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The Mystery and Magic of Glia: A Perspective on Their Roles in Health and Disease

Ben A. Barres[1,*]
[1]Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA
*Correspondence: barres@stanford.edu
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In this perspective, I review recent evidence that glial cells are critical participants in every major aspect of brain development, function, and disease. Far more active than once thought, glial cells powerfully control synapse formation, function, and blood flow. They secrete many substances whose roles are not understood, and they are central players in CNS injury and disease. I argue that until the roles of nonneuronal cells are more fully understood and considered, neurobiology as a whole will progress only slowly.

Introduction

Twenty years ago, George Somjen began his excellent review on the history of glia by noting how much of today's research is still directed toward answering questions first asked a century ago (Somjen, 1988). Indeed my students, so sure of the rapid pace of science today, are surprised when I tell them that they are still investigating exactly the same questions that my graduate student contemporaries investigated 25 years ago. Though there has been a great deal of progress, most fundamental questions about brain development, function, and disease are still relatively poorly understood. How do synapses form, stabilize, and achieve their specificity? How do we learn and remember? How are neurons and glia generated? How does myelination happen? Why don't severed CNS axons regenerate and why do synapses degenerate in Alzheimer's disease? Fortunately, thanks to the power of modern tools, the rate of progress is ramping up, so that the average student today can generally make mechanistic, rather than solely descriptive, steps forward.

Here I will focus on the role of glial cells in the development and function of neural circuitry, both in health and disease. As a young neurologist in training, I became interested in the function of glial cells during a neuropathology clerkship. As I looked at brain sections from various neurological diseases under the microscope, I realized not only that at least half the volume of the human brain is constituted by glial cells—astrocytes, oligodendrocytes, and microglial cells–but also how radically altered glial cell phenotype is in every brain injury and disease. I became captivated by the questions: what do glial cells normally do, and what is their role in disease? Might glial cells be important drug targets? Throughout this perspective, I will speculate often and without apology. When it comes to understanding the mystery and magic of glia, progress depends on guesswork. As Nobel Laureate Richard Axel has put it: ''Before you know, you must imagine.''

What Do Astrocytes Do?

A Role for Astrocytes in Synapse Formation and Plasticity

Astrocytes could be as heterogeneous as neurons. They fall into at least two main classes distinguished by morphology, antigenic phenotype, and location. Accordingly, they most likely differ in some of their main functions. Protoplasmic astrocytes are found in gray matter and their processes ensheath synapses as well as blood vessels. Fibrillary (or fibrous) astrocytes in white matter contact nodes of Ranvier and blood vessels. A single astrocyte extends thousands of fine membranous processes that ensheath synapses and fine blood vessels and help fill the neuropil (Araque et al., 1999; Ventura and Harris, 1999; Bushong et al., 2002). There are specialized astrocytes, including Müller glia in the retina and Bergmann glia in the cerebellum, that appear similar to protoplasmic astrocytes in antigenic phenotype in that they exist in gray matter and ensheath many synapses. Glia exhibit remarkably similar morphologies in *Drosophila*, suggesting that important glial functions are likely to be highly conserved.

What do astrocytes do at synapses? They help to control the levels of some ions such as K^+ ions and neurotransmitters in the extracellular space. This is said in every textbook, yet our understanding of exactly how astrocytes control extracellular K^+ and other ions is poorly understood and deserves fresh attention, particularly now that the full cassette of ion transporters, pumps, and channels they express has been elucidated (Lovatt et al., 2007; Cahoy et al., 2008). Astrocytes express receptors for a wide variety of neurotransmitters, and can release many known and unknown neuroactive substances, as well as trophic factors. The functional significance of most of this signaling is mysterious, but it is likely to help control development and function of synapses, blood vessel flow, and neuronal survival.

To try to better understand the role of astrocytes at synapses, Pfrieger and Barres (1997) asked whether a highly purified CNS neuron type, retinal ganglion cells, would form functional synapses when cultured in the absence of all glial cells. They used retinal ganglion cells because this is still one of the few CNS neuron types that can be highly purified and cultured in defined, serum-free medium at high survival in the total absence of glial cells. Although purified retinal ganglion cells elaborated dendrites and axons and are electrically excitable, they exhibit little synaptic activity. In contrast, when cultured with astrocytes, or culture medium that has been conditioned by astrocytes, their synaptic activity increases by nearly 100-fold. In contrast, coculture with other cell types such as fibroblasts and oligodendrocytes does not enhance their synaptic activity, and the requirement for astrocyte signals persists even when the retinal ganglion cells are cocultured with their normal target neurons from the superior

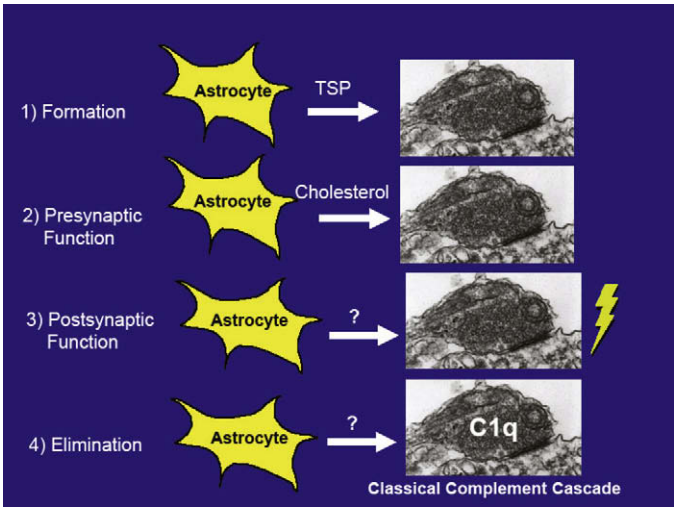

Figure 1. The Formation of Functional Synapses Is Controlled by Astrocytes in Multiple Steps

Astrocytes secrete proteins called thrombospondins (TSP) that induce neurons to form synapses. The presynaptic function of these synapses is strongly enhanced by astrocyte-secreted cholesterol. The postsynaptic function of synapses, determined by the level of synaptic AMPA glutamate receptors, is strongly enhanced by an as yet unidentified protein secreted by astrocytes. Finally, astrocytes also help to control synapse elimination by secreting an unidentified signal that induces neurons (and possibly also microglia) to express and secrete C1q, which becomes synaptically localized and leads to activation of the classical complement cascade (see text).

colliculus. Is the ability of astrocytes to enhance synaptic activity due to enhancement of synapse number, synapse function, or both? To find out, Ullian et al. (2001) used quantal analyses, FM1-43 imaging, immunostaining, and electron microscopy to determine that few synapses form between retinal ganglion cells in culture in the absence of astrocytes, and that the few synapses that do form are functionally immature. Astrocytes increase the number of synapses that form by nearly 10-fold, but also strongly enhance their presynaptic and postsynaptic function. Similarly, Schwann cells promote the formation and maintenance of the neuromuscular junction (Feng and Ko, 2008). These findings demonstrated novel, active roles for astrocytes and Schwann cells in promoting synapse formation and function in vitro. The possibility that astrocytes might act similarly in vivo is strongly suggested by the correlation between when and where synapses form and the timing and localization of astrocyte generation in vivo.

What are the signals that astrocytes release that promote CNS synaptogenesis? Christopherson et al. (2005) found that astrocytes release a large matrix-associated protein called thrombospondin (Figure 1). Thrombospondins consist of a family of five homologous proteins, which all share the ability to induce synaptogenesis. At least four of these family members are expressed by astrocytes in the brain, particularly during development and after injury. In addition TSP3 remains highly expressed in the normal adult hippocampus and thrombospondins are amongst the few genes that are far more highly expressed in human brain compared with primate brain (Caceres et al., 2006). Thrombospondin is sufficient to induce synapses that have normal presynaptic and postsynaptic ultrastructure as well as normal clustering of presynaptic and postsynaptic proteins, such as synapsin and PSD-95, respectively. These synapses, however, are postsynaptically silent, lacking glutamate sensitivity. Astrocytes secrete a different protein, not yet identified, that induces postsynaptic glutamate (AMPA) responsiveness (Christopherson et al., 2005). In addition, astrocyte-derived cholesterol powerfully enhances presynaptic function by nearly 100-fold (Mauch et al., 2001). Thrombospondin-1/2 null brains have significantly decreased synapse number, providing evidence that astrocytes normally help to promote synapse formation in vivo. Moreover the neuronal receptor that mediates thrombospondin-induced synaptogenesis has recently been identified, and antagonists of this receptor profoundly impair synaptogenesis in vitro and in vivo (C. Eroglu and B.B., unpublished data). Thus, astrocytes secrete many signals that promote synapse formation and function.

One of the great unsolved mysteries in understanding brain development is how short-term changes in sensory activity in neurons can permanently alter synaptic structure during a critical window of brain development. Do astrocytes play a role in critical period plasticity? The possibility that astrocytes may play an important role in this process has recently been reviewed (Eroglu et al., 2008). One of the most provocative experiments was performed by Christian Muller, who found that transplantation of immature astrocytes into adult primary visual cortex of cats robustly restored ocular dominance plasticity (Müller and Best, 1989). The secretion of thrombospondins by immature astrocytes is under the control of ATP and other neurotransmitters (Tran and Neary, 2006), suggesting the possibility that neuronal activity may control the ability of astrocytes to promote synaptogenesis. Moreover thrombospondin has been found to be one of the few genes that are highly upregulated in human brain compared with primate brain, suggesting that it may contribute to the greatly enhanced brain plasticity of humans. Removal of inappropriate synaptic connections is also a critical component of brain plasticity (Boulanger and Shatz, 2004), a process which astrocytes also participate in (see below). Thus, understanding the role of astrocytes in structural synaptic plasticity promises to be a fruitful area for future investigation in understanding how to build neural circuits as well as how to rebuild them after injury. What is as yet unclear is why a neuron-glial interaction is so critical for some, or all, of synaptogenesis. Presumably glial cells play a critical role in controlling the timing, location, number, function, and plasticity of synapses, and perhaps in the evolution of greater synaptic plasticity in human brains.

Glial Calcium Waves, Gliotransmission, and the Function of Neural Circuits

Do astrocytes actively control neural circuit function in the adult CNS? Astrocytes are highly secretory cells and given their proximity to synapses, it is not surprising that there is emerging evidence that glia secrete many different signals that control synaptic function. But exactly what these signals are, how they are released, and what their functional significance might be are still

open questions. Application of neurotransmitters has long been known to induce robust intracellular calcium waves that propagate between astrocytes in culture. Neurons release a variety of substances such as ATP and glutamate that activate G protein coupled receptors (GPCRs) in astrocytes, which leads to elevation of IP3 and IP3 receptors and calcium release from the endoplasmic reticulum (Agulhon et al., 2008). Only recently has it been conclusively shown that neuronal activity in awake, mobile mice is correlated with intracellular calcium transients in astrocytes (Wang et al., 2006; Dombeck et al., 2007; Petzold et al., 2008; Schummers et al., 2008). By imaging these calcium transients, it has been found that astrocytes, like neurons, respond to visual stimuli with distinct spatial receptive fields and sharp tuning to visual stimulus features including orientation and spatial frequency (Schummers et al., 2008). Surprisingly, Schummers et al. (2008) found that these astrocyte calcium waves generally did not propagate to other astrocytes in vivo, providing evidence that astrocytes can respond as individual cells, much like neurons, with their own unique response patterns.

All of these groups found that calcium waves in astrocytes correlate with increased microvascular blood flow. Although this might just be a correlation, a variety of pharmacological manipulations provide evidence that neuronal signals induce glial cells to elevate their calcium levels and to release signals that regulate vasodilation. Astrocytes can release either vasoconstrictors or vasodilators depending on context (Zonta et al., 2003; Metea and Newman, 2006; Gordon et al., 2007), but the nature of the signals, relevant contexts, and functional significance is not yet clear. Emerging data suggests that the extent of gap junction coupling between astrocytes is very region and astrocyte dependent, as well as strongly neuronal activity dependent, suggesting the existence of glial circuits (Houades et al., 2006). This suggests that glial gap junctions may help remove ions and toxin metabolites from synapses, deliver nutrients, or both. This close coupling between neurons, glia, and blood vessels has been termed the neurovascular unit. Neurons, glia, and blood vessels all work together in a close symbiosis to control our cognitive functions, and impairments of this symbiosis correlate with, and may well contribute to, diseases of cognitive dysfunction such as Alzheimer's disease (Takano et al., 2007). The significance of the neurovascular unit for normal brain function and brain dysfunction deserves much more attention.

A controversial question in glial biology has been whether neuronal activity, by inducing calcium waves in astrocytes, induces secretion of neuroactive substances from astrocytes back onto synapses in a process known as gliotransmission. Glutamate release at neuron-glial synapses onto NG2-positive glial cells, oligodendrocyte precursor cells (OPCs), has been conclusively demonstrated (Paukert and Bergles, 2006), although its function is mysterious. It has been repeatedly claimed, however, that astrocytes in vivo secrete quanta of glutamate by regulated vesicular release. There are many reasons to be skeptical. First, astrocytes, unlike neurons, are highly enriched in the enzyme glutamine synthetase, which degrades glutamate to glutamine. A variety of methods for measuring intracellular glutamate concentrations suggest that whereas levels of glutamate may approach 10 mM within neurons, glutamate does not exceed housekeeping levels within astrocyte cytoplasm. Consistent with this, it is easy to detect glutamate immunoreactivity in neurons, but not in astrocytes. Furthermore, astrocytes in vivo do not express any of the known vesicular glutamate transporters, nor do they express any of the components of regulated vesicular release that mediate glutamate release in neurons (Cahoy et al., 2008). Some labs have been unable to find evidence for calcium-induced release of glutamate onto postsynaptic neurons (reviewed in Agulhon et al., 2008). Most of the arguments that astrocytes release glutamate in response to elevated calcium in vivo are indirect and are, for instance, based on blockade of a response by mGluR5 pharmacological blockers. However, in the mature brain mGluR5 is primarily expressed by neurons. Overall, the case for regulated release of glutamate from astrocytes onto neurons at tripartite synapses is not convincing.

Although astrocytes do not appear to be capable of vesicular release of the kind used by neurons, recent studies reveal that elevated calcium in astrocytes does induce a special kind of regulated secretion from secretory lysosomes (Jaiswal et al., 2007; Zhang et al., 2007; Li et al., 2008). Secretory lysosomes are enriched in certain cell types such as immune cells and glia. In oligodendrocytes, secretory lysosomes secrete myelin proteins and likely play a critical role in myelination (Trajkovic et al., 2006). In astrocytes, secretory lysosomes release ATP, and blocking release of ATP from secretory lysosomes blocks propagation of calcium waves between neighboring astrocytes. Although these studies have so far focused on astrocytes in culture, a similar mechanism of release is likely to occur in vivo given that acutely isolated astrocytes express the genes involved in lysosome secretion (Cahoy et al., 2008). ATP release by astrocytes regulates CNS synaptic transmission in vivo (Pascual et al., 2005), and it is thus likely that this glial release in vivo occurs from secretory lysosomes.

There are many other substances released by astrocytes that are likely to regulate synaptic transmission. Perhaps the most interesting of these is D-serine, an important neurotransmitter that serves as a coagonist with glutamate at NMDA receptors (Mustafa et al., 2004; Panatier et al., 2006). Although mRNA for its synthetic enzyme serine racemase is expressed equally by neurons and astrocytes, only glial cells can synthesize serine, so synaptically available D-serine is likely to be primarily made and secreted by astrocytes. In addition, it is very likely that astrocytes serve as a primary supplier of the four-carbon backbone for de novo synthesis of neuronal glutamate and GABA because pyruvate carboxylase is primarily found in astrocytes (Hertz et al., 2007; Cahoy et al., 2008). Some evidence suggests that the rate at which astrocytes supply this precursor may limit the rate at which neurons can fire. Astrocytes also make and secrete many unique lipids, including PUFAs, whose possible roles at synapses have so far received little attention (Cahoy et al., 2008). In summary, although astrocytes probably do not conduct gliotransmission by secreting vesicular glutamate onto synapses, astrocytes secrete many neuroactive substances such as ATP and D-serine. Our understanding of how and why these substances regulate synaptic function is in its infancy.

The Astrocyte Transcriptome

In order to gain new clues to the mysterious function of astrocytes and related cells such as Müller glia, several labs have

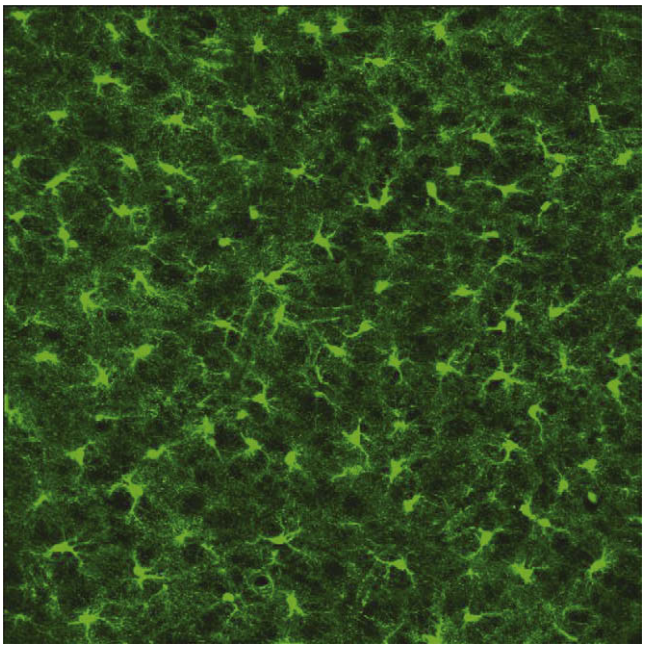

Figure 2. Protoplasmic Astrocytes in the Developing Cerebral Cortex
Protoplasmic astrocytes (green) throughout the postnatal day 7 mouse cerebral cortex are visualized by expression of green fluorescent protein (GFP) in a transgenic mouse in which GFP expression is driven from a bacterial artificial chromosome (BAC) using the Aldh1L1 promoter. Image from Gensat.org.

developed new methods to isolate highly purified glial cell types in order to extract their mRNA, gene profile it using Affymetrix gene chips, and compare it with neurons. This has allowed elucidation of the astrocyte, oligodendrocyte, neuron, and Müller glial transcriptomes (Lovatt et al., 2007; Cahoy et al., 2008; Roesch et al., 2008; these are available as supplemental Excel spreadsheets from the journal web sites; the mouse all-exon data set from Cahoy et al. consisting of 1.2 million probe sets worth of exon expression data is too large to be posted as an Excel spreadsheet but is now available at http://innateimmunity.mcb.harvard.edu/exonarray/cahoy.html; see Supplemental Data, available online, for further deatils). Only a brief coverage of a few of the interesting findings from analysis of these transcriptomes is possible here. Many new astrocyte-specific genes were identified. Aldh1L1 (Figure 2) was identified as a highly specific antigenic marker for astrocytes with a substantially broader pattern of astrocyte expression than the traditional astrocyte marker GFAP (a monoclonal that works well for staining rat Aldh1L1 is now available from Neuromab, Aldh1L1-GFP mice are available from Gensat, and Aldh1L1-Cre mice will soon be available from Jackson Labs). The transcriptomes of oligodendrocytes and astrocytes were not more similar to each other than to neurons, thus calling into question the concept of a glial cell class. Interestingly, several evolutionarily conserved phagocytic pathways were found to be highly enriched in astrocytes including the Draper/Megf10 and Mertk/integrin alpha(v)beta5 pathways, suggesting that mammalian astrocytes may be professional phagocytes. Similarly the Draper/Megf10 pathway has been previously localized to *Drosophila* astrocytes where it mediates axon pruning, and recently it has been localized to Schwann cells as well, which is interesting because Schwann cells mediate clearance of degenerating myelin as well as eliminated synapses at the developing neuromuscular junction (Bishop et al., 2004). These findings raise the interesting possibility that astrocytes are actively mediating synapse elimination by phagocytosis using these pathways during development, normal adulthood, or after injury. This function is reminiscent of the role of retinal pigment epithelial cells that mediate daily clearance of the shed outer segments of photoreceptors via the Mertk pathway.

One longstanding idea is that astrocytes may be critical in promoting neuronal survival by releasing neurotrophic factors. Known neurotrophic factors do not strongly promote the survival of most types of CNS neurons the way that they do for PNS neurons. It has been known for 30 years, however, that astrocytes in culture release neurotrophic signals that strongly promote CNS neuron survival (Banker, 1980), and that in vivo astrocyte survival is necessary for CNS neuron survival (Wagner et al., 2006). However, what the identities of these astrocyte-secreted signals are and how they promote neuronal survival are still unanswered questions. Astrocytes might promote neuron survival simply by virtue of inducing CNS neurons to form synapses, or they may secrete other signals that activate specific neuron survival pathways. The transcriptomes reveal a large variety of trophic factors made by astrocytes that suggest they may in fact contribute to neuronal survival, and this will be interesting to explore in future studies. One of the greatest mysteries surrounding the astrocyte transcriptome is that the functions of most of its most highly expressed specific genes are still relatively poorly understood. These genes include ApoE, ApoJ, MFGE8, and cystatin C. The first three of these, however, are likely to function as lipid or lipid-associated signal carriers in the lipoprotein particles that astrocytes secrete, and possibly also function as opsonins to coat unwanted debris or synapses and enable their phagocytic clearance by astrocytes.

There are many other fundamental questions about astrocytes that gene profiling should be helpful for in future studies. First, the gene profiles indicate a surprising amount of regional astrocyte heterogeneity. As one example, astrocytes largely present in the thalamus express high levels of the NMDA receptors 1 and 2C. Bergmann glia continue to express in the adult brain many astrocyte genes that are otherwise only expressed by immature astrocytes in postnatal brain, which suggests the possibility of some unusual sustained structural plasticity of adult cerebellar Purkinje cells. A better understanding of the nature of astrocyte heterogeneity will likely provide new insight into astrocyte functions. Second, gene profiling will help to understand how white matter (fibrous) and gray matter (protoplasmic) astrocytes compare and how they differ in function. Finally, gene profiling has great potential to provide new insight into the functional roles of reactive astrocytes.

Astrocytes, the Blood-Brain Barrier, and Disease

Vascular cells are a major cellular constituent of the brain (Figure 3) and have recently emerged as important, though relatively neglected, contributors to brain development and function. Vascular cells guide developing axons (Makita et al., 2008), provide

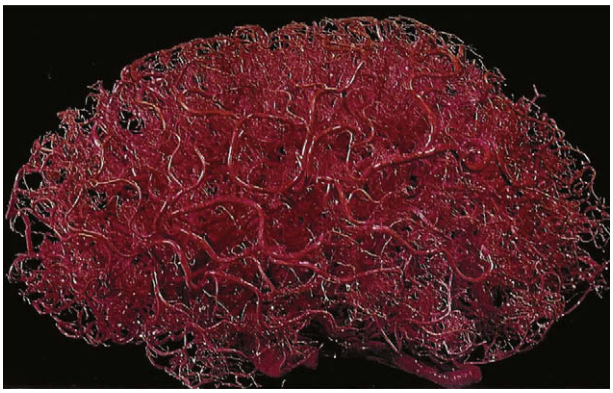

Figure 3. Vascular Cells Are a Major Cellular Constituent in the Human Brain
Blood vessels represent a substantial fraction of the volume of the brain. Vessels were visualized by filling them with a plastic emulsion, after which brain parenchymal tissue was dissolved (from Zlokovic and Apuzzo, 1998).

trophic support and differentiation signals to neurons and stem cells (Shen et al., 2004; Dugas et al., 2008), and provide a niche for neural stem cells (Tavazoie et al., 2008). One role commonly noted in textbooks for astrocytes is that they induce the blood-brain barrier, although there is still relatively little evidence that they do so in an uninjured brain. The blood-brain barrier is actually several different barriers that include tight junctions between brain endothelial cells, low brain endothelial rates of endocytosis, and a high level of multiple export and import transporters (Zlokovic, 2008). A role for astrocytes has been suspected because reactive astrocytes clearly play a critical role in sealing the barrier after brain injury (Bush et al., 1999) and because the barrier has long been believed to be formed postnatally concurrently with astrocyte generation. However, recent studies have demonstrated that the blood-brain barrier is fully intact from the earliest time that blood vessels enter the CNS parenchyma, about embryonic day 11 or 12 in mice (Saunders et al., 2008; R. Daneman and B.B., unpublished data). Different signaling pathways control different aspects of the barrier and include Wnt signaling, which derives from neural stem cells and drives CNS-specific angiogenesis, brain endothelial migration, and expression of at least some importers (R. Daneman, D. Agalliu, L. Zhou, F. Kuhnert, C. Kuo, and B.B., unpublished data). In postnatal development, after stem cells are largely depleted, these functions may be taken over postnatally by astrocytes, which may thus serve more of a maintenance function than an initial blood-brain-barrier-inducing function (Cahoy et al., 2008). Given the large number of signals and cell types that participate in controlling the blood-brain barrier, it is not surprising that so many different brain diseases can compromise this barrier.

Because astrocytes constitute nearly half of the cells in the human brain, there is no CNS disease that does not substantially involve astrocytes. Astrocyte swelling is a dramatic and very harmful component of any acute neurological injury including stroke and brain trauma, yet we still do not understand well why astrocytes are more likely to swell than neurons and how this swelling can be lessened. Neurological diseases, including dysmyelinating diseases and epilepsy, can result from mutations of astrocyte genes. Reactive gliosis (astrocytosis) also accompanies every neurological disease. Although reactive astrocytosis clearly is beneficial in that it can encapsulate infections and help seal a damaged blood-brain barrier, there are many ways in which it has been found to be harmful. Glial scarring contributes substantially to the glial cues that inhibit severed CNS axons from regenerating (Silver and Miller, 2004). Reactive astrocytes upregulate synapse-inducing genes such as thrombospondins, which have the potential to help repair the brain (Liauw et al., 2008) but may also induce unwanted synapses that can cause epilepsy or neuropathic pain (Boroujerdi et al., 2008). In addition, recent studies have found that sick astrocytes can release a profoundly neurotoxic signal. For instance, mutant astrocytes carrying the SOD1(G93A) allele release a toxic signal that rapidly kills wild-type motor neurons (Di Giorgio et al., 2007; Nagai et al., 2007; Lobsiger and Cleveland, 2007).

A critically important area of neuroscience research today is to understand the pathophysiology of stroke, one of the most common neurological diseases. Other than clot-busting drugs, there are not yet good treatments that minimize brain tissue loss and dysfunction after stroke. Perhaps before we can successfully treat stroke, we need to more fundamentally understand why the CNS is so much more vulnerable to ischemia than non-CNS tissues. Thirty years ago in medical school, I was taught that neurons are more vulnerable to ischemia than any other cell type in the body. Excitotoxicity is certainly a central element of ischemic damage that is unique to the CNS. But in the light of modern-day knowledge, I often wonder whether it is also possible that neurons have exactly the same intrinsic vulnerability to ischemia as any other cell type. The greater brain vulnerability to ischemia might simply reflect the lower redundancy and repair ability of the brain compared those of the liver or the kidney. Or alternatively it might reflect the special nature of the division of metabolic labor between neurons and glia. It is a good time for more graduate students and postdocs to begin addressing these questions.

How Do Oligodendrocytes Myelinate?

If the close association of astrocytes with neurons reflects the importance of their functional interactions, perhaps there is no more intimate cellular interaction than that of oligodendrocytes and Schwann cells wrapping their membranes around axons to form myelin. In addition to providing insulation and trophic support to neurons, myelinating glia are active participants in nervous system function, sculpting the structural and electrical properties of axons by controlling their diameter, as well as the spacing and clustering of ion channels at nodes and paranodes. Schwann cells also help promote the regeneration of axons and the formation and function of synapses at the neuromuscular junction. Surprisingly, our understanding of how Schwann cells and oligodendrocytes myelinate is still very limited. Oligodendrocytes are generated by OPCs that migrate from their germinal zones during development and after injury to regions where axons are unmyelinated, ensheath these axons, and then wrap them. Whereas the mechanisms of wrapping remain largely unknown, neuregulin-1 has been identified as a critical axonal signal controlling myelination in the PNS, and gliomedin has been identified as a key Schwann cell signal that triggers clustering

of sodium channels at nodes of Ranvier (Eshed et al., 2005; Brinkmann et al., 2008). Although it was long thought that the signals in the CNS and PNS would be the same, differential regulation of sensory axonal signals by NGF indicated that distinct mechanisms are involved (Chan et al., 2004). Indeed, the neuregulin-1 isoform controlling Schwann cell myelination turned out not to be essential for CNS myelination, and gliomedin has not been implicated in ion channel clustering induced by oligodendrocytes. Similarly, the molecular mechanisms that enable oligodendrocytes to recognize, ensheath, and wrap axons are not known. Progress has been slow in part because myelination is largely a vertebrate adaptation, so forward genetic screens have not been practical. In addition, knockout mice that do not express major myelin proteins and lipids are surprisingly good at myelinating, with the exception of myelin basic protein, which is clearly required for wrapping in the CNS (but not PNS). As for the astrocyte transcriptome, the oligodendrocyte transcriptome has revealed a large number of highly expressed, oligodendrocyte-specific molecules whose roles are mostly unknown (Nielsen et al., 2006; Cahoy et al., 2008). Zebrafish has emerged as a powerful new genetic model system for the study of myelination and node of Ranvier formation (Pogoda et al., 2006).

Another long-time limitation in studying the molecular basis of CNS myelination has been the lack of a rapidly myelinating culture system. In general, when CNS cultures consisting of mixed neurons and glia are prepared, they need to be cultured for at least 30 days before substantial myelination occurs. A recently developed coculture system that enables rapid myelination of CNS axons offers new opportunities for molecular dissection of multiple stages of myelination. Purified CNS neurons can be cultured as ''reaggregates'' so that they extend dense beds of axons, which can then be seeded with purified OPCs in a serum-free medium (Watkins et al., 2008; Figure 4). In this system, myelination occurs in three stages that are under differential control. First, OPCs are largely inhibited from differentiating into oligodendrocytes by axonal signals. Although all of these inhibitory axonal signals are not yet known, Notch ligands such as Jagged1 contribute, because genetic or pharmacologic disruption of Notch1 signaling favors oligodendrocyte differentiation. Despite expressing myelin proteins, these newly differentiated oligodendrocytes do not robustly ensheath axons unless gamma secretase activity within the oligodendrocytes is inhibited either genetically or pharmacologically. Interestingly, the ensheathment of multiple axons by each oligodendrocyte seems to be a coordinated event. Oligodendrocytes, observed by time-lapse microscopy, do not ensheath their various axons sequentially at different times, but rather ensheath them all within only a brief period, typically just 12–18 hr. For this concurrent ensheathment to be triggered, an OPC seems to need to contact a sufficient number of axons within the first 12 hr or so of its differentiation. This implies the existence of a nuclear program that controls ensheathment: cleavage of a gamma secretase substrate may release a C-terminal fragment that enters the nucleus and inhibits ensheathment; reduction of gamma secretase activity would then regulate this nuclear program, enabling ensheathment of multiple axons at once. One hypothesis suggested by these findings is that an unidentified axonal signal triggers ensheathment by inhibiting gamma secretase activity within oligodendrocytes.

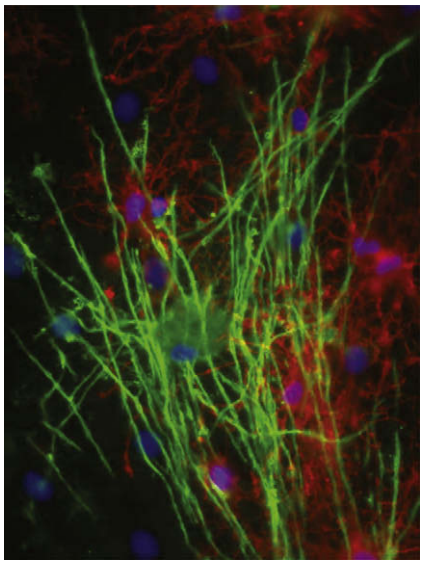

Figure 4. Myelinating Oligodendrocytes in Coculture with Retinal Ganglion Cells

Green represents myelin basic protein immunoreactivity, which labels oligodendrocytes and myelin. Blue is the DAPI nuclear dye. Red is NG2 chondroitin sulfate proteoglycan immunoreactivity, which labels oligodendrocyte precursor cells. Cocultures were prepared as described in Watkins et al. (2008).

Finally, simplified culture systems have revealed that myelinating oligodendrocytes receive a helping hand from astrocytes, particularly in the later stage of wrapping (Ishibashi et al., 2006; Sorensen et al., 2008; Watkins et al., 2008). The complete picture of the ways in which astrocytes promote more rapid wrapping is not yet clear, but could include providing both signals, such as LIF, and material contributions in the form of packaged lipids. Some evidence suggests that this support may be preferentially provided by white-matter- rather than gray-matter-derived astrocytes. By characterizing astrocyte functions, and by identifying the gamma secretase substrate within oligodendrocytes, which may connect an axonal signal with a nuclear program in oligodendrocytes, it should be possible to make future steps forward in understanding the molecular basis of CNS myelination.

Many diseases of the nervous system involve myelin. Multiple Sclerosis is one of the most common neurological diseases. It involves demyelination due to an autoimmune attack on myelin and oligodendrocytes. Although in most cases of relapsing and remitting Multiple Sclerosis, it appears that there is initial remyelination due to the generation of new oligodendrocytes and new myelin, at some point in the disease this repair process fails. It is not known whether repair fails because of exhaustion of new OPCs, a deficiency in the relevant axonal signals or electrical activity that induces OPC proliferation and myelination, a diversion of OPCs into an astrocyte differentiation pathway, or the development of inhibitors that prevent migration or myelination by OPCs. The answer may lead to new drugs that promote myelin repair. Oligodendrocytes are also lost in brain trauma and spinal cord injury either directly or as an indirect result of axon injury and degeneration. Axonal signals, not yet identified, are required for the survival of oligodendrocytes. Reciprocally, demyelinated

axons do not survive indefinitely when they lose their myelin, and this failure mechanism is also unclear.

Surprisingly, there is new evidence that major depressive disorder may involve a massive loss of oligodendrocytes and myelin within the temporal lobe. When mRNA extracted from human temporal lobes was analyzed by gene profiling, a 3-fold decrease in all oligodendrocyte genes was found (Aston et al., 2005). These findings corresponded well to a histological loss of oligodendrocytes that had been observed in previous studies, and occurred regardless of whether the patients had been treated with antidepressant medication. Over the past 50 years of intensive study, loss of myelin is the most dramatic abnormality to ever be reported in this very common disorder. A long-prevailing view has been that depression is a disorder caused by low CNS serotonin levels, but it is interesting to note that oligodendrocytes express high levels of dopa decarboxylase (Cahoy et al., 2008), a serotonin synthetic enzyme. These findings suggest the possibility that low serotonin levels are an effect rather than a cause of depression. It is critical that additional studies be conducted to confirm these findings, because if they are correct, there are important implications for developing new treatments. Although based on rodent studies, it has long been thought that oligodendrocytes do not turn over appreciably during a lifetime, it is possible in humans that they are slowly replaced, much as is now known to occur for hippocampal neurons. If this rate of new generation is even slightly lessened, for instance by stress, this could lead over time to substantial loss of myelin. If so, new treatments that promote the generation of new oligodendrocytes might be beneficial.

What Are the Roles of Microglia?

Immune system cells called microglia constitute about 10% of CNS glia (Hanisch and Kettenmann, 2007; Soulet and Rivest, 2008). Much mystery surrounds the functions of microglia in health and in disease. Like perivascular macrophages of the brain, microglia are derived from uncommitted myeloid progenitor cells that invade the brain neonatally (Santambrogio et al., 2001). In vitro, these myeloid progenitor cells are bipotential; depending on context, they can become phagocyte-like cells or immature dendritic-like cells. Within the normal brain, it is unclear exactly what their phenotype is; many or all may retain a relatively uncommitted state. Like other glial cell types, much of their function remains mysterious, and like reactive astrocytes, there has been much debate about whether their functions are helpful or harmful. There is increasing evidence for microglial heterogeneity within the brain, with antigen-presenting dendritic cell types present even within uninjured brain tissue (Carson et al., 2007; Bulloch et al., 2008; Bailey-Bucktrout et al., 2008; Gowing et al., 2008). Normally macrophages are situated in the perivascular space, whereas microglia are located within the brain parenchyma. Within the normal brain, microglia appear to act as sensors of the extracellular environment, rapidly responding to and potentially communicating changes or injury to surrounding neural cells or non-CNS immune cells. Recent in vivo time-lapse imaging has revealed dynamic interactions between microglia and neurons in the brain following lesion or injury (Davalos et al., 2005; Nimmerjahn et al., 2005). Although microglia display at least some phagocytic ability, so far they do not appear to have the strong professional phagocytic ability exhibited by activated macrophages. Many recent papers have found that amyloid deposits and degenerating CNS myelin are far more robustly phagocytosed by macrophages than microglia. Dendritic microglia have recently been demonstrated to present myelin antigens to T cells within the brain, where they play a critical role in driving the progression of relapsing experimental autoimmune encephalomyelitis, a mouse model of the demyelinating disease Multiple Sclerosis (Miller et al., 2007).

Activated microglia secrete high levels of many cytokines including TNFα, a proinflammatory cytokine involved in demyelinating and other diseases. TNFα signals directly to lymphocytes and macrophages to control their function, but recent studies have called attention to its actions on neural cells as well. Microglial-derived TNFα plays a critical role in promoting generation of new oligodendrocytes in mouse models of demyelination (Arnett et al., 2001). Cytokines released by microglia weaken the integrity of the blood-brain barrier in brain inflammation. TNFα even plays a role in controlling normal function and plasticity of neural circuits in vitro and in vivo (Stellwagen and Malenka, 2006; Kaneko et al., 2008). Blockade of activity in hippocampal neurons scales up the size of their synaptic inputs, an effect dependent on microglia-derived TNFα (although astrocytes in culture have frequently been suggested to secrete TNFα, it is likely that this TNFα derives from microglia, which generally heavily contaminate astrocyte cultures). Astrocytes and OPCs express TNFα receptors, but it is unclear whether neurons normally do. Thus, it is possible that microglial TNFα exerts its effects on neurons indirectly by acting on synaptic astrocytes. The effects of cytokines on neuronal activity, both normally and after injury, are worthy of much further attention.

In addition to affecting synaptic activity, emerging data point to an important role for microglia during CNS development in mediating the selective elimination of inappropriate synaptic connections during the formation of mature neural circuits. The initiating protein of the classical complement cascade called complement component 1 q (C1q) is highly deposited on many synapses throughout the developing CNS (Stevens et al., 2007). Little C1q is present in the adult CNS, but postnatally, immature astrocytes release a signal that induces neuronal (and possibly also microglial) expression and secretion of C1q. Although neuronal C1q was observed primarily within the retina, microglia throughout the developing, but not adult, CNS express extremely high levels of C1q. Secreted C1q binds to and tags developing synapses. Then, at some or all of these synapses, the classical complement cascade becomes activated, leading to the synaptic deposition of the complement component C3. Mice deficient in complement protein C1q or C3 fail to eliminate many CNS synapses, as shown by the failure of anatomical refinement of retinogeniculate connections and the retention of excess functional retinal innervation by lateral geniculate neurons. How do complement-tagged synapses get removed? It is likely they are phagocytosed by microglia. Microglia express high levels of C3 receptors, and binding of C3 to this receptor signals microglia and macrophages to phagocytose. In fact, microglia are well known to phagocytose synaptic terminals of spinal and hypoglossal motor neurons following injury in a process known as synaptic stripping, although it is not yet known whether

this process is also complement cascade dependent. These findings add to the growing evidence that immune system molecules are crucial for the patterning of neural circuits (Boulanger and Shatz, 2004; Huh et al., 2000) and support a model in which unwanted synapses are tagged by complement proteins for elimination by phagocytic cells.

These findings indicate that the immune system plays important roles in normal brain function and raises the question of whether it plays similar roles in brain disease. Interestingly, C1q levels have been demonstrated to be substantially elevated in most acute and chronic CNS diseases, particularly neurodegenerative diseases. For instance, C1q become elevated and localized to retinal synapses as the earliest manifestation of the disease process in a mouse model of glaucoma (Stevens et al., 2007). In Alzheimer's disease, C1q levels within the CNS have been found to be as much as 70-fold elevated. This is interesting because Alzheimer's disease is a disease of massive synapse loss. It has been estimated that by the time even the earliest cognitive loss can be detected in an Alzheimer's patient, some regions of their brain have already lost as many as 80% of their synapses. So far, mouse models of Alzheimer's disease have not exhibited such a profound synapse loss. Nonetheless, C1q deficiency has been shown to be predictive in a mouse model of Alzheimer's disease (Fonseca et al., 2004). Thus, classical-complement-cascade-mediated synapse loss may be a central feature of neurodegenerative diseases such as Alzheimer's disease, ALS, Multiple Sclerosis, and glaucoma. If so, drugs that block synaptic complement cascade activation have the potential to minimize neurodegeneration in these diseases.

The role of microglia in neurological disease is now the matter of much intrigue and debate. Microgliosis and reactive astrocytosis generally occur together, but it is not known whether there is a causal connection and if so in which direction. Astrocytes release signals such as CSF-1 and ATP that can signal to microglia, whereas microglia release signals such as TNFα that can signal to astrocytes. Nor is there agreement on whether lessening either type of gliosis will be helpful or harmful. The answer may well depend on the type and stage of each disease process. This is an emerging, understudied area of research that will undoubtedly remain fruitful for a long time, and it is likely to teach us much about normal and abnormal brain function.

Could Glial Cells Be Important Drug Targets?

As we have seen, virtually every aspect of brain development and function involves a neuron-glial partnership. Therefore, the answer to every important question about brain disease will also involve glia. The most common brain diseases include traumatic brain injury, stroke, spinal cord injury, Multiple Sclerosis, epilepsy, Alzheimer's disease, Parkinson's disease, and ALS, Down's syndrome, glioma, major depressive disorder, and autism. Other than palliative treatments, we currently have few effective treatments that block the underlying disease process for any of these disorders, and we cannot repair and restore a damaged brain yet. An obvious reason is that for each of these diseases we still do not understand many basic aspects of their pathophysiology. In every one of these diseases, glial cells are central contributors, yet their roles are often neglected (Miller, 2005). If we want to keep neurons from dying or misbehaving in these diseases, we must understand how glial pathology contributes to neuronal dysfunction and vice versa (Lobsiger and Cleveland, 2007). Over 1000 drug trials for stroke have now failed (O'Collins et al., 2006). In most of these trials, neurons were exclusively targeted, for instance using drugs that blocked neuronal glutamate receptors. Yet the extent to which astrocytes are killed in stroke or in neurodegenerative processes has received relatively little attention. If the glia that support the neurons are killed, how can the neurons be saved by just targeting the neurons? Quite possibly saving astrocytes from dying in neurological disease would be a far more effective strategy than trying to save neurons (glia already know how to save neurons, whereas neuroscientists still have no clue).

Therefore if we are to make progress in understanding normal and abnormal neurobiology, we have to start teaching neurobiologists more about how neurons interact with other cell types including glia, vascular cells, and immune cells. The explosion in interest in neurobiology among young people today has lead to the creation of an undergraduate major in neurobiology at most universities, which has arguably substantially undermined interdisciplinary training of neurobiologists. An undergraduate degree in engineering, physics, bioinformatics, immunology, or genetics, perhaps along with a minor in neuroscience, would make far more sense. One solution may be to offer undergraduates additional options, perhaps the option of a major in neuroengineering or neurogenetics. There is much to be said for the creation of a coterm masters degree in human biology and disease (the equivalent of the first year and a half or so of medical school) for undergraduates interested in translational research or obtaining a broader perspective of brain function in the context of a whole organism, but not interested in clinical practice (see http://msm.stanford.edu). Such programs would help to deploy our young scientists far more effectively. It is unfortunate that today's conservative funding climate encourages, indeed almost mandates, students to continue in their own labs working on exactly the same focused areas of research that they trained in. Having 20,000 neuroscientists that study LTP while only 20 are studying glia simply makes no sense. In the growth of knowledge, as in the growth of savings, diversification makes all the difference.

The pipelines are now starting to run dry at major pharmaceutical companies. A business model does not seem to work well when it comes to understanding pathophysiology because of the high risk nature of this research. But new treatments will only come when we have a deep mechanistic understanding of disease processes. It is therefore urgent that more academic scientists be involved in this quest. If we are to develop new treatments more rapidly, then academia, pharmaceutical companies, philanthropists, venture capitalists, and nonprofit foundations will have to innovate completely new ways of working together. Interesting new attempts at a more collaborative approach include the Adelson Medical Foundation, Fast Forward (www.fastforward.org), the CHDI Foundation (Pacifici and Rankin, 2008), and the Myelin Repair Foundation (www.myelinrepair.org). Let's all work together—if we can cure neurological diseases, there will be more than enough credit to share.

And please don't forget the glia! Quite possibly the most important roles of glia have yet to be imagined.

SUPPLEMENTAL DATA

The supplemental data for this article contain a set of instructions on how to use the All Exon Browser and can be found at http://www.neuron.org/supplemental/S0896-6273(08)00886-6.

REFERENCES

Agulhon, C., Petravicz, J., McMullen, A.B., Sweger, E.J., Minton, S.K., Taves, S.R., Casper, K.B., Fiacco, T.A., and McCarthy, K.D. (2008). What is the role of astrocyte calcium in neurophysiology? Neuron *59*, 932–946.

Araque, A., Parpura, V., Sanzgiri, R.P., and Haydon, P.G. (1999). Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. *22*, 208–215.

Arnett, H.A., Mason, J., Marino, M., Suzuki, K., Matsushima, G.K., and Ting, J.P. (2001). TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat. Neurosci. *4*, 1116–1122.

Aston, C., Jiang, L., and Sokolov, B.P. (2005). Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol. Psychiatry *10*, 309–322.

Bailey-Bucktrout, S.L., Caulkins, S.C., Goings, G., Fischer, J.A., Dzionek, A., and Miller, S.D. (2008). Cutting edge: central nervous system plasmacytoid dendritic cells regulate the severity of relapsing experimental autoimmune encephalomyelitis. J. Immunol. *180*, 6457–6461.

Banker, G.A. (1980). Trophic interactions between astroglial cells and hippocampal neurons in culture. Science *209*, 809–810.

Bishop, D.L., Misgeld, T., Walsh, M.K., Gan, W.B., and Lichtman, J.W. (2004). Axon branch removal at developing synapses by axosome shedding. Neuron *44*, 651–661.

Boroujerdi, A., Kim, H.K., Lyu, Y.S., Kim, D.S., Figueroa, K.W., Chung, J.M., and Luo, Z.D. (2008). Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. Pain, in press. Published online June 19, 2008. 10.1016/j.pain.2008.05.004.

Boulanger, L.M., and Shatz, C.J. (2004). Immune signalling in neural development, synaptic plasticity and disease. Nat. Rev. Neurosci. *5*, 521–531.

Brinkmann, B.G., Agarwal, A., Sereda, M.W., Garratt, A.N., Müller, T., Wende, H., Stassart, R.M., Nawaz, S., Humml, C., Velanac, V., et al. (2008). Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. Neuron *59*, 581–595.

Bulloch, K., Miller, M.M., Gal-Toth, J., Milner, T.A., Gottfried-Blackmore, A., Waters, E.M., Kaunzer, U.W., Liu, K., Lindquist, R., Nussenzweig, M., et al. (2008). CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult and injured mouse brain. J. Comp. Neurol. *508*, 687–710.

Bush, T.G., Puvanachandra, N., Horner, C.H., Polito, A., Ostenfeld, T., Svendsen, C.N., Mucke, L., Johnson, M.H., and Sofroniew, M.V. (1999). Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. Neuron *23*, 297–308.

Bushong, E.A., Martone, M.E., Jones, Y.Z., and Ellisman, M.H. (2002). Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J. Neurosci. *22*, 183–192.

Caceres, M., Suwyn, C., Maddox, M., Thomas, J.W., and Preuss, T.M. (2006). Increased Cortical Expression of Two Synaptogenic Thrombospondins in Human Brain Evolution. Cereb Cortex *17*, 2312–2321.

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P.A., Krupenko, S.A., et al. (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. *28*, 264–278.

Carson, M.J., Bilousova, T.V., Puntambekar, S.S., Melchior, B., Doose, J.M., and Ethell, I.M. (2007). A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease. Neurotherapeutics *4*, 571–579.

Chan, J.R., Watkins, T.A., Cosgaya, J.M., Zhang, C., Chen, L., Reichardt, L.F., Shooter, E.M., and Barres, B.A. (2004). NGF controls axonal receptivity to myelination by Schwann cells or oligo- dendrocytes. Neuron *43*, 183–191.

Christopherson, K.S., Ullian, E.M., Stokes, C.C., Mullowney, C.E., Hell, J.W., Agah, A., Lawler, J., Mosher, D.F., Bornstein, P., and Barres, B.A. (2005). Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell *120*, 421–433.

Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., and Gan, W.B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. *8*, 752–758.

Di Giorgio, F.P., Carrasco, M.A., Siao, M.C., Maniatis, T., and Eggan, K. (2007). Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. Nat. Neurosci. *10*, 608–614.

Dombeck, D.A., Khabbaz, A.N., Collman, F., Adelman, T.L., and Tank, D.W. (2007). Imaging large-scale neural activity with cellular resolution in awake, mobile mice. Neuron *56*, 43–57.

Dugas, J.C., Mandemakers, W., Rogers, M., Ibrahim, A., Daneman, R., and Barres, B.A. (2008). A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. J. Neurosci. *28*, 8294–8305.

Eroglu, C., Barres, B.A., and Stevens, B. (2008). Glia as active participants in the development and function of synapses. In Structural and Functional Organization of the Synapse, J.W. Ehlers and M.D. Hell, eds. (New York: Springer).

Eshed, Y., Feinberg, K., Poliak, S., Sabanay, H., Sarig-Nadir, O., Spiegel, I., Bermingham, J.R., Jr., and Peles, E. (2005). Gliomedin mediates Schwann cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron *47*, 215–229.

Feng, Z., and Ko, C.P. (2008). The role of glial cells in the formation and maintenance of the neuromuscular junction. Ann. N Y Acad. Sci. *1132*, 19–28.

Fonseca, M., Zhou, J., Botto, M., and Tenner, A. (2004). Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimers disease. J. Neurosci. *24*, 457–465.

Gordon, G.R., Mulligan, S.J., and MacVicar, B.A. (2007). Astrocyte control of the cerebrovasculature. Glia *55*, 1214–1221.

Gowing, G., Philips, T., Van Wijmeersch, B., Audet, J.N., Dewil, M., Van Den Bosch, L., Billiau, A.D., Robberecht, W., and Julien, J.P. (2008). Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. J. Neurosci. *28*, 10234–10244.

Hanisch, U.K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. *10*, 1387–1394.

Hertz, L., Peng, L., and Dienel, G.A. (2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. J. Cereb. Blood Flow Metab. *27*, 219–249.

Houades, V., Rouach, N., Ezan, P., Kirchhoff, F., Koulakoff, A., and Giaume, C. (2006). Shapes of astrocyte networks in the juvenile brain. Neuron Glia Biol. *2*, 3–14.

Huh, G.S., Boulanger, L.M., Du, H., Riquelme, P.A., Brotz, T.M., and Shatz, C.J. (2000). Functional requirement for class I MHC in CNS development and plasticity. Science *290*, 2155–2159.

Ishibashi, T., Dakin, K.A., Stevens, B., Lee, P.R., Kozlov, S.V., Stewart, C.L., and Fields, R.D. (2006). Astrocytes promote myelination in response to electrical impulses. Neuron *49*, 823–832.

Jaiswal, J.K., Fix, M., Takano, T., Nedergaard, M., and Simon, S.M. (2007). Resolving vesicle fusion from lysis to monitor calcium-triggered lysosomal exocytosis in astrocytes. Proc. Natl. Acad. Sci. USA *104*, 14151–14156.

Kaneko, M., Stellwagen, D., Malenka, R.C., and Stryker, M.P. (2008). Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. Neuron *58*, 673–680.

Li, D., Ropert, N., Koulakoff, A., Giaume, C., and Oheim, M. (2008). Lysosomes are the major vesicular compartment undergoing Ca2+-regulated exocytosis from cortical astrocytes. J. Neurosci. *28*, 7648–7658.

Liauw, J., Hoang, S., Choi, M., Eroglu, C., Choi, M., Sun, G., Percy, M., Widman-Tobriner, B., Bliis, T., Guzmann, R.G., et al. (2008). Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. J. Cereb. Blood Flow Metab. *28*, 1722–1732.

Lobsiger, C.S., and Cleveland, D.W. (2007). Glial cells as intrinsic component of non-cell-autonomous neurodegenerative disease. Nat. Neurosci. *10*, 1355–1360.

Lovatt, D., Sonnewald, U., Waagepetersen, H.S., Schousboe, A., He, W., Lin, J.H., Han, X., Takano, T., Wang, S., Sim, F.J., et al. (2007). The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. J. Neurosci. *27*, 12255–12266.

Makita, T., Sucov, H.M., Gariepy, C.E., Yanagisawa, M., and Ginty, D.D. (2008). Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. Nature *452*, 759–763.

Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A., and Pfrieger, F.W. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. Science *294*, 1354–1357.

Metea, M.R., and Newman, E.A. (2006). Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. J. Neurosci. *26*, 2862–2870.

Miller, G. (2005). The Dark Side of Glia. Science *308*, 778–781.

Miller, S.D., McMahon, E.J., Schreiner, B., and Bailey, S.L. (2007). Antigen presentation in the CNS by myeloid dendritic cells drives progression of relapsing experimental autoimmune encephalomyelitis. Ann. N Y Acad. Sci. *1103*, 179–191.

Müller, C.M., and Best, J. (1989). Ocular dominance plasticity in adult cat visual cortex after transplantation of cultured astrocytes. Nature *342*, 427–430.

Mustafa, A.K., Kim, P.M., and Snyder, S.H. (2004). D-Serine as a putative glial neurotransmitter. Neuron Glia Biol. *1*, 275–281.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., and Przedborski, S. (2007). Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat. Neurosci. *10*, 615–622.

Nielsen, J.A., Maric, D., Lau, P., Barker, J.L., and Hudson, L.D. (2006). Identification of a novel oligodendrocyte cell adhesion protein using gene expression profiling. J. Neurosci. *26*, 9881–9891.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science *308*, 1314–1318.

O'Collins, V.E., Macleod, M.R., Donnan, G.A., Horky, L.L., van der Worp, B.H., and Howells, D.W. (2006). 1,026 experimental treatments in acute stroke. Ann. Neurol. *59*, 467–477.

Pacifici, R., and Rankin, D. (2008). Foundation led drug discovery. Scientist *22*, 28–29.

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., and Oliet, S.H. (2006). Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell *125*, 775–784.

Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.Y., Takano, H., Moss, S.J., McCarthy, K., and Haydon, P.G. (2005). Astrocytic purinergic signaling coordinates synaptic networks. Science *310*, 113–116.

Paukert, M., and Bergles, D.E. (2006). Synaptic communication between neurons and NG2+ cells. Curr. Opin. Neurobiol. *16*, 515–521.

Petzold, G., Albeanu, D., Sato, T., and Murthy, V. (2008). Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. Neuron *58*, 897–910.

Pfrieger, F.W., and Barres, B.A. (1997). Synaptic efficacy enhanced by glial cells in vitro. Science *277*, 1684–1687.

Pogoda, H.M., Sternheim, N., Lyons, D.A., Diamond, B., Hawkins, T.A., Woods, I.G., Bhatt, D.H., Franzini-Armstrong, C., Dominguez, C., Arana, N., et al. (2006). A genetic screen identifies genes essential for development of myelinated axons in zebrafish. Dev. Biol. *298*, 118–131.

Roesch, K., Jadhav, A.P., Trimarchi, J.M., Stadler, M.B., Roska, B., Sun, B.B., and Cepko, C.L. (2008). The transcriptome of retinal Müller glial cells. J. Comp. Neurol. *509*, 225–238.

Santambrogio, L., Belyanskaya, S.L., Fischer, F.R., Cipriani, B., Brosnan, C.F., Ricciardi-Castagnoli, P., Stern, L.J., Strominger, J.L., and Riese, R. (2001). Developmental plasticity of CNS microglia. Proc. Natl. Acad. Sci. USA *98*, 6295–6300.

Saunders, N.R., Ek, C.J., Habgood, M.D., and Dziegielewska, K.M. (2008). Barriers in the brain: a renaissance? Trends Neurosci. *31*, 279–286.

Schummers, J., Yu, H., and Sur, M. (2008). Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science *320*, 1638–1643.

Shen, Q., Goderie, S.K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K., and Temple, S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science *304*, 1338–1340.

Silver, J., and Miller, J.H. (2004). Regeneration beyond the glial scar. Nat. Rev. Neurosci. *5*, 146–156.

Somjen, G. (1988). Nervenkitt: notes on the history of the concept of neuroglia. Glia *1*, 2–9.

Sorensen, A., Moffat, K., Thomson, C., and Barnett, S.C. (2008). Astrocytes, but not olfactory ensheathing cells or Schwann cells, promote myelination of CNS axons in vitro. Glia *56*, 750–763.

Soulet, D., and Rivest, S. (2008). Microglia. Curr. Biol. *18*, R506–R508.

Stellwagen, D., and Malenka, R.C. (2006). Synaptic scaling mediated by glial TNF-alpha. Nature *440*, 1054–1059.

Stevens, B., Allen, N.J., Vazquez, L.E., Howell, G.R., Christopherson, K.S., Nouri, N., Micheva, K.D., Mehalow, A.K., Huberman, A.D., Stafford, B., et al. (2007). The classical complement cascade mediates CNS synapse elimination. Cell *131*, 1164–1178.

Takano, T., Han, X., Deane, R., Zlokovic, B., and Nedergaard, M. (2007). Two-photon imaging of astrocytic Ca2+ signaling and the microvasculature in experimental mice models of Alzheimer's disease. (2007). Ann. N Y Acad. Sci. *1097*, 40–50.

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., Garcia-Verdugo, J.M., and Doetsch, F. (2008). A specialized vascular niche for adult neural stem cells. Cell Stem Cell *3*, 279–288.

Trajkovic, K., Dhaunchak, A.S., Goncalves, J.T., Wenzel, D., Schneider, A., Bunt, G., Nave, K.A., and Simons, M. (2006). Neuron to glia signaling triggers myelin membrane exocytosis from endosomal storage sites. J. Cell Biol. *172*, 937–948.

Tran, M.D., and Neary, J.T. (2006). Purinergic signaling induces thrombospondin-1 expression in astrocytes. Proc. Natl. Acad. Sci. USA *103*, 9321–9326.

Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., and Barres, B.A. (2001). Control of synapse number by glia. Science *291*, 657–661.

Ventura, R., and Harris, K.M. (1999). Three-dimensional relationships between hippocampal synapses and astrocytes. J. Neurosci. *19*, 6897–6906.

Wagner, B., Natarajan, A., Grünaug, S., Kroismayr, R., Wagner, E.F., and Sibilia, M. (2006). Neuronal survival depends on EGFR signaling in cortical but not midbrain astrocytes. EMBO J. *25*, 752–762.

Wang, X., Lou, N., Xu, Q., Tian, G.F., Peng, W.G., Han, X., Kang, J., Takano, T., and Nedergaard, M. (2006). Astrocytic Ca2+ signaling evoked by sensory stimulation in vivo. Nat. Neurosci. *9*, 816–823.

Watkins, T.A., Emery, B., Mulinyawe, S., and Barres, B. (2008). Distinct stages of myelination regulated by γ-secretase and astrocytes in a rapidly myelinating CNS coculture system. Neuron *60*, in press.

Zhang, Z., Chen, G., Zhou, W., Song, A., Xu, T., Luo, Q., Wang, W., Gu, X.S., and Duan, S. (2007). Regulated ATP release from astrocytes through lysosome exocytosis. Nat. Cell Biol. *9*, 945–953.

Zlokovic, B.V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron *57*, 178–201.

Zlokovic, B.V., and Apuzzo, M.L. (1998). Strategies to circumvent vascular barriers of the central nervous system. Neurosurgery *43*, 877–878.

Zonta, M., Angulo, M.C., Gobbo, S., Rosengarten, B., Hossmann, K.A., Pozzan, T., and Carmignoto, G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat. Neurosci. *6*, 43–50.