Glia as Functional Barriers and Signaling Intermediaries

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Glia play a crucial role in providing metabolic support to neurons across different species. To do so, glial cells isolate distinct neuronal compartments from systemic signals and selectively transport specific metabolites and ions to support neuronal development and facilitate neuronal function. Because of their function as barriers, glial cells occupy privileged positions within the nervous system and have also evolved to serve as signaling intermediaries in various contexts. The fruit fly, *Drosophila melanogaster*, has significantly contributed to our understanding of glial barrier development and function. In this review, we will explore the formation of the glial sheath, blood–brain barrier, and nerve barrier, as well as the significance of glia–extracellular matrix interactions in barrier formation. Additionally, we will delve into the role of glia as signaling intermediaries in regulating nervous system development, function, and response to injury.

A key evolutionarily conserved role of glia is to support neurons metabolically (Pellerin and Magistretti 2012; Volkenhoff et al. 2015). Glia control the flow of metabolites in the nervous system, ensuring that specific metabolites and ions reach neurons at the right time and place. Therefore, to support neurons as effectively as possible, glia first establish barrier properties to strictly separate neuronal compartments from external influences and, in addition, establish selective transport mechanisms for effective glia–neuron metabolic coupling.

Indeed, glia across different model systems and circuit types perform barrier functions, which require them to occupy key positions at biological interfaces across scales, from tissue to cellular to subcellular levels. In *Drosophila*, each glial cell type plays distinct barrier functions. Astrocytes shield synapses, ensheathing glia encase the entire neuropil, cortex glia wrap around neuronal cell bodies, wrapping glia shield peripheral axons and the surface glia or blood–brain barrier forming glia seal the whole nervous system from circulating hemolymph (Figs. 1 and 2; see also Coutinho-Budd et al. 2023). It is therefore unsurprising that in these specialized positions glia function not only as barriers, but also as signaling intermediaries to both regulate and integrate signals. Here we discuss the formation and function of the glial sheath, blood–brain barrier, and nerve

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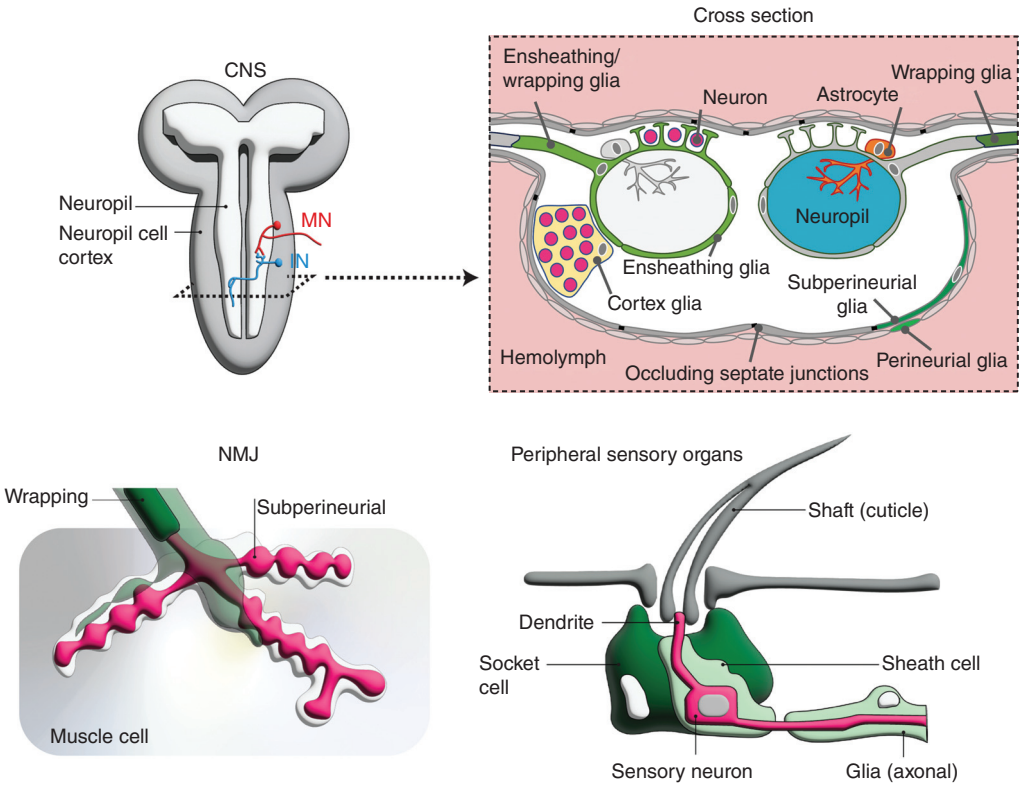

Figure 1. *Drosophila* glial classes.

barrier, as well as the role of glia–extracellular matrix (ECM) interactions in barrier formation. Finally, we discuss how their positions allow glia to act as signaling intermediaries to modulate nervous system development, function, and response to injury.

GLIA AS FUNCTIONAL BARRIERS

Formation of the Glial Sheath, Blood–Brain Barrier, and Nerve Barrier

The blood–brain barrier comprises the most established and best-studied barrier in the nervous system, as it represents the gatekeeper that regulates all entry into and also exit from the nervous system. In *Drosophila*, the blood–brain barrier is made up of two distinct cell types, perineurial glial cells and subperineurial glial cells; together called the surface glia (Figs. 1 and 2; Beckervordersandforth et al. 2008; Stork et al. 2008). Here, it appears that the transport and barrier functions are, in part, divided across the two cell types. Although the subperineurial glial cells are very large and flat polarized cells that establish occluding junctions (Figs. 1 and 2A; Stork et al. 2008), perineurial glia appear responsible for taking up nutrients from the hemolymph and do not form occluding junctions (Volkenhoff et al. 2015).

Both blood–brain barrier–forming glial cells are born during embryonic stages. *Drosophila* neurogenesis is distinct from vertebrate neurogenesis, as individual progenitor cells delaminate into the interior of the animal in five waves beginning about 30 min after gastrulation. In total, 31 such neuroblasts are formed in each thoracic hemineuromer, one of which is a pure glioblast and six are neuroglioblasts (Doe and Technau 1993; Beckervordersandforth et al. 2008). The perineurial glial cells are formed by neuroglio-

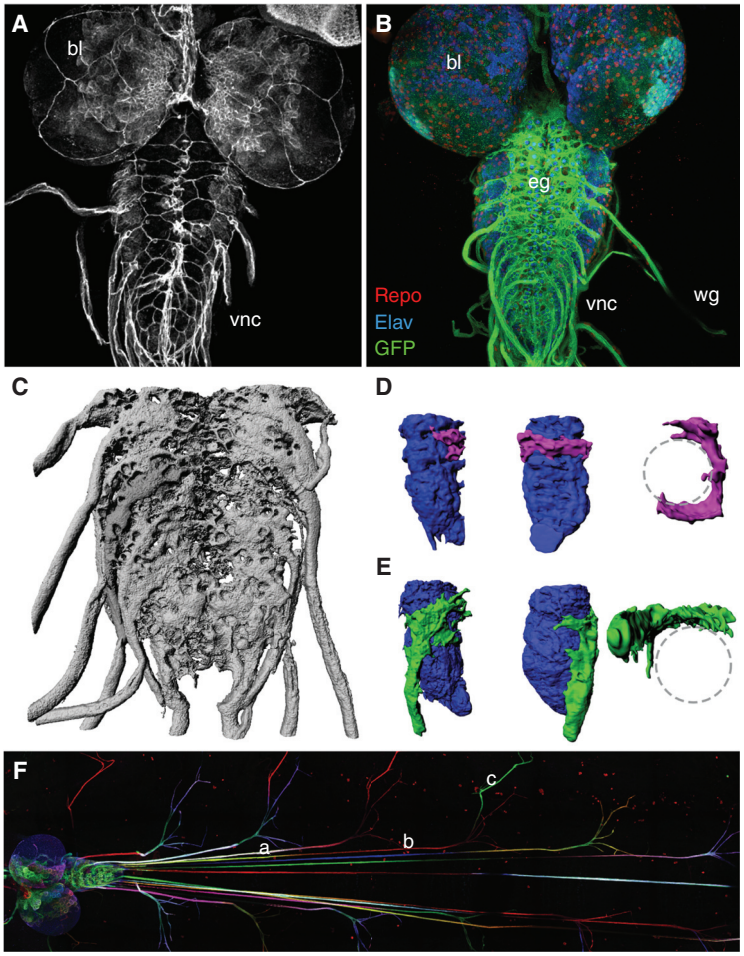

Figure 2. Glial cells as functional barriers. (*A*) The glial isoform of the cell adhesion protein NeurexinIV is specifically expressed by the subperineurial glial cells where it localizes to the pleated septate junctions. The tiling of subperineurial glial cells, which form the blood–brain barrier, becomes visible. (bl) Brain lobe, (vnc) ventral nerve cord. (*B*) Ensheathing glial cells are visualized by membrane-bound green fluorescent protein (GFP) expression directed by *83E12-Gal4*. (wg) Wrapping glia. (*C*) Surface reconstruction of the ensheathing glia (gray) using Imaris. Note the dense coverage of the neuropil. (*D*) Reconstruction of a single ensheathing glial cell (magenta) that covers part of the neuropil (blue). Note, that no projections into the neuropil are formed. (*E*) Reconstruction of a single ensheathing/wrapping glial cell (green) that covers part of the neuropil (blue) and also follows the nerve toward the periphery. No projections into the neuropil are formed. (*F*) Multicolor flipout labeling of the peripheral wrapping glia. In most segments three wrapping glial cells can be identified by a distinct color each (a,b,c). Wrapping glial cells are very large; polyploid cells can reach 2–3 mm in length.

blasts and cover the outermost surface of the nervous system, the peripheral nervous system (PNS), as well as the central nervous system (CNS). All subperineurial glial cells of the CNS also stem from distinct neuroglioblasts, whereas in the PNS some subperineurial glia are also generated by sensory organ precursor cells (Beckervordersandforth et al. 2008; von Hilchen et al. 2008).

The organization of the different surface glial cell types is best analyzed for the peripheral nerves in larvae (Figs. 1 and 2F). Along each of the abdominal nerves (except A8), three perineurial and three to four subperineurial glial

cells are formed. Once specified, perineurial and subperineurial glial cells follow distinct modes of development. The perineurial glial cells divide and expand their cell numbers enormously. Depending on the length of the peripheral nerve, 20 to 100 perineurial glial cells are formed (Matzat et al. 2015). Therefore, it appears possible that perineurial glial cell division may be regulated in a cell–cell contact–dependent manner to generate the appropriate number of cells covering the nervous system. In contrast, the number of subperineurial glial cells stays the same until pupal development. The majority of subperineurial glia arise within the embryonic CNS and some of these cells then migrate into the periphery along the developing motor and sensory axons (Sepp et al. 2000; Sepp and Auld 2003) and take up highly stereotypic positions along each nerve (von Hilchen et al. 2008). To cover the entire nervous system, the subperineurial glia must grow enormously, which is supported by a switch to polyploidy (Unhavaithaya and Orr-Weaver 2012).

In a typical embryonic abdominal hemineuromere, eight subperineurial glial cells, and three to four perineurial glial cells can be identified (Beckervordersandforth et al. 2008; von Hilchen et al. 2008; Schwabe et al. 2017). The number of perineurial glial cells has not yet been determined for the brain lobes. The relatively few embryonic perineurial glial cells (~150 cells) divide to generate more than 2000 cells, which evenly cover the surface of the entire adult CNS (Awasaki et al. 2008; Kremer et al. 2017). The mitotic potential of the perineurial glial cells can be increased by the activation of receptor tyrosine kinase signaling pathways (Franzdóttir et al. 2009; Avet-Rochex et al. 2012).

The subperineurial glia covering the embryonic brain have not been counted but it is assumed to be 16 subperineurial glial cells per neuromere (Beckervordersandforth et al. 2008); 288 cells are expected by the end of embryogenesis (nine abdominal, three thoracic, and six head segments). This number matches the approximately 300 subperineurial glial cells that were reported to cover the adult nervous system (Kremer et al. 2017) suggesting that subperineurial glial cells do not divide, but instead grow in size and stay intact throughout life. In fact, lineage analysis failed to obtain any indication of cell division (Awasaki et al. 2008), and the use of photoconvertible dendra demonstrates that subperineurial glia stay intact until midpupal stages (Winkler et al. 2021). Almost 10 times as many perineurial cells as subperineurial glial cells cover the nervous system; thus, subperineurial cells are very large to match the surface area of the perineurial layer.

A key feature of subperineurial cells is their flattened shape and the formation of occluding junctions between neighboring cells. The formation of the characteristic flat shape starts at ~10 h after egg laying, after individual subperineurial glial cells have moved to the surface of the brain. Here, following a mesenchymal to epithelial transition, they grow extensively in a synchronous and isometric manner with neighboring cells, allowing them to tightly tile the brains surface by ~13 h after egg laying (Kremer et al. 2017). The lateral growth of the subperineurial glial cells is controlled by an orphan G-protein-coupled receptor Moody (Bainton et al. 2005; Schwabe et al. 2005, 2017). Moody acts via $G\alpha_i$, the RGS-protein Loco, and the cAMP effector PKA (Granderath et al. 1999; Schwabe et al. 2005; Li et al. 2021). Moody localizes to the domain of the subperineurial glia facing the cortex glia, suggesting that the signal-activating Moody might originate from the cortex glia or the CNS neurons. In absence of *moody* signaling, lateral expansion of the subperineurial glial cells is reduced during embryonic stages; however, a functional blood–brain barrier is established that allows survival of *moody* mutants until adulthood.

When subperineurial glial cells establish contacts with their neighbors, different junctional contacts are established. On the one hand, subperineurial glia form gap junctions with each other, which leads to metabolic coupling in the population and on the other hand, they also form occluding junctions, which separate the hemolymph from all neural cells. Gap junctional coupling is not needed for barrier formation, but, interestingly, is required for formation of neurons during larval stages and thus the growth of the brain lobes (discussed more below).

Subperineurial glial cells form occluding pleated septate junctions (pSJs). These are generated by a bewilderingly large number of membrane and membrane-associated proteins that require endocytic recycling to properly form (Tepass and Hartenstein 1994; Baumgartner et al. 1996; Tiklová et al. 2010; Petri et al. 2019; Böhme et al. 2021). pSJs form belts surrounding the entire cell, which fence paracellular diffusion. To generate a fully tight barrier, these belts need to be formed in continuous bands that connect different cell vertices (Babatz et al. 2018). The process of septate strand generation requires vesicular traffic as septate junction components are preassembled before being integrated into the pSJ strands and the actin cytoskeleton (Hatan et al. 2011; Babatz et al. 2018; Li et al. 2021). It is possible that septate junction strand formation is initiated at a tricellular junction and spreads to link each cell vertex. In addition to its role in lateral subperineurial growth, Moody signaling is needed to ensure the formation of intact pSJ strands connecting the different cell vertices. Surprisingly, growth of pSJ strands is not needed as subperineurial glial cells increase their diameter during development, because strand formation during embryonic stages already matches the size requirements of later larval and adult stages. In *moody* mutants, septate junction formation initially proceeds normally; however, breaks can be seen in individual pSJ strands, which renders the blood–brain barrier slightly leaky (Babatz et al. 2018). Interestingly, subperineurial glial cells are able to sense the level of occluding junctions that are established and initiate compensatory growth and the formation of specific cellular interdigitations that increase the length of the diffusion path and thus counteract the reduced tightness of the barrier caused by misarranged septate junctions in *moody* mutans (Babatz et al. 2018). In conclusion, the blood–brain barrier adapts well to the growing brain tissue by regulation of cell division in the outer perineurial glial layer and by expansion of cell size by the subperineurial glia.

As aforementioned, subperineurial glia coordinate their own cell size with the perineurial glia cell number through polyploidization (Unhavaithaya and Orr-Weaver 2012). Polyploidization is common in excessively large cells (e.g., muscle cells). The level of polyploidy is controlled by the amino-terminal asparagine amidohydrolase homolog Öbek that controls the N-end rule protein degradation pathway (Zulbahar et al. 2018). In blood–brain barrier glia, Öbek counteracts fibroblast growth factor (FGF) and Hippo signaling to differentially affect cell growth and cell number. The Hippo pathway is a central regulator of cell growth and proliferation. In subperineurial glial cells, a double-negative feedback loop comprising the microRNA miR-285, the Hippo signaling effector Yorkie (vertebrate Yap), and the multiple ankyrin repeats single KH domain (Mask) regulates not only subperineurial glial growth but also pleated septate junction strand integrity (Li et al. 2017).

Ploidy can result from endocycling and from endomitosis—the former increases ploidy in mononucleated cells and the latter increases ploidy via multinucleation. The switch between endocycling to endomitosis is, in part, Notch-dependent, with Notch inhibiting this transition. Interestingly, subperineurial glial cells that cover the brain lobes undergo endomitosis (multinucleation) during larval development to accommodate brain growth (Von Stetina et al. 2018); whereas subperineurial glia in the ventral nerve cord (VNC) are polyploid through endocycling reduced Notch activity can also trigger endomitosis in subperineurial glia covering the VNC (Von Stetina et al. 2018). However, how Notch is differentially regulated in the VNC versus the brain lobe subperineurial glia and how this signaling contributes to later barrier functions is currently unknown.

To summarize, the blood–brain and nerve barriers are generated by the close apposition of two distinct glial cell types—perineurial and subperineurial. Although perineurial glia loosely tile with one another and have a direct interface with the nutrient-rich hemolymph, subperineurial glia play more classic "barrier" functions by tightly tiling the surface of the brain and sealing it from the external environment. Together, these cells perform the function of perineurial glia in mammals. In both cases, loss of barrier glia can have profound consequences for brain health and function, which will be discussed in more detail below.

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Glial–ECM Interactions Drive External and Internal Glial Barrier Formation

A key glial interaction that mediates nervous system integrity is the interaction between glia and the ECM. The ECM surrounding the nervous system, or neural lamella, is composed of a range of ECM components including laminin, perlecan, collagen, nidogen, and SPARC (Hynes and Zhao 2000). These ECM components are secreted by the fat body, hemocytes, and the glia themselves (Broadie et al. 2011; Petley-Ragan et al. 2016). The surface glia interact with the ECM around the VNC during embryogenesis (subperineurial glia) and larval stages (perineurial glia), and these interactions are essential for nervous system morphology, function, and integrity. During embryogenesis, the CNS and, in particular, the VNC, drastically condenses in size. Although both glia and neurons contribute to the active contraction of the VNC, glia have a major contribution to this critical process (Karkali et al. 2022), which relies on the ECM surrounding the VNC and depends on the glia–ECM interface. The ECM at this stage is deposited by circulating hemocytes, a key function of hemocytes. Thus, hemocyte function is required for proper CNS morphogenesis, including condensation of the VNC (Sears et al. 2003; Olofsson and Page 2005; Martinek et al. 2008; Defaye et al. 2009; Evans et al. 2010). A key element to the organization and integrity of the ECM around the VNC is laminin. Loss of integrin, which is a major membrane-bound receptor of laminins, results in abnormal nervous system condensation (Brown 1994) and loss of integrin in glia causes the elongation of the VNC and deformation of the brain lobes (Xie and Auld 2011; Meyer et al. 2014). In the peripheral nerves, loss of integrin in the perineurial glia results in incomplete ensheathment of the nerve and individual perineurial glia appear to become detached from one another. Similarly, loss of laminins themselves disrupts collagen and perlecan accumulation around the VNC and blocks VNC condensation (Urbano et al. 2009) as does loss of collagen (Martinek et al. 2008). Furthermore, loss of expression of GlcAT-P, glucuronosyltransferase P, which is required for proper ECM formation, causes an elongated larval VNC phenotype (Pandey et al. 2011). Beyond embryonic development, continued glia–ECM interactions are necessary to maintain the structure of the VNC. For example, directed degradation of the ECM in late larvae stages through expression of matrix metalloproteinases (MMPs) in the perineurial glia also leads to nerve cord extension and lethality (Xie and Auld 2011; Meyer et al. 2014). Modulation of the ECM is also important for nervous system integrity and function. MMP or protease remodeling of the ECM is key to the proper development of the nervous system including regulation of expression of MMPs, proteases such as kuzbanian, and ADAMTS-like family proteases (Meyer et al. 2014; Skeath et al. 2017; Calderon et al. 2022).

Although MMP activity is required for proper brain integrity, its levels must be tightly regulated. When Mmp1 activity is too high, septate junction strands are disrupted around peripheral nerves, compromising neurotransmitter release downstream at the neuromuscular junction (NMJ). Interestingly, matrix metalloproteinase 1 (*Mmp1*) expression is also regulated by Delta-dependent Notch activation, which inhibits c-Jun amino-terminal kinase (JNK) to reduce *Mmp1* levels. These results provide mechanistic insight into the regulation of neuronal health and function via glial-initiated signaling and open a framework for understanding the complex relationship between ECM regulation and the maintenance of barrier function (Calderon et al. 2022).

Glia–ECM interactions are also important for glial migration. During larval stages in the optic stalk, perineurial glia migrate between the basal ECM and cortex glia along the optic stalk into the eye disc (Silies et al. 2007). Pan-glial knockdown of integrin and talin, components of the focal adhesion complex, impaired perineurial glia migration along the optic stalk (Xie et al. 2014). ECM stiffness can be a modulator of glial migration. In a *Drosophila* glioma model, in which glial migration is increased by overexpression of PDGF receptor (PVR), glial overmigration is suppressed upon knockdown of *Drosophila* Lysyl oxidase (Lox), indicating that glia migrate less when ECM stiffness is reduced

(Kim et al. 2014). Lox oxidizes peptidyl lysine residues on ECM proteins such as collagen, a process critical for covalent cross-linking, and loss of Lox function leads to immature or less stiff ECM (Lucero and Kagan 2006; Kim et al. 2014). Of note, changes to ECM stiffness does not appear to alter normal migration of the perineurial glia during development but rather PVR activation changes integrin–ECM dynamics in this glioma model.

Beyond creating the external barriers that protect, insulate, and support nervous system integrity, glia play critical roles in creating internal barriers. For instance, the cortex–neuropil barrier is formed by ensheathing glia and contributes to glutamate homeostasis in the neuropil (Otto et al. 2018). For macrophage-like functions of the ensheathing glia, see Coutinho-Budd et al. (2023). As with the subperineurial glia, ensheathing glia have a polarized cell morphology but do not form special cell–cell junctions (Fig. 2B–E; Pogodalla et al. 2021). Within the CNS, ensheathing glia are necessary to separate the neuropil from the neuronal cell bodies, generating an internal diffusion barrier, a process that is complete by the larval third instar (Pogodalla et al. 2021). Integrin subunits and ECM components including the heparan sulfate proteoglycan Dally are enriched around the neuropil in larval stages, and in adults, both perlecan and collagens flank ensheathing glia (Pogodalla et al. 2021), suggesting the ensheathment of the neuropil requires glia–ECM interactions. Disruption of this internal glial barrier results in changes to larval locomotion pointing to an important role for internal glial barriers in both the CNS and peripheral nerves in maintaining nervous system function (Pogodalla et al. 2021).

The ensheathing glia comprises two related cell types. Two ensheathing glial cells are found in each abdominal hemineuromer that only encase the neuropil (Fig. 2B–D). In addition, two ensheathing/wrapping glial cells are found in each abdominal hemineuromer (Peco et al. 2016), which, in addition, encase dorsal neurons as well as they ensheath axons of the nerves connecting the neuropil with the periphery (Figs. 1 and 2F).

Ensheathment of axons in the PNS is done by the innermost layer of glia in the peripheral nerve generated by the wrapping glia. Similar to subperineurial glia, wrapping glia are polyploid and do not undergo mitosis; instead, three to four wrapping glia are present along the entire nerve length and send long extensive processes to cover the nerve interior (von Hilchen et al. 2013). In an MCFO-type single-cell labeling experiment, individual cells can be identified by their specific color (Nern et al. 2015; Kottmeier et al. 2020). A single wrapping glia can reach a length of 2 mm, demonstrating the extreme hypertrophic growth of this cell type resembling a nonmyelinating Schwann cell of a Remak fiber (von Hilchen et al. 2013; Matzat et al. 2015). Wrapping glia development occurs relatively late in comparison to the other glial types where differentiation of the wrapping glia begins in the first-instar larval stage and continues throughout the rest of the larval stages such that by the late third-instar stage, most individual axons and groups of axons are in contact with some wrapping glia processes (Matzat et al. 2015). Although loss of wrapping glial ensheathment is not lethal during larval stages, ablation does cause a reduction in axon diameter and nerve conduction velocity, along with larval locomotion defects (Kottmeier et al. 2020). Similarly, loss of nonmyelinating Schwann cells, the equivalent cell type in vertebrate peripheral nerves, impairs sensorimotor behaviors but is similarly viable (Harty and Monk 2017). During the pupal stage, axonal wrapping is reorganized (Subramanian et al. 2017). In adult leg nerves, a large number of wrapping glial cells wrap axons according to their size (Rey et al. 2023b). At the axon initial segment of large motor axons, glial cells form lacunar structures that possibly serve as an ion reservoir and participate in blocking ephaptic coupling between axons (Kottmeier et al. 2020; Rey et al. 2023a,b).

Several factors have been identified as important for wrapping glia differentiation and/or ensheathment. Inhibition of the epidermal growth factor (EGF) and FGF signaling reduces wrapping glia ensheathment (Matzat et al. 2015; Kottmeier et al. 2020). Proper ionic homeostasis, sphingolipid biosynthesis, and intracellular

transport are all important for wrapping glia ensheathment, as demonstrated by the loss of the serine/threonine kinase Fray, the serine palmitoyltransferase subunit Lace, and kinesin heavy chain (Khc), respectively (Leiserson et al. 2000, 2011; Schmidt et al. 2012; Ghosh et al. 2013). Wrapping of axons by glia also requires the presence of ECM components and their receptors including laminins and the integrin receptor (Xie and Auld 2011; Petley-Ragan et al. 2016), the Discoidin domain receptor, and the XV/XVIII collagen Multiplexin (Corty et al. 2022).

GLIA AS SIGNALING INTERMEDIARIES

By virtue of their roles as barriers and partitions, glia occupy privileged positions in the nervous system and have been co-opted to also function as signaling intermediaries that gate and integrate signals during nervous system development and function and in response to injury.

Glia as Signaling Intermediaries during Nervous System Development

In *Drosophila*, neural stem cells or neuroblasts in the CNS proliferate during embryogenesis, but most enter a state of reversible cell-cycle arrest termed quiescence toward the end of embryogenesis. Following hatching and feeding during the first larval instar, neuroblasts exit quiescence and reenter the cell cycle in a process termed reactivation (Hartenstein et al. 1987; Truman and Bate 1988; Prokop and Technau 1991; Ito and Hotta 1992). These neuroblasts are isolated from the circulating hemolymph by several glial barriers including the perineurial glia and subperineurial glia, as well as the cortex glia, which envelop neuroblast and neuronal cell bodies within the CNS (Freeman 2015). Necessarily, systemic signals that promote neuroblast reactivation must act through these glia that shield neuroblasts. In *Drosophila*, the fat body, which performs both adipose and liver functions, senses nutrient status and dietary amino acids to stimulate larval growth (including neuroblast reactivation) (Britton and Edgar 1998). Tissue-specific genetic manipulations showed that an amino acid transporter called Slimfast (SLIF) and target of rapamycin (TOR) signaling function autonomously within the fat body to produce yet-to-be-identified signal(s) required to stimulate larval growth and neuroblast reactivation (Sousa-Nunes et al. 2011). Blocking vesicular trafficking specifically in the fat body was sufficient to inhibit neuroblast reactivation, suggesting that the fat body–derived signal is likely secreted into the hemolymph where it acts systemically. Neuroblast reactivation was also shown to depend on phosphatidylinositol 3-kinase (PI3K) and TOR signaling pathway activity cell-autonomously in neuroblasts downstream of the insulin-like receptor (InR) (Chell and Brand 2010; Sousa-Nunes et al. 2011). Indeed, larvae deficient for various combinations of insulin-like peptides (ILPs) displayed defects in the timing of neuroblast reactivation (Chell and Brand 2010; Sousa-Nunes et al. 2011). Interestingly, ILPs are produced by multiple sources in *Drosophila* including the ILP-producing median neurosecretory cells in the brain as well as subsets of glia (Brogiolo et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002). Although the most prominent source of ILPs is the median neurosecretory cells, which respond to signals from the fat body to secrete ILPs into the hemolymph, overexpression of ILPs in these cells under conditions of nutrient restriction rescued body growth but not neuroblast reactivation (Sousa-Nunes et al. 2011). In contrast, surface and cortex glia–specific overexpression of ILPs under nutrient restriction rescued neuroblast reactivation but not body growth (Chell and Brand 2010; Sousa-Nunes et al. 2011). Together these data support a model in which surface and cortex glia respond to a systemic fat body–derived signal(s) to produce and secrete ILPs, which stimulate neuroblast reactivation (Fig. 3A). Moreover, surface glial cells form an extensive network with each other through gap junctions, and this gap-junction coupling enables coordinated and synchronized calcium oscillations across the surface glial network, which in turn promotes synchronized secretion of ILPs for appropriately timing neuroblast reactivation (Spéder and Brand 2014). A recent characterization of cell-type-specific responses and growth over short intervals found that, initially, cortex glia do not fully envelop qui-

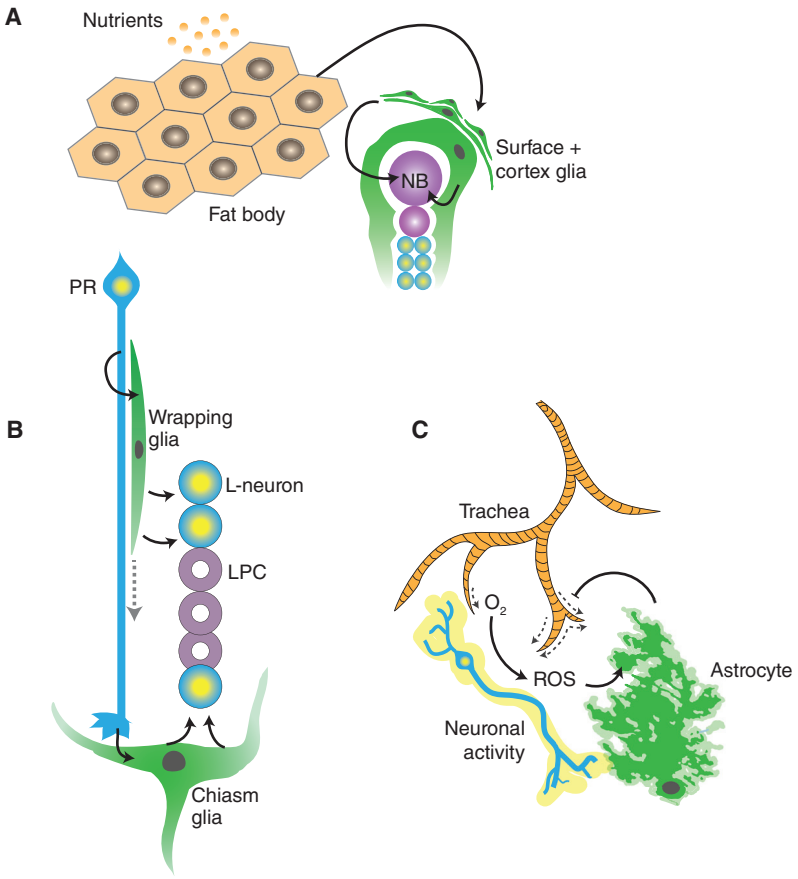

Figure 3. Examples of glia as signaling intermediaries. (*A*) Surface and cortex glia relay feeding-dependent signals from the fat body to reactivate quiescent neuroblasts (NBs) in the first-instar central nervous system (CNS). (*B*) Wrapping glia and outer chiasm giant glia respond to signals from photoreceptors (PRs) and relay these to lamina precursor cells (LPCs) to induce their differentiation into neurons. (*C*) Trachea are gas-filled vasculatures responsible for delivering oxygen to the brain. Astrocytes monitor their local environments and under local hyperoxic conditions signal to trachea to retract their branches and filopodia, which reduces local oxygen delivery.

escent neuroblasts, but instead grow their membranes in a nutrient-dependent manner to ensheath quiescent neuroblasts and promote their reactivation (Yuan et al. 2020). Thus, the full extent of interorgan and intercellular signals in this process is still ambiguous and may involve signaling relays between multiple glial cell types.

Another prime example of glia functioning as signaling intermediaries occurs in the developing visual system where glia maturing to establish barriers have been co-opted to act as signaling intermediaries that coordinate development across neuropils. During visual system development, photoreceptor and wrapping glia morphogenesis are intricately coordinated. Photoreceptors from the developing eye disc are born sequentially as a wave of differentiation sweeps across the disc along the anteroposterior axis (Roignant and Treisman 2009). Photoreceptors grow their axons into the optic stalk, which connects the eye disc with the optic lobe, and into the developing lamina and medulla neuropils (Roignant and Treisman 2009). A population of wrapping glial cells ensheath photoreceptor axon bundles from individual ommatidial clusters (unit eyes) progressively from the eye disc

through the optic stalk and into the lamina (Rangarajan et al. 1999; Franzdóttir et al. 2009). Wrapping glial morphogenesis (i.e., axonal ensheathment) is driven by photoreceptor-derived Thisbe, an FGF that activates the FGF receptor, Heartless, in wrapping glia (Franzdóttir et al. 2009). Thus, as photoreceptor axons arrive sequentially in the optic lobe, they are progressively ensheathed by wrapping glia from the eye disc with ensheathment delayed relative to axonal arrival.

In addition to promoting wrapping glial morphogenesis, photoreceptors also induce their neuronal target field, the lamina, such that every ommatidium has a corresponding lamina unit (cartridge or column) composed of five neuronal types (Huang and Kunes 1996, 1998; Huang et al. 1998). Photoreceptor-derived Hedgehog directly induces lamina precursors, their terminal divisions, and their assembly into columns (i.e., stacked ensembles of postmitotic precursors) (Huang and Kunes 1996, 1998). In addition, photoreceptors induce neuronal differentiation of postmitotic lamina precursors in columns indirectly through glial signaling intermediaries—the wrapping glia and a second ensheathing-like glial population positioned below the lamina called the outer chiasm giant glia (xg^O) (Fernandes et al. 2017; Rossi and Fernandes 2018; Prasad et al. 2022). The xg^O ensheaths neuronal projections between the lamina and medulla neuropils, including a subset of photoreceptor axons (Edwards and Meinertzhagen 2010). Both glial populations respond to Spitz, an EGF produced by photoreceptors, and relay this signal by producing either ILPs, in the case of the wrapping glia or Spitz and a type IV collagen, in the case of the xg^O (Fernandes et al. 2017; Prasad et al. 2022). These glial-derived signals nonautonomously activate mitogen-activated protein kinase (MAPK) signaling in lamina precursors, which drives their differentiation into neurons. The result is a striking spatiotemporal pattern of neuronal differentiation, which reflects the morphogenesis and positioning of both wrapping glia and xg^O (Fig. 3B). Interestingly, although each lamina column eventually contains five neurons, extra precursors, which do not differentiate, incorporate during column assembly (Huang and Kunes 1996). To ensure that only the correct number of neurons differentiate, differentiation signals from xg^O set up an additional relay between the neurons induced to differentiate and their neighboring undifferentiated precursors, such that the newly differentiating neurons antagonize differentiation signaling to prevent the "extra" precursors from differentiating, resulting in their death (Prasad et al. 2022). Thus, by relaying signals from photoreceptors to lamina precursors, wrapping glia and xg^O, coordinate neuronal differentiation between the developing eye disc and the developing lamina and set neuronal number and stoichiometry. Surprisingly, xg^O are born in the central brain from DL1 type II neuroblasts and migrate from the central brain and through the optic lobe before settling in their final position below the lamina (Viktorin et al. 2013; Ren et al. 2018). This raises the intriguing possibility that xg^O coordinates aspects of optic lobe development with that of central brain development, and that other glia, which migrate between brain regions, may also coordinate aspects of development. Although macroglia are not thought to be present during vertebrate embryonic neurogenesis, yolk sac–derived microglia, which migrate into the CNS during early embryonic development, have been shown to secrete mitogenic factors that induce neural stem cell proliferation in a PI3K and Notch-dependent manner in mammals (Morgan et al. 2004) and to regulate the timing of neural differentiation in the developing zebrafish retina (Huang et al. 2012). Whether microglia coordinate developmental processes by acting as signaling intermediaries in these contexts remains to be explored.

Glia as Signaling Intermediaries during Nervous System Function and during Injury

Beyond coordinating development, glia also maintain normoxic conditions in the brain by acting as intermediaries that regulate local gas exchange. In *Drosophila*, a dynamic branched tubular network called the tracheal system supplies oxygen to various organs including the brain. Astrocytes in the larval CNS exhibit TRP channel (TrpML)-mediated Ca^{2+} transients in microdomains that are spontaneous, activity independent, and regulated by reactive

oxygen species (ROS) (Ma and Freeman 2020; see Coutinho-Budd et al. 2023). Interestingly, disrupting microdomain Ca^{2+} transients in astrocytes through loss of TrpML leads to tracheal overgrowth and increased ROS in the CNS (Ma and Freeman 2020). Thus, astrocytes monitor the brain environment, respond to local hyperoxic conditions, and signal to tracheal branches and filopodia for local branch retraction and reduction of local oxygen delivery (Fig. 3C). Importantly, vertebrate astrocytes are similarly coupled to vascular networks where they regulate metabolite and gas exchange, in addition to classic barrier functions (Petzold and Murthy 2011).

In addition to modulating local microenvironments, the compartmentalization of the brain by glia can also regulate circuit function and animal behavior. In addition to their role in neurovascular coupling, astrocytes tile with one another to establish a dense meshwork of gap junction–coupled processes, which individually envelop and compartmentalize synapses from their neighbors. In the larval CNS, tyrosine decarboxylase 2 (Tdc2)-expressing neurons are activated by olfactory neurons (Ma et al. 2016). Active Tdc2 neurons signal to astrocytes through the invertebrate analogs of norepinephrine, octopamine, and tyramine (Ma et al. 2016). In response, astrocytes, which are gap junction–coupled, increase whole-cell Ca^{2+} signaling synchronously (Ma et al. 2016). This, in turn, is required to inhibit downstream dopaminergic neuron activity with a substantial delay, because the astrocytic Ca^{2+} response occurs on a much slower timescale relative to Tdc2 neurons (see Singhvi et al. 2023 for a more extensive review). Parallel work in zebrafish showed that radial astrocytes also integrate information from neuromodulatory neurons over slower timescales to stop motor output (Mu et al. 2019).

Another context in which glia sense, integrate, and transmit information to neuronal circuitry occurs during sleep regulation. Sleep is defined by periods of reversible behavioral inactivity, which normally occur at set times of the day in a species-dependent manner, and which are subject to the homeostatic influence of sleep pressure, where sleep pressure builds up as a function of time spent awake (Artiushin and Sehgal 2020). Although the genetic, molecular, and cellular basis of sleep and sleep pressure are still being elucidated, it is increasingly clear that glia play a central role in sleep regulation (Artiushin and Sehgal 2020). Although numerous glial cell types, including astrocytes, ensheathing glia, cortex glia, and surface glia, have been implicated in homeostatic or baseline sleep regulation in *Drosophila* (reviewed extensively by Artiushin and Sehgal 2020), the role has been best studied in astrocytes (see Coutinho-Budd et al. 2023). Blum et al. (2021) showed that calcium dynamics in astrocytes encode sleep pressure and depend on Ca-α1D, an L-type voltage-gated calcium channel, which enables astrocytes to monitor neuronal activity. Increases in neuronal activity during wakefulness drives, in a calcium-dependent manner, astrocytic increases in expression of a monoamine-activated G-protein-coupled receptor called tyramine receptor II (TyrRII) (Blum et al. 2021). Together with Ca-α1D, TyrRII acts in a positive feedback loop to increase astrocytic calcium (and therefore its own expression), thus sensitizing astrocytes to extracellular monoamines that accumulate during wakefulness (Blum et al. 2021). In turn, astrocytes transmit sleep pressure information to a homeostatic sleep circuit by activating sleep-promoting neurons (R5 neurons) and inhibiting arousal-promoting neurons, in part, through Spätzle–Toll signaling. *spätzle (spz)*, which encodes a cytokine and signals through the Toll receptor, is transcriptionally up-regulated in astrocytes in response to elevated calcium signaling (Blum et al. 2021). Thus, astrocytes monitor their environments, integrate sleep pressure information, and regulate neuronal circuitry that controls state transitions during sleep.

Finally, the positioning of glia at specialized brain interfaces allows them to not only respond to neuronal damage rapidly for debris clearance (Doherty et al. 2009; Purice et al. 2017), but also to modulate the physiology of neighboring uninjured neurons called "bystander" neurons (Hsu et al. 2021). Axon transport is suppressed rapidly in both injured and bystander neurons following an injury. Moreover, bystander neurons also display reduced mechano- and chemosensory signal transduction following injury (Ma et al. 2003;

Meyer and Ringkamp 2008; Hsu et al. 2021). Although initially suppressed, bystander neurons eventually recover while injured axons degenerate (Hsu et al. 2021). Interestingly, glia (wrapping and subperineurial) appear to spread injury signals and suppress bystander neuron function transiently, in a process requiring Draper/MEGF10 signaling in glia (Hsu et al. 2021).

In summary, although glia play prominent roles as barriers, their unique positions in the nervous system are often repurposed to facilitate other diverse processes. In some instances, glia may function as signaling intermediaries out of necessity because glia insulate the CNS from systemic signals (Chell and Brand 2010; Sousa-Nunes et al. 2011; Spéder and Brand 2014). In addition to necessity, gap junction–coupled glial networks, such as the subperineurial glia involved in neuroblast reactivation or astrocytes in modulating circuit activity, may be uniquely poised to survey and coordinate a synchronous response to global metabolic/nutritional status or circuit activity more effectively. Thus, in both developing and functioning nervous systems, glia intercept, integrate, and relay signals both locally and globally, in a manner that is intimately tethered to their function as barriers and insulators.

REFERENCES

*Reference is also in this subject collection.

Artiushin G, Sehgal A. 2020. The glial perspective on sleep and circadian rhythms. *Annu Rev Neurosci* **43:** 119–140. doi:10.1146/annurev-neuro-091819-094557

Avet-Rochex A, Kaul AK, Gatt AP, McNeill H, Bateman JM. 2012. Concerted control of gliogenesis by InR/TOR and FGF signalling in the *Drosophila* post-embryonic brain. *Development* **139:** 2763–2772. doi:10.1242/dev.074179

Awasaki T, Lai SL, Ito K, Lee T. 2008. Organization and postembryonic development of glial cells in the adult central brain of *Drosophila*. *J Neurosci* **28:** 13742–13753. doi:10.1523/JNEUROSCI.4844-08.2008

Babatz F, Naffin E, Klämbt C. 2018. The *Drosophila* blood–brain barrier adapts to cell growth by unfolding of pre-existing septate junctions. *Dev Cell* **47:** 697–710.e3. doi:10.1016/j.devcel.2018.10.002

Bainton RJ, Tsai LT, Schwabe T, DeSalvo M, Gaul U, Heberlein U. 2005. *moody* encodes two GPCRs that regulate cocaine behaviors and blood–brain barrier permeability in *Drosophila*. *Cell* **123:** 145–156. doi:10.1016/j.cell.2005.07.029

Baumgartner S, Littleton JT, Broadie K, Bhat MA, Harbecke R, Lengyel JA, Chiquet-Ehrismann R, Prokop A, Bellen HJ. 1996. A *Drosophila* neurexin is required for septate junction and blood–nerve barrier formation and function. *Cell* **87:** 1059–1068. doi:10.1016/S0092-8674(00)81800-0

Beckervordersandforth RM, Rickert C, Altenhein B, Technau GM. 2008. Subtypes of glial cells in the *Drosophila* embryonic ventral nerve cord as related to lineage and gene expression. *Mech Dev* **125:** 542–557. doi:10.1016/j.mod.2007.12.004

Blum ID, Keleş MF, Baz ES, Han E, Park K, Luu S, Issa H, Brown M, Ho MCW, Tabuchi M, et al. 2021. Astroglial calcium signaling encodes sleep need in *Drosophila*. *Curr Biol* **31:** 150–162.e7. doi:10.1016/j.cub.2020.10.012

Böhme MA, McCarthy AW, Blaum N, Berezeckaja M, Ponimaskine K, Schwefel D, Walter AM. 2021. Glial Synaptobrevin mediates peripheral nerve insulation, neural metabolic supply, and is required for motor function. *Glia* **69:** 1897–1915. doi:10.1002/glia.24000

Britton JS, Edgar BA. 1998. Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* **125:** 2149–2158. doi:10.1242/dev.125.11.2149

Broadie K, Baumgartner S, Prokop A. 2011. Extracellular matrix and its receptors in *Drosophila* neural development. *Dev Neurobiol* **71:** 1102–1130. doi:10.1002/dneu.20935

Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E. 2001. An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr Biol* **11:** 213–221. doi:10.1016/S0960-9822(01)00068-9

Brown NH. 1994. Null mutations in the αPS2 and βPS integrin subunit genes have distinct phenotypes. *Development* **120:** 1221–1231. doi:10.1242/dev.120.5.1221

Calderon MR, Mori M, Kauwe G, Farnsworth J, Ulian-Benitez S, Maksoud E, Shore J, Haghighi AP. 2022. Delta/notch signaling in glia maintains motor nerve barrier function and synaptic transmission by controlling matrix metalloproteinase expression. *Proc Natl Acad Sci* **119:** e2110097119. doi:10.1073/pnas.2110097119

Chell JM, Brand AH. 2010. Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell* **143:** 1161–1173. doi:10.1016/j.cell.2010.12.007

Corty MM, Hulegaard AL, Hill JQ, Sheehan AE, Aicher SA, Freeman MR. 2022. Discoidin domain receptor regulates ensheathment, survival and caliber of peripheral axons. *Development* **149:** dev200636. doi:10.1242/dev.200636

* Coutinho-Budd J, Freeman MR, Ackerman S. 2023. *Drosophila* central nervous system glia. *Cold Spring Harb Perspect Biol* doi:10.1101/cshperspect.a041347

Defaye A, Evans I, Crozatier M, Wood W, Lemaitre B, Leulier F. 2009. Genetic ablation of *Drosophila* phagocytes reveals their contribution to both development and resistance to bacterial infection. *J Innate Immun* **1:** 322–334. doi:10.1159/000210264

Doe CQ, Technau GM. 1993. Identification and cell lineage of individual neural precursors in the *Drosophila* CNS. *Trends Neurosci* **16:** 510–514. doi:10.1016/0166-2236(93)90195-R

Doherty J, Logan MA, Taşdemir OE, Freeman MR. 2009. Ensheathing glia function as phagocytes in the adult *Drosophila* brain. *J Neurosci* **29:** 4768–4781. doi:10.1523/JNEUROSCI.5951-08.2009

Edwards TN, Meinertzhagen IA. 2010. The functional organisation of glia in the adult brain of *Drosophila* and other insects. *Prog Neurobiol* **90:** 471–497. doi:10.1016/j.pneurobio.2010.01.001

Evans IR, Hu N, Skaer H, Wood W. 2010. Interdependence of macrophage migration and ventral nerve cord development in *Drosophila* embryos. *Development* **137:** 1625–1633. doi:10.1242/dev.046797

Fernandes VM, Chen Z, Rossi AM, Zipfel J, Desplan C. 2017. Glia relay differentiation cues to coordinate neuronal development in *Drosophila*. *Science* **357:** 886–891. doi:10.1126/science.aan3174

Franzdóttir SR, Engelen D, Yuva-Aydemir Y, Schmidt I, Aho A, Klämbt C. 2009. Switch in FGF signalling initiates glial differentiation in the *Drosophila* eye. *Nature* **460:** 758–761. doi:10.1038/nature08167

Freeman MR. 2015. *Drosophila* central nervous system glia. *Cold Spring Harb Perspect Biol* **7:** a020552. doi:10.1101/cshperspect.a020552

Ghosh A, Kling T, Snaidero N, Sampaio JL, Shevchenko A, Gras H, Geurten B, Göpfert MC, Schulz JB, Voigt A, et al. 2013. A global in vivo *Drosophila* RNAi screen identifies a key role of ceramide phosphoethanolamine for glial ensheathment of axons. *PLoS Genet* **9:** e1003980. doi:10.1371/journal.pgen.1003980

Granderath S, Stollewerk A, Greig S, Goodman CS, OKane CJ, Klämbt C. 1999. loco encodes an RGS protein required for *Drosophila* glial differentiation. *Development* **126:** 1781–1791. doi:10.1242/dev.126.8.1781

Hartenstein V, Rudloff E, Campos-Ortega JA. 1987. The pattern of proliferation of the neuroblasts in the wild-type embryo of *Drosophila melanogaster*. *Rouxs Arch Dev Biol* **196:** 473–485. doi:10.1007/BF00399871

Harty BL, Monk KR. 2017. Unwrapping the unappreciated: recent progress in Remak Schwann cell biology. *Curr Opin Neurobiol* **47:** 131–137. doi:10.1016/j.conb.2017.10.003

Hatan M, Shinder V, Israeli D, Schnorrer F, Volk T. 2011. The *Drosophila* blood brain barrier is maintained by GPCR-dependent dynamic actin structures. *J Cell Biol* **192:** 307–319. doi:10.1083/jcb.201007095

Hsu JM, Kang Y, Corty MM, Mathieson D, Peters OM, Freeman MR. 2021. Injury-induced inhibition of bystander neurons requires dSarm and signaling from glia. *Neuron* **109:** 473–487.e5. doi:10.1016/j.neuron.2020.11.012

Huang Z, Kunes S. 1996. Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the *Drosophila* brain. *Cell* **86:** 411–422. doi:10.1016/S0092-8674(00)80114-2

Huang Z, Kunes S. 1998. Signals transmitted along retinal axons in *Drosophila*: hedgehog signal reception and the cell circuitry of lamina cartridge assembly. *Development* **125:** 3753–3764. doi:10.1242/dev.125.19.3753

Huang Z, Shilo BZ, Kunes S. 1998. A retinal axon fascicle uses spitz, an EGF receptor ligand, to construct a synaptic cartridge in the brain of *Drosophila*. *Cell* **95:** 693–703. doi:10.1016/S0092-8674(00)81639-6

Huang T, Cui J, Li L, Hitchcock PF, Li Y. 2012. The role of microglia in the neurogenesis of zebrafish retina. *Biochem Biophys Res Commun* **421:** 214–220. doi:10.1016/j.bbrc.2012.03.139

Hynes RO, Zhao Q. 2000. The evolution of cell adhesion. *J Cell Biol* **150:** F89–F96. doi:10.1083/jcb.150.2.F89

Ikeya T, Galic M, Belawat P, Nairz K, Hafen E. 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr Biol* **12:** 1293–1300. doi:10.1016/S0960-9822(02)01043-6

Ito K, Hotta Y. 1992. Proliferation pattern of postembryonic neuroblasts in the brain of *Drosophila melanogaster*. *Dev Biol* **149:** 134–148. doi:10.1016/0012-1606(92)90270-Q

Karkali K, Tiwari P, Singh A, Tlili S, Jorba I, Navajas D, Muñoz JJ, Saunders TE, Martin-Blanco E. 2022. Condensation of the *Drosophila* nerve cord is oscillatory and depends on coordinated mechanical interactions. *Dev Cell* **57:** 867–882.e5 doi:10.1016/j.devcel.2022.03.007

Kim SN, Jeibmann A, Halama K, Witte HT, Wälte M, Matzat T, Schillers H, Faber C, Senner V, Paulus W, et al. 2014. ECM stiffness regulates glial migration in *Drosophila* and mammalian glioma models. *Development* **141:** 3233–3242. doi:10.1242/dev.106039

Kottmeier R, Bittern J, Schoofs A, Scheiwe F, Matzat T, Pankratz M, Klämbt C. 2020. Wrapping glia regulates neuronal signaling speed and precision in the peripheral nervous system of *Drosophila*. *Nat Commun* **11:** 4491. doi:10.1038/s41467-020-18291-1

Kremer MC, Jung C, Batelli S, Rubin GM, Gaul U. 2017. The glia of the adult *Drosophila* nervous system. *Glia* **65:** 606–638. doi:10.1002/glia.23115

Leiserson WM, Harkins EW, Keshishian H. 2000. Fray, a *Drosophila* serine/threonine kinase homologous to mammalian PASK, is required for axonal ensheathment. *Neuron* **28:** 793–806. doi:10.1016/S0896-6273(00)00154-9

Leiserson WM, Forbush B, Keshishian H. 2011. *Drosophila* glia use a conserved cotransporter mechanism to regulate extracellular volume. *Glia* **59:** 320–332. doi:10.1002/glia.21103

Li D, Liu Y, Pei C, Zhang P, Pan L, Xiao J, Meng S, Yuan Z, Bi X. 2017. miR-285-Yki/Mask double-negative feedback loop mediates blood-brain barrier integrity in *Drosophila*. *Proc Natl Acad Sci* **114:** E2365–E2374.

Li X, Fetter R, Schwabe T, Jung C, Liu L, Steller H, Gaul U. 2021. The cAMP effector PKA mediates moody GPCR signaling in *Drosophila* blood-brain barrier formation and maturation. *eLife* **10:** e68275. doi:10.7554/eLife.68275

Lucero HA, Kagan HM. 2006. Lysyl oxidase: an oxidative enzyme and effector of cell function. *Cell Mol Life Sci* **63:** 2304–2316. doi:10.1007/s00018-006-6149-9

Ma Z, Freeman MR. 2020. TrpML-mediated astrocyte microdomain Ca^{2+} transients regulate astrocyte-tracheal interactions. *eLife* **9:** e58952. doi:10.7554/eLife.58952

Ma C, Shu Y, Zheng Z, Chen Y, Yao H, Greenquist KW, White FA, LaMotte RH. 2003. Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons. *J Neurophysiol* **89:** 1588–1602. doi:10.1152/jn.00855.2002

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Ma Z, Stork T, Bergles DE, Freeman MR. 2016. Neuromodulators signal through astrocytes to alter neural circuit activity and behaviour. *Nature* **539:** 428–432. doi:10.1038/nature20145

Martinek N, Shahab J, Saathoff M, Ringuette M. 2008. Haemocyte-derived SPARC is required for collagen-IV-dependent stability of basal laminae in *Drosophila* embryos. *J Cell Sci* **121:** 1671–1680. doi:10.1242/jcs.021931

Matzat T, Sieglitz F, Kottmeier R, Babatz F, Engelen D, Klambt C. 2015. Axonal wrapping in the *Drosophila* PNS is controlled by glia-derived neuregulin homolog vein. *Development* **142:** 1336–1345.

Meyer RA, Ringkamp M. 2008. A role for uninjured afferents in neuropathic pain. *Sheng Li Xue Bao* **60:** 605–609.

Meyer S, Schmidt I, Klämbt C. 2014. Glia ECM interactions are required to shape the *Drosophila* nervous system. *Mech Dev* **133:** 105–116. doi:10.1016/j.mod.2014.05.003

Morgan SC, Taylor DL, Pocock JM. 2004. Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. *J Neurochem* **90:** 89–101. doi:10.1111/j.1471-4159.2004.02461.x

Mu Y, Bennett DV, Rubinov M, Narayan S, Yang CT, Tanimoto M, Mensh BD, Looger LL, Ahrens MB. 2019. Glia accumulate evidence that actions are futile and suppress unsuccessful behavior. *Cell* **178:** 27–43.e19. doi:10.1016/j.cell.2019.05.050

Nern A, Pfeiffer BD, Rubin GM. 2015. Optimized tools for multicolor stochastic labeling reveal diverse stereotyped cell arrangements in the fly visual system. *Proc Natl Acad Sci* **112:** E2967–E2976. doi:10.1073/pnas.1506763112

Olofsson B, Page DT. 2005. Condensation of the central nervous system in embryonic *Drosophila* is inhibited by blocking hemocyte migration or neural activity. *Dev Biol* **279:** 233–243. doi:10.1016/j.ydbio.2004.12.020

Otto N, Marelja Z, Schoofs A, Kranenburg H, Bittern J, Yildirim K, Berh D, Bethke M, Thomas S, Rode S, et al. 2018. The sulfite oxidase Shopper controls neuronal activity by regulating glutamate homeostasis in *Drosophila* ensheathing glia. *Nature Commun* **9:** 3514. doi:10.1038/s41467-018-05645-z

Pandey R, Blanco J, Udolph G. 2011. The glucuronyltransferase GlcAT-P is required for stretch growth of peripheral nerves in *Drosophila*. *PLoS ONE* **6:** e28106. doi:10.1371/journal.pone.0028106

Peco E, Davla S, Camp D, Stacey SM, Landgraf M, van Meyel DJ. 2016. *Drosophila* astrocytes cover specific territories of the CNS neuropil and are instructed to differentiate by Prospero, a key effector of Notch. *Development* **143:** 1170–1181.

Pellerin L, Magistretti PJ. 2012. Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* **32:** 1152–1166. doi:10.1038/jcbfm.2011.149

Petley-Ragan LM, Ardiel EL, Rankin CH, Auld VJ. 2016. Accumulation of laminin monomers in *Drosophila* glia leads to glial endoplasmic reticulum stress and disrupted larval locomotion. *J Neurosci* **36:** 1151–1164. doi:10.1523/JNEUROSCI.1797-15.2016

Petri J, Syed MH, Rey S, Klämbt C. 2019. Non-cell-autonomous function of the GPI-anchored protein Undicht during septate junction assembly. *Cell Rep* **26:** 1641–1653.e4. doi:10.1016/j.celrep.2019.01.046

Petzold GC, Murthy VN. 2011. Role of astrocytes in neurovascular coupling. *Neuron* **71:** 782–797. doi:10.1016/j.neuron.2011.08.009

Pogodalla N, Kranenburg H, Rey S, Rodrigues S, Cardona A, Klämbt C. 2021. *Drosophila* β (Heavy)-Spectrin is required in polarized ensheathing glia that form a diffusion-barrier around the neuropil. *Nat Commun* **12:** 6357. doi:10.1038/s41467-021-26462-x

Prasad AR, Lago-Baldaia I, Bostock MP, Housseini Z, Fernandes VM. 2022. Differentiation signals from glia are fine-tuned to set neuronal numbers during development. *eLife* **11:** e78092. doi:10.7554/eLife.78092

Prokop A, Technau GM. 1991. The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila melanogaster*. *Development* **111:** 79–88. doi:10.1242/dev.111.1.79

Purice MD, Ray A, Münzel EJ, Pope BJ, Park DJ, Speese SD, Logan MA. 2017. A novel *Drosophila* injury model reveals severed axons are cleared through a draper/MMP-1 signaling cascade. *eLife* **6:** e23611. doi:10.7554/eLife.23611

Rangarajan R, Gong Q, Gaul U. 1999. Migration and function of glia in the developing *Drosophila* eye. *Development* **126:** 3285–3292. doi:10.1242/dev.126.15.3285

Ren Q, Awasaki T, Wang YC, Huang YF, Lee T. 2018. Lineage-guided notch-dependent gliogenesis by *Drosophila* multi-potent progenitors. *Development* **145:** dev160127. doi:10.1242/dev.160127

Rey S, Ohm H, Klämbt C. 2023a. Axonal ion homeostasis and glial differentiation. *FEBS J* **290:** 3737–3744. doi:10.1111/febs.16594

Rey S, Ohm H, Moschref F, Zeuschner D, Praetz M, Klämbt C. 2023b. Glial-dependent clustering of voltage-gated ion channels in *Drosophila* precedes myelin formation. *eLife* **12:** e85752. doi:10.7554/eLife.85752

Roignant JY, Treisman JE. 2009. Pattern formation in the *Drosophila* eye disc. *Int J Dev Biol* **53:** 795–804. doi:10.1387/ijdb.072483jr

Rossi AM, Fernandes VM. 2018. Wrapping glial morphogenesis and signaling control the timing and pattern of neuronal differentiation in the *Drosophila* lamina. *J Exp Neurosci* **12:** 117906951875929. doi:10.1177/1179069518759294

Rulifson EJ, Kim SK, Nusse R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* **296:** 1118–1120. doi:10.1126/science.1070058

Schmidt I, Thomas S, Kain P, Risse B, Naffin E, Klämbt C. 2012. Kinesin heavy chain function in *Drosophila* glial cells controls neuronal activity. *J Neurosci* **32:** 7466–7476. doi:10.1523/JNEUROSCI.0349-12.2012

Schwabe T, Bainton RJ, Fetter RD, Heberlein U, Gaul U. 2005. GPCR signaling is required for blood–brain barrier formation in *Drosophila*. *Cell* **123:** 133–144. doi:10.1016/j.cell.2005.08.037

Schwabe T, Li X, Gaul U. 2017. Dynamic analysis of the mesenchymal–epithelial transition of blood–brain barrier forming glia in *Drosophila*. *Biol Open* **6:** 232–243.

Sears HC, Kennedy CJ, Garrity PA. 2003. Macrophage-mediated corpse engulfment is required for normal *Drosoph-*

ila CNS morphogenesis. *Development* **130:** 3557–3565. doi:10.1242/dev.00586

Sepp KJ, Auld VJ. 2003. Reciprocal interactions between neurons and glia are required for *Drosophila* peripheral nervous system development. *J Neurosci* **23:** 8221–8230. doi:10.1523/JNEUROSCI.23-23-08221.2003

Sepp KJ, Schulte J, Auld VJ. 2000. Developmental dynamics of peripheral glia in *Drosophila melanogaster*. *Glia* **30:** 122–133. doi:10.1002/(SICI)1098-1136(200004)30:2<122::AID-GLIA2>3.0.CO;2-B

Silies M, Yuva Y, Engelen D, Aho A, Stork T, Klämbt C. 2007. Glial cell migration in the eye disc. *J Neurosci* **27:** 13130–13139. doi:10.1523/JNEUROSCI.3583-07.2007

* Singhvi A, Shaham S, Rapti G. 2023. Glia development and function in the nervous system of *Caenorhabditis elegans*. *Cold Spring Harb Perspect Biol* doi:10.1101/cshperspect.a041316

Skeath JB, Wilson BA, Romero SE, Snee MJ, Zhu Y, Lacin H. 2017. The extracellular metalloprotease AdamTS-A anchors neural lineages in place within and preserves the architecture of the central nervous system. *Development* **144:** 3102–3113.

Sousa-Nunes R, Yee LL, Gould AP. 2011. Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. *Nature* **471:** 508–512. doi:10.1038/nature09867

Spéder P, Brand AH. 2014. Gap junction proteins in the blood-brain barrier control nutrient-dependent reactivation of *Drosophila* neural stem cells. *Dev Cell* **30:** 309–321. doi:10.1016/j.devcel.2014.05.021

Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, Klämbt C. 2008. Organization and function of the blood–brain barrier in *Drosophila*. *J Neurosci* **28:** 587–597. doi:10.1523/JNEUROSCI.4367-07.2008

Subramanian A, Siefert M, Banerjee S, Vishal K, Bergmann KA, Clay C, Curts CM, Dorr M, Molina C, Fernandes J. 2017. Remodeling of peripheral nerve ensheathment during the larval-to-adult transition in *Drosophila*. *Dev Neurobiol* **77:** 1144–1160. doi:10.1002/dneu.22502

Tepass U, Hartenstein V. 1994. The development of cellular junctions in the *Drosophila* embryo. *Dev Biol* **161:** 563–596. doi:10.1006/dbio.1994.1054

Tiklová K, Senti KA, Wang S, Gräslund A, Samakovlis C. 2010. Epithelial septate junction assembly relies on melanotransferrin iron binding and endocytosis in *Drosophila*. *Nat Cell Biol* **12:** 1071–1077. doi:10.1038/ncb2111

Truman JW, Bate M. 1988. Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Dev Biol* **125:** 145–157. doi:10.1016/0012-1606(88)90067-X

Unhavaithaya Y, Orr-Weaver TL. 2012. Polyploidization of glia in neural development links tissue growth to blood–brain barrier integrity. *Genes Dev* **26:** 31–36. doi:10.1101/gad.177436.111

Urbano JM, Torgler CN, Molnar C, Tepass U, López-Varea A, Brown NH, de Celis JF, Martín-Bermudo MD. 2009. *Drosophila* laminins act as key regulators of basement membrane assembly and morphogenesis. *Development* **136:** 4165–4176. doi:10.1242/dev.044263

Viktorin G, Riebli N, Reichert H. 2013. A multipotent transit-amplifying neuroblast lineage in the central brain gives rise to optic lobe glial cells in *Drosophila*. *Dev Biol* **379:** 182–194. doi:10.1016/j.ydbio.2013.04.020

Volkenhoff A, Weiler A, Letzel M, Stehling M, Klämbt C, Schirmeier S. 2015. Glial glycolysis is essential for neuronal survival in *Drosophila*. *Cell Metab* **22:** 437–447. doi:10.1016/j.cmet.2015.07.006

von Hilchen CM, Beckervordersandforth RM, Rickert C, Technau GM, Altenhein B. 2008. Identity, origin, and migration of peripheral glial cells in the *Drosophila* embryo. *Mech Dev* **125:** 337–352. doi:10.1016/j.mod.2007.10.010

von Hilchen CM, Bustos AE, Giangrande A, Technau GM, Altenhein B. 2013. Predetermined embryonic glial cells form the distinct glial sheaths of the *Drosophila* peripheral nervous system. *Development* **140:** 3657–3668. doi:10.1242/dev.093245

Von Stetina JR, Frawley LE, Unhavaithaya Y, Orr-Weaver TL. 2018. Variant cell cycles regulated by Notch signaling control cell size and ensure a functional blood–brain barrier. *Development* **145:** dev157115. doi:10.1242/dev.157115

Winkler B, Funke D, Benmimoun B, Spéder P, Rey S, Logan MA, Klämbt C. 2021. Brain inflammation triggers macrophage invasion across the blood-brain barrier in *Drosophila* during pupal stages. *Sci Adv* **7:** eabh0050. doi:10.1126/sciadv.abh0050

Xie X, Auld VJ. 2011. Integrins are necessary for the development and maintenance of the glial layers in the *Drosophila* peripheral nerve. *Development* **138:** 3813–3822. doi:10.1242/dev.064816

Xie X, Gilbert M, Petley-Ragan L, Auld VJ. 2014. Loss of focal adhesions in glia disrupts both glial and photoreceptor axon migration in the *Drosophila* visual system. *Development* **141:** 3072–3083. doi:10.1242/dev.101972

Yuan X, Sipe CW, Suzawa M, Bland ML, Siegrist SE. 2020. Dilp-2-mediated PI3-kinase activation coordinates reactivation of quiescent neuroblasts with growth of their glial stem cell niche. *PLoS Biol* **18:** e3000721. doi:10.1371/journal.pbio.3000721

Zulbahar S, Sieglitz F, Kottmeier R, Altenhein B, Rumpf S, Klambt C. 2018. Differential expression of Obek controls ploidy in the *Drosophila* blood–brain barrier. *Development* **145:** dev164111. doi:10.1242/dev.164111

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