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Glia as mediators of growth cone guidance: studies from insect nervous systems

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Abstract. Growth cones experience many different cues in their journey to their final target. They can respond to a variety of attractive and repulsive cues that can be secreted or cellular. These cues are generated by a wide range of cell types. One subset of cells that play an important role in growth cone guidance are glial cells. Glia secrete guidance cues and express cellular cues on their surface that guide axonal outgrowth. In doing so, glia can act as intermediate targets in growth cone guidance, a process that is conserved between vertebrate and invertebrate nervous systems. Recent work in grasshopper, *Drosophila* and moth nervous system development has underscored the importance of the instructive role glia play during axonal outgrowth.

Key words. Glia; intermediate targets; *Drosophila*; grasshopper; *Manduca*; growth cone guidance.

Glia and the guidance of growth cones

A series of classic experiments carried out in vertebrates led to the hypothesis that glia act as intermediate targets for growth cone guidance and neuronal migration and this role is essential for the proper formation of the vertebrate nervous system. For example, axon outgrowth in the vertebrate optic nerve and corpus callosum occurs along primitive glial pathways and these glia pathways are necessary for axon guidance [1, 2]. Neuronal migration occurs along the preformed processes of radial glia in the cerebellum [3], as well as in the developing cerebral cortex [4]. In *Xenopus*, chick, and mouse, primitive glia may form channels that direct axon growth within the spinal cord by supplying a preferred environment for axonal growth [5, 6]. A number of in vitro studies have shown that glial surfaces are a preferred substrate for neurite extension [7, 8]. Therefore, glia can provide a surface which facilitates growth cone guidance.

Glia may also play an inhibitory role in axonal guidance. In vertebrates, astrocytes express a number of molecules that act as inhibitors of growth cone guidance and thus help form barriers or boundaries that contain axonal outgrowth [9]. Astrocytes in the dorsal root entry zone and in the roof plate are thought to act as a barrier to axonal projection and express a number of proteins including chondroitin 6-sulfate proteoglycans known to be inhibitory to axonal outgrowth [10–12]. During the formation of retinocollicular projections, the radial glia that form the tectal midline are necessary to prevent the retinal axons from crossing the midline [13]. Thus some glia work to form physical barriers to block migration into inappropriate regions.

In early stages of *Drosophila* and grasshopper nervous system development, the growth cones that pioneer many axonal pathways progress towards and extend along a variety of glial cells. Some of the first experimental evidence supporting the instructive role of glia during axonal outgrowth came from work done on the developing grasshopper nervous system. During the development of this system, pioneering growth cones make extensive contacts with glial cells which act as intermediate targets on the path to the final destination of the neuron [14]. These glial cells at this stage of development are in a compact, undifferentiated state. During the development of the *Drosophila* nervous system, glia also prefigure the pathways taken by some pioneering growth cones [15]. Similar to the grasshopper studies, growth cones make extensive contacts with the glia which are in the right place to act as intermediate targets in the formation of a number of axonal tracts. These papers led to the hypothesis that as in the

vertebrate nervous system, the glia in insect nervous system, also provide instructive information to guide the migration of growth cones.

This work has been continued by a number of laboratories to study more closely whether glia do play an instructive role in growth cone guidance. Their combined effort has lead to convincing evidence that glia act as important intermediate targets for:

(1) growth cone guidance across the midline of the central nervous system (CNS);
(2) growth cone migration during the formation of the longitudinal connectives or tracts;
(3) growth cone migration as motor neurons exit the CNS and enter the periphery;
(4) growth cone migration as sensory neurons enter the CNS.

Glia as intermediate targets for growth cones crossing the midline

During embryonic development, commissural axons cross the CNS midline to form the nerve bundles or commissures. It has been shown in *Drosophila* that a cluster of glia called the midline glia are necessary for the proper formation of these axon pathways. The midline glia are contacted by the growth cones of the neurons that pioneer the anterior commissure [15]. During these early stages, the glial cells are undifferentiated, being compact and oval shaped. Later they develop the characteristic morphology of ensheathing glia and enclose and then wrap the axon tracts of the commissures [15] (fig. 1). The midline of the CNS arises from a specialized set of precursor cells, the mesectoderm. The anterior part of the segment contains the midline glia precursors which divide once each to give rise to the pairs of medial and anterior midline glia (MGM, MGA) [16]. The midline glia have been shown to play an important role in the guidance of the axons that pioneer the anterior and posterior commissures.

The formation of the midline projections and the role played by the glia in this process are discussed in greater detail in the review by Tear in this issue. Briefly, the midline glia express a number of proteins important for the proper guidance of growth cones across the midline. For example, the midline glia express the netrin guidance molecules, in the absence of which, the commissures fail to form [17, 18]. The midline glia also express another class of guidance molecules that dynamically interact to mediate either the attraction or inhibition of growth cones across the midline. Mutations in these proteins results in either the failure to extend across the midline, as with *commissureless* [19], or excessive migration across the midline due to the absence of inhibitory signals, as with *slit* and *roundabout* [20, 21].

Mutations that disrupt the formation of the midline or specifically the midline glia result in a fused commissure phenotype and a collapse of the longitudinal connectives towards the midline. This phenotype can be attributed to the removal of the inhibitory guidance cues expressed on the midline glia and to the later role of the midline glia in physically separating the commissures. For example in the mutants, *Star*, *pointed*, *spitz*, and *rhomboid*, the midline glia are specifically affected and the fused commissure phenotype correlates with these defects in the midline glia [16, 22]. In addition, mutations in the SOX domain protein Dichaete, which is necessary for midline glia differentiation, also result in the fusion of the commissures [23]. Selective ablation of the midline glia by expression of the Reaper or Grim proteins also results in a fused commissure phenotype [24, 25]. Of interest in all these experiments is that the number of commissural axons crossing the midline is not reduced. This has led to the proposal that the role of the midline glia is not to attract commissural growth cones across the midline but to act as a control point to determine which growth cones are allowed to cross [26]. In the absence of the midline glia, growth cones are still attracted to the midline [27] but either fail to cross or cross indiscriminately.

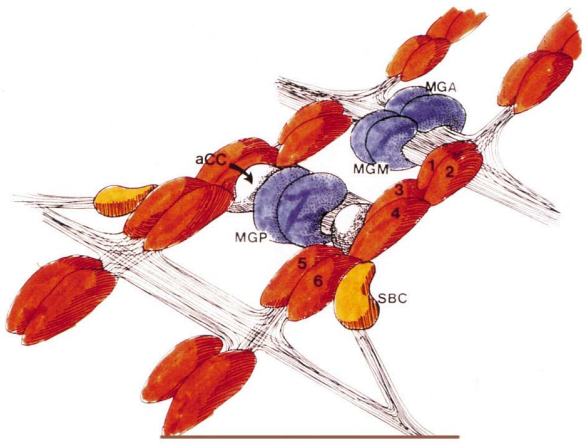

Figure 1. Some classes of glia and their position in the *Drosophila* embryonic nervous system. Six of the eight to ten longitudinal glia (orange cells, labeled 1–6) are shown positioned on the longitudinal connectives. The three pairs of midline glia—MGA, MGM, and MGP (purple cells)—are positioned on the anterior and posterior commissures. The position of one of the nerve root glia, the segment boundary cell (SBC, yellow cell) is also shown on the intersegmental nerve root. The cell body of the anterior corner cell (aCC, white cell) motor neuron is shown at the corner of the posterior commissure and the longitudinal connective [taken from ref. 15].

Glia as intermediate targets in the formation of longitudinal tracts

The longitudinal glia are a subclass of glia associated with the longitudinal tracts or connectives of the CNS (fig. 1). The longitudinal glia were first described in grasshopper and later in *Drosophila*. Using a molecular lineage marker specific for the longitudinal glia, the development of this class of glia was followed during *Drosophila* CNS development [28]. From this work it was observed that a glioblast appears in the lateral neurogenic region and divides once symmetrically to produce two glial cells. The two cells then migrate medially and arrive at a position associated with the developing longitudinal connectives. These precursors then further divide to generate between six to eight longitudinal glia cells [15]. From electron micrograph (EM) studies in grasshopper and *Drosophila*, it was observed that these glia are initially found in a primitive (immature) state spread along the pathway where the longitudinal connectives form [14, 15]. At later stages, the glia mature to wrap the axon tracts that form the longitudinal connectives (fig. 1).

There are a number of axons that pioneer the longitudinal tracts by extending processes in either an anterior or posterior direction. There are glial cells spaced in an array that act as intermediate targets for the guidance of some of these pioneering growth cones (fig. 2). During the early stages of longitudinal tract formation, the pioneer growth cones of these tracts make extensive contacts with the glial cells in their path (fig. 2E). In *Drosophila*, the growth cones of the vMP2 and pCC neurons extend in an anterior direction and eventually meet up with the growth cones of the dMP2 and MP1 axons that extend in a posterior direction [30]. As the growth cones of both groups migrate, they make extensive contacts with the LGX glial cell and the axons growing down or up from the next segment [30] (fig. 2F). Once contact is made, the axons will fasciculate to form the vMP2 and MP1 pathways. The location of the glia and their interactions with pioneer growth cones suggests that they play a role in the formation of the first longitudinal axonal pathways (fig. 2A–D). Yet in the absence of the Fasciclin II protein, this interaction with the LGX glia does not occur and the defasciculated growth cones migrate normally and in the correct directions [30]. Therefore, the interaction with the LGX glial cells is not essential but facilitates the formation of the longitudinal connectives.

Additional evidence that supports these conclusions came from observations that mutations in a number of transcription factor genes that are expressed in the longitudinal glia lead to defects in the formation of the CNS. For example, mutations in *pointed*, *prospero*, *hindsight*, *midline*, and *orthodenticle* result in abnormal longitudinal glial phenotypes with associated problems in the development of the longitudinal connectives [31, 32]. These results give an indication that longitudinal glia play an important role in determining CNS structure.

Further and more conclusive evidence to support a role for longitudinal glia in axonal guidance comes from a series of experiments that removed the longitudinal glia by genetic or physical means. The longitudinal glia are physically removed using a toxin ablation method to block protein synthesis in these cells which results in their death. This is carried out using the GAL4 system to drive the expression of the ricin toxin in the longitudinal glia [33]. Removal of these glia results in the absence of longitudinal connectives in the majority of segments in the embryonic CNS (fig. 3C–F). Due to the timing of expression of these lines, the removal of the longitudinal glia occurs at stages after the outgrowth of the first pioneering axons. These results indicate that the longitudinal glia play an important role in later axonal outgrowth [33].

The specific removal of these glia at the earliest stages of CNS development became possible with the cloning and mutagenesis of the gene *glial cells missing* (*gcm*) [34–36]. Gcm is expressed in all glial cells (with the exception of the midline glia) and removal of this protein results in the absence of the glia. Gcm appears to be essential for the determination of glial cells. In the absence of *gcm*, many presumptive glial cells take on neuronal characteristics [34–36]. Therefore, in *gcm* mutants which are characterized by the loss of most glia, the possible role of glia as intermediate targets for axonal outgrowth could be addressed. In *gcm* mutants, the formation of the longitudinal tracts is disrupted in a manner similar to the phenotypes observed with the glial ablation studies (fig. 3C). There are numerous segments with breaks in the longitudinal connectives and the anterior and posterior commissures appear to be thicker. The defects though are most dramatic at older stages of embryogenesis. In the earliest stages, there appears to be little or no misguidance of the pioneer axons in the absence of the glial cells. For example, the vMP2 and MP1 pathways are normal in 82% of hemisegments at stage 13 (fig. 4D) with only 18% being incomplete or absent [34]. By stage 16, the number of hemisegments with abnormal longitudinal connectives increases to 35%, again giving support to the hypothesis that the longitudinal glia play an important role in later longitudinal connective formation.

With the *gcm* mutation, neuronal apoptosis at later embryonic stages appears to increase [34]. This has led to the suggestion that the longitudinal glia may support neurons by providing trophic or structural support. For example, glia have previously been shown to secrete factors necessary to control neuronal differentiation

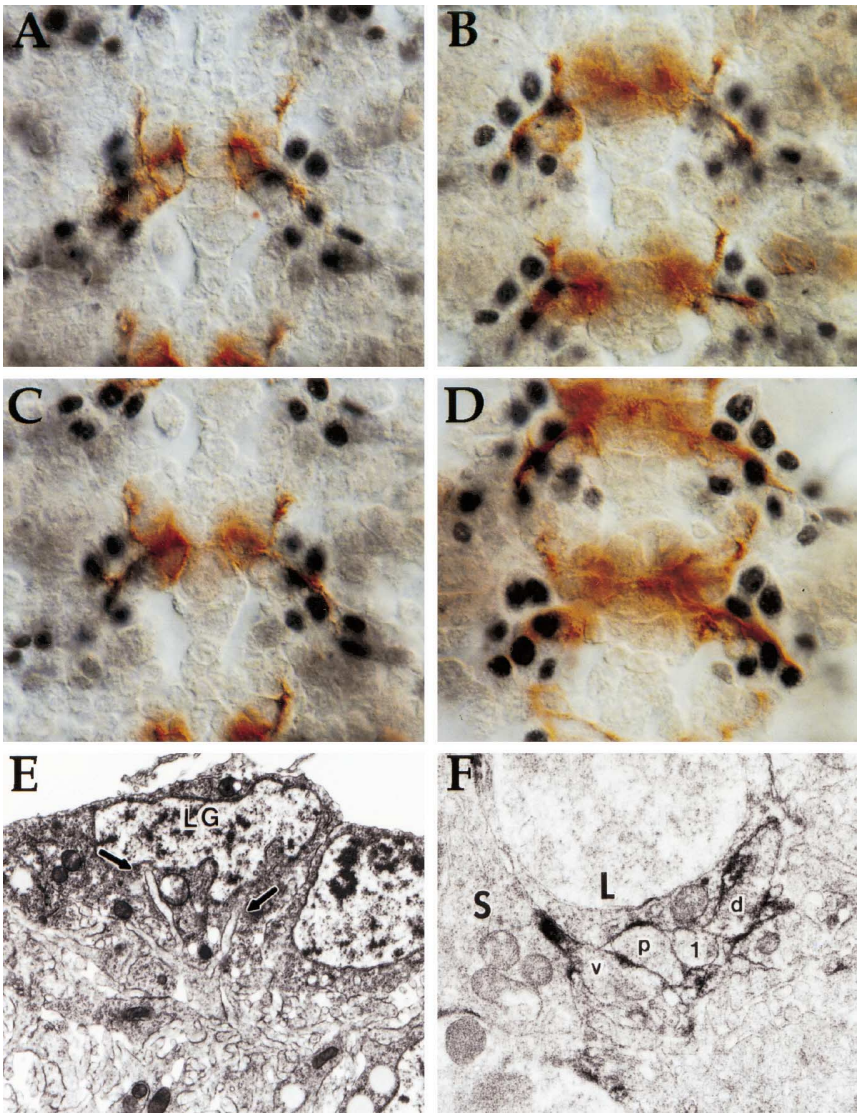

Figure 2. Longitudinal glia act as intermediate targets for axonal guidance during *Drosophila* nervous system development. Glia are present and preform some of the pathways taken by neurons that form the longitudinal connectives. (*A–D*) The position of glial cells with respect to the pioneering growth cone of the dMP2 neuron. Each panel represents a slightly older embryo during stage 12 of embryogenesis. The glial nuclei (black) are stained using the anti-repo antibody [29] and the neurons (brown) are stained using the 22C10 monoclonal antibody which at this stage labels the vMP2 and dMP2 neurons. (*A*, *B*) At the earliest stages of outgrowth, the dMP2 growth cone contacts a number of glial cells. (*C*) The dMP2 growth cone extends laterally along the cluster of glial cells before making a posterior turn. (*D*) The vMP2 and pCC (not shown) growth cones do not contact glial cells as they extend in an anterior direction prior to meeting the descending dMP2 and MP1 at the LGX glial cell. (*E*) An EM showing the interaction between the growth cone and longitudinal glial cell. The arrows indicate two filopodial insertions into the overlying glial cells [taken from ref. 15]. (*F*) An EM showing the interactions between the neurons that form the longitudinal connectives and the LGX glial cell. vMP2 (v), pCC (p), MP1 (1) and dMP2 (d) make extensive contact with this cell and each other [taken from ref. 30].

[38]. In the adult brain, defective glia increase neurodegeneration in a number of mutants [39]. Glia also play a role in the removal of apoptotic neurons from the embryonic nervous system in a manner similar to vertebrate microglia [40]. Therefore, in the absence of glia, neurons may degenerate and persist in the CNS.

The combined evidence from EM studies, molecular biology, and genetics indicates that the longitudinal glia are important for guidance during the development of the longitudinal connectives of the CNS. But rather than playing an essential role in growth cone guidance, these glia appear to facilitate guidance such that in the *Drosophila* embryo, the longitudinal connectives can form but are subject to more errors in the absence of

The longitudinal glia play an important function in later embryonic development in a supportive rather than an instructive role.

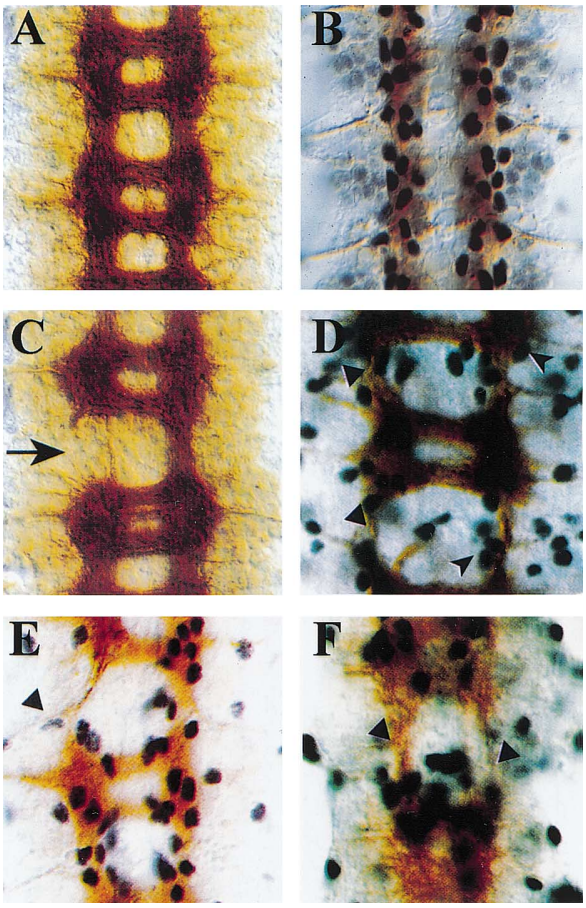

Figure 3. Ablation of longitudinal glia results in defects in longitudinal tract formation during *Drosophila* nervous system development. Removal of the longitudinal glia by either physical or genetic means results in breaks in the longitudinal connectives but these phenotypes are more apparent at later stages of CNS development. In all panels, the axons of the CNS (brown) are stained with the monoclonal antibody bp102 and the nuclei of the glia (black) are stained black using the anti-repo antibody [29]. (*A*) The wild-type pattern of axonal tracts in the CNS of a late *Drosophila* embryo. The neurons show the stereotypic pattern of two longitudinal connectives and the segmentally repeated anterior and posterior commissures [taken from ref. 34]. (*B*) The wild-type pattern of the nuclei of the longitudinal glia (or interface glia) overlying the CNS of a late-stage embryo. (*C*) The *glial cells missing* mutant has numerous breaks in the longitudinal connectives in a late-stage embryo [taken from ref. 34]. (*D–F*) Toxin ablation of the longitudinal glia also results in defects in the formation of the longitudinal connectives. All three longitudinal glia GAL4 lines (*D* MZ1131, E 12M, *F* C321c) have defects in the formation of the longitudinal connectives when used to drive expression of the ricin toxin. These defects are again more apparent later in CNS development, most likely due to the late onset of expression of all three longitudinal glial lines. Arrowheads point to the longitudinal glia and the triangles point to the longitudinal connectives [taken from ref. 33].

Glia as intermediate targets in the formation of peripheral nerves

In both grasshopper and *Drosophila*, glia cells act as intermediate targets at specific points along the pathway taken by the motor neurons that form the major nerves of the peripheral nervous system (PNS), the intersegmental and segmental nerves (fig. 1). Primitive glial cells, one of which is called the segmental boundary cell (SBC), are positioned at the point where the nerve root of the peripheral intersegmental nerve leaves the longitudinal connective in both *Drosophila* and grasshoppers (fig. 4). The growth cones that pioneer the intersegmental nerve display a selective affinity for the segmental boundary cell as they turn laterally within the CNS and travel along the surface of the SBC [14, 15]. These growth cones then extend distally over other glial cells before they exit the CNS (fig. 4C). At these early stages, the glial cells have a flattened, undifferentiated morphology. Later they will differentiate into mature glial cells and enwrap the axons of the intersegmental nerve root.

One of the first demonstrations that glial cells can act as intermediate targets for growth cone guidance came from cell ablation of the SBC in grasshopper embryos [14]. Deletion of the SBC causes misrouting of the growth cones that pioneer the intersegmental nerve root (fig. 4E) [14]. In the absence of the SBC, the growth cones of the pioneering motor neurons (the Us and aCC) fail to turn laterally at the segment boundary and continue migrating within the CNS in a posterior direction along the longitudinal connective. Therefore, the SBC is an important intermediate target guiding the direction of outgrowth of these pioneering growth cones. This interaction is highly selective in that the growth cones of other axons contact these same glial cells and yet do not change their path of migration [14]. In *Drosophila*, the SBC cell is also contacted by the motor neurons that pioneer the intersegmental nerve, but in this case it is the growth cone of the aCC motor neuron that first contacts the SBC (fig. 4A, B). Removal of the glial cells in the mutant *gcm* has only a minor effect on the outgrowth of aCC which appears to be normal in the majority of segments (fig. 4D). Therefore in *Drosophila*, it appears that aCC and the other motor neurons are able to find their correct pathways to exit the CNS in the absence of the SBC and its neighbors. As before, rather than being essential, the glia appear to facilitate axonal guidance such that there are errors in pathfinding in their absence but not complete disruption.

The intersegmental and segmental nerves are the major nerves of the PNS in *Drosophila*. These nerves are formed by the motor neurons as they extend to their muscle targets and are expanded later with the addition

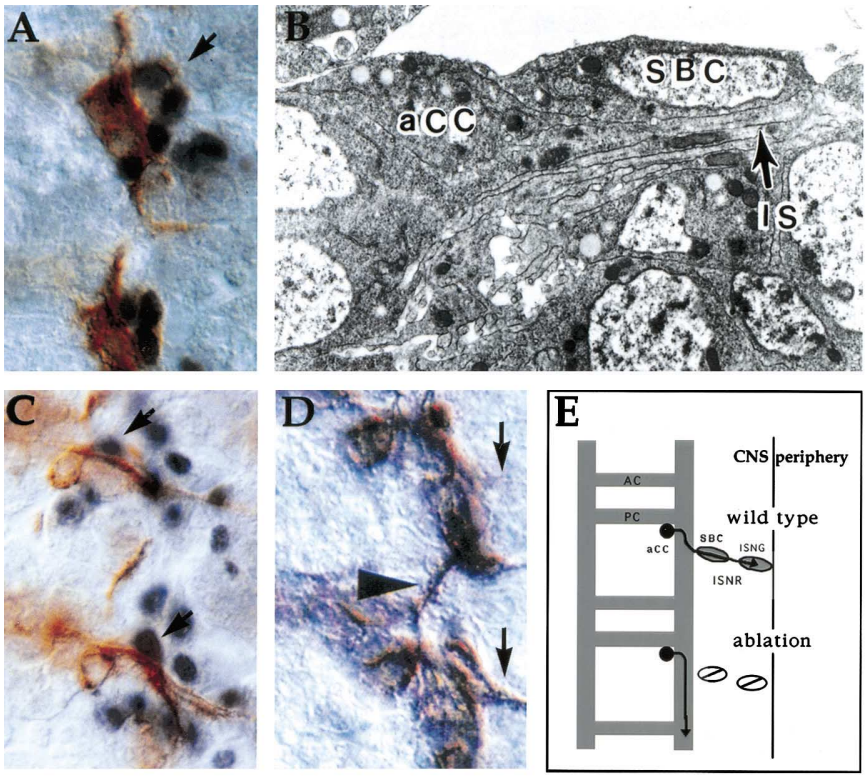

Figure 4. The segment boundary cell (SBC) can act as an intermediate target during motor neuron outgrowth. (A) In *Drosophila*, the initial extension of aCC is lateral (stained brown with the anti-Fasciclin II antibody) and contacts the labeled glial cells in the region (stained black using the anti-repo antibody). The arrow indicates the point of contact between the growth cone and the glial cells. The sister cell pCC ignores the neighboring glia and extends in an anterior direction. In grasshopper, a similar process occurs but it is the growth cones of the U motor neurons that are the first to contact the SBC. (*B*) EM showing that the neurons that form the intersegmental nerve root (IS) including aCC (arrow) make extensive contacts with the SBC glial cell in *Drosophila* [taken from ref. 15]. (*C*) The intersegmental axons appear to use a number of glial cells as guidance cues as they extend laterally during *Drosophila* CNS formation. The arrows indicate the position of the first glial cell that these neurons contact which are followed by a series of other glia as the growth cone extends to the periphery. This is similar to what is seen in grasshopper where the U pioneer axons use a number of glial cells including the SBC as intermediate targets. (*D*) The presence of the glial cells is not essential for the correct formation of the intersegmental nerve in *Drosophila*. The removal of the glia in the mutant *glial cells missing*, *gcm*, does not disrupt the formation of the majority of the intersegmental (lower arrow) or segmental nerves. There is the occasional example of an absent or abnormal nerve (upper arrow). The extension of the pCC growth cone is also not disrupted (arrowhead) [taken from ref. 34]. (*E*) Ablation of the SBC in the grasshopper CNS results in the misguidance of the aCC. When the SBC is removed by laser ablation before the arrival of the U growth cones, the aCC motor neuron fails to turn and continues in a posterior direction [taken from ref. 37].

of the incoming sensory nerves. Associated with these neurons is a class of glia called the peripheral glia which will later provide the glial sheath that enwraps the axons of the peripheral nerves and is responsible for the formation of the peripheral blood nerve barrier [41]. In the *Drosophila* embryo, the two major nerve roots, the intersegmental and segmental nerves, come together just outside the CNS at a point where four glial cells are located. This region has also been termed the exit junction and the presence of these glia at this transition between the CNS and the periphery has led to the speculation that these glia may play a role in the formation of the peripheral nerves.

There are two lines of evidence to support this idea. Removal of exit glia in the mutant *gcm* increases the number of defects in the peripheral nerves. While the normal number of neurons appear to exit the CNS, they often exit at aberrant positions and can have abnormal morphologies [35]. The intersegmental and segmental nerves in the *gcm* mutant consistently fail to contact at the exit junction and enter the periphery independently [34–36]. This provides evidence that the glial cells at the boundary of the CNS are essential for the close association of the intersegmental and segmental nerves. These glia may also facilitate the correct migration of the peripheral neurons out of the CNS in the periphery but are not essential for this process, as the majority of neurons exit in the correct position.

The second line of evidence comes from work with enhancer trap lines that label these glia. A series of enhancer trap lines inserted into the same gene are expressed early during nervous system development in glia that mark the boundary between the CNS and PNS [42]. The growth cone of the aCC motor neuron appears to head towards and contact these glia as it exits the CNS (fig. 5A, B). Exit glia will later differentiate to enwrap the intersegmental and segmental nerve bundles at the boundary of the PNS and CNS. Removal of these glia in the *gcm* mutant results in pathfinding errors at the exit junction and in the initial formation of the peripheral nerves (fig. 5D, E). These errors are not absolute, as the neurons can recover such that by the stages of embryogenesis in the *gcm* mutant, the motor neuron pattern has corrected to some extent [34–36]. The conclusive test of the role played by glia at the exit point as intermediate targets will come from cell-specific ablation of these glia. This will test the function of these glial cells specifically, by not removing all glia wholesale.

Glia as intermediate targets for sensory neuron projections

Glia have been shown to play an important role during the development of a number of sensory projections in insects. During the development of the *Drosophila* visual system, the axons of the photoreceptor cells will project into the brain lobe to make connections with either the first or second optic ganglia (lamina or medulla). It is thought that guidance of the retinal axons is mediated by local guidance cues. The retinal axons arrive at their target prior to the generation of the lamina neurons (which require retinal input for differentiation) and appear to contact the lamina glia that are found in the target region [43, 44]. This suggests a role for lamina glia as intermediate targets during the formation of the retinal projections. The immature glial cells may function as a transient target for these axons in a manner analogous to the subplate neurons of the developing vertebrate cortex [45].

Glia are known to be important for the formation of the antennal lobe in insects. Sensory axons leave the antenna (the major olfactory organ of the insect) and project to the antennal lobe of the brain. The lobe is made up glomeruli which contain the synaptic connections between the arbors of the sensory and antennal lobe neurons. The role of glia in the development of the antennal lobe of the moth, *Manduca sexta*, has been extensively studied [46, 47]. The arrival of the sensory axons into the antennal lobe triggers a series of changes in the shape and position of the antennal glia. These glial cells proliferate, migrate, and finally extend pro-

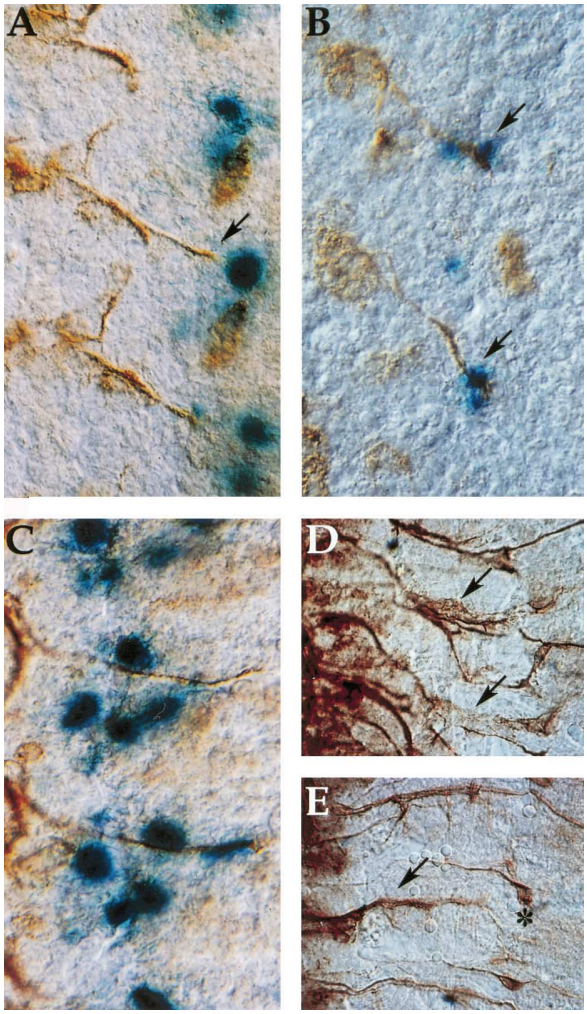

Figure 5. The exit glia can act as intermediate targets during peripheral nerve formation. The exit glia are specifically labeled by a series of enhancer traps inserted into the same gene. These enhancer traps drive the expression of the *E. coli* lacZ which can be detected in the presence of Xgal (blue cells). (*A*) The lacZ expression labels a series of glial cells (blue) found at the boundary of the CNS and periphery before the outgrowth of the aCC motorneuron growth cone (brown). At this stage (stage 12/1) the aCC growth cone, as it exits the CNS, heads directly for the labeled glial cell. (*B*) The aCC growth cone makes contact with the glial cells. At a slightly older stage (early stage 13) the aCC growth cone has now contacted the labeled glial cells. The cell has divided to produce two daughter cells. (*C*) The aCC growth cone has now extended beyond the glial cells into the periphery. At this stage (stage early/mid 13) the glial cells have divided again for a total of four. For panels *A–C* embryos were staged, fixed, and stained using a combination of Xgal (blue) to detect lacZ expression and monoclonal antibodies 22C10 or 1D4 (brown) to detect neurons. (*D–E*) In the *glial cells missing* mutant some of the peripheral nerve roots have defects. Removal of the glia results in the defasciculation of the major peripheral nerves (arrows). The absence of glia also results in a higher incidence of abnormal path-finding of the motorneurons. For instance in *E*, the intersegmental nerve (ISN) is absent (asterisk). The motorneurons (brown) are stained using the anti-Fasciclin II antibody 1D4 in a $gcm^{\Delta P1}$ background.

cesses to create a scaffolding within which the antennal glomeruli will form. If sensory axons are blocked from entering the lobe, the glial cells still proliferate but do not generate this scaffolding which blocks the formation of the glomeruli. When glial cell numbers are significantly reduced, even in the presence of the inducing sensory axons, the antennal lobe fails to develop glomeruli [48, 49]. Analysis of the development of the antennal lobe of the bee, *Apis mellifera*, confirms the results obtained from the moth studies [50]. These results suggest that antennal glial cells are necessary for the formation of the olfactory glomeruli in the antennal lobes of insects. These glia are proposed to act as intermediary targets by passing the incoming signal from the sensory axons to their target neurons in the antennal lobe.

Summary

Through the analysis of glial function in a variety of insects there is strong evidence to support a role for glial cells in the formation of the nervous system. Glia can act as intermediate targets in the formation of a number of neuronal pathways such as the commissures, the longitudinal tracts, and the peripheral nerves. The glia are present prior to the extension of the pioneering growth cones and these growth cones make contact with the glia en route to their final targets. The role of these glia, though, appears to be facilitatory rather than essential. In the absence of all glia during *Drosophila* embryogenesis, many of the neuronal pathways form correctly. The glia could be providing preferred substrates that help guide axons correctly, the absence of which increases the number of errors but does not totally disrupt guidance. Perhaps the glia are working to physically separate axonal tracts to regulate axonal fasciculation/adhesion by providing a physical barrier to interactions. The glia could provide inhibitory boundaries such that in their absence, axons will migrate into inappropriate regions. The glia could also provide 'trophic' support to the developing neurons, such that in their absence the neurons fail to differentiate or even survive. Resolution of these possibilities lies in determining the molecular nature of the interactions that occur between the glia and the growth cone during the development of the nervous system in insects and in vertebrates.

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