

# Role for Glia in Synaptogenesis

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**KEY WORDS** astrocytes; CNS synapses; development

**ABSTRACT** Nearly one-half of the cells in a human brain are astrocytes, but the function of these little cells remains a great mystery. Astrocytes form an intimate association with synapses throughout the adult CNS, where they help regulate ion and neurotransmitter concentrations. Recent *in vitro* studies, however, have found that astrocytes also exert powerful control over the number of CNS synapses that form, are essential for postsynaptic function, and are required for synaptic stability and maintenance. Moreover, recent studies increasingly implicate astrocytes *in vivo* as participants in activity-dependent structural and functional synaptic changes throughout the nervous system. Taken together, these data force us to rethink the role of glia. We propose that astrocytes should not be viewed primarily as support cells, but rather as cells that actively control the structural and functional plasticity of synapses in developing and adult organisms. © 2004 Wiley-Liss, Inc.

## INTRODUCTION

It has been estimated that individual astrocytes in the adult rodent brain may ensheath and interact with as many as 10,000 synapses (Bushong et al., 2003, 2004). While our understanding of these cells has grown tremendously during the past decade, a great deal of mystery still surrounds the possible synaptic functions of astrocytes. The traditional textbook view is that these functions are primarily passive, regulating ion concentrations, taking up neurotransmitters, and providing substrates for energy metabolism. Although this view greatly simplifies our thinking about how the brain may develop and work, it is the purpose of this review—indeed, of all the reviews in this issue—to show that this view is no longer tenable.

In this review, we will focus on recent studies that provide evidence that astrocytes actively control synaptogenesis in the developing rodent nervous system, by regulating synapse number, function, and stability. The synaptic structure of the brain has largely been sculpted by the end of the third week of postnatal development. We will argue that astrocytes are active participants in this synaptic sculpting, and that this participation culminates in a matching of astrocyte processes to synapses. By the end of this sculpting period, mature astrocytes are highly coupled in syncytial networks that propagate  $Ca^{2+}$  waves (see review by Schipke and Kettenmann, 2004, *this issue*), and

modulate the functioning of synaptic circuits by releasing gliotransmitters (see reviews by Haydon, 2001, *this issue*). These new studies provide strong evidence that astrocytes actively mold neural circuits during development and then continue to actively participate in the proper functioning and plasticity of these circuits.

We will begin by reviewing *in vitro* studies demonstrating that glial cells enhance the formation of functional synapses in the CNS and PNS, and then summarize recent studies that provide support for the idea that glia also regulate synaptogenesis in the developing brain.

## ASTROCYTES PROMOTE SYNAPTOGENESIS BETWEEN CNS NEURONS IN VITRO

The ability of neurons to form synapses has long been assumed to be intrinsic to neurons. Neurons constitutively express a large number of synaptic proteins needed for synaptogenesis (Scheiffele, 2001). However, most CNS neurons innervate their target areas at least

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Received 28 November 2003; Accepted 15 April 2004

DOI 10.1002/glia.20082

Published online  
in Wiley InterScience (www.interscience.wiley.com).

in Wiley InterScience (www.interscience.wiley.com).

one week before they form most of their synapses (Pfrieger and Barres, 1996). Interestingly, synaptogenesis is delayed until about the same time that astrocytes are generated. For instance, retinal ganglion cells (RGCs) reach their target in the superior colliculus beginning around embryonic day 16 (E16) and most neurons have reached the colliculus by the day of birth, postnatal day 0 (P0). Despite the arrival of axons in their target area, there is a delay of about a week before the majority of synapses have formed in the superior colliculus (Lund and Lund, 1972). Precisely during this delay period, during the first postnatal week, astrocytes are born and proliferate in the superior colliculus (Ullian et al., 2001). This delay period, along with the spatiotemporal correlation between synaptogenesis and astrocyte appearance, strongly suggests the possibility that extracellular signals from astrocytes could provide a signal necessary to trigger synapse formation. Such a delay might greatly help synchronize the competitive interactions between presynaptic neurons as they fight to establish their ultimate synaptic territory, by ensuring that all axons have reached their targets before the competition begins.

To test directly whether neurons by themselves are able to form synapses, Pfrieger and Barres (1997) studied the ability of purified CNS neurons to form synapses when cultured in the presence or absence of astrocytes. These investigators used retinal ganglion cells (RGCs) because methods were available to purify them to greater than 99.5% purity and then culture them in defined serum-free medium in the absence of other cell types. Importantly, the serum-free medium contained several neurotrophic factors that ensured equally high rates of survival and neurite outgrowth, regardless of the presence of astrocytes. These investigators found that RGCs cultured in the absence of astrocytes, even after many weeks of culture, exhibited very little spontaneous synaptic activity when excitatory postsynaptic currents (EPSCs) were measured by patch-clamp. In contrast, the RGCs exhibited high levels of synaptic activity when they were cultured in the presence of a feeding layer of astrocytes or in astrocyte-conditioned medium (ACM) (Pfrieger and Barres, 1997). Even when RGCs were co-cultured with purified superior collicular neurons (their normal target neuron), little synaptic activity was observed unless astrocytes were present. The ability of astrocytes to enhance synaptic activity depends on their release of a soluble proteinaceous signal and is mimicked by Schwann cells (Ullian et al., 2003b), but not fibroblasts or microglia (Pfrieger and Barres, 1997; Ullian et al., 2004). Similarly, purified spinal motor neurons exhibit little synaptic activity unless astrocytes or Schwann cells are present (Ullian et al., 2004).

Taken together, these findings reveal that astrocytes and other perisynaptic glia, such as Schwann cells, release soluble proteinaceous signals that profoundly enhance synaptic activity by nearly 100-fold. Is the ability of astrocytes to increase synaptic activity ac-

counted for by an increase in synapse number or synapse function, or both? To determine this, these investigators then examined the effects of astrocytes on the number of synapses that formed between RGCs *in vitro*. As measured by immunostaining with pre- and postsynaptic markers, as well as by electron microscopy, astrocytes were found to induce a 7-fold increase in synapse number between RGCs (Nagler et al., 2001; Ullian et al., 2001). These synapses were ultrastructurally normal (Ullian et al., 2001) and they were also functional. When their function was investigated by patch-clamp recordings from autaptic neurons, clonal density neurons that have been allowed to synapse upon themselves, there was a very close correlation between the number of synapses counted by immunostaining and the number of synapses estimated by measuring the quantal content of the evoked response—a measure of the number of functional synapses on each neuron. This close agreement between the number of synapses estimated by these two procedures indicated that each of the synaptic puncta observed by double immunostaining likely corresponds to the site of a single functional synapse. These synapses were presynaptically functional as shown by FM1-43 imaging and uptake of an anti-luminal domain synaptotagmin antibody, both measures of vesicular recycling. They were also postsynaptically functional, as shown by the amplitudes of mini-excitatory postsynaptic currents (mini-EPSCs) measured in the presence of the sodium channel blocker tetrodotoxin (TTX).

Taken together, these findings show that astrocytes profoundly increase the number of synapses that form between CNS neurons *in vitro*. They also provide striking evidence that the number of synapses a neuron can form is not solely determined by the intrinsic properties of that neuron but can be powerfully controlled by extracellular signals. The 7-fold increase in synapse number, however, is not as large as the nearly 100-fold increase in synaptic activity induced by astrocytes, raising the question of whether astrocytes also enhance synaptic function. This question will be further addressed below.

While the studies discussed above raise the prospect that glia are important regulators of synaptogenesis during development, the question remains whether glia can have similar roles in the adult brain; indication that they might, comes from work on neuronal stem cells. Stem cells from the adult hippocampus need to be signaled to differentiate into neurons. One source of this signal comes from hippocampal astrocytes (Song et al., 2002a). Once the stem cells have differentiated into their appropriate neuronal cell types, however, the neurons need an additional signal from astrocytes in order to integrate into synaptic circuits (Song et al., 2002a,b). This astrocyte signal appears to be instructive for an increase in synapse number in the fully differentiated neurons. This work suggests that astrocytes continue to play a role in the synaptogenesis and plasticity occurring in the adult brain where stem cells are continuously generating new neurons in the hip-

pocampus that presumably need astrocyte signals to make synapses.

### SCHWANN CELLS ALSO ENHANCE SYNAPTOGENESIS

New studies indicate that glia also play a role in PNS synaptogenesis. Schwann cells (SCs) strongly induce the formation of functional glutamatergic synapses between spinal motor neurons (MNs) in culture (Ullian et al., 2004). Similarly, *Xenopus* MNs in culture form few synapses with muscle cells, but SC-conditioned medium induces many synaptic puncta between the MNs and muscle (Peng et al., 2003). These results indicate that SCs secrete synapse-promoting factors that can increase the number of neuromuscular junction (NMJ) synapses in vitro. How do SCs increase synapse number in MN-muscle co-cultures? The authors show that SC factors lead to an increase in agrin expression by the cultured MNs. Agrin is a critical protein for the clustering and maintenance of postsynaptic nicotinic acetylcholine receptors (nAChRs) (McMahan, 1990). Thus SC may promote synaptogenesis by instructing neurons to synthesize a factor necessary for mature NMJ formation. Indeed, Peng et al. (2003) speculate that SCs switch MNs from a growth state to a synaptogenic state. This result raises the question of whether glia in vivo can act as switches, converting axons from growth states to synaptogenic states. If so, then glia could play roles in the both the formation and stabilization of synapses by switching and maintaining axons in the proper state.

Do SCs also play a role in promoting synaptogenesis in vivo? Trachtenberg and Thompson (1997) found that the addition of the neuregulin glial growth factor II (GGF2) to developing NMJs resulted in SC process extension and migration away from the synapse. In conjunction with the migration of SCs away from synaptic sites, the nerve terminals retracted from the synapse and regrew along with the SCs away from postsynaptic sites. The motor neuron axons were unable to make synapses onto muscle until the GGF2 was removed. Is GGF2 separately affecting both the SCs and MN axons to leave the synapse and reenter a plastic growth state, or is GGF2 affecting only SCs by inducing them to become synaptogenic? The SCs are thought to directly instruct the synaptic remodeling that occurs in vivo because SCs growing onto terminals after transplantation onto the muscle, and in the absence of additional GGF2, also cause the withdrawal and remodeling of synapses. Thus, these investigators concluded that SCs play an important role in the formation of the NMJ synapse. If this is true then synaptogenesis at the NMJ should be impaired if Schwann cells are directly eliminated. Two recent studies provide evidence that this is the case. First, in *erbB2* deficient mice there is a failure of the Schwann cell lineage to develop, which is accompanied by impaired development of the junctional folds of NMJ synapses

(Lin et al., 2000). Second, when perisynaptic Schwann cells are ablated by complement lysis in live frog muscles, formation of synapses was dramatically reduced and existing synapses underwent retraction (Reddy et al., 2003). Taken together, these results indicate that SCs play a part in synaptic plasticity by guiding, forming, and maintaining the mature NMJ in vivo.

### WHAT IS THE MECHANISM BY WHICH ASTROCYTES AND OTHER PERISYNAPTIC GLIA INCREASE SYNAPSE NUMBER IN VITRO?

We have used the term “synaptogenesis” to refer to any mechanism that increases synapse number. Do astrocytes enhance synaptogenesis by inducing the formation of new synapses or by stabilizing existing synapses? To find out, Ullian et al. (2001) first asked whether the glial-induced synapses would be maintained if the glial cells were removed. They allowed synapses to form by co-culturing purified RGCs with a feeder layer of astrocytes cultured on a removable insert. After 1 week, many synapses had formed, and they then removed the astrocyte insert. After an additional week, they examined the neurons by immunostaining and patch clamp recording. Using both measures, they found that the majority of synapses disappeared when the astrocytes were removed. These findings provide evidence that astrocytes are required for synapse stability, ensuring synaptic maintenance. Thus one way that astrocytes may increase synapse number is to enhance synapse stability. It remains likely that astrocytes also enhance the rate of formation of synapses, the relative contribution of astrocytes to synapse formation and stabilization need to be further studied in the future by time-lapse recording. However, since synapses are specialized cell adhesions, distinguishing formation and maintenance may be a semantic issue if what glial cells are doing is secreting a protein necessary to both induce and maintain this adhesive event.

Thus, the molecular identity of this proteinaceous activity is an important remaining question. A number of neurotrophic signals, including some released by astrocytes, have previously been suggested to enhance synapse formation; however because the strong survival and neurite outgrowth promoting effects of many of these signals is typically a confounding issue, it is not entirely clear if these signals are simply enhancing the number of synapses by increasing the number of neurons or processes. This is generally a difficult issue to address experimentally because the signals that control the survival of most CNS neurons are still poorly understood. Thus the identification of astrocyte-derived signals that promote synaptogenesis is an important area for further research. In recent studies, astrocyte-derived cholesterol bound to apolipoprotein E has been suggested to play a role in inducing functional synapses (Mauch et al., 2001; see below). Recent stud-

ies have also lead to the identification of an astrocyte-derived protein that is necessary and sufficient for their ability to enhance synaptogenesis by RGCs in vitro (Christopherson et al., 2003 submitted). Manipulation of the levels of this protein, or its receptors, promises to be a useful approach in future studies directed at investigating the role of astrocytes in synaptogenesis in vivo.

### ASTROCYTES CONTROL THE FUNCTION OF DEVELOPING SYNAPSES IN VITRO

We have so far reviewed the ability of astrocytes to induce the formation of structural synapses in vitro. In this section, we will next turn to recent studies providing evidence that astrocytes are also essential for the functioning of these newly-formed synapses (other reviews in this issue will address the continued role of astrocytes in regulating synaptic function within a mature CNS). As we will see, there is evidence that astrocytes can powerfully control both pre- and postsynaptic function.

#### Presynaptic Function

In a recent paper, Mauch et al. (2001) identified cholesterol as one of the astrocyte-derived signals that induce the formation of functional synapses between RGCs in culture. They found that astrocyte-derived cholesterol enhances synaptic activity by markedly increasing the quantal content of evoked responses in autaptic RGCs. This can be explained by an increase in the number of release sites. Cholesterol likely functions by increasing the number of synaptic vesicles, as it is a limiting factor in vesicle formation and is required for synaptic vesicle curvature (Thiele et al., 2000), and by increasing the formation of the vesicle release machinery, which is transported along axons and associated with vesicles being sequestered at mature synapses (Zhai et al., 2000). Because cholesterol is also necessary for lipid raft formation, its concentration may control raft-associated signaling processes that regulate synaptic function. The authors proposed that there is a cholesterol shuttle between astrocytes and neurons and that astrocytes are the primary source of cholesterol when co-cultured with neurons in vitro (Pfrieger, 2003).

One limitation of these studies is that most of the experiments were performed on autaptic neurons cultured at clonal density and thus were possibly in highly cholesterol-starved condition. Both neurons and astrocytes can synthesize cholesterol, but as neurons actively import and export cholesterol (Dietschy and Turley, 2001), any cholesterol produced by the autaptic neurons might have been released and diffused away quickly. Thus an important question for future studies is the extent to which astrocyte-derived cholesterol is an important source for neurons in vivo, where all the

cell types are normally at high density. Another important finding in the Mauch et al. (2001) work is that whereas cholesterol greatly enhanced presynaptic function it had no effect on postsynaptic function, and its effects in increasing synapse number appear to be smaller than the effects of live astrocyte feeding layers.

#### Postsynaptic Function

These observations suggest that many astrocyte-derived signals remain to be identified that regulate synapse formation, maintenance, and postsynaptic function. In this regard, an astrocyte-derived protein that is sufficient to induce the formation of ultrastructurally normal synapses that are presynaptically active, but postsynaptically silent has recently been identified (Christopherson et al., 2003; Ullian et al., 2003; submitted). The identification of this astrocyte-derived signal allows the effects of astrocytes on synapse number and synapse function to be clearly separated, and raises the question of the astrocyte-derived signal(s) necessary to induce postsynaptic function (see Figure 1). By studying postsynaptic function of hippocampal neurons in culture and in slices (Beattie et al., 2002) found that the glial-derived cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) significantly increases postsynaptic efficacy by increasing surface levels of GluR1 AMPA receptors at synapses and that neutralizing TNF- $\alpha$  had the opposite effect both in hippocampal culture and slices. Synaptic strengthening through mechanisms such as LTP are currently thought to involve the recruitment of GluRs to postsynaptic sites, ultimately leading to the strengthening and stabilizing of synapses. Are astrocytes actively contributing to LTP through TNF- $\alpha$ , or can astrocytes bypass the NMDAR-dependent LTP that occurs during development and learning, perhaps acting in concert with more established, NMDAR-dependent mechanism of synaptic strengthening? This result raises interesting questions about the role of glia in the formation, stabilization, and elimination of synapses during development as well as after injury when microglia become important contributors of TNF- $\alpha$ . Further studies will reveal the role of glial cytokines in the developmental and activity-dependent mechanisms that change synaptic structure and number.

The ability of astrocytes to regulate postsynaptic function is easily discernable in our preparation of highly purified RGCs in culture. Astrocytes enhance the amplitude of mini-EPSCs by 3- to 5-fold (Pfrieger and Barres, 1997; Ullian et al., 2001). Glutamate is the mediator of these synaptic events, but the postsynaptic receptor detecting the released glutamate is not likely GluR1 but other AMPA receptors such as GluR2 as well as kainate receptors (unpublished observations). TNF- $\alpha$  is unable to enhance postsynaptic function of the synapses between RGCs, indicating that an as yet to be identified astrocyte-derived signal is responsible. Interestingly, neuronal activity can apparently en-

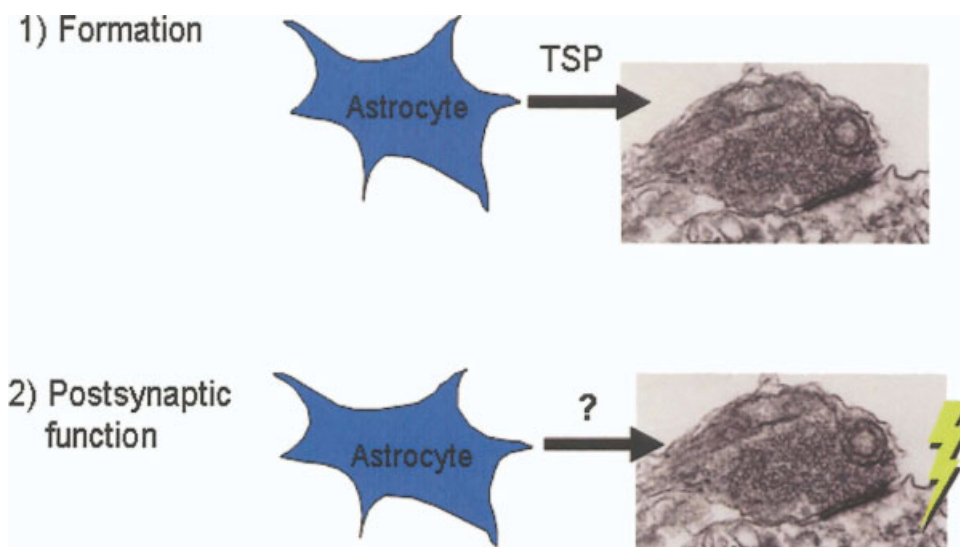


Fig. 1. Astrocytes induce formation of fully functional synapses in two steps. In a first step in synapse formation, astrocytes secrete a proteinaceous signal thrombospondin (TSP), that increases synapse number in vitro (Signal 1). This protein has also been suggested to be cholesterol bound to ApoE, which appears to be particularly important for presynaptic maturation. In addition, another astrocyte-derived protein is needed to induce synaptic adhesions (Christopherson

et al., 2003). In a second step, astrocytes produce an as yet unidentified signal(s) that increases the function of synapses by increasing the postsynaptic response of neurons (Signal 2), converting them from a "silent" state to a fully active state. Astrocytes may also detect neuronal activity and respond to activity by increasing their release of synapse-promoting factors.

hance release of astrocyte-derived signals that promote the formation of functional synapses (Wark et al., 2002), suggesting that activity-dependent neuron-glia interactions may help control the formation and function of synapses.

Astrocytes can also regulate  $\gamma$ -aminobutyric acid (GABA)ergic transmission in vitro. Astrocytes in vitro can alter the chloride ion gradient in developing CNS neurons converting GABAergic inputs from excitatory to inhibitory and providing a mechanism whereby astrocytes could profoundly alter circuit properties in the developing brain (Li et al., 1998). In addition, astrocyte contact in vitro leads to an increase in GABA currents on developing neurons (Liu et al., 1996). Astrocytes appear to do this by increasing receptor density with no change in the channel properties. These results are noteworthy because of the profound effect inhibitory currents can have on the modulation of plasticity. For instance, the appearance of inhibitory inputs has an instructive role in terminating critical period plasticity (reviewed in Hensch, 2003).

#### REGULATION OF SYNAPTOGENESIS BY ASTROCYTES WITHIN A DEVELOPING BRAIN

The developing brain undergoes profound changes in synapse number, function, and stability that are temporally and spatially coincident with the development of astrocytes. Above, we have reviewed the many recent in vitro studies that together show that astrocytes are powerful controllers of changes. Could astrocytes play

a role in the sculpting of synaptic structure and function within the developing or even adult brain? In this section, we review some recent studies in several different regions of the brain that provide strong support for this possibility.

#### Synaptic Plasticity in the Hypothalamus

An intriguing example of the role of astrocytes in regulating synaptic plasticity comes from the study of the hypothalamic hypothalamo-neurohypophysial system (HNS). Activation of this system by physiological stimuli such as parturition or lactation leads to a fast coordinated withdrawal of astrocytic processes that normally lie between neuronal cell bodies and innervating dendrites and the somatodendritic synapses (Hatton, 1997). One consequence of astrocyte withdrawal is a buildup of glutamate concentration at the synapse, as astrocytes are no longer present to clear the glutamate. The increased glutamate concentration in the synaptic cleft activates presynaptic glutamate receptors, dramatically altering neuronal excitability (Piet et al., 2002). Moreover, astrocyte processes cover the postsynaptic membrane, after astrocyte withdrawal there is a significant increase in the number of GABAergic, glutamatergic, and noradrenergic synapses as postsynaptic target sites are revealed (Theodosis and Poulain, 2001). This suggests that there may normally be competition between astrocytes and neurons for postsynaptic target space. Thus, astrocyte response to environmental stimuli may be a critical component of some forms of structural and functional

plasticity *in vivo*. This system demonstrates the potentially powerful role astrocytes play *in vivo* in regulating structural and functional plasticity.

Another interesting aspect to hypothalamic astrocytes is that they highlight the diversity of astrocytes throughout the brain, and the potential significance of that diversity. For instance, astrocytes in the arcuate nucleus undergo morphological changes in response to testosterone during development, coincident with a reduction in the number of spines on arcuate neurons. Interestingly, in the adjacent ventromedial nucleus astrocytes do not respond to testosterone and there is no concurrent reduction in spine density in response to testosterone (Mong and McCarthy, 1999). This indicates that distinct regions may contain distinct astrocytes with very different responses to factors and functional properties. This illustrates something not often considered, which is that not all astrocytes are the same. Indeed, astrocytes may play different roles throughout the brain much as neurons do. Understanding the diversity of astrocytes may ultimately help us to understand differences in the functions of various brain regions.

### Synapse Elimination in the Cerebellum

Another example of the role of glia in regulating synaptic structure comes from studies in the cerebellum. Previous culture studies have shown that glia dramatically control synaptogenesis and synapse elimination in cultured cerebellar neurons (Seil, 2001). A recent study suggests that glia function similarly *in vivo*. Bergmann glia normally surround climbing fiber (CF) synapses on Purkinje cells in the cerebellum. These glia respond to synaptic activity with  $\text{Ca}^{2+}$  transients (Matyash et al., 2001) and contribute to synaptic function by clearing synaptically released glutamate (Bergles et al., 1997; Bordey and Sontheimer, 2003). But do they control synaptic structure? To find out, Iiono et al. (2001) took advantage of the fact that Bergman glia express  $\text{Ca}^{2+}$ -permeable glutamate receptors (GluRs) containing GluR1 and 4 but not GluR2 subunits. Expression of GluR2 is sufficient to make these receptors  $\text{Ca}^{2+}$ -impermeant. They expressed the GluR2 subunit in Bergman glia in 3-week-old rats *in vivo* using an adenoviral vector, thus inducing them to express  $\text{Ca}^{2+}$  impermeable GluRs. Remarkably, this expression of GluR2 led to the retraction of glial processes that normally surround synapses. During normal development, Purkinje neurons are multiply innervated by CFs but undergo synapse elimination to a mono-innervated state. However this glial retraction lead to persistent multiple innervation by CF inputs on single Purkinje cells. What is particularly interesting about this observation is that this synapse elimination process is strongly activity-dependent (Rabacchi et al., 1992; Kakizawa et al., 2000; Hashimoto and Kano, 2003). Taken together with the hypothalamic findings discussed above, they also raise the possibility that glia

may eliminate synapses by competing with synapses for postsynaptic target area, as withdrawal of glia is sufficient to increase synapse number. Most importantly, these findings directly implicate Bergmann glia as actively involved in activity-dependent synapse elimination within the developing brain.

### Spine Morphology in the Hippocampus

Astrocytes ensheath synapses throughout the hippocampus (Ventura and Harris, 1999) and new work shows that they help to control dendritic spine morphology *in vivo* (Murai et al., 2003). These investigators found that, in the hippocampus, astrocytes and their processes express ephrin A3, while neurons express the ephrin receptor ephA4. They also found that this receptor-ligand interaction regulates spine shape by inhibiting spine extension. Soluble ephrin A3 is sufficient to cause spine retraction in hippocampal slice cultures whereas transfecting neurons with a kinase inactive ephA4 results in longer spine extension. An ephA4 knockout mouse shows similar extension of spines in hippocampal slice cultures, indicating that this receptor is required to maintain spine morphology. This work raises the possibility that by regulating spine shape, astrocytes may play a role in maintaining synaptic structure and number. This study is also important because it illustrates that bidirectional, contact-mediated, signaling may be occurring between astrocytes and neurons, since the ephrin/eph system is bidirectional. Could there be signaling from neurons to astrocytes through ephrins or other bidirectional signaling systems that maintains astrocyte morphology, function, or even survival?

In addition to the astrocyte effect on spine morphology, there is evidence that astrocytes can regulate synapse number in function in hippocampal neurons as well. For example, hippocampal cultures grown in the absence of astrocytes display low levels of synaptic activity, as measured by whole cell patch-clamp recordings. The addition of astrocyte feeding layers or astrocytes in contact with the neurons greatly increases activity. Interestingly, only astrocyte contact with the hippocampal neuron is sufficient to allow long-term potentiation of synaptic inputs, apparently through the release of D-serine from the astrocyte (Yang et al., 2003). Another example of the importance of astrocyte contact comes from the recent work of (Hama et al., 2004), who found that hippocampal neurons grown at low density will not form numerous synapses unless the neuron contacts an astrocyte. Contact of any part of the neuron with an astrocyte appears to be able to allow synapse formation along the entire neuron. Astrocyte contact apparently activates neuronal protein kinase C (PKC), leading to an enhancement of synaptogenesis on the cultured neurons.

### Critical Period Plasticity in the Visual Cortex

One of the most striking examples supporting an active role for glia in synaptic plasticity in the developing brain comes from studies of the role of astrocytes in ocular dominance plasticity in the primary visual cortex. During development, inputs corresponding to the visual inputs from both eyes converge and establish permanent connections in a shared area of cortex. This sharing of cortex is an activity-dependent event, requiring active inputs from both eyes. If the input from one eye is reduced by forcing that eye closed (monocular deprivation), the input from the open eye takes over the territory normally shared by the two eyes, while the input from the closed eye is lost. This plasticity of synaptic inputs, termed ocular dominance plasticity, only occurs during the first 4–7 weeks in cat visual cortex, indicating that there is a critical period for plasticity to occur. If, for example, the deprived eye is opened again after the critical period extending to 7 weeks, the deprived eye cannot regain the territory it lost, whereas it can if reopened before the end of the critical period. These events are well studied and known to depend on neuronal activity, NMDA receptors, and inhibitory input (Hensch, 2003).

The participation of glia is not generally invoked in models of critical period plasticity, yet there is striking evidence that astrocytes could play an important, even essential, role. To test their possible involvement (Muller and Best, 1989), injected immature astrocytes from newborn kitten into the visual cortex of adult cats long after termination of the critical period. Amazingly, this transplantation of immature astrocytes into adult cortex induced the reappearance of ocular dominance plasticity. In addition, the immature phenotype of astrocytes correlates temporally with the critical window for ocular dominance plasticity and maneuvers such as dark rearing that prolong ocular dominance plasticity also prolong astrocyte immaturity, as assessed by the expression of glial fibrillary acid protein (GFAP), a marker that appears in older astrocytes (Muller, 1990). The implication of this work is that astrocytes play two roles: immature astrocytes are instructive for synaptic plasticity, while mature astrocytes may help limit synaptic plasticity.

Is there any evidence that mature astrocytes limit plasticity? Pizzorusso et al. (2002) examined the role of an extracellular matrix (ECM) molecule secreted by mature astrocytes, chondroitin sulfate proteoglycan (CSPG), in the mature visual cortex. These investigators found that the appearance of CSPGs in the visual cortex is temporally correlated with the end of the critical period and that their appearance could be delayed by dark rearing, suggesting that astrocyte maturation and the termination of ocular dominance plasticity may be linked. To test this possibility, Pizzorusso et al. (2002) dissolved the CSPG network in the brain with a specific enzyme. Dissolving CSPGs lead to the reemergence of the critical period, much as Muller and Best (1989) found in injecting immature astrocytes. This work implies that astrocytes can signal the end of the critical period by laying down the ECM molecule

CSPG and altering synaptic plasticity. What accounts for the opposing actions of the two stages of the astrocyte lineage, in one case extending the critical period and in the other stopping it? Immature astrocytes, which correspond closely to the astrocytes used in most culture studies of synaptogenesis reviewed above, may promote synaptic plasticity by secreting molecules that promote synapse formation and function, whereas mature astrocytes may lay down ECM molecules that lock synapses in place, thus terminating structural plasticity. Astrocytes thus appear to produce some powerful stuff that can dramatically control synaptic plasticity.

### CONCLUSIONS

In summary, recent studies have shown that astrocytes are necessary for the formation, function, and stability of CNS synapses *in vitro* and have provided increasingly provocative evidence that astrocytes also actively participate in synaptic plasticity within the developing brain. The molecular mechanisms by which astrocytes control synapse formation and function *in vitro* are beginning to unfold and will provide a crucial avenue for further exploration of the synaptic roles of astrocytes *in vivo* in future studies.

It is increasingly hard to resist the conclusion that astrocytes are actively involved in activity-dependent synaptic plasticity within the developing brain. Current models cite the critical role of neuronal activity-dependent changes that lead to strengthening and elimination of synapses (Malenka and Nicoll, 1997). While neuronal activity plays a decisive role in much of the structural plasticity throughout the nervous system, the precise mechanisms leading to the formation and withdrawal of synapses remains a mystery. Synaptic modeling in the developing brain happens in three sequential steps. First, structural synapses are formed. Second, some of these synapses are converted from silent to active synapses. Initially, silent synapses lack AMPA receptors but, through activity-dependent changes, some of these synapses can insert postsynaptic AMPA receptors and become active (Malenka and Nicoll, 1997; Petralia et al., 1999). Third, unwanted synapses are eliminated. The studies we have reviewed provide evidence that astrocytes may be necessary for each of these three steps, and that neuronal activity may regulate the ability of astrocytes to control synapse number, function, and elimination.

Why bring glia into plasticity at all? Why complicate things with a third party? One reason may be that astrocytes provide an important mechanism to integrate signals from many different neurons, providing a local mechanism to coordinate which synapses are strengthened or eliminated during development, and then to help coordinate the function of groups of neurons in adulthood. Perhaps astrocytes themselves are actively matched to synapses, allowing specific glial circuits to influence neuronal function. Although astrocytes are highly coupled to each other by gap junctions, it is not necessarily the case

that these glial interconnections are random. Another possibility might simply be that as the complexity of the brain increases so does the need for additional regulation of the timing and modulation of synaptic plasticity. Immature astrocytes are present only during developmental synaptogenesis, initially inducing functional synapses. But as they differentiate into mature astrocytes, synaptic structure and perhaps to some extent postsynaptic function become stabilized and the mature astrocytes begin to secrete gliotransmitters such as D-serine that modulate synaptic function (Boehning and Snyder, 2003). Finally, astrocyte heterogeneity in different regions of the brain may allow synaptic structure or function to be molded differently in various brain regions. Bringing this diverse population of glial cell types into the plasticity loop may provide an additional level of control in a highly complex system. Whatever the case, the studies we have reviewed provide compelling evidence that glial cells can no longer be regarded as passive support cells, but are active participants interacting together with neurons to control synaptic development and function.

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