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Keith K. Murai and Donald J. Van Meyel

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Neuron–Glial Communication at Synapses: Insights From Vertebrates and Invertebrates

KEITH K. MURAI and DONALD J. VAN MEYEL
Centre for Research in Neuroscience, Department of Neurology and Neurosurgery, McGill University the Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada

Glial cells are instrumental for many aspects of nervous-system function. Interestingly, complex neuron–glial interactions at synapses are commonly found in both invertebrates and vertebrates. Although these interactions are known to be important for synaptic physiology, the cellular processes and molecular mechanisms involved have not been fully uncovered. Identifying the common and unique features of neuron–glial interactions between invertebrates and vertebrates may provide valuable insights into the relationship of neuron–glial cross-talk to nervous-system function. This review highlights selected studies that have revealed structural and functional insights into neuron–glial interactions at synapses in invertebrate and vertebrate model systems. NEUROSCIENTIST 13(6): 657–666, 2007. DOI: 10.1177/1073858407304393

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The complex architecture of glia has been observed for more than a century (Somjen 1988), and glial cells are now recognized as key elements in the nervous systems of many invertebrate and vertebrate organisms. Golgi impregnation and other staining techniques have revealed that distinct glial subtypes are present in many organisms ranging from flies and other insects to crustacea and leeches to rodents and humans. A remarkable property of many of these glial cells is their intimate association with neuronal connections. The concept that glial cells could have a valuable and active role at sites of contact between neurons was realized after their initial anatomical description by neuroanatomists in the mid- to late 1800s. Even then, it was proposed

> that the neuroglia abounds in regions with numerous and complicated intercellular connections, not only because of the existence of contacts, but with the purpose of regulating and directing these contacts, so that each dendritic process may become intimately related with a special group of terminal axonal branches. (Ramon y Cajal 1899, translated to English by Pasik and Pasik 1999)

Today, the idea that glia help *regulate* and *direct* neural connections and play defined roles at neural circuitry is clear, although it took decades for glia to overcome the unfortunate stigma that they indiscriminately fill gaps between neurons and simply glue the nervous system together (Somjen 1988). Indeed, early neuroanatomists would be astounded by the number of studies that have shed light on the myriad roles of glia in modulation, maintenance, and protection of the nervous system. The importance of glia at *complicated intercellular connections* (i.e., synapses) has been further illuminated by studies showing that glia participate in synaptic function and plasticity and may be involved in a number of neurological diseases that cause cognitive and behavioral deficits.

The progress of neuroscientists in understanding the properties of neurons has been impressive in that we now have broad insights into how neurons develop, diversify, and intercommunicate. However, comparatively less is known about the glial subtypes that thrive in the central nervous system and the extent of their actions. Complex interplay between neurons and glia can be found in both invertebrates and vertebrates, indicating important and evolutionarily conserved roles for neuron–glial interactions. The intent of this review is to discuss parallels across phyla with respect to the structural and functional intricacies of neuron–glial interactions at neural connections. The review focuses mainly on research in fruit flies and rodents, in which genetic approaches have offered added advantages. As has been found for other fields of neuroscience (i.e., axon guidance), identifying the common and unique features of neuron–glial interactions between invertebrates and vertebrates may provide valuable insights into the cellular and molecular mechanisms that underlie nervous-system function. Many fascinating properties of glia remain to be resolved, and unlike the early neuroanatomists, we are fortunate to be writing this review in an era when advanced experimental tools are available to help answer key questions regarding the function of glia at neuronal circuitry.

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Address correspondence to: Dr Keith K. Murai or Dr Van Meyel, McGill Centre for Research in Neuroscience, 1650 Cedar Avenue, Montreal, QC H3G 1A4, Canada (e-mail: keith.murai@mcgill.ca or don.vanmeyel@mcgill.ca).

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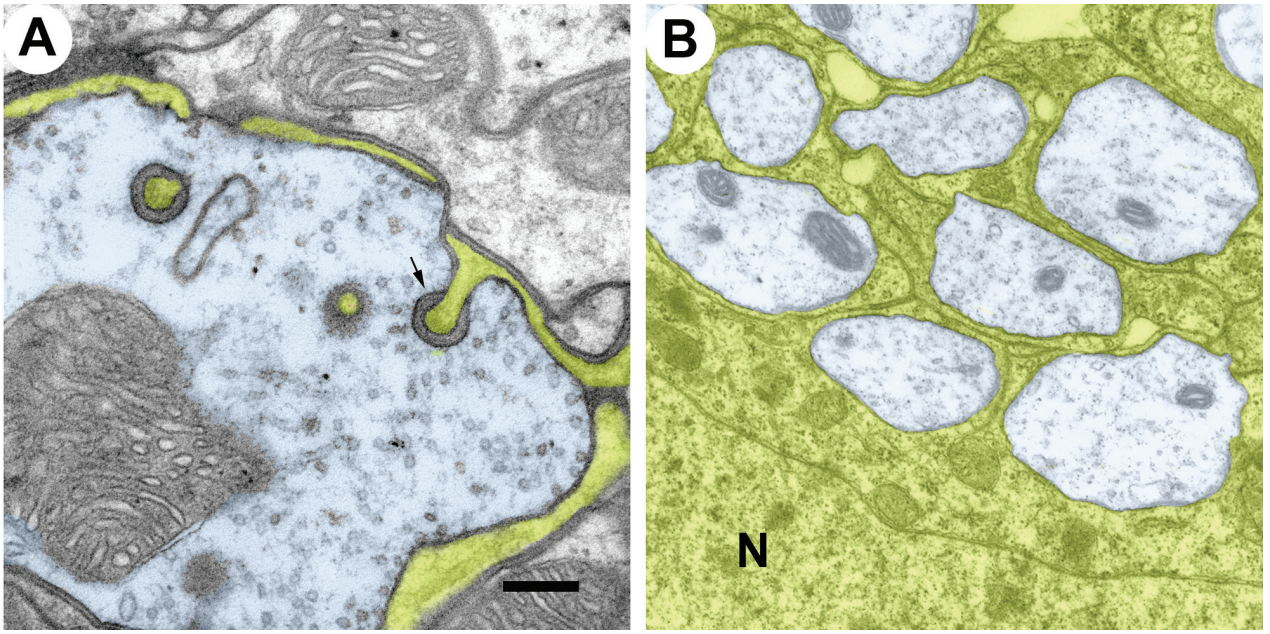

Fig. 1. Examples of neuron–glial interactions in invertebrates. (*A*) Capitate projections in the *Drosophila* visual system. These structures are formed by glial cell processes (yellow) in neuropil regions where photoreceptor (R-cell) axons (blue) synapse with target neurons in the optic lobe. Capitate projections have an interesting shape composed of a stalk and head that are embedded into the axon terminals of R-cells (arrows). Image courtesy of IA Meinertzhagen. Scale bar = 200 nm. (*B*) Processes of glial cells in insects are often highly interdigitated between neuronal elements within the neuropil. Electron micrograph (21,000×) shows a cross-section through the neuropil of the thoracic ganglion of *Manduca sexta*. The axon tracts (blue) are ensheathed by glial-cell processes (yellow). A large, flattened glial-cell nucleus (N) lies at the periphery of the neuropil. Image courtesy of R Cantera. Reproduced from Cantera and Trujillo-Cenoz (1996), copyright 1996 by Wiley-Liss, Inc.

Morphological Diversity of Neuron–Glial Interactions at Synapses

The degree of structural interplay between neurons and glia near synapses can be diverse, ranging from highly extensive and stable relationships to those that are more variable and capricious. Here, we provide illustrative examples of sites where extensive physical contact is made between glia and synaptic compartments. This is followed with instances in which the degree of contact is known to be more limited. From examples such as these, the heterogeneous appearance of neuron–glial associations at synapses strongly suggests that glial cells in both invertebrates and vertebrates are adapted to fit the functional properties of the particular neural circuitry in which they are integrated.

An excellent example of a specialized site where glial processes show extensive interaction with synapses is found in the visual system of *Dipteran* species such as fruit flies and house flies. In the neuropil regions where photoreceptor (R-cell) axons synapse with target neurons in the optic lobe, there are intriguing structures called capitate projections, which are specialized extensions of neighboring glial cells (Fig. 1*A*; Frohlich and Meinertzhagen 1982; Stark and Carlson 1986). Ultrastructural analysis has revealed that capitate projections have a peculiar shape composed of a stalk and head that is actually embedded into the axon terminals of R-cells. Capitate projections are dynamic, moving piston-like into and from sites adjacent to collections of presynaptic vesicles. In flies, there are as many as 800 capitate projections per R-cell terminal ($3/\mu m^2$; Stark and Carlson 1986). Their abundance and intimate relationship with presynaptic terminals likely accommodate glial-mediated recovery of neurotransmitters at R-cell terminals (Fabian-Fine and others 2003). As we will discuss in more detail, R-cells tonically release the neurotransmitter histamine, and capitate projections likely provide the means by which R-cells achieve adequate neurotransmitter turnover and recycling.

In mammals, Bergmann glia in the cerebellum show extensive interplay with both the presynaptic and postsynaptic terminals of climbing and parallel fiber connections onto the spines of Purkinje cell dendrites (Fig. 2*A*; Bellamy 2006). Although these interactions in the cerebellum are obviously anatomically distinct from invertebrate capitate projections, they nonetheless provide examples of sites of close juxtaposition between neurons and glia. The Bergmann glia elaborate complex lateral appendages that encapsulate synapses onto Purkinje cells. On average, 87% of any climbing fiber synapse and 65% of any parallel fiber synapse is likely to be ensheathed by glial membrane (Xu-Friedman and

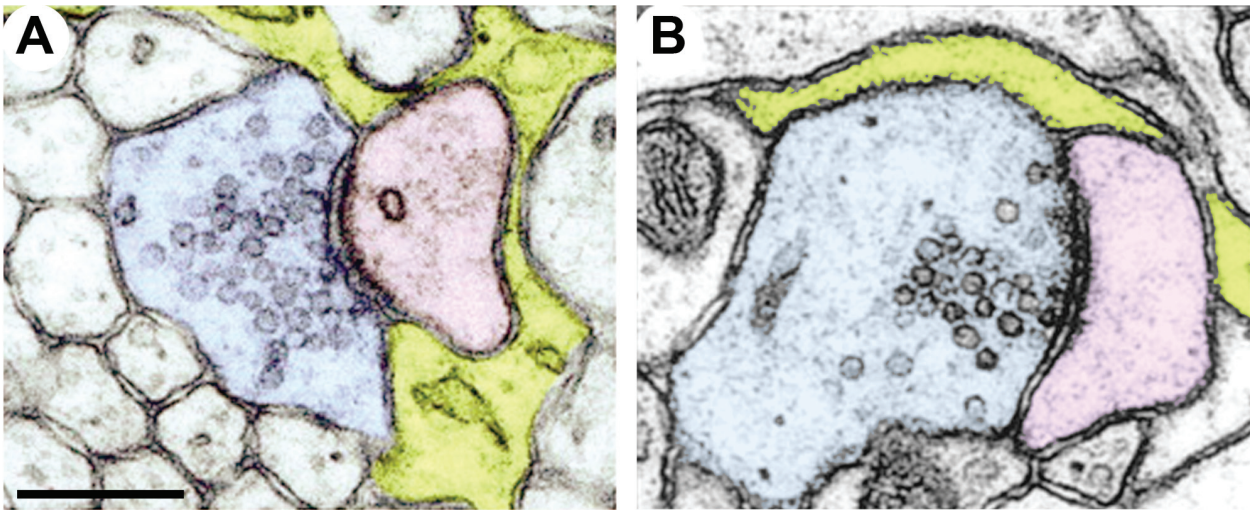

Fig. 2. Examples of neuron–glial interactions at vertebrate synapses. (*A*) A Bergmann glial cell process (yellow) encompasses a synapse made between a parallel fiber (blue) and a Purkinje neuron spine (red) in the rat cerebellum. Reproduced from Xu-Friedman and others (2001), copyright 2001 by the Society for Neuroscience. Scale bar = 300 nm. (*B*) Astrocytic processes (yellow) show more variable coverage around presynaptic (blue) and postsynaptic (red) elements in the CA1 region of the hippocampus. Scale bar = 300 nm. Reproduced from Ventura and Harris (1999), copyright 1999 by the Society for Neuroscience.

others 2001). Measured another way, more than 90% of the synapse surface is encapsulated in more than half of all climbing fiber synapses and a third of all parallel fiber synapses (Xu-Friedman and others 2001). Interestingly, the elaborations of a single Bergmann glial cell can be separated into distinct microdomains (Grosche and others 1999). It has been shown that these glial microdomains respond independently to subsets of parallel fiber synapses onto Purkinje cell dendrites. Stimulation of a few parallel fibers reveals calcium transients that are restricted to discrete microdomains on a single Bergmann glial arbor. Thus, Bergmann glia have local interactions with individual synapses while perhaps partitioning groups of synapses into microdomains.

Invertebrate glia also likely play a role in partitioning and coordinating neuronal connectivity. In the CNS of arthropods, glial cells are highly interdigitated between neurons and their processes (Fig. 1*B*; Horridge 1965). In many insect species, CNS glia have complex membrane arborizations, often sending thin glial processes deep into synapse-rich regions of the neuropil (Cantera and Trujillo-Cenoz 1996; Radojcic and Pentreath 1979). These arborizations can be clustered in discrete compartments circumscribing groups of synaptic terminals. In *Drosophila* embryos and larvae, a subclass of neuropil-associated glia called the longitudinal glia (LG, also called interface glia) are in intimate contact with the neuropil of the ventral nerve cord (Stacey and others 2007). LG ensheath axons within the longitudinal connectives and likely extend processes near synapses, although the postdevelopmental extent of these glial–synapse associations warrants detailed investigation. However, the emerging molecular profile of these cells indicates a role for LG in neuromodulation and neuroprotection (Altenhein and others 2006; Egger and others 2002; Freeman and others 2003), in addition to the established roles of these cells in neuron proliferation, survival, and axon guidance during development (Stacey and others 2007). Because LG ensheath axonal projections and likely extend processes into synapse-bearing regions, they illustrate how glia perform multiple tasks to accommodate distinct requirements of neural connectivity.

Perhaps the most striking example of glial-cell morphology that indicates functional multitasking is provided by the Müller glia of the mammalian retina (Bringmann and others 2006; Robinson and Dreher 1990). Each Müller glial cell spans multiple layers of the retina, and hence, it is exposed to different anatomical compartments, each with their own requirements for synaptic transmission and connectivity (Reichenbach and others 1989). These layers include the cell-body regions of the inner and outer nuclear layers and the neuropil regions of the inner and outer plexiform layers. The morphology of Müller glia is regionally customized to accommodate these distinct anatomical compartments of the retina. Indeed, a single Müller glial cell extends processes that envelop the cell bodies in the nuclear layers and projects fine processes into the synapse-rich plexiform layers. Furthermore, there is additional regional complexity because the same cell wraps ganglion cell axons in the nerve-fiber layer and also extends finger-like protrusions toward the vitreous body (Reichenbach and others 1989). Thus, the unique morphological properties of Müller glia are fully compatible with the anatomical layout of the retina and appear to be patterned by distinct spatial cues in the immediate environment

(Reichenbach and others 1989; Robinson and Dreher 1990). Interestingly, somewhat akin to the epithelial glia that form capitate projections in the visual system of the fly, the Müller glia encapsulate photoreceptor cell terminals where they participate in neurotransmitter recycling (Bringmann and others 2006).

In contrast to persistent and pervasive interactions with synapses exemplified by glia in the visual system and CNS of the fly and the Bergmann or Müller glia in mammals, other glial cells interact with synapses in perhaps a more limited yet no less vital manner. For example, protoplasmic astrocytes in the mammalian forebrain have less contact with synapses, and these interactions are surprisingly dynamic and variable (Fig. 2*B*; Haber and Murai 2006; Ventura and Harris 1999). Detailed reconstructions of glial associations with neurons in the hippocampus show that astrocytes extend fine processes toward axon terminals and dendritic spines, which are the postsynaptic regions of glutamatergic synapses (Ventura and Harris 1999). At a given moment, only about 57% of the synapses have astrocytic contact near the synaptic cleft, and about 43% of the perimeter of the synapse is covered by the astrocyte. At such excitatory synapses, astrocytic processes appear to preferentially surround dendritic spines, where there is a threefold to fourfold increase in glial coverage compared to the closest presynaptic terminals (Lehre and Rusakov 2002). The size and/or architecture of dendritic spines may influence the degree of astrocytic contact, because spines containing multiple postsynaptic densities tend to have closer associations with astrocytes than those with a single postsynaptic density (Ventura and Harris 1999). Based on studies that have characterized the effect of spine size on the molecular and physiological properties of excitatory synapses, this indicates that astrocytes are attracted to larger synapses that contain more glutamate receptors and have a higher probability of neurotransmitter release (Ganeshina and others 2004; Harris and Sultan 1995; Witcher and others 2007). Thus, the degree of association between astrocytes and excitatory synapses likely reflects the extent of glial modulation over the synapse and is an indication of the level of synaptic efficacy.

As described earlier, the microdomains formed by Bergmann glia may help compartmentalize subsets of synapses within the molecular layer of the cerebellum. Forebrain astrocytes are also likely to coordinate the function of groups of synapses, in part because of their intercellular spacing. During early postnatal development, astrocytes promote synapse formation and maturation while elaborating their own complex morphology (Bushong and others 2004; Christopherson and others 2005; Slezak and Pfrieger 2003). Protoplasmic astrocytes mature to display highly ramified arbors that occupy distinct territories by excluding the processes of neighboring astrocytes (Bushong and others 2002; Distler and others 1991; Ogata and Kosaka 2002). In the hippocampus, it has been suggested that an individual astrocyte is surrounded by up to 14 neighboring astrocytes (Ogata and Kosaka 2002). Inter-astrocyte spacing may contribute to the functional organization of glial networks in the mature brain because astrocytes intercommunicate through gap junctions and ATP-induced calcium signaling (Volterra and Meldolesi 2005). The fact that each astrocyte occupies an individual territory indicates that a single astrocyte modulates the functional properties of a collection of synapses, which has been estimated at 140,000 per astrocyte domain in the hippocampus (Bushong and others 2002; Kirov and others 1999).

Compartmentalization of synapses by glia can also occur without direct contact, as illustrated in the main olfactory systems of both invertebrates and vertebrates. In general, the functional organization of olfactory systems has been conserved among species, with sensory neurons sending their axons into the olfactory bulb (mammals) or the antennal lobe (insects), where they form synapses with second-order neurons in rounded regions of synaptic neuropil called glomeruli. In both systems, glial cells reside mainly on the periphery of individual glomeruli (Tolbert and others 2004). In mammals, some of these cells extend processes into the glomerular neuropil, where they contact dendrites of mitral, tufted, and periglomerular neurons but have limited association with synapses made by the presynaptic terminals of olfactory neurons. In this way, they may segregate intraglomerular synaptic connections from those of the incoming sensory axons (Bailey and Shipley 1993; Valverde and others 1992). In insects, glial coverage of synapses in glomeruli is also sparse (Tolbert and others 2004). As in mammals, these glia form envelopes around individual glomeruli, perhaps preventing the spread of potassium and reducing cross-communication between adjacent glomeruli (Goriely and others 2002).

Thus, the elaborate and diverse morphologies of glial cells are customized to fit the functional properties of particular synapse types. In the section Diversification of Glial Subtypes, we will discuss how glial cells may diversify to accommodate differences in neural connectivity. Then, we will focus on neuron–glial communication, and in particular, on the role of invertebrate and vertebrate glia in handling neurotransmitters at synapses, detecting neural activity, and secreting factors that modulate neurotransmission. Although their critical role for cellular nutrition, metabolism, and ion homeostasis (i.e., buffering extracellular K^+) is not addressed here, neuron–glial interactions can have broad effects on both neuronal and glial physiology. Interested readers can consult reviews on these subjects in invertebrate (Coles 1989; Tsacopoulos and Veuthey 1993) and vertebrate model systems (Laming and others 2000; Walz 2000b).

Diversification of Glial Subtypes

In the mammalian CNS, major subdivisions in glial classes such as astrocytes and oligodendrocytes are known, and broad differences within a particular glial class, such as protoplasmic astrocytes in grey matter versus fibrous astrocytes in white matter, have been recognized (Somjen 1988). For astrocytes in brain structures such as the hippocampus, regional variations in gap junctional coupling and distinct physiological profiles of

cells have also emerged (Matthias and others 2003; Steinhauser and others 1994). However, the true extent of the functional diversity of glial cells in the mammalian CNS remains an important yet unanswered question for the field. Currently, there are no known signals that promote subtype specification of astrocytes (Matthias and others 2003; Walz 2000a).

Some appreciation of the diversity of glial subtypes has been achieved in insects. For example, six morphologically distinct glial subtypes are recognizable in just the visual system of adult flies alone (Kretzschmar and Pflugfelder 2002; Saint Marie and Carlson 1983). Roughly the same number of glial subtypes exists in the metathoracic ganglion of locusts (Hoyle 1986) and in the *Drosophila* ventral nerve cord (Ito and others 1995).

How are glia subtypes specified? Although it is an underdeveloped area of glial research, glial-subtype specification likely is directed by a combination of intrinsic genetic programs and extrinsic inductive cues, allowing glial subtypes to achieve the position, morphology, and molecular profile needed to meet the demands of the neurons with which they associate. Evidence for this comes from the genetically tractable fruit fly *Drosophila*, in which most glial cells are specified by a transcription factor known as Glial cells missing (Gcm). Gcm sits atop a regulatory cascade that promotes glial fate and/or represses neuronal fate (Jones 2005). In *gcm*-mutant embryos, glia are lost and there are extra neurons, whereas *gcm* overexpression has opposite effects. Three research groups have exploited these observations to identify glial-specific genes on a large scale (Altenhein and others 2006; Egger and others 2002; Freeman and others 2003). They found that the expression of many of these genes was restricted to glial subtypes, suggesting unique molecular profiles that could underlie functional diversification. Although all of the genes identified in these surveys were controlled by Gcm, these subtype-specific patterns of expression likely arise from additional, unknown factors that are spatially or temporally restricted (Altenhein and others 2006; Freeman and others 2003). In search of such factors, one study has shown that gene expression in a subtype of LG is influenced by neuron-to-glial signaling through the Notch-receptor pathway (Thomas and van Meyel 2007). The anterior six LG in each hemisegment use the glycosyltransferase Fringe as a means to sustain signaling through the Notch receptor and promote the subtype-specific gene expression of the transcription factor Prospero. Fringe adds carbohydrates to specific epidermal growth factor repeats of the Notch extracellular domain, sensitizing Notch receptors in these glia to activation by the ligand Delta, which is expressed on a subset of axons. The restricted expression of Fringe and Delta, coupled with the timing of neuron–glial contact, provides spatial and temporal context to limit Notch signaling specifically to the anterior LG subtype. This illustrates the close interplay between intrinsic and extrinsic factors for glial-subtype specification. In vertebrates, Notch signaling regulates the differentiation of multiple glial-cell types (for references, see Thomas and van Meyel 2007), and the Fringe ortholog Lunatic Fringe is expressed in glial progenitors (Ishii and others 2000) and mature glia of different subtypes (GENSAT, the Gene Expression Nervous System Atlas Project, NINDS Contract # N01NS02331 to the Rockefeller University, New York), although its function there remains unknown.

Glia and Neurotransmitter Uptake and Recycling

Clearly, some classes of glia are skilled at the uptake, metabolism, and recycling of neurotransmitters at synapses (Fig. 3). As described earlier, capitate projections are dynamic sites of interaction between specialized glial processes and R-cell axon terminals that tonically release the neurotransmitter histamine onto synaptic targets in the optic neuropil. In *Drosophila*, genetic mutations in two genes, *ebony* and *tan*, impair histamine metabolism and synaptic transmission. Histamine released at the photoreceptor terminals is captured by glial cells and converted by the product of the *ebony* gene to B-alanyl-histamine, an inactive derivative of histamine also known as carcinine (Borycz and others 2000; Richardt and others 2002). Capitate projections are specialized regions for membrane retrieval by clathrin-mediated endocytosis, and it is likely that they are sites of rapid recycling of inactive carcinine back to R-cell axon terminals. Once there, carcinine is hydrolyzed to histamine by the product of the *tan* gene, replenishing histamine levels for neurotransmission (True and others 2005; Wagner and others 2007). Thus, through the *ebony–tan* circuit of histamine inactivation and recovery, neuron–glial interactions at capitate projections regulate the maintenance and perhaps the effectiveness of synaptic connections in the fly's visual system.

The Prospero-expressing LG subtypes in the *Drosophila* ventral nerve cord that were mentioned earlier also appear to be suited for neurotransmitter processing because of their selective expression of glutamate-processing molecules such as the enzyme Glutamine synthetase 2 (Gs2; Freeman and others 2003; Thomas and van Meyel 2007). Gs2 is responsible for converting glutamate to synaptically inert glutamine. Some LG (perhaps the anterior LG subset) and other glial subtypes also express EAAT1, a transporter for the uptake of glutamate (Freeman and others 2003; Soustelle and others 2002). Normally, EAAT1 and its orthologs in other species allow glia to recover glutamate from the extracellular space, which can then be converted to glutamine and safely recycled back to neurons. Reduced EAAT1 function in flies causes neuronal excitotoxicity that likely results from the accumulation of glutamate (Rival and others 2004). In the ventral ganglion of *Drosophila* embryos and larvae, where the LG reside, the presynaptic vesicular glutamate transporter VGlut (Mahr and Aberle 2006) and the postsynaptic glutamate receptor GluRIID (Featherstone and others 2005) are both expressed. Therefore, glutamatergic transmission at CNS synapses in *Drosophila* could be influenced by an LG subtype, as it is by glia at glutamatergic neuromuscular synapses of adult flies (Rival and others 2006).

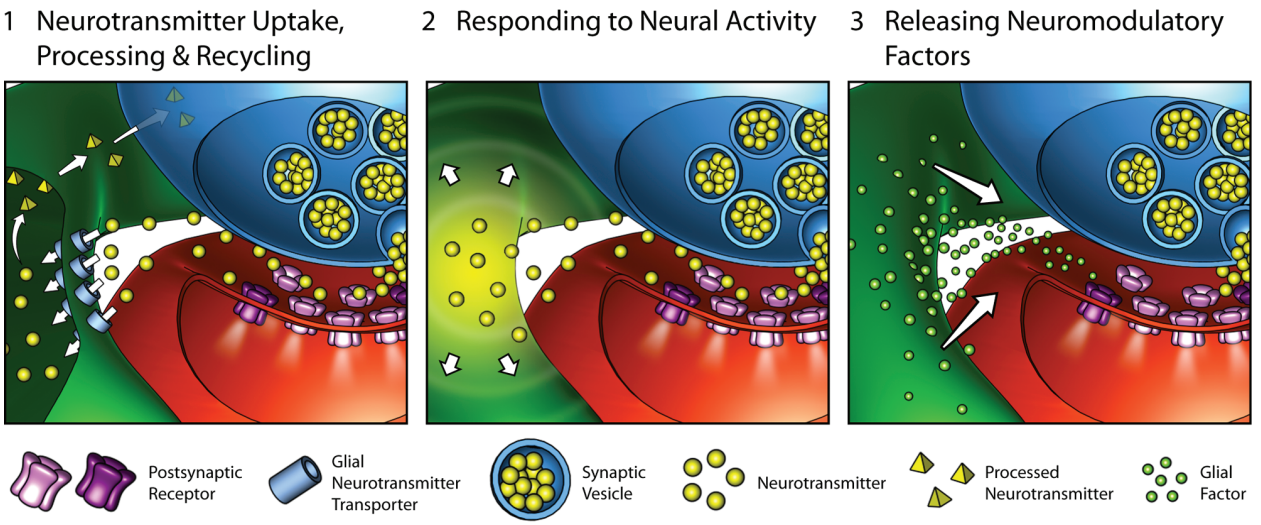

Fig. 3. Neuron–glial communication at synapses. Examples of roles of glia in (*1*) neurotransmitter uptake, processing, and recycling, (*2*) responding to neural activity, and (*3*) releasing neuromodulatory factors. Illustration created by Michael Haber.

Mammalian glia also express glutamine synthetase and the transporters EAAT1/GLAST and EAAT2/GLT-1, which are the primary transporters for glutamate homeostasis in the CNS (Huang and Bergles 2004). Interestingly, EAAT1/GLAST and EAAT2/GLT-1 appear to be enriched in different brain regions (Lehre and others 1995). For example, EAAT2/GLT-1 is the predominant protein for glutamate recovery in forebrain astrocytes. In mice, antisense oligonucleotide-dependent knockdown of EAAT2/GLT-1 increases extracellular glutamate levels and causes neurodegeneration believed to be linked to excitotoxicity (Rothstein and others 1996). Consistent with this, GLT-1 knockout mice exhibit seizures and have increased brain damage following injury (Tanaka and others 1997).

Glial Responsiveness to Neuronal Activity

Besides controlling neurotransmitter levels at synapses, glia themselves are also responsive to neuronal activity (Fig. 3). Although glia do not fire action potentials, many glia are endowed with specialized forms of calcium excitability (Fiacco and McCarthy 2004). Indeed, glial calcium signaling is now believed to be an important response mechanism to neural activity. Because of considerations of size and accessibility, calcium signaling in invertebrate glia was first studied in the leech *Hirudo medicinalis* (Lohr and Deitmer 2006). More recently, it has also been examined in the olfactory system of the moth *Manduca sexta*, in which cholinergic neurotransmission from olfactory receptors stimulates calcium signaling in glia that correlates well with the migration of glia to correct positions within the developing glomeruli (Lohr and others 2005).

In the leech CNS, the so-called giant glial cells depolarize and increase cytosolic calcium in response to stimulated neuronal activity (Lohr and Deitmer 2006). Evoked neuronal activity causes transient changes in calcium levels that are largest in the processes of these cells, suggesting that locally restricted calcium signals may occur in subcompartments of the giant glial cell (Lohr and Deitmer 1999). Similar observations have been described in mouse Bergmann glia (Grosche and others 1999), which show spontaneous and evoked calcium signaling in response to activation of parallel fibers and afferent inputs into the cerebellum. Interestingly, the strength and location of the stimulation of distinct synaptic terminals can affect the degree of calcium signaling. Repeated stimulation of the parallel fiber pathway or stimulation of the granule cell layer evoke broad calcium-signaling events in the Bergmann glial-cell processes and soma (Grosche and others 1999; Kulik and others 1999; Matyash and others 2001). On the other hand, weak stimulation of only a few parallel fibers can restrict the calcium signaling to discrete microdomains (Grosche and others 1999; Matyash and others 2001). Some of the substances that trigger calcium signaling in Bergmann glia appear to be nitric oxide released from parallel fibers and noradrenaline from afferent inputs into the cerebellum (Kulik and others 1999; Matyash and others 2001). Further studies are required to differentiate the effects of global versus regionalized calcium signaling in regulating the functional properties of Purkinje neuron synapses.

Astrocytes in the cortex and hippocampus also show interesting calcium-signaling properties. Calcium signaling in astrocytes is initiated by many factors including glutamate, ATP, and direct neuronal stimulation (Fiacco and McCarthy 2006). For example, glutamate delivery to cultured astrocytes elicits intracellular calcium transients that can propagate to neighboring cells (Cornell-Bell and others 1990). Calcium signaling in glia is also induced by ATP and activation of purinergic receptors and along, with gap-junctional signaling, it

contributes to calcium "waves" across glial networks (Scemes and Giaume 2006). In slices, stimulation of Schaffer collaterals, which synapse onto CA1 pyramidal neurons in the hippocampus, leads to increased astrocytic intracellular calcium, which has been attributed to activation of signaling cascades downstream of metabotropic glutamate receptors (Porter and McCarthy 1996).

Astrocytic calcium signaling has also been detected in vivo, using calcium-indicator dyes and two-photon laser scanning microscopy to study the intact rodent brain (Hirase and others 2004; Wang and others 2006). Spontaneous calcium transients in astrocytes can be observed and further provoked by enhancing neural activity with blockers of GABAergic transmission or by external sensory manipulation in which whisker stimulation in the 3- to 7-Hz frequency range induces astrocytic calcium signaling in the barrel cortex (Wang and others 2006). Consistent with other studies in acute slices (Porter and McCarthy 1996), this signaling is mediated by activation of metabotropic glutamate receptors and does not require the function of postsynaptic N-methyl-D-aspartate (NMDA) or AMPA receptors, indicating that it is likely a direct glial response to presynaptic neuronal activity.

Glial Modulation of Synapse Function

The detection of calcium signaling in glia is an intriguing concept; however, its connection to neuromodulation remains to be fully explored. Glial calcium signaling is likely tied into the modulation of synaptic transmission by the release of neuroactive substances (Fig. 3; Montana and others 2006; Nedergaard and others 2003; Volterra and Meldolesi 2005). Neurotransmission may elicit the release of glial factors that increase or decrease synaptic efficacy. Furthermore, the finding that astrocytes exhibit intrinsic calcium dynamics in the absence of neural activity may indicate that astrocytes can initiate and help direct neurotransmission (Parri and others 2001).

Accumulating evidence indicates that astrocytes secrete neuromodulatory factors through a variety of mechanisms (Montana and others 2006). For example, neural activity triggers vesicular-mediated release of glutamate from astrocytes. Glial glutamate may have many effects on neuronal glutamate receptors and regulate synaptic efficacy. Indeed, Jourdain and others (2007) showed that vesicular-mediated glutamate release from astrocytes activates NMDA receptor subunit 2B (NR2B) containing NMDA receptors to modulate presynaptic release properties of perforant path terminals onto granule cells in the hippocampus. Interestingly, vesicular-mediated release of ATP from astrocytes, which is readily broken down to adenosine, is critical for dampening synaptic transmission in the brain. Among other things, this is important for preventing the saturation of synaptic potentiation to maintain a useful working range of plasticity (Pascual and others 2005).

Activity-dependent release of D-serine from vesicular and nonvesicular pathways provides another route for glial modulation of synaptic function (Oliet and Mothet 2006). D-serine is an NMDA receptor coagonist that can potently regulate NMDA channel properties. Astrocytic release of D-serine has been shown to be important for regulating long-term synaptic plasticity (Yang and others 2003; Panatier and others 2006) and can accompany morphological changes in neuron–glial interactions, as discussed in the section Structural Plasticity of Neuron–Glial Interactions and Neuromodulation.

Other factors secreted by glia also have a significant effect on synaptic properties (Volterra and Meldolesi 2005). For example, glial-derived tumor necrosis factor-α (TNF-α) is important for increasing surface AMPA receptors at synapses and elevating miniature excitatory postsynaptic current (mEPSC) amplitude during chronic activity blockade of cultured neurons (Beattie and others 2002; Stellwagen and Malenka 2006). This form of glial-mediated homeostatic plasticity may have important implications for activity-dependent remodeling of developing and established neuronal circuits and immune-related changes to synaptic efficacy following brain injury.

In *Drosophila*, neurotransmitter release from glia has also been found to alter the properties of synapses. Nonvesicular release of glutamate influences the number of postsynaptic glutamate receptors at the neuromuscular junction (NMJ; Featherstone and others 2002). The gene *genderblind* (*gb*) encodes a cystine/glutamate transporter (xCT) that is required to maintain extracellular levels of glutamate (Augustin and others 2007). In *gb* mutants, the number of postsynaptic glutamate receptors increases by 200% to 300%, and electrophysiological studies found a concomitant increase in postsynaptic response. *Genderblind* is expressed in peculiar subsets of central and peripheral glia, some of which extend processes immediately next to synaptic boutons of the NMJ. It has been proposed that extracellular glutamate maintains at least one-half of the ionotropic glutamate receptors at neuromuscular synapses in a desensitized state, effectively suppressing their ability to cluster. By regulating systemic levels of glutamate in the hemolymph, or perhaps by acting locally at synapses, *gb* may modulate the availability of functional glutamate receptors and thereby alter membrane excitability and synaptic strength (Augustin and others 2007).

Additional evidence for insect glia-releasing factors that modulate synaptic efficacy is lacking. However, at least one example illustrates how *Drosophila* glia can secrete factors that affect neuronal excitability. During development, a subset of LG expresses Axotactin, a secreted member of the neurexin protein superfamily (Yuan and Ganetzky 1999). As developing axons grow past the LG, they accumulate glial-derived Axotactin protein (Yuan and Ganetzky 1999). Direct electrophysiological recordings of the motor nerves of *axotactin* mutants showed temperature-sensitive abnormalities and a reduction of axonal conduction, although neurotransmitter release and postsynaptic responses were shown to be normal. Because *axotactin* mutants resemble mutants with reduced sodium-channel function, it has been proposed that Axotactin is involved in the expression or localization of ion channels (Yuan and Ganetzky 1999).

Structural Plasticity of Neuron–Glial Interactions and Neuromodulation

Control of the degree of glial-mediated neuromodulation may be further achieved by anatomical changes in neuron–glial contact. The organization of glial-cell processes around presynaptic and postsynaptic terminals and neuronal cell bodies can be remodeled in brain slices (acute and organotypic) and in vivo (Haber and Murai 2006; Hatton 1997). The structural plasticity of glial processes may provide an added layer of sophistication to regulatory mechanisms that govern synaptic plasticity.

Dramatic yet reversible changes in astrocytic morphology are known to occur in the hypothalamic region following dehydration and lactation, in which astrocytes retract their processes over long distances (Hatton 1997). This has a significant effect on neuronal physiology on multiple levels. It increases the juxtaposition of neuronal cell bodies while potentially reducing the glial-mediated uptake of potassium that regulates neuronal excitability. Furthermore, glial retraction prohibits neurotransmitter removal from the extracellular milieu, which affects glutamate transmission (Hatton 1997; Oliet and Piet 2004). Glial withdrawal also changes the number of glial-derived factors that can regulate synaptic function. The degree of glial coverage at hypothalamic synapses controls the amount of glial-derived D-serine that regulates the threshold for inducing long-term potentiation (LTP) and long-term depression (LTD), two forms of synaptic plasticity implicated in information storage at synapses (Panatier and others 2006). Thus, the restructuring of glial contacts with synapses is linked to long-term physiological changes of synapses and to behavioral modifications.

Glial remodeling may also coordinate with other synaptic plasticity mechanisms that operate within a short time frame of seconds to minutes. This includes mechanisms of ion-channel phosphorylation and trafficking, regulation of presynaptic release machinery, and structural modifications of dendritic spines. Rapid and dynamic astrocyte changes have been reported in the hippocampus and brain stem. As described previously, most synapses in the hippocampus are only partially contacted by astrocytic membranes (Ventura and Harris 1999), yet astrocytes have a complex morphological interplay with active presynaptic terminals and motile dendritic spines in both acute and organotypic brain slices (Haber and others 2006; Hirrlinger and others 2004; Nishida and Okabe 2007). The motility of astrocytic processes may allow reorganization of the synaptic microenvironment, quickly mobilizing substances to or from synapses and perhaps controlling synapse morphology (Murai and others 2003; Nishida and Okabe 2007). Astrocytes continuously redefine their juxtaposition to dendritic spines, and the extent of this reorganization correlates with spine size (Haber and others 2006). As spines get larger, nearby astrocytic processes become stabilized. This structural plasticity may be important for localizing key molecules such as glutamate transporters and gliotransmitters such as glutamate near larger synapses. This motility may also allow astrocytes to dynamically control the amount of extracellular space near synapses (Sykova 2004) and modulate the degree of intersynaptic communication (Kullmann and Asztely 1998; Serrano and others 2006). Structural changes in astrocytes have also been reported following the induction of LTP, kindling (Wenzel and others 1991; Hawrylak and others 1993) and after raising rodents in an enriched environment (Jones and Greenough 1996), suggesting an intriguing connection between glial remodeling and cognitive processes such as learning and memory formation.

Conclusion

Collectively, studies of diverse organisms have secured the concept that neuron–glial communication is essential for the proper function of neural circuits. Based on careful observations and rigorous experimentation, it is today clear that glia play a prominent role in regulating and directing neural connections as once envisioned by early neuroanatomists. This concept has a rich history, and the examples presented here represent only a small portion of research that has revealed the morphology and function of glia at synapses. We regret all exclusions caused by space constraints. Nevertheless, it is evident from the research discussed here that a solid incorporation of glial-cell biology is fundamental to our understanding of how invertebrate and vertebrate neural circuits work. It is also evident that in our era of advanced experimental resources, many important questions remain to be addressed as to how glia influence synaptic function through reuptake mechanisms, detecting neural activity, and modulating neuronal function through glial-derived factors.

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