

Roles of glial cells in synapse development

Frank W. Pfrieger

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Abstract Brain function relies on communication among neurons via highly specialized contacts, the synapses, and synaptic dysfunction lies at the heart of age-, disease-, and injury-induced defects of the nervous system. For these reasons, the formation—and repair—of synaptic connections is a major focus of neuroscience research. In this review, I summarize recent evidence that synapse development is not a cell-autonomous process and that its distinct phases depend on assistance from the so-called glial cells. The results supporting this view concern synapses in the central nervous system as well as neuromuscular junctions and originate from experimental models ranging from cell cultures to living flies, worms, and mice. Peeking at the future, I will highlight recent technical advances that are likely to revolutionize our views on synapse–glia interactions in the developing, adult and diseased brain.

Keywords Astrocytes · Neurodegeneration · Neuroglia · Neuromuscular junction · Schwann cells · Synaptogenesis

Introduction

Establishing and maintaining connections is crucial throughout life and often requires help from third parties. Increasing evidence suggests that this also applies to neurons: they seem to require support from glial cells to get

and stay in touch via synaptic contacts. These contacts, also called chemical synapses, probably represent the most highly specialized form of intercellular connections. They allow for the intercellular transmission of electrical signals with remarkable spatial and temporal precision in the micrometer and millisecond range, respectively, and an enormous bandwidth [1].

Synapse assembly requires a cascade of precisely timed and coordinated processes in two different partners, very much like what is needed during ordinary dating. In a first seek-and-find phase, neuronal partners meet their match, which depends on both attraction and good timing. In a second construction phase, the pre- and postsynaptic partners assemble the complex structures for transmitter-based communication. During a third phase, connections are fine-tuned to acquire their mature qualities. Finally, a fourth, break-up phase results in the elimination of unfit liaisons. Our understanding of the molecules and mechanisms that induce and mediate these different phases has greatly advanced in the last few years [2–14]. Here, I summarize the experimental evidence for glial contributions to each of the phases that has emerged since the last overviews were published [15, 16].

Glial cells in touch with synapses

The generic term “glial cells” is often used in this review, although in reality only subclasses of these cells are in touch with synapses. The terms glial cells or neuroglia, originally introduced by Rudolf Virchow to label “nerve glue” [17, 18], describe non-neuronal cells in nervous systems. In vertebrates, the four core types are astrocytes, oligodendrocytes, microglial cells in the central nervous system (CNS), and Schwann cells in the peripheral nervous

F. W. Pfrieger (✉)
Institute of Cellular and Integrative Neurosciences (INCI),
CNRS UPR-3212, University of Strasbourg, 5, rue Louis
Pasteur, 67084 Strasbourg, France
e-mail: fw-pfrieger@gmx.de; pfrieger@neurochem.u-strasbg.fr

system. In addition, there are ependymal cells and radial glia comprising cerebellar Bergmann glia, retinal Müller cells and hypothalamic tanycytes. Each of the glial cell types serves distinct functions (see [19–21] for astrocytes; [22–24] for microglia; [25] for oligodendrocytes; [26, 27] for radial glia, Müller cells).

Of these glial cell types, only astrocytes and perisynaptic (or terminal) Schwann cells (PSCs) contact synaptic connections in the CNS and peripheral nervous system, respectively, with micrometer-sized processes. This spatial arrangement was first described more than 30 years ago in pioneering ultrastructural studies [28] and has now been revealed in three dimensions (3D) in the cerebellum [29] and the hippocampus, where the size of the synapses and the extent of astrocytic contacts appear to correlate [30]. The intimate vicinity of synapses and astrocytes has led to speculations about the functional implications and inspired the concept of the “tripartite synapse”, which regards glial cells as integral elements of synaptic connections [31]. There is increasing experimental support for this hypothesis. Recent studies show, for example, that glial cells influence synaptic function from the level of individual contacts [32] up to network activity that generates complex behavior [33, 34]. Overviews on this steadily growing field (Fig. 1) can be found in recent reviews [35–50].

Do glial cells help to find a partner? Glia as match-maker

The first hurdle to form a contact is to meet a (potential) partner. Glial cells facilitate this phase by providing

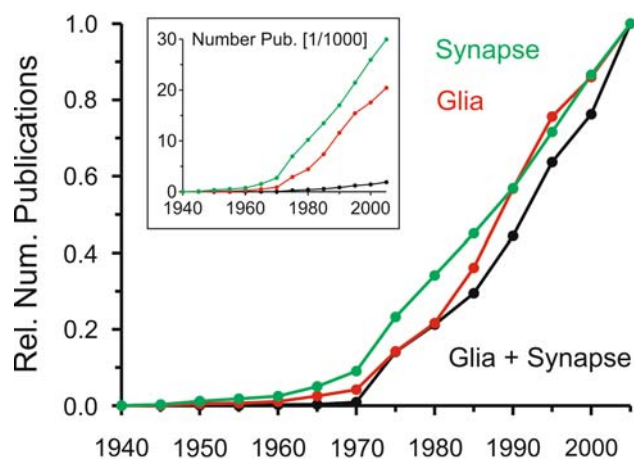


Fig. 1 Research on glia-synapse interactions. Number of publications in 5-year intervals normalized to paper counts in the last interval (2005–2010) related to synapses (green), glial cells (red), and synapses combined with glial cells (black). The numbers of different publications were extracted from PubMed searches on the indicated terms plus variations appearing in the titles or abstracts. *Inset* Absolute numbers of publications for each keyword

guidance and promoting growth [51–53]. Direct confirmation that Schwann cells induce and guide the axons of motoneurons to muscle fibers was provided by an in vivo study on transgenic mice, where a constitutively active neuregulin receptor, ErbB2, was reversibly induced in Schwann cells [54]. This manipulation triggered the formation and growth of PSC processes and the concomitant formation of nerve terminal sprouts along glial processes.

Glial cells also guide and promote the growth of axons and dendrites in the CNS. The most recent advances are based on studies of transgenic mice, which allow for selective labeling and gene ablation in glial cells. Ango et al. [55] reported that axons of stellate, but not basket, cells grow along processes of Bergmann glia and establish their synaptic connections with Purkinje cells (PCs) at intersections with glial fibers. Ablation of Ch11, a member of the L1CAM family that is expressed by axons of stellate cells and by Bergmann glia, perturbed arborization and terminal formation of stellate cell axons, reduced their synaptic contacts with PCs, and led to age-dependent axonal degeneration [55]. A study on fluorescently labeled PCs and Bergmann glia in cultured cerebellar slices from postnatal mice showed that the dendritic tips of PCs grow along processes of radial/Bergmann glia [56], thus extending previous findings [57]. Glial promotion of dendrite growth and branching has been observed in cultures of neurons derived from human stem cells [58] and of immuno-isolated retinal ganglion cells (RGCs) [59] and PCs from postnatal rodents (Buard and Pfrieger, unpublished observation).

Results from several recent studies help to explain how glia influence neuronal growth. Further evidence for the involvement of the L1CAM family comes from a study in *Drosophila*, where the elimination of neuroglian (Nrg), a L1-like adhesion molecule, caused ectopic axonal sprouting and dendrite deformation in a specific sensory neuron [60]; this phenotype was only rescued if neuroglian was re-expressed in both neurons and associated glial cells. These results indicate that glial cells prevent the formation of ectopic sprouts and help establish the stereotypic morphology of neurons. A genetic screen for abnormal synapse distribution in mutant *Caenorhabditis elegans* revealed that contact establishment between two specific types of interneurons, AIY and RIA, requires the netrin receptor/UNC-40 on axons of presynaptic AIY cells and its ligand netrin/UNC-6 on the so-called cephalic sheath (CEPsh) glia [61]. The netrin-dependent function of CEPsh glia as guideposts can explain at least in part why growth and branching of axons and dendrites fail following the elimination of these cells [62].

Signals on the surface of glial cells are an obvious mechanism to promote and direct neuronal growth. The involvement of intracellular calcium signaling is indicated

by a recent study on cocultures of hippocampal neurons and astrocytes from embryonic rats [63]. Retrovirus-mediated overexpression of an enzyme that hydrolyzes inositol triphosphate in astrocytes blocked calcium transients and reduced the growth of dendrites and axons by 60–70%. Wild-type and mutant forms of the enzyme differentially affected spontaneous and evoked calcium oscillations, while the growth-promoting activity of astrocytes required spontaneous transients. The decreased neurite growth on astrocytes with deficient calcium signaling was caused by lower levels of the cell adhesion molecule *N*-cadherin, which is well-known to promote neurite growth.

A new mechanism by which glial cells stimulate neurite outgrowth has recently been discovered in olfactory ensheathing glia [64]. This special type of glial cell in the olfactory bulb guides and promotes the growth of axons from olfactory receptor neurons, which are generated throughout life and integrate into the olfactory circuit [65]. Olfactory ensheathing glia prepared from embryonic mice released osteonectin/secreted protein acidic rich in cysteine (Sparc). Sparc in turn enables Schwann cells to promote neurite outgrowth from explants of dorsal root ganglia from mouse embryos [64]. This effect required transforming growth factor beta ($Tgf\beta$) and was promoted by laminin [64]. These data show that neurite outgrowth requires the cooperative actions of multiple signals, whose cellular sources and targets remain to be clarified.

Why should glia influence synapse development? It's the timing, stupid!

The idea that synaptogenesis is controlled by glial cells derives from a temporal correlation between synapse formation and astrocyte development in the rodent CNS [66], where the large majority of synaptic connections are generated during a protracted phase that spans from the first to the third postnatal week. Curiously, this happens after the formation of astrocytes [67], suggesting that bulk synaptogenesis requires glia. Fluorescent labeling of individual astrocytes by dye injection plus immunohistochemical staining revealed drastic morphological changes in astrocytes at the time of postnatal synapse development, which supports speculations about their role in synaptogenesis [68]. It should be noted that the temporal coincidence of synapse development and astrocyte differentiation applies mainly to glutamatergic connections. γ -Aminobutyric acid (GABA)-ergic neurons establish a functional network in the embryonic brain well before astrocytes are generated [13, 69], indicating glia-independent formation of GABAergic contacts.

Can neurons form contacts autonomously? Models matter!

The requirement of glial cells for synaptogenesis has been studied mainly in culture preparations: glia-free cultures allow to address a key question, namely whether neurons can form synapses without glia. Such cultures can be obtained by three methods: (1) neuronal cultures can be prepared from rodent brains on embryonic day 15–17, before glial cells are generated; (2) neurons can be prepared from postnatal brains after active separation from glia by immunoisolation [70–72] or by fluorescence-activated cell sorting [73–75]; (3) neurons can be generated from stem cells by promoting their neuronal differentiation [58, 76, 77]. These methods reach purities of up to 99.5% and thereby establish virtually glia-free conditions. Studies on such preparations have revealed that there is no absolute requirement for glia: some neurons can make connections (Fig. 2a), whereas others cannot (Fig. 2b). RGCs, motoneurons, and PCs immunoisolated from postnatal rodents formed only very few synapses under glia-free conditions [71, 78, 79] (Buard and Pfrieger, unpublished observation). Synapses among these neurons may be regarded as artificial, as they form their connections in vivo with partners that were not present in the cultures. However, few synapses were also observed in glia-free cultures of subplate neurons from embryonic rats or mice, which normally form synapses among each other [80]. On the other hand, strong glutamatergic and GABAergic synaptic activity was found in serum- and glia-free cultures of neurons from superior cervical ganglia of newborn rats [81], from spinal cords of embryonic mice [82], and from hippocampi and cerebella of postnatal mice [72]. In some of these preparations, a glial influence due to presence a few glial cells cannot be excluded. Nonetheless, the data suggest that the requirement for glia varies with the neuronal cell type: long-projecting neurons may need glia to form or receive synapses, whereas locally connecting cells may not.

Interestingly, the results from two studies on distinct culture preparations suggest that the competence of neurons to form and to receive synapses develop independently, although the reports disagree on the sequence of events. RGCs immunoisolated from embryonic rats could form, but not receive synapses, and the latter ability required contact with astrocytes [83]. A reversed sequence was observed in neurons that were generated by genetic re-programming of glial fibrillary acid protein-positive cells from the cerebellar cortex of postnatal mice. These neurons were able to receive glutamatergic inputs but were unable to form presynaptic contacts [77].

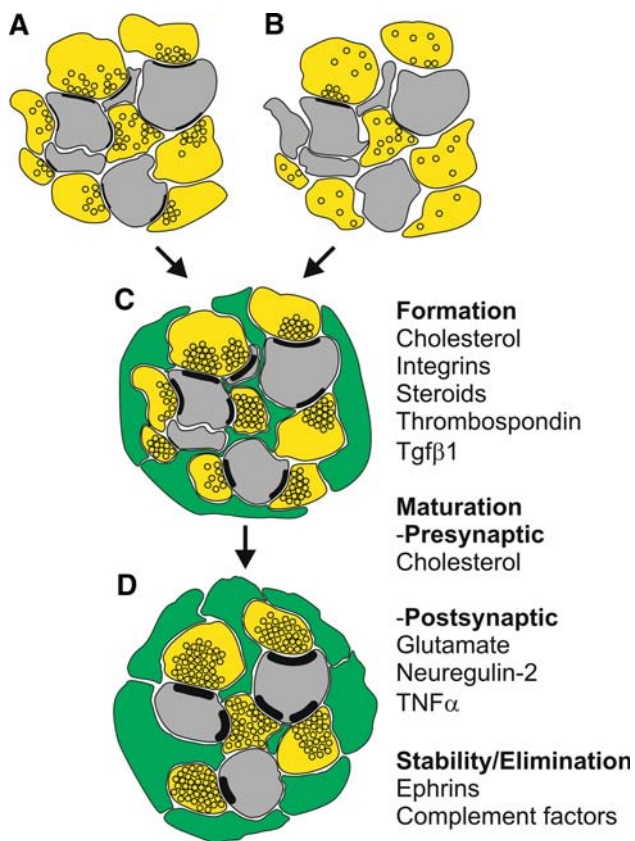


Fig. 2 Influences of glia on synapse development. Diagram illustrating the different effects of glia (green) on the development of synaptic connections between pre- (yellow) and postsynaptic (grey) partner neurons. Some neurons form synapses in the absence of glial cells (a), whereas others do not (b). Glia-derived factors promote synapse formation in the latter and enhance the pre- and postsynaptic efficacy of connections (c). In addition, they contribute to the stabilization or destruction of synaptic connections (d). Molecules implicated in glia-synapse interactions during development are indicated

The discrepancy may be due to the different neuronal cell types or culture preparations.

The most important question, namely, whether synaptogenesis requires glial cells in living animals, can only be addressed in invertebrate and vertebrate species, but not in mammals. Genetic ablation of astrocytes in developing and adult mice causes massive damage to neurons [84, 85], which precludes conclusions on their relevance for synaptogenesis. In *C. elegans*, whose cells are fully inventoried, four of its 50 glia-like cells, the so-called CEPsh glia, are in contact with dendrites of sensory neurons and with synaptic connections in the nerve ring [40]. Their ablation by optical and genetic methods does not impair the survival of associated sensory neurons, but it does perturb their morphology [62] and impair chemotactic behavior [86]. However, it remains unclear whether synapse formation is affected. Selective

ablation of PSCs in developing and adult frogs has been accomplished by a PSC-specific antibody combined with complement-mediated cell lysis. These experiments revealed that PSCs are required for the formation and growth of neuromuscular junctions (NMJs) in developing frogs in vivo [87].

Do glial cells promote the formation of synaptic contacts? Yes, but be aware!

Whether or not glial cells are required for the establishment of synaptic contacts, there is evidence that they increase their formation based on electron microscopic and immunocytochemical studies of different primary culture preparations (Fig. 2c). These include cocultures of frog spinal cord neurons and myocytes [88, 89], cultured rat and mouse RGCs [72, 78, 79, 90], PCs (Buard and Pfrieger, unpublished observation), motoneurons [71, 89], neurons derived from human embryonic stem cells [58], as well as cortical [80, 91] and hippocampal neurons [92, 93] from embryonic rats, where glia also increased the frequency of action potential-independent (miniature) postsynaptic currents [80, 91, 94]. All of the cited studies have focused on excitatory synapses. Astrocytes also enhanced the number of inhibitory connections among cultured hippocampal neurons from embryonic rats, an effect that required brain-derived neurotrophic factor signaling in neurons [95]. On the other hand, Steinmetz and colleagues [72] did not observe glial effects on inhibitory synapses in hippocampal neurons immunisolated from postnatal mice. The diverging results may be due to age- and preparation-dependent differences.

A caveat in these studies is that the observed increase in synapse numbers may be secondary to glial promotion of neuronal survival and/or growth. Glia-induced dendritogenesis, which facilitates synapse formation, was detected in stem cell-derived neurons [58] and in RGCs [59]. A glia-induced increase in the number of surviving neurons can explain the enhanced level of synaptic activity [93], whereas other studies have excluded this possibility [71, 72, 80, 90, 95].

Remarkably, glial cells can also have the opposite effect and impede synapse formation, for example by preventing physical contact between neurons. In vivo studies showed that decreased glial ensheathment of cerebellar PCs enhances the number of synaptic inputs [96, 97]. Alternatively, astrocytes may suppress synaptogenesis by secreted signals: fibroblast growth factor 2, which is produced by astrocytes, lowers the number of synapses in cultured mesencephalic neurons from neonatal rats [98], but the mechanism remains unclear.

Do glial cells promote synapse maturation? Glia foster fitness

There is evidence that glial cells promote the maturation of synapses, when the connections attain their characteristic transmission properties (Fig. 2c). In single-cell microcultures of neurons from rat superior cervical ganglia, Schwann cells were dispensable for the formation of cholinergic connections, but they did enhance the frequency of action potential-independent release and paired-pulse depression [81]. Both changes indicate a glial enhancement of release efficacy, which has also been observed in cultured RGCs [59, 78, 99]. Glial signals also enhanced the size of miniature excitatory postsynaptic currents in primary cultures of immunoisolated hippocampal neurons [72], cortical subplate neurons [80], RGCs [72, 78, 79], and spinal cord motoneurons [71]. This latter effect points to an enhancement of postsynaptic glutamate sensitivity, although presynaptic causes, such as an increased vesicular glutamate content, cannot be excluded. Notably, in dissociated and organotypic cultures of hippocampal neurons, glia-derived tumor necrosis factor alpha regulates activity-dependent changes in postsynaptic receptor density [100].

Transcript profiling of cultured RGCs by oligonucleotide microarrays revealed that glial signals enhance mRNA levels of glutamate receptor subunits and of components that regulate their clustering and stability [101]. Similar changes were observed in a microarray analysis of cortical subplate neurons [80]. Further evidence that glial signals promote postsynaptic maturation comes from studies on NMJs. Neuregulin 2 is produced by PSCs covering NMJs of adult rats and stimulates the transcription of acetylcholine receptors (AChR) by activating the ErbB4 receptor in a muscle cell line [102, 103]. Whether NMJ formation per se depends on the subsynaptic transcription of AChRs is unclear [104]. In *Drosophila*, elimination of a cysteine/glutamate transporter, which is expressed in NMJ-associated glial cells, reduces the extracellular glutamate level by half and increases the number of glutamate receptors in NMJs by two- to threefold [105], indicating that glia-controlled glutamate levels regulate the postsynaptic sensitivity of NMJs.

Glial-synapse communication: mind the signals!

A key to understand how glial cells influence synapse development is to identify the molecular basis of this process. Within the last years, there has been some progress in this area, namely the identification of synaptogenic components from glia.

Previous studies showed that Schwann cells promote the formation of NMJs in vitro [88]. A candidate approach recently identified *Tgf β 1* as one of the factors involved in this process [89]. The authors further reported that *Tgf β 1* mediates the previously reported increase in neuronal agrin levels [88] which, in turn, control AChR expression and clustering. These results substantiate the glial control of AChR levels in myotubes. However, *Tgf β 1* did not mimic the potentiation of spontaneous transmitter release from motoneuron terminals that was observed after acute application of Schwann cell-conditioned medium [106].

In the CNS, a few secreted glial factors that promote synapse formation have been uncovered. Thrombospondin was identified as an astrocyte-derived matrix component that promotes the formation of ultrastructurally normal, but postsynaptically silent synapses in cultured RGCs. Transgenic mice lacking thrombospondins showed a reduced density of immunohistochemically identified synapses in the cortex [99]. Another glial factor that contributes to this process is cholesterol [107]. Cholesterol promotes several aspects of synaptogenesis in RGCs. It enhances the efficacy of presynaptic transmitter release, enables dendrite differentiation and promotes the redistribution of glutamate receptors. Externally supplied cholesterol also sustained continuous synapse development and the stability of evoked release [59]. Neurons may require external cholesterol as building material for dendrites and synapses because they cannot produce sufficient amounts on their own [108]. Cholesterol can also influence signaling pathways—albeit indirectly. Our data suggest that cholesterol treatment modifies the membrane structure of cultured RGCs (Thiebaut et al., unpublished observation), which may affect the properties of membrane-resident signaling components. A synaptogenic effect of neurosteroids, which are produced from cholesterol, was excluded in the case of RGCs [59]. However, in primary cultures of cortical neurons, estradiol mimics the glia-induced increase in synapse number and function [91]. Moreover, estradiol controls the density of synapses and the level of synaptophysin in hippocampal slice cultures [109], although the cellular source of the steroid remains unknown. Progesterone enhances synapse formation in cultured hippocampal neurons. This effect required the presence of astrocytes and was possibly mediated by an increase in the glial production of agrin [92]. Cholesterol, estrogen, and creatine enhance the number and activity of synapses in cultured hippocampal neurons [110], but it remains unclear which factor induced the effect. Contact-dependent signals may also promote synapse development. In hippocampal microisland cultures, local contact with an astrocyte increases the number of synapses across the entire neuron, and integrins and protein kinase C are involved in this process [111]. As

described above, RGCs from E17 rats can only receive synaptic inputs when in contact to astrocytes, and this effect may involve changes in the subcellular distribution of neurexins [83]. In both cases, however, the astrocytic signals that initiate these changes remain unknown.

The identification of glial signals that influence synaptogenesis by biochemical purification and candidate guesswork is laborious and not always crowned by success. Fortunately, new techniques have emerged within the last years that complement these traditional approaches. For example, transcriptional profiling of neurons cultured with or without glial factors can provide a global view on the influence of glia on neurons and uncover unexpected pathways. Three recent reports have proven the validity of this approach [80, 101, 112]. Two studies on cultured RGCs revealed a glia-induced upregulation of components that had not yet been discussed in a neuronal context. This included a complement factor that contributes to synapse elimination (see below) [112] and matrix gla protein (Mgp), a component of the extracellular matrix, which regulates calcification in the body [101]. Interestingly, a recent study showed that Mgp modifies neuronal signaling by bone morphogenetic proteins, which belong to the Tgf family [113].

Should it stay or should it go? Glia live and let die!

Like many partnerships, synaptic connections have a limited life-time. Their turnover is particularly high during development, when neurons form promiscuous connections, and dump them subsequently. In adult mice, synapses appear to be more stable and last for many months, as shown by *in vivo* two-photon imaging of fluorescently labeled neurons [114]. On the other hand, synapse loss may spearhead the cascade of pathological changes in neurodegenerative diseases [115, 116].

There is now evidence that glial cells enhance the stability of synaptic connections (Fig. 2d): simultaneous time-lapse imaging of fluorescently labeled astrocytes and dendritic spines in organotypic hippocampal cultures revealed that contact to astrocytes enhances the life-time of dendritic protrusions and promotes their conversion to spines. This effect was possibly mediated by ephrin-A3/EphA4 signaling [117]. Ablation of PSCs in adult frogs *in vivo* caused the loss of NMJs [87], indicating that their maintenance depends on the glial sheath. There are also indications for the opposite, namely, that glial cells promote the death of synapses. A striking mechanism was uncovered by a study on immunisolated RGCs [112]. Coculture with glial cells strongly enhanced the neuronal level of C1q, a component of the complement cascade, which was present at synapses. Mice lacking C1q showed

defects in the eye-specific segregation of synaptic inputs to the lateral geniculate nucleus [112]. These observations suggest that astrocytes command the onset and extent of synapse execution by as yet unknown signals.

Synapse destruction can involve the removal of axonal branches that form obsolete connections. In the peripheral nervous system, motoneurons form supernumerary NMJs, most of which are destroyed postnatally [118]. Repeated imaging of NMJs in living transgenic mice combined with electron microscopy revealed that axon remnants, the so-called axosomes, end up in PSCs [119]. Moreover, the elimination of unwanted NMJs and climbing fiber inputs to cerebellar PCs was accompanied by enhanced lysosomal activity in glial cells [120]. Whether glial cells play an active or passive role in this process remains unclear.

Direct evidence for an active glial contribution to axon—and thereby synapse—removal comes from studies on the olfactory system of flies, which undergoes substantial remodeling during metamorphosis [121]. Selective labeling of neurons and glial cells in the mushroom body and genetic disturbance of glial membrane function revealed an active role of glial cells in the pruning of axons [122–124].

What's in store for astrocyte–synapse interactions? Don't miss the buzz!

The research progress documented in this review is to a great part driven by technical advances, which will continue to propel the field. Three “techno-tracks” seem to be of particular importance.

First, advanced genetic and proteomic approaches will divulge the molecular setup of astrocytes. An important first step has been the transcriptional profiling of cultured and acutely isolated astrocytes [125–128], retinal Müller cells [129], and Schwann cells [130] of developing and adult rodents. Moreover, the proteome and secretome of cultured astrocytes have been inventoried [64, 131–133]. Evidently, the molecular signature of isolated or cultured cells may not reflect the situation *in vivo*. This caveat can be overcome by exciting new approaches to transcriptional profiling *in vivo*, based on new molecular baits for transcription complexes [134] and on the bioinformatic analysis of coexpression [135]. Together, these techniques should provide a comprehensive molecular definition of astrocytes, which—up to now—has been based on very few markers. Ultimately, such new approaches may lead to a molecular classification of astrocytes that is of a similar complexity as that for neurons.

Second, glial cells and their interactions with synapses can now be visualized in a much more refined and dynamic manner than ever before thanks to technical advances in

cell labeling and microscopy. First, astrocytes can be visualized *in vivo* or *in situ* by fluorescent molecules. Dyes can be applied by micro-iontophoretic injection in lightly fixed tissue [136] or by topical application to the brain surface of living animals [137]. These methods, in combination with confocal or two-photon microscopy, have brought unprecedented insight in the morphology and spatial arrangement of astrocytes under normal and pathological conditions [136, 138] and have allowed, for example, to separate astrocytic from neuronal calcium signals [137]. An alternative method that circumvents dye-related problems is based on the glia-specific expression of fluorescent proteins in transgenic animals. Time-lapse imaging of fluorescently labeled neurons and astrocytes in intact preparations has revealed a high motility of glial filopodia [139, 140] and their dynamic interactions with dendritic spines [97, 117, 141, 142]. Astrocytes interact with synapses via extremely delicate processes in the micrometer range, whose visualization evades conventional light microscopy. These key structural domains and their dynamics can be reconstructed by serial section electron microscopy [143] and advanced 3D computer electron tomography [144]. More insight at the ultrastructural level will probably come from new light [145] and electron microscopy techniques [146, 147].

Third, new animal models allow to test the functional relevance of glial cells for synapse development and function *in vivo*. Several transgenic mouse lines enable astroglia-specific somatic mutagenesis based on the Cre/loxP system and different astrocyte-specific promoters contained in short transgenesis vectors (hGfap: [148, 149]), bacterial artificial chromosomes (see [150] for Glast and Cx30; [151] for Gfap; [129] for Pdgfra; [152] for Blbp) or genomic DNA ([153] for Glast). Moreover, transgenic mice have been generated to overexpress specific proteins in astrocytes [152, 154] and to reversibly target Schwann cells [54] and astrocytes [155, 156] by the Tet-on/off system. Alternatively, virus-based transfection of astrocytes *in vivo* is now possible by a lentiviral construct that achieves preferential targeting of astrocytes [157].

Finally, visualization of glial cells can be combined with functional manipulation in genetically modified worms and flies, where the characterization of glial cells and their interactions with synapses are progressing quickly [40, 158]. These invertebrate models will certainly continue to provide important insights in glia-synapse interactions and to inspire further research on the situation in vertebrates.

Summary and outlook

Three lessons can be learnt from this review. First, the plot thickens: neurons require help from their glial friends to

find their match, to form strong, enduring connections, and to eliminate obsolete liaisons. Second, the chances to understand these interactions are increasing thanks to relentless technical advances in areas such as molecular characterization, visualization, and functional interference in glial cells. Finally, a *sine qua non* for further progress remains the identification of signaling pathways, as this is vital to determine the relevance of glia for the development and maintenance of synaptic connections and to explore their potential to repair brain damage. Whatever is in store, the times are exciting for those interested in glia-synapse interactions.

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