-fold. A subsequent paper revealed the long-sought identity of the synaptogenic activity [37••]. Surprisingly, it turned out to be cholesterol, which is produced by astrocytes and secreted in apolipoprotein E-containing lipoproteins.

This finding suggests that neurons require glia-derived cholesterol to form numerous and efficient synapses. Importantly, it raises new questions about the link between cholesterol and synaptogenesis and about brain cholesterol metabolism in general (for detailed discussions see [38,39]). Does cholesterol mimic previously described glial effects in other culture preparations [40–43,44••]? How does cholesterol promote synapse formation: does it act as a synaptogenic signal, possibly after conversion to steroids [45], or does it serve as building material? Does synaptogenesis *in vivo* depend on glia-derived cholesterol? Experimental evidence indicates that synapse formation *per se* does not require glial signals: RGCs form ultrastructurally defined synapses in the complete absence of glia [34]. The massive increase in synapse number during postnatal development, however, may require large amounts of cholesterol that neurons must import from astrocytes. This may explain why most synapses are formed after differentiation of astrocytes [1,36••,46,47], which have been shown to secrete cholesterol-rich lipoproteins [48].

A next important step will be to test these hypotheses *in vivo*. Unfortunately, ablation of astrocytes [49–51] and oligodendrocytes [52,53] in living animals causes neurodegeneration, thus precluding an analysis of synapse development. Consequently, new transgenic animal models are required to examine the link between glia-derived cholesterol and synaptogenesis. The detection of other glial factors that influence central nervous system (CNS) synaptogenesis relies on the development of new culture preparations, where glial effects on synapses can be separated from changes in neuronal survival and growth.

Glia help synapses to mature

Newborn synapses undergo a maturation process, which endows each connection with its specific transmission

properties. Recent work indicates that glia-derived signals regulate the maturation of the postsynaptic density (PSD). The aforementioned studies on purified RGCs showed that glial cells enhance the quantal size, which represents the magnitude of postsynaptic responses to individual quanta of transmitter [34,35••,36••]. In principle, this result can stem from a higher intravesicular transmitter concentration or from an enhanced postsynaptic receptor clustering. Ullian *et al.* [36••] reported that glial cells increase the size of glutamate-induced whole-cell currents in RGCs, which points clearly to a postsynaptic effect. The glial signals that promote postsynaptic differentiation in RGCs are unknown. Neuron–glia contact enhances quantal size more strongly than do soluble factors from glia-conditioned medium (GCM) [35••] and the effects of the latter are not fully mimicked by cholesterol [37••]. This suggests that soluble and membrane-delimited factors play a role in postsynaptic differentiation.

Several interesting candidates for these factors have appeared on the scene recently. One of them is tumor necrosis factor α (TNFα), which appears to be released by glial cells and to control the postsynaptic insertion of functional glutamate receptors in hippocampal neurons [54••]. Application and removal of TNFα induced a rapid increase (within minutes) and a slow decline (within hours) of the glutamate receptor density at synapses, respectively, suggesting that its continued presence is necessary to maintain functional transmission. TNFα probably does not mediate the GCM-induced increase in quantal size in RGC cultures, because this effect developed with a much slower time course [35••].

Blondel *et al.* [55] recently proposed an intriguing pathway by which glia may regulate postsynaptic receptor clustering. They showed that activity-dependent neurotrophic factor (ADNF), which is released from astrocytes upon treatment with vasoactive intestinal polypeptide [56], strengthens glutamatergic synaptic transmission in cultured hippocampal neurons by increasing the density of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors. This pathway may involve autocrine actions of neurotrophin-3, whose secretion from neurons is enhanced by ADNF and which mimics the ADNF-induced effects on NMDA receptors. Future experiments will show whether this complicated neuron–glia interplay is implemented *in vivo*.

A glia-derived signal that controls the expression of transmitter receptors has been detected in the chick retina [57]. Cultured Müller glia secrete a protein that selectively raises the expression of the M2 subtype of muscarinic acetylcholine receptors in retinal neurons *in vitro* and *in ovo*. This may explain why, during development, the M2 receptor appears after differentiation of Müller glia. To date, the identity of the glial protein is unknown.

Finally, a recent study suggests a link between glial cells and the most prominent synaptogenic factor, agrin, which is essential for the formation of neuromuscular junctions (NMJs) [25] and which may play a role in synaptogenesis in the CNS [58]. Lesuisse *et al.* [59] showed that glial signals regulate the expression of agrin. Growing rat hippocampal neurons in contact with mouse glia led to a reduction in agrin-encoding mRNA, whereas soluble factors from mouse glia halved the expression of a specific isoform, but left the total level of agrin unaffected. Interestingly, there is also evidence that Schwann cells produce and secrete agrin isoforms with receptor-clustering activity during development and after nerve injury [60•], suggesting that Schwann cells may influence the maintenance of NMJs and their re-establishment after injury.

To date, there is little evidence that glia promote presynaptic maturation. Different growth factors including neurotrophins induce this process, but it is not known whether they are secreted by glial cells *in vivo*. Soluble glial factors enhanced the efficacy of transmitter release and augmented the pool of presynaptic vesicles in cultures of purified RGCs [35••,36••]. Mauch *et al.* [37••] showed, however, that these presynaptic effects are mimicked by cholesterol, possibly by promoting the formation of synaptic vesicles.

Glia live and let die synapses

There is increasing evidence that individual synaptic connections have an intrinsic lifetime [61•,62,63], which is modulated by electrical activity and probably other, still largely unknown factors (for recent reviews see [25,64,65]). Several papers suggest that glial cells may control synaptic stability and participate in their elimination. The pioneering work of Trachtenberg and Thompson [66] showed that, in young rats, withdrawal of the Schwann cells that cover NMJs, also called terminal Schwann cells (TSCs), leads to nerve terminal loss and dispersal of postsynaptic receptor clusters. Their conclusion that TSCs are required for synapse maintenance has been corroborated by a different line of experiments. Transgenic mice, which do not generate Schwann cells due to genetic disruption of neuregulin/ErbB receptor signaling, form ultrastructurally defined NMJs during the late embryonic stage. However, these junctions disappear a few days later and the mice die just after birth. They cannot breathe because of the absence of neuromuscular transmission [67–69]. The idea that TSCs support NMJs is further underlined by the fact that the number of TSCs scales with the size of muscle endplates during development [70] and after testosterone treatment [71,72]. The observation that TSCs do not save NMJs from elimination at androgen-sensitive muscles [72] suggests that NMJs (and synapses) may differ in their stability requirements. Experimental evidence for such differences at NMJs has been presented recently [73].

A first hint that signals from glial cells stabilize interneuronal synapses came from Ullian *et al.* [36••]. Removal of glial feeding layers from cultured RGCs decreased the quantal content of evoked synaptic transmission and the number of immunocytochemically defined synapses. Future

experiments are required to identify the stabilizing factors and to determine how they work. It appears possible that they maintain synapses indirectly, for example by supporting the integrity of axons and dendrites.

Selective elimination of synapses is an important step during brain development and may contribute to structural remodeling in the adult brain [65]. A classic example for synapse elimination has been described in the cerebellum, where surplus synapses between climbing fibers and Purkinje cells are pruned to leave all but one input. A recent study showed that experimentally induced retraction of Bergmann glia processes from Purkinje cells, which had attained monosynaptic innervation, leads to reinnervation by multiple fibers, in a quarter of neurons [74••]. A still unanswered question is whether the glial processes also played a role in the prior fiber elimination. In any case, this observation supports previous hints that the astrocytic sheath around neurons limits the density of synaptic inputs (reviewed in [75–77]). To date, it is not known whether glia mark synapses for elimination. One could speculate, however, that glia release soluble factors, for example proteases, which in turn destroy the extracellular matrix components that maintain synaptic stability [78,79]. This would allow glial processes to invade the synaptic cleft and to eliminate the synapse [80].

Conclusions

Taken together, the results summarized above shed new light on the synapse–glia affair. The establishment of a synaptic contact probably relies on neuronal signals, but the massive increase in synapse number and the diverse presynaptic and postsynaptic maturation processes appear to require glia-derived components. Notably, the various types of synapses may differ in their reliance on glial components. Clearly, the next step is to define the molecular details of these interactions and to determine their relevance *in vivo*. Whatever lies ahead, we have come to realize that the intimate relationship between glia and synapses starts much earlier than suspected.

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