Drosophila Central Nervous System Glia

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Molecular genetic approaches in small model organisms like *Drosophila* have helped to elucidate fundamental principles of neuronal cell biology. Much less is understood about glial cells, although interest in using invertebrate preparations to define their *in vivo* functions has increased significantly in recent years. This review focuses on our current understanding of the three major neuron-associated glial cell types found in the *Drosophila* central nervous system (CNS)—astrocytes, cortex glia, and ensheathing glia. Together, these cells act like mammalian astrocytes: they surround neuronal cell bodies and proximal neurites, are coupled to the vasculature, and associate closely with synapses. Exciting recent work has shown essential roles for these CNS glial cells in neural circuit formation, function, plasticity, and pathology. As we gain a more firm molecular and cellular understanding of how *Drosophila* CNS glial cells interact with neurons, it is becoming clear they share significant molecular and functional attributes with mammalian astrocytes.

Invertebrate preparations have contributed enormously to our understanding of fundamental principles of nervous system biology, including the chemical and electrophysiological basis of the action potential, synaptic vesicle release, neural cell fate specification, and axon pathfinding. This is largely thanks to the high experimental accessibility, ease of culture, rapid growth, and the panoply of molecular genetic tools with which to manipulate individual cells *in vivo* in organisms like *Drosophila* and *Caenorhabditis elegans*. The focus of many neuroscientists has shifted in recent years toward careful exploration of how glial cells participate in nervous system development, neural circuit function and plasticity, and neurological disease. Based on the remarkable success

with which invertebrates were used to dissect fundamental aspects of the cell biology of the neuron, interest in the potential of small genetic model organisms to contribute to unraveling the mysteries of glial cells has grown significantly. This article will provide a brief overview of *Drosophila* glial cell biology, then focus on fly glial cell subtypes that are tightly associated with neurons in the central nervous system (CNS)—astrocytes, ensheathing glia, and cortex glia. A growing body of work argues strongly that these glia share a range morphological and functional features with mammalian astrocytes, and recent molecular studies indicate that conservation of basic glial cell biology extends, perhaps not surprisingly, to the molecular level.

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OVERVIEW OF *Drosophila* NERVOUS SYSTEM HISTOLOGY

In total, the adult fly brain and thoracic ganglion (the fly equivalent of the mammalian spinal cord) houses ~200,000–300,000 neurons. *Drosophila* neurons are quite similar in terms of electrophysiological properties to mammalian neurons. They fire proper Na^+/K^+ -based action potentials; they use highly conserved mechanisms for synaptic vesicle release of conserved neurotransmitters, such as γ -aminobutyric acid (GABA), glutamate, and acetylcholine, and neuromodulators, such as biogenic amines and neuropeptides; and they modulate a diverse behavioral repertoire that can be studied in the intact organism that shows both electrophysiological and behavioral plasticity. The histology of the adult *Drosophila* nervous system is relatively complex. The brain houses multiple anatomically distinct brain lobes, which are connected to one another by fasciculated nerves. The CNS can be subdivided into two histological regions: the neuronal cell cortex, where all CNS neuronal cell bodies reside; and the neuropil, to which axons and dendrites project and form neural circuits (Fig. 1A, top).

As in mammals, glial cells in *Drosophila* are characterized in large part by their morphology and association with neurons (Fig. 1A, bottom). The precise number of glia in the fly nervous remains unclear, but likely represents 5%–10% of the total population of cells within the CNS. The outermost layer of cells associated with the surface of the CNS is composed of a subset of glia termed perineural glia (PG), which together with macrophages are thought to secrete a dense carbohydrate-rich lamella that covers the CNS and peripheral nerves and acts as a chemical and physical barrier for the CNS (Carlson et al. 2000; Leiserson et al. 2000). The PG layer is discontinuous, with small gaps, but below this is a layer of subperineural glial cells (SPGs), which show a flattened morphology, cover the entire CNS surface, and establish a blood–brain barrier (BBB) by forming pleated septate junctions with one another (Auld et al. 1995; Baumgartner et al. 1996; Schwabe et al. 2005). SPGs make contact with only the most

superficial layer of neuronal cell bodies in the cortex; whether they make any contact with neurites has not been carefully studied, but seems unlikely. Deeper in the CNS, a number of specialized glial subtypes—cortex glia, ensheathing glia, and astrocytes—associate closely with neurons. These will be the focus of this review and discussed in detail below, along with a comparison of these cells to their mammalian counterparts.

Drosophila also have a number of glial subtypes outside of the CNS that ensheath, support, and modulate the development and function of peripheral sensory neurons, and motoneuron axons and terminals (Fig. 1A–C) (Freeman 2012; Stork et al. 2012). Peripheral nerves are covered by the PG- and SPG-based BBB similar to the CNS, but additionally house a population of glia termed wrapping glia that ensheath motor and sensory axons and whose histology is very similar to that of mammalian Remak bundles (Leiserson et al. 2000; Becker-vordersandforth et al. 2008; Stork et al. 2008). At the neuromuscular junction, SPGs extend processes that interact with motoneuron synaptic contacts on muscles (Fig. 1B) where they perform many key functions, including recycling neurotransmitters (Rival et al. 2004; Danjo et al. 2011), sculpting growing presynaptic morphology by engulfing shed axonal/synaptic debris during development (Fuentes-Medel et al. 2009), and secreting transforming growth factor (TGF)- β molecules that modulate retrograde muscle $\rightarrow$ presynapse signaling and thereby neuromuscular junction (NMJ) growth (Fuentes-Medel et al. 2012) and regulating synaptic physiology by secreting Wnts that modulate postsynaptic glutamate receptor clustering (Kerr et al. 2014). Finally, external sensory organ neurons responsible for receiving mechanical, chemical, or other stimuli from the environment are closely associated with socket glial cells, sheath glial cells that wrap the neuronal dendrite and cell body, and an axon-associated glial cell (Fig. 1C). The biology of these sensory organ precursors will likely be very similar to *C. elegans* glia (Shaham 2006), but their functions have not been studied extensively.

Figure 1. Subtypes, positions, and morphology of *Drosophila* glia. (A) Overview of the *Drosophila* larval central nervous system (CNS). The neuronal cell cortex (gray) houses all neuronal and most glial cell bodies. CNS synaptic contacts between neurons are found within the neuropil (light gray). Interneurons (IN) (blue) maintain all projections within the neuropil: motoneurons (MN) (red) extend axon terminals into the peripheral muscle field. (Bottom) cross-sectional view of glial subtypes (green). Morphological arrangement in the adult brain is similar. See text for details. (B) Glia at the *Drosophila* larval neuromuscular junction (NMJ). MN terminals (red) penetrate the muscle; subperineurial glia (light green) enter the space between the MN and muscle. (C) Sensory organs in *Drosophila* contain at least three glial types: the socket cell, sheath cell, and an axon-associated glial cell. (From Freeman 2012; reprinted, with permission, from the author.)

CNS GLIAL SUBTYPES CLOSELY ASSOCIATED WITH NEURONS

Glial cells that are directly associated with neurons likely mediate key events that allow glia to modulate neural circuit assembly, function, plasticity, or degeneration. In *Drosophila*, cortex glia, ensheathing glia, and astrocytes (Fig. 1A) constitute the majority of glial subtypes present in the CNS beneath the BBB, and together these fully cover the CNS scaffold of neuron cell bodies, neurites, and synapses. Cortex glia surround

neuronal cell bodies, ensheathing glia surround and compartmentalize the neuropil and nerves as they project out of the CNS, and astrocytes densely infiltrate the synaptic neuropil. The remainder of this review will discuss our current understanding of these CNS glial cells and how they interact with neurons *in vivo*. I note that an additional mesectodermally derived subset of glia, termed midline glia, are also present in the *Drosophila* CNS. Midline glia play a central role in axon pathfinding, separation of the major commissures of the CNS axon scaffold,

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and ultimately they ensheath axons at the CNS midline. These have been the subject of excellent reviews (Jacobs 2000; Crews 2010) and will not be covered here.

ASTROCYTES

Morphology, Polarity, and Growth of Astrocytes

Drosophila astrocytes bear remarkable morphological, molecular, and functional similarities to mammalian protoplasmic astrocytes (Fig. 2) (Awasaki et al. 2008; Doherty et al. 2009; Muthukumar et al. 2014; Stork et al. 2014; Tasdemir-Yilmaz and Freeman 2014). Of the three glial subtypes discussed in this chapter astrocytes by far are the most heavily studied. Astro-

cyte cell bodies reside at the cortex/neuropil interface, but they extend major processes into the neuropil that then branch repeatedly to form a dense meshwork of very fine processes, with the finest membranes very close to synapses (Stork et al. 2014). Fly astrocytes are highly polarized. The cell body and primary branches of astrocytes are microtubule (MT)-rich with MT plus ends oriented toward the fine processes, which are actin rich (Stork et al. 2014). In the larval and adult nervous system, these cells seem to extend processes that cover the vast majority of the neuropil synaptic space (Muthukumar et al. 2014; Stork et al. 2014). Astrocytes appear to talk to one another to ensure full coverage of the neuropil. They tile with one another to establish unique spatial domains (Stork et al. 2014) similar to astrocyte–astrocyte tiling ob-

Figure 2. Astrocytes in *Drosophila*. (A) A single cell clone of a larval astrocyte. Green, astrocyte membranes; blue, astrocyte nuclear marker; red, neurons. (From Tasdemir-Yilmaz and Freeman 2014; reprinted, with permission, from the authors.) (B) Astrocyte membrane processes (green) in the larval neuropil associate with nearly all regions of the neuropil containing synapses (red). (From Stork et al. 2014; reprinted, with permission, from the authors.) (C) *Drosophila* astrocytes recycle neurotransmitters using molecular pathways similar to those in mammals. See text for details. (D) Astrocyte membranes (green) associate closely with tracheal cells (blue), which are gas-filled tubes that allow for gas exchange with the environment. EAAT, excitatory amino-acid transporters; GABA, γ -aminobutyric acid; GAT, GABA transporter; GS, glutamine synthetase.

served in mammals (Bushong et al. 2004), and ablation of astrocytes from regions of the neuropil leads to the expansion of remaining astrocytes into the astrocyte-depleted regions (Stork et al. 2014). Similar results were found in studies of zebrafish muller glia (MG); MG tiled with one another and ablation of single MG led to compensatory growth by surrounding MG to fill in MG-depleted areas (Williams et al. 2010). The molecular basis of how astrocytes tile with one another remains completely unexplored in any organism. That *Drosophila* astrocytes show tiling behavior like their vertebrate counterparts opens the door to a forward genetic analysis of the mechanisms of astrocyte tiling and its importance in neural circuit function.

Most of what astrocytes are thought to do in the brain depends on their close physical relationship with synapses. Understanding how astrocytes acquire their remarkable morphology and closely associate with synapses remains a major challenge for the field. Stork and colleagues (Stork et al. 2014) recently found that early astrocyte morphogenesis critically depends on a neuron–astrocyte fibroblast growth factor (FGF) signaling cascade. The *Drosophila* FGF receptor (FGFR) Heartless is expressed early in astrocyte development (Shishido et al. 1997). Interestingly, *heartless* mutants showed defects in the migration of astrocyte cell bodies to their appropriate positions around the neuropil, and a failure of astrocyte membrane extension into the neuropil (Stork et al. 2014). Thus, without Heartless/FGFR signaling, astrocytes are born but fail to elaborate their tufted morphology. The level of Heartless/FGFR signaling appears to have a strong regulatory effect on astrocyte growth rates, as an expression of an activated version of this receptor in a single astrocyte led to an increase in its size relative to its wild-type neighbors, and partial blockade of Heartless signaling in a single astrocyte by RNAi-mediated knockdown had the opposite effect, making the Heartless-deficient astrocyte smaller than its neighbors. Elimination of the ligands for Heartless, Pyramus, and Thisbe, led to similar defects in astrocyte morphogenesis, and based on RNA in situ hybridizations and rescue experiments, it is believed that Pyramus

and Thisbe are released by neurons (Stork et al. 2014). It is interesting to note that mouse astrocytes also express high levels of FGFR3 (Pringle et al. 2003; Cahoy et al. 2008), the ortholog of *Drosophila* Heartless. Initial studies of an FGFR3 mutant mouse suggested that it negatively regulated GFAP expression (Pringle et al. 2003), but it would be interesting to revisit its role in astrocyte morphological elaboration based on the newly described and critical role for Heartless in *Drosophila* astrocyte development.

Astrocyte Roles in Neural Circuit Remodeling: Pruning and Synapse Formation

During metamorphosis, the *Drosophila* CNS undergoes a dramatic transformation from a simple larval neural tissue to the much more architecturally complex adult brain and thoracic ganglion. Neural circuit reorganization entails, first, the elimination of a significant number of neurons by apoptosis and the pruning of many larval-specific neurites, and then wiring of new adult and retained larval neurons into adult-specific neural circuits (Truman 1996). Astrocytes and other CNS glia mediate both of these phases by engulfing and eliminating neuronal cell corpses, axons, dendrites, and synapses, and then promoting synapse formation in adult neural circuits.

Drosophila mushroom body (MB) γ neurons have served as a very useful model for local neurite pruning—where only selected neurites and their synapses are eliminated but the parent neuron is retained and reorganized (Lee et al. 1999). In the larva, γ neurons extend both medial and dorsal axonal projections into the MB. At metamorphosis medial axon and their synapses fragment and are cleared from the CNS, and subsequently adult-specific MB axonal projections are elaborated. Glial cells invade the mushroom body lobes at the initiation of axon pruning (Awasaki and Ito 2004; Watts et al. 2004) and prime the MB γ neurons for pruning through secretion the TGF- β molecule Myoglianin (Myo). Elimination of Myo from glial cells leads to blockade of MB γ neuron pruning, providing direct evidence that *Drosophila* glial cells actively signal to initiate neuronal pruning

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(Awasaki et al. 2011). This is reminiscent of retinal ganglion pruning in mammals, where astrocytes secrete TGF- β to activate C1q expression in retinal ganglion cells (RGCs), and C1q subsequently opsinizes weak synapses for elimination (Bialas and Stevens 2013). As *Drosophila* MB axons fragment, glial cells engulf and degrade degenerating axonal debris. Genetic blockade of glial engulfing activity potently blocks axonal pruning (Awasaki and Ito 2004), and genetically labeled axon fragments can be found within phagocytic glial cells (Watts et al. 2004). Precisely, how much axonal and dendritic material is pruned during *Drosophila* metamorphosis remains unclear, but it seems likely that glial cells are the primary cell type responsible for clearing most neuronal debris in the CNS.

Exactly which glial cells engulf pruned neurites and synapses during metamorphosis was not clear, but recent work shows that astrocytes transform at the initiation of metamorphosis from a cell that nourishes neurons and synapses to a highly phagocytic cell type that engulfs perhaps the majority of pruned debris within the neuropil (Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014). Before metamorphosis, astrocytes in the larva do not express detectable levels of the engulfment receptor Draper. However, within 6 h after puparium formation (APF) steroid-dependent signaling events in astrocytes result in their dramatic increase Draper expression, transformation into phagocytes, and initiation of engulfment of pruned axons, dendrites, and synapses (Tasdemir-Yilmaz and Freeman 2014). Blockade of signaling through the ecdysone receptor, or Draper, suppresses axon clearance of MB γ neurons (Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014). Surprisingly, careful genetic studies reveal that there is not a single engulfment pathway responsible for clearance of all neurite debris during pruning. In contrast, it appears that astrocytes engage unique molecular programs to engulf different subsets of neurites. For instance, clearance of MB γ neuron axons requires Draper signaling (Awasaki et al. 2006; Hoopfer et al. 2006; Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014) and the guanine

nucleotide exchange factor (GEF) Crk/Mbc/dCed12 in a partially redundant fashion (Tasdemir-Yilmaz and Freeman 2014). In contrast, the clearance of neurites from ventral corazonin-expressing ($vCrz^+$) neurons requires Crk/Mbc/dCed12, although Draper is completely dispensable (Tasdemir-Yilmaz and Freeman 2014). This latter result was unexpected as it is the first example of a glial-mediated engulfment event that can occur in a Draper-independent fashion in *Drosophila*. Future studies will be necessary to identify the additional signaling pathways required for astrocyte clearance of pruned neurites in these contexts.

The first 2 d of *Drosophila* metamorphosis is the time during which larval neural circuits are deconstructed. Astrocytes actively prune neurites and synapses during this developmental window and their membranes become progressively less prominent in the neuropil, such that by 2 d APF they are absent from the neuropil, and almost no synapses are present in the CNS (Muthukumar et al. 2014). During the next 2 d of metamorphosis, adult neural circuits are assembled. Muthukumar et al. (2014) examined the timing of synapse formation compared with astrocyte infiltration of the adult CNS. The major wave of CNS synaptogenesis (scored by classical electron microscopy-based identification of synapses) began ~ 72 h APF, and coordinate with initiation of astrocyte infiltration into the CNS. Synapse numbers continued to increase over developmental time but largely plateaued by 84 h APF. Immature astrocyte membranes were also initially found in the neuropil at 72 h APF, and they continued to infiltrate the neuropil more densely over time such that, by eclosion as adults (~ 96 h APF), astrocytes were found throughout the neuropil and had taken on their mature morphology. Interestingly, timed ablation of astrocytes after pruning was complete, but before synapses formed, led to a 40%–50% decrease in the number of synapses formed in the late pupal brain, although gross brain histology and neuronal numbers remained largely unchanged (Muthukumar et al. 2014). These data argue that *Drosophila* astrocytes, like their mammalian counterparts, appear to be impor-

tant for CNS synapse formation. Whether similar presynaptic molecules, such as thrombospondins (Christopherson et al. 2005), Hevin (Kucukdereli et al. 2011), or glypicans (Allen et al. 2012), are secreted by *Drosophila* astrocytes to promote synapse formation remains to be explored.

Although much is known regarding how astrocytes control of synapse formation, much less is understood about how synapses or neurotransmission might reciprocally regulate astrocyte development. In mammals, glutamate signals directly through metabotropic glutamate receptors on astrocytes to modulate levels of excitatory amino acid transporters in astrocytes (Yang et al. 2009; Benediktsson et al. 2012; Devaraju et al. 2013), thereby allowing direct regulation the astrocyte glutamate buffering capacity by glutamatergic neurotransmission. *Drosophila* astrocytes express excitatory amino acid transporters, such as excitatory amino acid transporters (EAAT1) (Freeman et al. 2003) and the sole GABA transporter Gat (Thimman et al. 2006). Whether glutamatergic signaling regulates EAAT1 in *Drosophila* has not been explored; however, Gat levels in astrocytes are regulated by local GABAergic circuits during late pupal development (Muthukumar et al. 2014). Gat expression in astrocytes is up-regulated ~72 h APE, the time when astrocytes have begun to infiltrate the neuropil and synapses are forming, and reaches its peak 96 h APE. Ablation or silencing of GABAergic neurons resulted in a significant decrease in astrocytic Gat levels at late pupal stages, indicating that GABAergic synaptic activity can regulate astrocytic Gat. This effect is mediated in part by GABA_B-R1/R2 signaling, because astrocyte-specific depletion of GABA_B-R1 or GABA_B-R2, or blockade of downstream G-protein signaling led to a similar decrease in Gat (Muthukumar et al. 2014). Interestingly, knockdown of GABA_B-R1/R2 in adult astrocytes had no effect on Gat levels, suggesting that this regulatory signaling event is specific to the late pupal developmental window when circuits are forming. Nevertheless, late pupal knockdown of GABA_B-R1/R2 signaling was sufficient to strong suppression seizure activity in a *Drosophila* model of hyperexcitability,

arguing that establishing appropriate Gat levels during development is essential for maintenance of excitatory–inhibitory balance in the adult nervous system.

Roles for Astrocytes in Neural Circuit Function and Behavior

Drosophila astrocytes are important for clearance of neurotransmitters from the synaptic space and loss of this activity can have profound effects on animal behavior and survival. Fly astrocytes express EAATs for glutamate (Rival et al. 2004; Stacey et al. 2010) and GABA (Gat) (Stork et al. 2014) as well as enzymes, such as glutamine synthetase (Freeman et al. 2003) and GABA transaminase (BDGP) (T Stork and M Freeman, unpubl.) for their metabolic breakdown. Depletion of EAAT1 from glial cells in adult *Drosophila* led to age-dependent behavioral defects and neuron loss that was suppressed by drugs used to suppress excitotoxicity in humans (Rival et al. 2004). Similarly, astrocyte-specific RNAi-depletion of the GABA transporter Gat led to severe motor defects in larvae and adults (Stork et al. 2014). Astrocytic expression of Gat is, in fact, essential for animal survival: null mutations in *gat* led to late embryonic or early larval lethality around the time these animals would emerge as larvae from the egg case, and these animals could be rescued to adulthood by resupplying Gat only in astrocytes (Stork et al. 2014).

Great interest has developed in the potential role of astrocyte Ca²⁺-signaling events in the regulation of neural circuit function (Araque et al. 2014) and this has been explored at some level in *Drosophila*. Adult brain astrocytes were found to show spontaneous Ca²⁺ activity in many brain regions, including the antennal lobe. In the olfactory circuit, stimulation of astrocytes using channel rhodopsin 2 (ChR2) inhibited odor-evoked responses of second-order olfactory projection neurons. Astrocyte activation decreased the amplitude and slope of excitatory postsynaptic potentials after antennal nerve stimulation (Liu et al. 2014). These data begin to make a case for astrocytes directly reg-

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ulating neuronal physiology, but caution should be taken in interpreting these results, as the means by which ChR2 “activates” astrocytes is not clear and could represent a nonphysiological event. Nevertheless, other studies support the notion that *Drosophila* glia can regulate neurotransmission and behavior. Suh and Jackson (2007) made the exciting discovery that the β -alanine transferase *Ebony* was expressed in CNS glia and was required for normal circadian rhythmicity (Suh and Jackson 2007). Subsequent work showed that manipulation of vesicle dynamics or Ca^{2+} signaling specifically in astrocytes in the intact adult brain could reversibly alter circadian motor output (Ng et al. 2011). Defining the precise Ca^{2+} -dependent signaling pathways that mediate these effects will be an exciting avenue for future investigation.

ENSHEATHING GLIA

Morphology and Role in CNS Compartmentalization

Ensheathing glial cells extend flattened processes along the edges of the neuropil and subdivide brain lobes and major commissures into anatomically discrete compartments (Hartenstein 2011). This arrangement derives from the close association of both cortex and ensheathing glia with neuroblasts and newly born neurons in the larval and pupal brain (Dumstrei et al. 2003). As neuroblasts generate daughter cells, they extend axons toward the neuropil. Ensheathing glia surround fiber tracts once they enter the neuropil and likely establish early boundaries that demarcate brain lobes and separate the neuropil from the cell cortex. Cortex glia (see below) remain in the cortex and associate closely with neuronal cell bodies and neuroblasts. Both of these cell types appear critical for proper CNS morphogenesis (Dumstrei et al. 2003; Pereanu et al. 2010). For instance, expression of a dominant negative *Drosophila melanogaster* epithelial (DE)-cadherin in cortex glia led to misplacement of neuroblasts and neuronal cell bodies, which in turn altered fiber tract morphology (Dumstrei et al. 2003); and ablation neuropil-associated glia, including ensheathing

glia led to defects in the formation of axon tracts and alterations of their trajectories (Spindler et al. 2009).

Responsiveness to Injury—Engulfing Debris and Modulating Plasticity

In the adult brain, ensheathing glia potently respond to injury and clear axonal debris from the neuropil. MacDonald et al. (2006) examined glial responses to axonal injury using a simple nerve injury assay in which olfactory receptor neurons (ORNs) were severed by removal of the antenna. Within hours after axon injury, ensheathing cells up-regulated expression of the engulfment receptor Draper, extended membranes directly to degenerating axonal debris, and phagocytosed axonal debris. Elimination of Draper blocked all glial response to axonal injury (MacDonald et al. 2006), indicating that Draper is a central regulator of glial clearance of axonal debris, and it appears to function in this context primarily in ensheathing glia (Doherty et al. 2009).

Draper-dependent activation of glial responses to axonal injury occurs through activation of a Src-family signaling cascade composed of Src42a and Shark, which, together with the PTB-domain protein dCed-6, promote engulfment of axon debris (Fig. 3) (Awasaki et al. 2006; Ziegenfuss et al. 2008; Doherty et al. 2009). Additional signaling molecules required for glial clearance of degenerating axonal materials include Rac1 and the Rac1 guanine nucleotide exchange factor (GEF) Crk/Mbc/dCed-12, which is required for glial internalization of axonal debris (Ziegenfuss et al. 2012), and the additional Rac1 GEF Drk/Dos/Sos, which appears to act in a partially redundant fashion with Crk/Mbc/dCed-12 to activate Rac1 downstream from Draper (Lu et al. 2014). Transcriptional activation of genes required for engulfment, for instance *draper*, is also a key feature of glial activation downstream from the Draper receptor, and involves signaling through the c-Jun kinase cascade and the transcriptional activators dAP-1 (MacDonald et al. 2013) and STAT92E (Doherty et al. 2014). That Draper signaling can regulate transcriptional activa-

Figure 3. Glial engulfment signaling in the adult *Drosophila* CNS after axotomy. (Step 1) Axonal debris activates signaling downstream from Draper. Activation includes signaling to the nucleus via dJNK/dAP-1 and STAT92E to activate engulfment gene expression, including *draper*. (Step 2) Glial membranes are recruited to axonal debris via dCed-6 and the Src family kinase cascade and Rac1. The GEFs Drk/Dos/Sos and dCed-12/Mbc/Crk are proposed to act redundantly upstream of Rac1. (Step 3 and Step 4) Internalization of axonal debris and its subsequent acidification for degradation requires Rac1 and the GEFs dCed-12/Mbc/Crk and Drk/Dos.

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tion of the *draper* gene suggests a simple model for how glia might gauge their activation state in accordance with the severity of axonal injury—A more severe injury should lead to the production of more axonal debris, which in turn will activate Draper signaling more strongly, and ultimately lead to enhanced activation of engulfment gene expression (Doherty et al. 2014).

Work on *Drosophila* Draper represents a good example of how basic cellular mechanisms can be elucidated in *Drosophila* glia and will teach us about mammalian glial cell biology. Recent work has shown that DRG satellite cells also use MEGF10/Jedi signaling (the mammalian orthologs of Draper/CED-1) to engulf cell corpses during mammalian nervous system development (Wu et al. 2009), and that MEGF10 also activates an Src family signaling cascade similar to Src42a and Shark (Scheib et al. 2012). Moreover, Chung et al. (2013) recently discovered an exciting and conserved role for MEGF10 in astrocyte engulfment of synapses during neural circuit refinement in the mammalian visual system (Chung et al. 2013).

When glia are activated in the adult *Drosophila* brain, they not only act to clear axonal debris, but they also somehow modulate synaptic plasticity in local circuits. ORNs project into the antennal lobe of the brain from either the antennae or maxillary palps synapse onto highly stereotyped target projection neurons (PNs) within defined glomerular structures. Local interneurons also interconnect different glomeruli within the antennal lobe, although excitatory connections between glomeruli are normally weak. Axotomy of ORN sensory afferents from the antenna resulted in a strong potentiation of interglomerular excitatory connections, indicating that injury somehow induced plasticity of interglomerular PNs (Kazama et al. 2011). Silencing ORN activity was not sufficient to induce this plasticity, rather degeneration of the severed ORN axon terminals turned out to be essential. Blocking ORN axon degeneration by expression of the neuroprotective Wld^s molecules suppressed the plasticity of interglomerular PNs after axotomy. What is the cellular mechanism for this plas-

ticity? Blockade of endocytosis in local ensheathing glia also suppressed induction of PN plasticity after ORN axotomy, indicating that ensheathing glia somehow mediate injury-induced plasticity of excitatory PN connections in the olfactory circuit, perhaps to increase activity in remaining neurons to compensate for lost sensory input (Kazama et al. 2011). Defining the mechanisms by which ensheathing glia exert these or other effects on synaptic function will be a very important line of investigation in the future.

CORTEX GLIA

Development, Morphology, and Functions

Cortex glial cells are the most understudied of *Drosophila* CNS glial subtypes. Cortex glial cells densely infiltrate the neuronal cell cortex and by late embryonic stages appear to ensheath each neuronal cell body individually (Ito et al. 1995). Impressively, a single cortex glial cell can encase around 100 neuronal cell bodies (Awasaki et al. 2008), and together they form the “trophospongium”—the honeycomb-like structure of glial membranes that surround and presumably support neuronal cell bodies and the proximal regions of neurites as they extend toward the neuropil. Cortex glial cells associate closely with the SPGs that form the blood–brain barrier and are likely conduits for the efficient transfer of nutrients from the hemolymph to neuronal cell bodies. Gas exchange is also likely occurring through cortex glia and astrocytes as these glial cell types make significant contact with the *Drosophila* vasculature as it penetrates the CNS (Pereanu et al. 2007).

Despite their remarkable morphology, cortex glial development or function has not been intensively studied. Heartless/FGF signaling plays a critical role in cortex glial development, particularly with respect to cortex glial proliferation, and this seems to require neuronally derived FGFs (Avet-Rochex et al. 2012). But how and why these cells elaborate their morphology and associate so closely with neuronal cell bodies remains a mystery. It appears that cortex glia

are very important for maintenance of neuronal firing properties. In a screen for temperature-sensitive conditional seizure mutants, mutations in the cortex glia-specific $\text{Na}^+/\text{Ca}^{2+}$, K^+ exchanger *Zydeco* were identified that resulted in a rapid and reversible induction of seizures and hyperexcitability (Melom and Littleton 2013). Intriguingly, Ca^{2+} transients were shown to occur broadly in cortex glia, and these were eliminated in *zydeco* mutants at restrictive temperature. *zydeco* mutant phenotypes could be significantly suppressed by depletion of calmodulin, suggesting that *Zydeco* functions in part through Ca^{2+} /calmodulin-dependent signaling (Melom and Littleton 2013). Emerging evidence also supports an important role for cortex glia, in concert with SPGs, in regulating neuroblast proliferation in the larva (Dumstreit et al. 2003) in response to nutritional control (Chell and Brand 2010; Sousa-Nunes et al. 2011; Coutinho-Budd and Freeman 2013). Cortex glia likely have a rich biology in vivo and interact with neurons in many yet-to-be-identified ways. Defining additional roles for cortex glia in the *Drosophila* nervous system should teach us a great deal about how glial cells interact with neuronal cell bodies and proximal neurites and, in turn, key interactions between protoplasmic astrocytes and neuronal cell bodies in the mammalian CNS.

ADDITIONAL FUNCTIONAL ROLES FOR CNS GLIA—LOCATIONS TO BE DETERMINED

In recent years, reliable tools (e.g., Gal4 driver lines) have finally become available to study subsets of glia in vivo and assign functional properties to each glial subtype. In the past, however, most manipulations were performed on the entire glial population because of the availability of a very strong and glial-specific driver (*repo-Gal4*). Many very interesting glial functions have been described (too many to be summarized here), including the engulfment of neuronal cell corpses during embryonic development (Sonnenfeld and Jacobs 1995; Freeman et al. 2003; Kurant et al. 2008), and neurotrophic support through the generation of a neurotrophin-like family of molecules, includ-

ing *Drosophila* neurotrophin 1 (DNT1), neurotrophin 2 (DNT2), Spatzle (Spz) (Zhu et al. 2008), and dmMANF (Palgi et al. 2009), which appear to act through the TLRs Toll6 and Toll7 receptors (McIlroy et al. 2013). Future studies should be aimed not only at further defining the molecular pathways mediating these neuron–glia signaling events, but also the precise glial subtype(s) involved.

CONCLUDING REMARKS

In many ways, the collection of *Drosophila* CNS glial cells discussed here—astrocytes, ensheathing glia, and cortex glia—might be thought of as having subdivided the functional roles of mammalian protoplasmic and fibrous astrocytes. Protoplasmic astrocytes associate closely with neuronal cell bodies and neural circuits, and fibrous astrocytes primarily make contact with axons at nodes of Ranvier in white matter axon tracts. *Drosophila* cortex glia appear to provide supportive and probably other functions for neuronal cell bodies and proximal neurites in the cortex, whereas fly astrocytes associate closely with and regulate the vast majority of axons, dendrites, and synapses. How we should think of the ensheathing glial subtype in the context of mammalian glial subtypes is not completely clear. Ensheathing glia have a more flattened morphology, separate the cell cortex from the neuropil, and compartmentalize brain lobes. Ensheathing glia may be astrocyte like based on their roles in synaptic plasticity, their expression of EAATs (Rival et al. 2004), and the fact that they engulf synaptic debris through Draper/MEGF10 (MacDonald et al. 2006), similar to mammalian astrocytes (Chung et al. 2013). However, further studies will be needed to clarify these functional relationships.

Will *Drosophila* glia prove to be exactly the same as mammalian glia? This will certainly not be the case, but it does not have to be for studies of fly glia to be extremely useful to the glial field. In the last decade, it has become increasingly clear that *Drosophila* CNS glia are quite similar to mammalian astrocytes in many ways, from how they clear neurotransmitters or promote synapse formation and plasticity to the mo-

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lecular pathways they use to engulf neuronal debris. A continued rigorous analysis of glial biology in *Drosophila*, along with a direct comparisons to mammalian glia, should highlight the similarities and the differences in the cellular and molecular biology, and allow us to prioritize the study of ancient and conserved neuron–glia interactions.

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