Published in final edited form as:
Glia. 2011 September ; 59(9): 1237–1252. doi:10.1002/glia.21162.

Structure and Development of Glia in *Drosophila*

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Abstract

Insect glia represents a conspicuous and diverse population of cells and plays a role in controlling neuronal progenitor proliferation, axonal growth, neuronal differentiation and maintenance, and neuronal function. Genetic studies in *Drosophila* have elucidated many aspects of glial structure, function and development. Just as in vertebrates, it appears as if different classes of glial cells are specialized for different functions. Based on topology and cell shape, glial cells of the central nervous system fall into three classes (Fig. 1A–C): (i) surface glia that extend sheath-like processes to wrap around the entire brain; (ii) cortex glia (also called cell body-associated glia) that encapsulate neuronal somata and neuroblasts which form the outer layer (cortex) of the central nervous system; (iii) neuropile glia that are located at the interface between the cortex and the neuropile, the central domain of the nervous system formed by the highly branched neuronal processes and their synaptic contacts. Surface glia is further subdivided into an outer, perineurial layer, and an inner, subperineurial layer. Likewise, neuropile glia comprises a class of cells that remain at the surface of the neuropile (ensheathing glia), and a second class that forms profuse lamellar processes around nerve fibers within the neuropile (astrocyte-like or reticular glia). Glia also surrounds the peripheral nerves and sensory organs; here, one also recognizes perineurial and subperineurial glia, and a third type called “wrapping glia” that most likely corresponds to the ensheathing glia of the central nervous system. Much more experimental work is needed to determine how fundamental these differences between classes of glial cells are, or how and when during development they are specified. To aid in this work the following review will briefly summarize our knowledge of the classes of glial cells encountered in the *Drosophila* nervous system, and then survey their development from the embryo to adult.

Glial Morphology in the Adult and Larval Brain

Glia at the brain surface: perineurial and subperineurial cells

The surface of the adult and late larval CNS is surrounded by two layers of glial cells, an outer layer of perineurial glia, and an inner layer of subperineurial glia. Cells of the subperineurial layer, which form extensive pleated septate junctions among themselves, function as an effective blood-brain barrier which excludes molecules of a size over from entering the CNS (Lane and Swales, 1979; Saint Marie et al., 1984; Hoyle, 1986; Cantera, 1993; Juang and Carlson, 1994; Tepass and Hartenstein, 1994; Baumgartner et al., 1996; Pereanu et al., 2005; Bainton et al., 2005; Schwabe et al., 2005; Stork et al., 2008; Fig. 2B′). Aside from septate junctions, spot adherens junctions (called spot desmosomes in the older literature; e.g., Lane, 1981; 1982), as well as gap junctions form contacts between subperineurial cells and between these cells and the underlying cortex glia lamella (Pereanu et al., 2005). Cell bodies and nuclei of subperineurial glial cells are large and flattened. Cell bodies measure more than 20μm in the tangential plane, and less than 0.3μm across (Stork et al., 2008; Awasaki et al., 2008; Fig. 2H–K). Given their size, subperineurial glia cells are very low in number. One early larval brain hemisphere contains less than 20 subperineurial cells, and this number does not increase significantly towards later larval stages (Pereanu et

al., 2005; Stork et al., 2008). Less than 50 cells surround one adult brain hemisphere (VH, unpublished).

Perineurial glia is located outside the subperineurial sheath. Perineurial cells are smaller than subperineurial cells and have an elongated or multilobulated shape, which can be best appreciated when looking at clonally labeled cells (Stork et al., 2008; Awasaki et al., 2008; Fig. 2D–G). Perineurial cells are more numerous than subperineurial cells, counting in the hundreds for the adult and late larval brain (Awasaki et al., 2008; Stork et al., 2008). The cell bodies of perineurial glia, though generally flattened, do not form a tight layer, but rather a cellular mesh, with cells sending out complex filopodial and lamellar processes.

The terms perineurial and subperineurial glia have been used inconsistently in the past, which causes problems when trying to review the literature and synthesize the findings in one cohesive picture. In many classical papers on insect glia, septate junctions are assigned to the perineurium (perineurial glia), which indicates that the authors of these papers used the term “perineurial” for what is called “subperineurial” in the recent *Drosophila* literature. To illustrate the point, panel A of Fig. 3 shows an image from the review of Carlson et al. (2000), depicting a late larval *Drosophila* brain hemisphere labeled with anti-Neurexin (Nrx) to visualize septate junctions. The cells outlined by Nrx expression (arrow) are called perineurial glia. Panel B of Fig. 3 (from Stork et al., 2008) shows a similar view of a larval brain with a Neurexin-Gal4 construct driving GFP in what evidently is the same cell type as the one shown in panel A. Here, in accordance with most of the recent papers focusing on elements of the *Drosophila* blood brain barrier, the labeled cell type is referred to as subperineurial glia. The argument for the changed usage of the term lies in the fact that the septate junction-possessing cells do not form the outermost layer, but are located underneath an external layer formed by a different cell type. This cell type, which is not explicitely discussed by Carlson et al. (2000) or other reviews of the classical literature, is now called perineurial glia (Stork et al., 2008; Awasaki et al., 2008). The function of the perineurial cells remains unknown to this date. It is well possible that they contribute to the blood-brain-barrier function. Thus, recent experimental data (Stork et al., 2008) demonstrate that molecules of 80kDa and above enter a larval brain that lacks all surface glia due to a mutation in *gcm* within seconds, whereas it takes about 20min to penetrate into brains that merely lack septate junctions. This indicates that cell contacts other than those mediated by septate junctions contribute to the barrier.

The confusion of perineurial and subperineurial glia is probably caused in part by the gradual maturation of surface glia as development proceeds. In the late embryo and early larva, perineurial glial cells are rare and confined to more medial positions, whilst most of the brain surface is covered only by the layer of subperineurial glia with septate junctions (Juang and Carlson, 1994; Tepass and Hartenstein, 1994; Ito et al., 1994; Pereanu et al., 2005; see Fig. 2C). Perineurial glial cells appear to proliferate profusely during the early larval period (Pereanu et al., 2005), as discussed in more detail below. It is not before the mid third instar that this cell population has reached a density that would justify to call it a cell layer.

Cortex glia

The cell bodies of differentiated neurons, which form the outer layer (cortex or rind) of the brain, are individually wrapped by the processes of cortex glial cells [Hoyle, 1986; called satellite glia in *Drosophila* optic lobe (Edwards and Meinertzhagen, 2009); Fig. 4A, B]. The adult and larval brain contain approximately 150 cortex glial cells per hemisphere (Pereanu et al., 2005; VH unpublished). Cortex glial cells have a small, rounded cell body and nucleus which is intermingled with neuronal cell bodies (Pereanu et al., 2005; Awasaki et al., 2008). From the cell body emanate multiple lamelliform processes that intercalate in between

neurons (Fig. 4B, E). Processes are mostly extremely thin (less than 0.1μm) and electron dense (Fig. 4C). Septate and adherens junctions interconnect cortex glial cells and neurons (Fig. 4D).

Lamellae of cortex glia surround the outer brain surface underneath the subperineurial glia (Fig. 4E), and are also found around part of the neuropile. Thus, at least in the early larva, the sheath covering the surface of the lateral brain neuropile is formed by cortex glia, rather than specialized neuropile glia (Pereanu et al., 2005). During the larval stage when neuroblasts proliferate to form adult secondary neurons, cortex glial cells form chambers that contain neuroblasts, ganglion mother cells and newly generated neurons (arrowhead in Fig. 4E). Thus, neuroblasts, which are located at the brain surface, are ensheathed by cortex glia, not surface (i.e., subperineurial) glia. Also the bundles of axons that are formed by newly generated secondary neurons and have to cross the cortex before entering the neuropile, are wrapped by cortex glia (Pereanu et al., 2005; Spindler et al., 2010).

A number of genetic studies attest to the important role of cortex glia in the maintenance of neurons, as well as in proper layering of neuronal cell bodies in the developing brain. Adult flies carrying mutations in the *drop dead* (*drd*) gene suffer from neuronal degeneration which sets in several days after eclosion. This phenotype is associated with a lack of cortex glial processes around neuronal somata (Buchanan and Benzer, 1993). Disruption of DEcad function in larval cortex glia also results in abnormal glial cell shape, followed by abnormal positioning of neurons within the cortex (Dumstrei et al., 2003).

Glia in and around the neuropile

Neuropile glia surrounds the neuropile; the flattened cell bodies and nuclei of this type of cell are located along the interface between cortex and neuropile, as well as in between neuropile compartments. Neuropile glia is large in cell number, with approximately 200 cells in one adult brain hemisphere, and 90–100 cells in a late larval hemisphere (Pereanu et al., 2005). Cell bodies are clustered around the neuropile surface and are heavily concentrated around the compartments of the central complex (Fig. 5A). Neuropile glia is also the most diverse class of glia. Thus, different neuropile compartments of the brain and ventral nerve cord are populated by subclasses of neuropile glia that differ in molecular characteristics, morphology, and function. This is particularly true for the populations of neuropile glia associated with specialized nerve tracts, such as the midline glia (commissures of the ventral nerve cord; Jacobs and Goodman, 1989), the interhemispheric glia (brain commissures and developing central complex; Simon et al., 1998), and chiasm glia (chiasms of the optic lobe; see below; for review see Edwards and Meinertzhagen, 2009). In the classical literature, focusing on insects other than *Drosophila* (e.g., Hoyle, 1986; Cantera, 1993), numerous different types of neuropile glia, specifically related to certain neuronal cell types such as motor neurons or interneurons, have been described. This diversity (which requires much more study to be fully appreciated) not withstanding, two main types of neuropile glia that differ fundamentally in their structure and expression of molecular markers can be distinguished in *Drosophila*. One class, called ensheathing glia in the recent literature (Awasaki et al., 2008; Doherty et al., 2009; Fig. 5A–C, G), has flattened cell bodies and lacks processes that penetrate deep into the neuropile compartments (a possible exception are the glial processes surrounding the brain tracheae; these processes are mostly formed by ensheathing glia; Pereanu et al., 2006). The second type of neuropile glia sends out widely branched, filiform or lamelliform processes that pervade the neuropile, forming a network (reticulum) of glial tissue that surrounds terminal axons, dendrites and synapses (Pereanu et al., 2005; Awasaki et al., 2008; Spindler et al., 2009; Doherty et al., 2009; Fig. 5D–G). The term “astrocyte-like glia” was recently proposed for this cell type (Awasaki et al., 2008). It may be advisable to avoid terms that suggest potentially

unwarranted homologies with vertebrate cell types (see below); we suggest a more descriptive term such as “reticular glia”.

Aside from the blood-brain barrier function of the subperineurial glia, that has received a lot of attention in the recent literature, the diverse roles of neuropile glia have been the topic of numerous genetic studies in *Drosophila*. Neuropile glia are in contact with neuronal processes in the developing and mature brain, which puts them in a position to act as a guidance mechanism for axons (Jacobs, 2000; Parker and Auld, 2004; Hidalgo et al., 2006; Chotard and Salecker, 2007; Fung et al., 2009; Spindler et al., 2009). Neuropile glia also are centrally involved in neuronal apoptosis (Sonnenfeld and Jacobs, 1995; Ziegenfuss et al., 2008; Kurant et al., 2008; Doherty et al., 2009), and in the removal/reorganization of neuronal processes during metamorphosis (Mac Donald et al., 2006; Awasaki et al., 2006). In the mature brain, neuropile glia play an important role for synaptic transmission, by taking up transmitters from the synaptic cleft (Grosjean et al., 2008; Jackson and Haydon, 2008).

Glia around the peripheral nerves

The glial layers that surround the peripheral nerves of the late larva and adult fly bear a high degree of resemblance to their counterparts, described above, in the CNS. In the late larva, one can distinguish perineurial glia and subperineurial glia, the latter forming the septate junction seal (Banerjee et al., 2006; Stork et al., 2008; Blauth et al., 2010). As in the CNS, the subperineurial layer is responsible for the blood brain barrier. Furthermore, similar to the central brain, perineurial glia forms a complete layer only in late larval stages (Fig. 6B), whereas, early, cell bodies are attached to the surface of the nerve without actually ensheathing it (Stork et al., 2008; Fig. 6A). Axons form bundles in the center of the nerve that are surrounded by a third type of glia termed “wrapping glia” by Stork et al. (2008). Given its direct contact with axons, wrapping glia should be considered as a subtype of neuropile glia. Just like perineurial glia at the surface of the peripheral nerves or the CNS, wrapping glia also undergoes a pronounced maturation process during larval development. In the early larva (Fig. 6A), wrapping glia has not yet extended many processes; in the late larva, glial processes are abundant and enwrap most axons individually (Fig. 6B). All three types of peripheral glial cells are large and low in number. Thus, a segmental nerve of the larval ventral nerve cord is accompanied by only 12 peripheral glial cells (von Hilchen et al., 2008). Numbers for adult nerves have not been established so far, but will most likely not exceed those in the larva.

The comparison of glial organization at different stages of larval development supports two conclusions. One is that the outer layer of surface glia, i.e., the perineurial glia, is not vital for neural function, since the early larva is able to live without it. The second is that studies of glial structure, and subsequent speculations regarding glial function, can be misleading if they focus on a single developmental stage.

Specification and early migration of glia in the *Drosophila* embryo

Glial cells are derived from a relatively small set of uniquely identifiable glioblasts and neuro-glioblasts that delaminate from the neurectoderm of the early embryo. Glioblasts are defined as progenitors that produce only glia, whereas neuro-glioblasts have both neurons and glia among their progeny (Jacobs et al., 1989; Nambu et al., 1990; Klämbt and Goodman, 1991; Klämbt et al., 1991; Udolph et al., 1993; Beckervordersandforth et al., 2008). Glial progeny can be recognized at an early stage by the expression of the genes *gmc* and *repo*, which represent the key molecular factors of glial fate and differentiation (Campbell et al., 1994; Xiong et al., 1995; Halter et al., 1995; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Jones, 2005). Embryonic glioblasts and neuro-glioblasts

divide and produce the populations of glial cells that differentiate in the late embryo, forming processes around the surface, cell bodies, and neuropile of the larval brain (Fig. 7A). During the larval period, most, if not all, of the subtypes of glia divide to increase their number (Awad and Truman, 1997; Pereanu et al., 2005; Colonques et al., 2007; Awasaki et al., 2008). In addition, a few neuro-glioblasts become active and add substantially to the number of neuropile glia (Izergina et al., 2009; Fig. 7A). Proliferation continues until the early pupal stage. Even in the adult, glial cells keep dividing at a low rate; brain injury can stimulate glial proliferation (Kato et al., 2009).

Glial progenitors of the embryonic ventral nerve cord

Each thoracic and abdominal neuromere of the ventral nerve cord possesses 30 left-right symmetrical pairs of glial cells. Surface glia of each thoracic/abdominal segment is comprised of 11 pairs, cortex glia has 4 pairs, and neuropile glia 15 pairs (Beckervordersandforth et al., 2008). Given that these glial cells form a fixed pattern that can be recognized in every segment of every embryo, the nomenclature for theses cells was further refined; for example, surface glia includes two medial pairs of cells, called channel glia; neuropile glia includes the longitudinal glia which encloses the longitudinal connectives, midline glia around the commissures, and nerve glia around the roots of the two nerves that enter each segment (Beckervordersandforth et al., 2008; Fig. 7B–E). There are minor differences between the abdominal and thoracic segments which will not be further considered here. The three gnathal segments, forming the anterior tip of the embryonic and larval ventral nerve cord, have glial patterns that also resemble the thoracic/abdominal pattern (Hartenstein et al., 1998), but have not been investigated in detail thus far.

With the help of specific molecular markers, glial cells of the ventral nerve cord could be followed back to their individual progenitors (Ragone et al., 2003; Beckervordersandforth et al., 2008). Glial cells of each thoracic/abdominal segment are derived from 10 pairs of progenitors, including four glioblasts and six neuro-glioblasts. Neuro-glioblasts show two different modes of divisions in which neuronal and glial progeny are separated (Udolph et al., 2001). According to the first mode, the neuro-glioblast performs an initial division whose two daughter cells then continue as a pure neuroblast and glioblast, respectively. These type I neuro-glioblasts (e.g., Nb6-4), express the glial fate determinant, *gcm*, at an early stage. In the second mode, neurons and glial cells derive as siblings from the division of ganglion mother cells. Here, the neuro-glioblast does not yet express *gcm*; instead, the gene appears later in the ganglion mother cells producing glial cells.

Glioblasts and neuro-glioblasts are arranged around the borders of each neuromere (Ragone et al., 2003; Fig. 7B). Five cells, most of them type II neuro-glioblasts (Nb1-1, Nb2-2, Nb5-6, Nb1-3, and Nb7-4), generate surface glia; they are located near the boundary in between two adjacent segments. Correspondingly, at the time when surface glia can be first recognized, it forms a crescent-shaped array at the inter-neuromeric boundary (Hartenstein et al., 1998). From there it spreads around the surface of the neuromere (Fig. 7G, H).

Most neuropile glia, namely longitudinal glia (9 pairs of cells) and midline glia (3 pairs of cells), are generated by dedicated glioblasts, the lateral glioblast (LGB; Jacobs et al., 1989) and three midline progenitors, respectively (Jacobs and Goodman, 1989; Bossing and Technau, 1994). As discussed above, the LGB expresses *gcm* at an early stage; paradoxically, midline progenitors do not express *gcm*, or its downstream target, *repo*, at any stage. The LGB migrates medially over the basal (dorsal) surface of the neural primordium (Fig. 7G, H) and, during this time, performs several rounds of divisions giving rise to the longitudinal glial cells. In the mid stage embryo (stage 13, after gernband retraction), these cells form elongated clusters which dorsally cover the pioneer neurons of the connectives, which consist of longitudinal tracts of nerve cell fibers (Jacobs et al., 1989). The

longitudinal glial clusters of either side are connected by the three pairs of midline glial cells which are associated with the commissural axon tracts. In the late embryo, longitudinal and midline glia form processes that grow around the connectives and commissures (Fig. 7G, H).

The neuropile glia wrapping the nerve roots (segmental and intersegmental nerve glia; Beckervordersandforth et al., 2008), as well as part of the peripheral glia, is derived from Nb1-3 (Fig. 7B, F). This cell is the neighbor of LGB, but unlike LGB, Nb1-3 and its progeny remain laterally; the cells destined to become peripheral glia even migrate laterally, following the axons that pioneer the segmental nerve.

Cortex glia is formed by the neuro-glioblast Nb6-4 (Fig. 7B), which expresses Gcm throughout its cytoplasm (Freeman and Doe, 2001). After the first division of Nb6-4, Gcm becomes restricted to one of the daughter cells, which from then onward only produces (cortex) glial cells (Akiyama-Oda et al., 1999; Bernardoni et al., 1999). During early stages, cortex glial cells form part of the “crescent” of surface glial precursors. In the late embryo, differentiation of the cortex glia follows a very different pathway than that of surface glia. Thus, nuclei and cell bodies of cortex glia remain in the center of the cortex (Fig. 7G, H), and extend a radial process towards the neuropile, as well as in the opposite direction towards the surface. The characteristic “bipolar” phenotype of developing cortex glia, not unlike the structure of radial glial cells in the vertebrate neural tube, has been documented for the embryonic brain (Younossi-Hartenstein et al., 2006). Following the bipolar stage, lamellar process extend in all directions, intercalating in between and then wrapping around the neuronal somata.

The last class of glia, peripheral glia, is derived in large part from the same neuroblasts that also form CNS glia. Thus, Nb1-3, which generates the neuropile glia surrounding the nerve roots, also contributes four cells to the peripheral nerve itself; Nb5-6 forms one peripheral glial cell. Nb2-5, which does not contribute to CNS glial cells, produces two peripheral glial cells (von Hilchen et al., 2008). The remainder of peripheral glial cells are the descendants of sensory organ precursors (SOPs), another set of uniquely identifiable neural progenitors located in the lateral ectoderm (Hartenstein, 1988; Bodmer et al., 1989; Younossi-Hartenstein and Hartenstein, 1997). SOPs, produce several “bona fide” glial cells that become associated with the sensory nerve formed by the corresponding sensory organ (sensillum). In addition, SOPs form sensory neurons and support cells of the sensilla. The support cells form specialized sheaths around dendrites and cell bodies of sensory neurons, and produce the cuticular apparatus involved in stimulus transduction (e.g., the hair or bristle of external sensilla). Support cells are sometimes referred to as glia in the literature; however, it is unclear to what extent biochemical and functional characteristics of glial cells and sensillum support cells are shared.

One important question that has so far not been answered is whether or not the two subclasses of surface glia (perineurial and subperineurial) and of neuropile glia (ensheathing and reticular) have separate pools of embryonic progenitors. In case of the neuropile glia, both the reticular and ensheathing variety can be distinguished in the freshly hatched larva (Pereanu et al., 2005). The studies that address the origin of glia in the embryo (see above) treat the surface glia as a single layer, called “subperineurial glia”, since these cells form septate junctions. Do progenitors of perineurial glia form a subset of these cells? Or, if not, where are they derived from? It was reported by Edwards et al. (1993) that a population of cells, surrounding the surface of the late embryonic brain, represents the embryonic counterpart of the perineurial glia. These cells were claimed to be of mesodermal origin. In other studies, notably those using dissected early larval brains, such a layer was not observed; here, the surface glia is formed by first one (embryo: Ito et al., 1994; Bhat et al.,

2006; early larva; Pereanu et al., 2005) and then two (late larva; Stork et al., 2008) cell layers. None of these had the cuboideal structure of the cells described by Edwards et al. (1993) for the embryo. As pointed out in previous discussions (Pereanu et al., 2005), the mesodermally derived cells observed by Edwards et al (1993) most likely were hemocytes which, in the embryo, also adhere as a dense layer to parts of the brain surface. That leaves the question of the origin of the perineurial cells open. The same can be said for the reticular neuropile glia: these cells either form a subset of the known neuropile glial cells (e.g., the longitudinal glia of the ventral nerve cord; see below), or they form a group of cells whose progenitors have not yet been discovered in the embryo.

Glial progenitors of the embryonic brain

The individiual progenitors that give rise to the different classes of glia associated with the brain has not yet been established. However, using the pan-glial marker Repo, the domains within the neurectoderm of the head that give rise to glia, as well as their subsequent migration and differentiation, was followed (Hartenstein et al., 1998). There are a number of clear parallels between the spatio-temporal patterns of glial development in the ventral nerve cord and brain.

1. As noted for the ventral nerve cord above, most of the neuropile glia derive from a single focus at an early stage of development. In case of the brain, these cells form a single cluster, called basal procephalic neuropile glia (BPLG), that appears among the neuroblasts of the tritocerebrum, the most basal part of the brain (Fig. 8A, B, D). It is likely that the neuropile glia progenitors are descendants of the tritocerebral neuroblast Td7 (Urbach and Technau, 2003), which expresses *gcm*. BPLGs increase in cell number by division and spread ventrally, along the connectives in between brain and ventral nerve cord, as well as dorsally, where the commissure interconnecting the two brain hemisphere forms (Fig. 7B, C, E, F). It is likely that that most, if not all, of the brain neuropile glial cells of the early larva are derived from the BPLG cluster. Thus, cell counts of neuropile glia for freshly hatched larvae yield between 15 and 20 cells (Pereanu et al., 2005); this number matches the count for embryonic, BPLG derived cells.

2. Progenitors of the surface and cortex glia of the brain form two major and several minor clusters that flank the primordium of the brain anteriorly and dorso-posteriorly (Fig. 7A, B, D, E). The ventral major cluster, called ventral procephalic subperineurial glia (VPSG), lies anteriorly, around the boundary between proto- and deutocerebrum and comprises approximately 10–15 cells. The dorsal procephalic subperineurial glia cluster (DPSG) has 5–10 cells located in the dorsal protocerebrum bordering the optic lobe placode. Several minor groups of glial progenitors (ADSG, PDSG) line the boundary between protocerebrum and deutocerebrum. At the time when differentiation of glia begins, cells of all clusters flatten and extend over the brain surface; VPSG cells cover the anterior - lateral surface of the deutocerebrum and basal protocerebrum, ADSG and PDSG spread out over the deutocerebrum and ventral protocerebrum, and DPSG over the dorso-lateral protocerebrum (Fig. 7C, F). By the end of embryogenesis and in the early larva, counts of surface glia yielded 30–35; cortex glia amounts to 10–15 cells in the early larva, but the number counted for late embryo is approximately 20, which suggests that, similar to what has been found for neurons (Larsen et al., 2009), cell death at the time around hatching reduces cortex glia.

3. Peripheral glia associated with the nerves contacting the brain are of both central and peripheral origin, similar to what has been found for the ventral nerve cord (see previous section). At the stage when Repo first comes on in glial precursors, small groups of labeled cells are clustered around the exit point of the Bolwig's nerve

(axons of larval eye, or Bolwig's organ) and antennal nerve (Hartenstein et al., 1998). Subsequently the glial cells migrate centrifugally along these nerves. In addition, Repo-positive peripheral glia arises peripherally, among the neurons of the antennal sensory organ, indicating that the SOPs forming the sensory neurons and support cells of the antennal organ also give rise to peripheral glia.

Glial development during the larval period

All classes of glia, as described in the first sections of this review, can be recognized in the early larva right after hatching. During subsequent larval development, the basic structure of the different types of glial cells does not change. However, glia of all classes increase in number, with the possible exception of the subperineurial glia, which may merely grow in surface area (Stork et al., 2008). Overall, glial cell counts for the late larva yielded approximately 360 cells (per brain hemisphere), as opposed to around 60 cells in the early larva (Pereanu et al., 2005). During metamorphosis, glial cell numbers increase further to approximately 450 (V.H. and Wayne Pereanu, unpublished).

The increase in glial cell number is due to two mechanisms. First, differentiated glial cells themselves divide (Pereanu et al., 2005; Colonques et al., 2007). Application of BrdU or anti-Phosphohistone 3 antibody to larvae at different stages results in labeling of all three classes of glial cells, albeit at a relatively low frequency (Fig. 9A, B). Mitotic division of glial cells is highest among the perineurial glia; as discussed above, this cell type is very rare in the early larva, but increases to more than 100 in the late larva. The fact that induction of clones in the early larva results in purely glial clones in the adult (Awasaki et al., 2008) also supports a mechanism where glial divide. It should be noted that, at least for surface glia, mitosis never ceases; it continues in the adult, and can be stimulated by brain injury (Kato et al., 2009).

In addition to glial division, the increase in glial number that occurs between early larval and early pupal development is due to the division of neuro-glioblasts. Neuroblasts (and neuro-glioblasts), that produced a first set of (primary) neurons during the embryonic period reactivate and produce a second, much larger set of (secondary) neurons in the larva (reviewed in Hartenstein et al., 2008; Larsen et al., 2009). Secondary neurons differentiate during metamorphosis and, together with remodeled primary neurons, form the adult nervous system. Several of these larvally active progenitor cells have glia among their progeny. Evidence for this notion was first provided by Pereanu et al. (2006) who observed numerous clones that encompassed a neuroblast, neuronal progeny, and numerous cortex glial cells or surface glial cells which were directly adjacent to the neuronal cluster, and for that reason most likely belonged to the clone (Fig. 9E, F). More recently, it was shown that a set of lineages in the dorso-medial brain express the glial determinant *gcm* in some of their progeny, and contribute to the neuropile glia associated with the midline compartments, i.e., the central complex, of the adult brain (Izergina et al., 2009; Fig. 9C, D). These larval neuro-glioblasts belong to a small subset of cells that proliferate in a peculiar manner, producing daughter cells that undergo a series of symmetric divisions, before producing ganglion mother cells. To distinguish them from the "classical" (type I) neuroblasts, asymmetyrically dividing cells producing only ganglion mother cells (and thereby ending up with considerably smaller lineages), the novel type of neuroblast was called transient amplifying (TAN or type II) neuroblast (Boone and Doe, 2008; Bello et al., 2008). Please note that the type I ("classical") and type II (transient amplifying) neuroblasts of the larval brain are unrelated to the type I and type II neuro-glioblasts which were differentiated by Udolph et al (2001) in reference to the embryonic ventral nerve cord.

Peripheral glia surrounding nerves also increase in number during the larval period. As in the embryo, most, if not all of these glia are derived from the division of sensory organ

progenitor cells (SOPs) which arise at late larval and early pupal stages in the imaginal discs (eye-antenna, wing and haltere, leg, genital) and abdominal histoblasts. Many imaginal SOPs have a glia cell among their progeny, as described in detail for the SOPs generating the microchaetes of the thorax (Gho et al., 1999; Reddy and Rodrigues, 1999) and wing. Glial cells migrate from their peripheral site of origin along the sensory nerve (Giangrande et al., 1993; Aigouy et al., 2008) and during late pupal stages differentiate into the various types of glia described above for the larva. The mechanism controlling these cell types (e.g., wrapping glia vs subperineurial glia) has not yet been addressed.

Glia of the optic lobe

The optic lobe, both in neuropile colume and cell number, represents the by far largest part of the *Drosophila* brain. Development of the optic lobe follows a route that is different from that of the central brain, or the ventral nerve cord. Thus, in the embryo and early larva, progenitors of the optic lobe form two neuroepithelia, called inner optic anlage (IOA) and outer optic anlage (OOA), which are attached to the lateral surface of the larval brain. Neuroepithelial cells divide symmetrically, leading to an increase in size of the IOA and OOA. Subsequently, the neuroepithelia convert into thousands of neuroblasts. Neuroblasts embark on a series of asymmetric divisions (similar to the neuroblasts of the central brain or ventral nerve cord), and produce lineages of neurons that form the ganglia of the optic lobe. These comprise, from peripheral (adjacent to the eye) to central (adjacent to the central brain), the lamina, medulla, and lobula/lobula plate (for review of optic lobe development, see Meinertzhagen and Hanson, 1993; Fischbach and Hiesinger, 2008; Ngo et al., 2010).

The development of the glia of the optic lobe is complicated by the fact that there appear to be a large number of distinct subpopulations of glia, in particular within the neuropile glia subgroup; in addition, the optic lobe undergoes complicated morphogenetic movements during its development, which make it difficult to follow the history of glial cells (and neurons as well) in this part of the brain. The recent detailed review by Edwards and Meinertzhagen (2010) lists several groups of optic lobe glia identified in the larva, and assigns them to defined glial populations in the adult. Panels A and B of Fig. 10 are adapted from Edwards and Meinertzhagen's review; Panels C–E show confocal sections of a late larval brain where glia is labeled by anti-Repo and/or the Nrv2-Gal4 line driving GFP.

Surface glial cells, including perineurial and subperineurial cells, form a layer around the surface of the larval and adult optic lobe. It is likely that, as reported above for the central brain, these cells simply divide in the larva and pupa as the optic lobe grows, thereby increasing their overall number in the adult, compared to larva. Another source of adult optic lobe surface glia is the larval eye disc optic stalk, which is formed by the photoreceptor axons connecting the eye disc with the outermost optic ganglion, the lamina. These larval structures are associated with several distinct glial progenitor populations which, during the pupal period, differentiate into the specialized surface glia (fenestrated glia, pseudocartridge glia) which covers the surface of the lamina, and at the same time surrounds bundles of photoreceptor axons entering the lamina (Chotard and Salecker, 2007; Edwards and Meinertzhagen, 2010; Silies et al., 2010).

Neuropile glial cells of the adult optic lobe fall into many different categories. In the lamina, one finds epithelial glia and marginal glia; in addition, at least two different types of cells ensheath the axons between lamina and medulla in the outer optic chiasm; and both medulla and lobula contain several types of neuropile glia that have not yet been characterized in detail. Most of these cells appear to derive from glial progenitors that appear relatively early in development, during the second half of larval life, and form distinctive, crescent-shaped assemblies in the late larva (Fig. 10A, C–F) These cells (at least the ones associated with the

lamina and distal medulla) are derived from two specialized domains at the dorsal and ventral tip of the outer optic anlage, called the glial precursor center (Dearborn and Kunes, 2004; Chotard and Salecker, 2005; Fig. 10A). This zone coincides with the domain of Wingless and Decapentaplegic expression, involved in the growth of the optic anlage, that had been discovered by Kunes and colleagues earlier (Kaphingst and Kunes, 1994). A similar restricted zone of origin may also exist for the neuropile glia of the adult lobula complex, but this has not been investigated so far. The various types of neuropile glia of the optic lobe, as well as the surface glia derived from the optic stalk, have been subject of numerous studies that investigate the developmental role of glia for axonal pathfinding and target selection (reviewed in Parker and Auld, 2006).

Cortex glia of the adult optic lobe, commonly referred to as satellite glia, most likely derives from different domains within the optic anlagen. It has been suggested that the satellite glia of the lamina cortex also originates in the glia precursor center, mentioned above, that generates neuropile glia (Edwards and Meinertzhagen, 2010). In addition, one can distinguish, from late larval stages onward, many small, scattered, Repo-positive cells among the progeny of the neuroblasts that give rise to the medulla (Fig. 10C–E, arrows). In other words, similar to the neuro-glioblasts of the central brain, these cells may produce both neurons and glial cells for the medulla and lobula complex (V.H., unpublished). Much work is still required to complete our picture of glia assembly in the optic lobe of the fly.

Conclusion and brief foray into glial evolution

What can one conclude from the developmental studies, reviewed above, about the separation between structurally different classes of glia? At what stage of development do glial cells adopt different shapes or migration patterns? Overall, it seems that different types of glia are predominantly generated by different embryonic progenitors. For example, neuropile glia of the larval CNS originates from glioblasts that may not give rise to any other cell type. For the ventral nerve cord, one neuroblast forms most of the cortex glia; four other neuroblasts generate surface glia. However, there clearly are exceptions; for example, neuroblast 7-4 generates predominantly surface glia, but also has one cortex and one neuropile glial cell among its progeny (see Fig. 7B). In the larva, it becomes even more complex; individual neuro-glioblasts, such as the dorsomedial type II neuroblasts, may produce multiple types of glia. Thus, it remains a largely open question when and how glial types (rather than neuron vs glia, a step which is well understood) are distinguished, and whether or not this distinction is irreversible. Since glial class specific transcriptional regulators have so far not been identified, it is difficult to follow the early steps (prior to structural differentiation) in which glial cells embark on different fates.

It seems useful to end this review with a brief look at homologies between glia of insects and vertebrates, and glial evolution in general (please also refer to the review by David Hartline on evolution of glia in this special issue). A number of different homology scenarios between types of vertebrate and *Drosophila* glia have been proposed in the recent literature, based on molecular homologies and/or functional similarities. Most notably, subperineurial glia with their septate junctions, responsible for the blood brain barrier, have been likened to vertebrate Schwann cells which, at the nodes of Ranvier, form similar junctions, composed (in part) of homologous proteins (Tepass et al., 2001; Daneman and Barres, 2005). Other homologies that have been discussed, mostly based on cell shape and topology, are between vertebrate astrocytes and *Drosophila* reticular/astrocyte-like neuropile glia (Awasaki et al., 2008), or, alternatively, between astrocytes and cortex glia (Pereanu et al., 2005; Freeman and Doherty, 2006). The first proposal emphasizes the fact that both vertebrate astrocytes and the fly neuropile glia contacts terminal neurites and synapses, and play a role in synaptic transmission (e.g., neurotransmitter re-uptake). The second proposal,

to consider astrocytes homologous to cortex glia, is based on developmental similarities: similar to the radial glia in the vertebrate embryo, which later differentiates into astrocytes (Mission et al., 1991), *Drosophila* cortex glia starts out as a radially oriented, bipolar cell that, like radial glia, plays a role in the migration/packing of neuronal cell bodies (Dumstrei et al., 2003). Another speculation has stressed the similarity between oligodendrocytes and reticular neuropile glia, since both wrap axons (Pereanu et al., 2005; Freeman and Doherty, 2006).

Rather than discussing the merits or shortcomings of any of these proposals, it should be emphasized that homology presupposes that the vertebrate-fly ancestor, which is to say, the bilaterian ancestor, possessed glia of different types (i.e., astrocyte-like, oligodendrocyte-like; Schwann cell-like). This may not have been the case; it is not even clear whether the bilaterian ancestor had any glia at all. Thus, surveying the extant invertebrate taxa for the presence or absence of structurally detectable glia provides an ambiguous answer in regard to this question (see accompanying review by David Hartline). For example, among the platyhelminths (flatworms), basal lophotrochozoans, most taxa have no cells that resemble glia (i.e., form sheet-like processes around nerve tracts, or neuronal cell bodies (Younossi-Hartenstein et al., 2000; 2001; Younossi-Hartenstein and Hartenstein, 2000; Morris et al., 2004). This is also true for many other phyla (reviewed in Radojcic and Pentreath, 1979). In acoels, a taxon which may represent the sister group of bilaterians, glia-like cells have been recently reported (Bery et al., 2010). In descriptions of developing basal deuterostome nervous systems (e.g., Dupont et al., 2009; Nakano et al., 2009) cells with glial morphology have not yet been described; adult echinoderm nerve cords appear to have cells with the characteristics of radial glia of chordates (Mashanov et al., 2009; 2010). One should realize that the “formation of sheet-like processes around neighboring cells”, used as a hallmark for glial cells in studies that have no other, molecular, markers at their disposal, is a fairly soft criterion for glial cells. Many types of cells that form layers, for example epidermal cells in *Drosophila*, form quite elaborate sheet-like processes around their neighbors, even if (in case of this example) only transiently (Hartenstein, 1988). We are currently left with the notion that the similarities that can be observed between certain types of vertebrate and *Drosophila* glia have most likely arisen independently, and do not represent homologies. This does not mean that the conservation, on the molecular level, of protein complexes associated with certain types of glia is not homologous. This is particularly evident for the septate junctions that form a crucial element of the blood brain barrier in *Drosophila*. In vertebrates, this role is taken over by tight junctions. Septate junctions do exist in vertebrates, but apparently are more limited in distribution. The paranodal junction, formed at the nodes of Ranvier between Schwann cells and axons, forms the clearest example of a vertebrate septate junction (Tepass et al., 2001). Protein complexes, forming part of septate junctions in *Drosophila* and paranodal junctions in vertebrates are homologous, based on the degree of sequence conservation (neurexin IV/Caspr; Contactin/Contactin; Protein 4.1/Coracle; Nf155/Neuroglian; reviewed in Daneman and Barres, 2005). At least some of the proteins found in *Drosophila* septate junctions (e.g., Sinuous) are homologous to tight junctions proteins (e.g., Claudins; Wu et al., 2004). Septate junctions connecting cells of the epidermal layer have been observed in most, if not all bilaterian phyla, including the platyhelminths that lack glial cells (Hartenstein and Ehlers, 2000; Younossi-Hartenstein and Hartenstein, 2000; Ramachandra et al., 2002; Morris et al., 2004; reviewed in Magie and Martindale, 2008). In conclusion, specialized membrane junctions formed by dedicated membrane-associated proteins were in existence at the stage of the bilaterian ancestor. They may have functioned to isolate the interstitial compartment within the animal from the exterior milieu. At the same time, glial cells acting as an effective blood brain, or serving to make synaptic transmission, signal conduction, or neural development more efficient, may not yet have been “invented”. As such cells arose, independently in different phyla, during subsequent evolution from epidermal cells and/or neurons, they incorporated the pre-

existing protein complexes and subcellular specializations. It has been reported frequently that molecular pathways controlling a certain morphogenetic event, such as forming a junctional contact, or subdividing an elongated structure into smaller segments, can be recruited to play this role in completely different developmental contexts (i.e., in structures that are not ancestrally related to the ones in which these molecular pathways originally evolved; see for example Bowsher and Nijhout, 2009).

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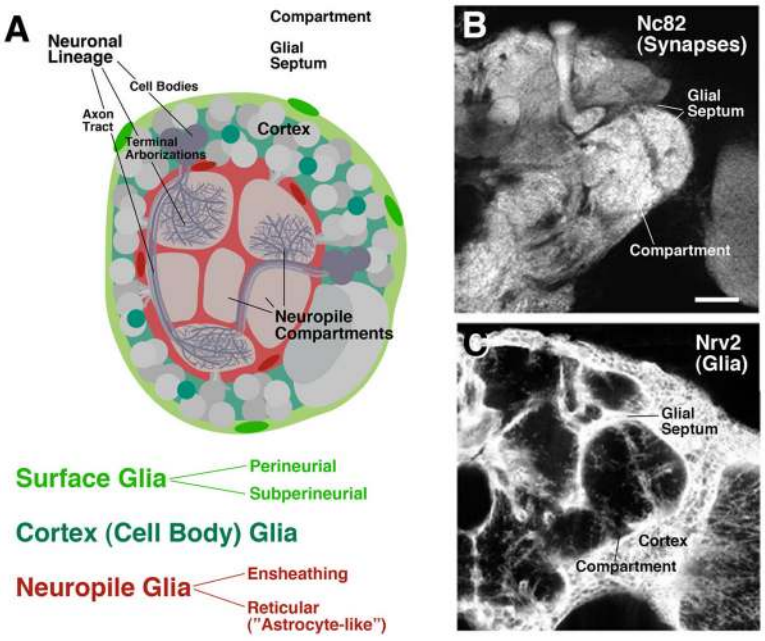

Fig. 1.

Classification and distribution of glia in the *Drosophila* brain. A. Schematic cross section of brain showing exterior cortex (neuronal and glial cell bodies), central neuropile (neuronal and glial processes), and glial cells. Neurons are subdivided into discrete lineages; each lineage is formed by one neuronal progenitor cell (neuroblast). Cell bodies and processes of neurons that belong to the same lineage form a structural unit, as schematically depicted. The five major classes of glial cells are represented in different colors. B, C. Confocal section of one adult brain hemisphere labeled with synaptic marker Nc82 (B) and glial marker Nervana 2-Gal4>UAS-GFP (Nrv2; C). In B, the interior of compartments shows strong label due to the high density of synapses. Compartment boundaries, formed by glial septa, express a low signal. In C, compartment boundaries which are rich in glial processes are strongly signal-positive. Note also Nrv2-positive cortex glial sheaths surrounding neuronal cell bodies.

Bar: 20μm

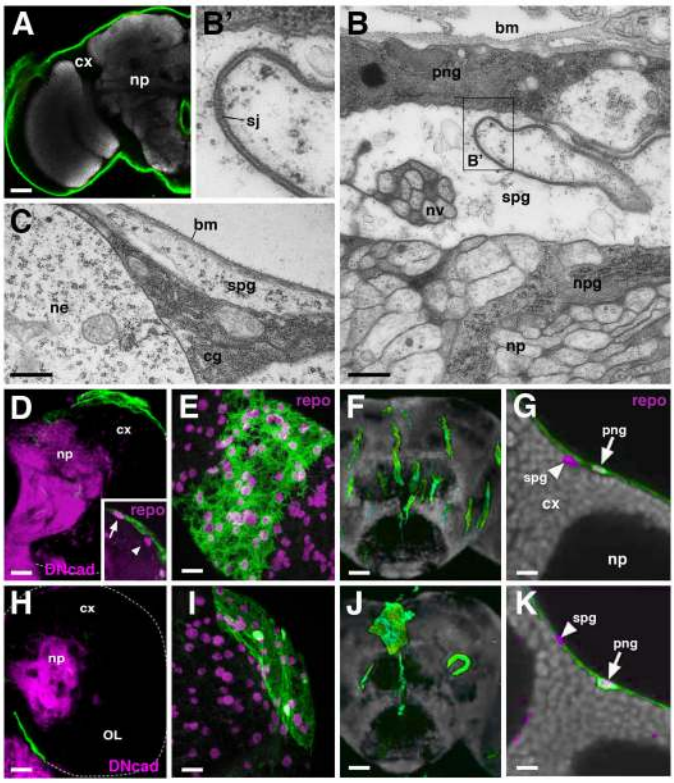

Fig. 2.

Surface glia. A: Confocal section of adult brain hemisphere. GFP is activated by surface glia-specific driver a250-Gal4 (kindly provided by Dr. Julie Simpson, JFRC). Cx cortex; np neuropile. B, B′: Transmission electron microscopic (TEM) photograph of medial part of first instar larval brain. Surface glia comprises an outer layer of perineurial cells (png), covered by a basement membrane (bm), and an inner layer of subperineurial cells (spg). Septate junctions (sj) are formed between subperineurial cells (B′). Note thin nerve (nv) traversing surface glial layer. At the medial position shown, neuronal fiber bundles (np) extending towards the brain commissure are lying directly underneath the surface glial layer. These bundles are surrounded by neuropile glia (npg). C: TEM section showing surface of lateral part of first instar brain. A perineurial layer is lacking; the superineurial glia, recognizable by its characteristic low electron density and the presence of septate junctions (not shown in this picture), forms the outermost layer of glia. Underneath superineurial layer one can distinguish the electron-dense cortex glia (cg) which surrounds neuronal cell bodies (ne). D–K: confocal sections showing clones of surface glia. Upper row (D–G): Perineurial glia. Lower row (H–K): Subperineurial glia. Left two columns (D, E, H, I): Third instar larva. Right two columns (F, G, J, K): Adult. All clones were induced at early first instar larval stage. Perineurial glial cells are elongated, relatively small cells that form the outer layer of surface glia. Arrows in D (inset) and G point at Repo-positive nuclei located within the GFP-labeled perineurial cells; arrowheads indicate Repo-positive, GFP-negative nuclei underneath which represent subperineurial glia. D and G show labeled clones in cross section; E and F are surface views of clones. The series of photographs of clones of subperineurial glia shown in panels H–K is constructed in the same manner as D–G above. Note large size (I, J) and small thickness (H, K) of subperineurial cells. E, I: from Stork et al., 2008. F, G, J, K: from Awasaki et al., 2008.

Bar: 20μm (A, D, F, H, J); 10μm (E, I); 5μm (G, K); 0.5μm (B, C)

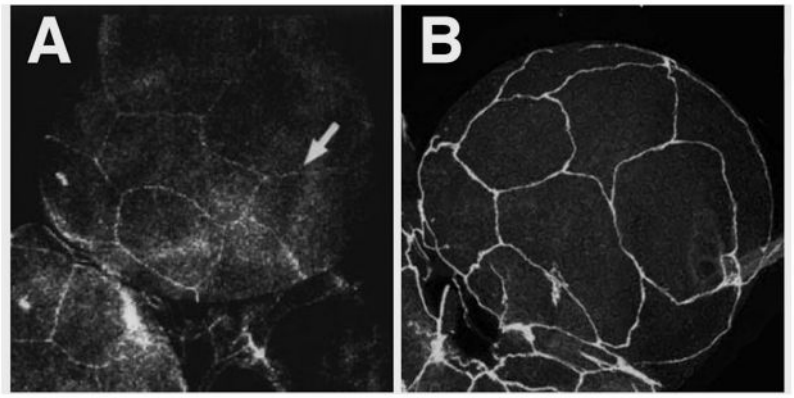

Fig. 3.

Septate junctions in surface glia: perineurial or subperineurial? A: Part of Fig. 10 of Carlson et al. (2000), showing *Drosophila* larval brain hemisphere labeled with anti-Neurexin (anti-Nrx) to visualize septate junctions. Cells expressing Nrx are designated as "perineurial glia). B: Part of Fig. 2E of Stork et al. (2008), showing brain hemisphere in which cells forming septate junctions are labeled by Nrx-Gal4>UAS-GFP; this cell type is referred to as "subperineurial glia".

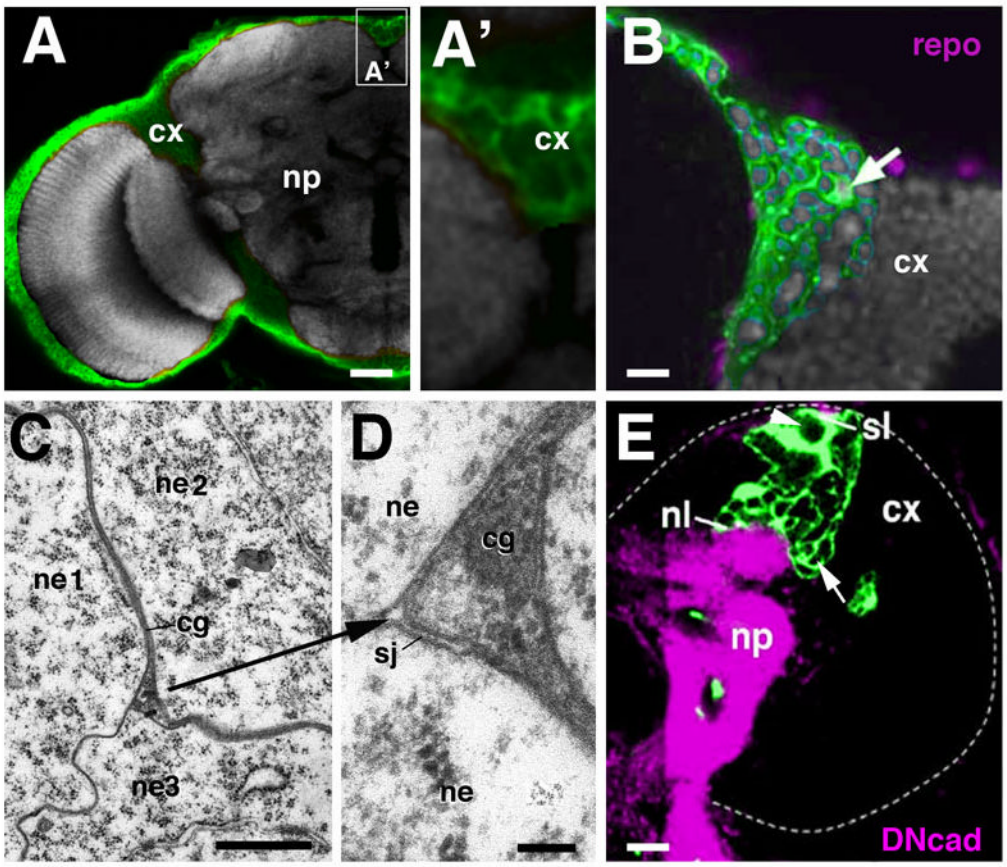

Fig. 4.

Cortex glia. A, A′: Confocal section of adult brain hemisphere. Cx cortex; np neuropile. GFP is activated by cortex glia-specific driver CG4288-Gal4 (kindly provided by Dr. Julie Simpson, JFRC).

B: Clone of cortex glia in adult brain. Note location of glial cell body, containing Repo-positive nucleus [arrow; from Awasaki et al., 2008 (with permission)]

C, D: TEM photograph of part of first instar larval brain. Thin, electron-dense process of cortex glial cell (cg) separates three neighboring neuronal somata (ne1-3). High magnification view (D) illustrates small septate junction (sj) between cortex glia and neuron.

E: Confocal section of larval brain hemisphere showing clone of cortex glia (from Pereanu et al., 2005). Neuropile is labeled with antibody against *Drosophila* N-cadherin (DNcad). Note surface lamella (sl) and neuropile lamella (nl). In the deep cortex, primary neurons and early born secondary neurons are individually wrapped by cortex glia; note small diameter of "holes" (each one corresponding to one neuron) surrounded by cortex glia (arrow). In superficial layers, neuroblasts and clusters of ganglion mother cells/late born neurons are all lodged together in larger chambers surrounded by cortex glia (arrowhead). Bar: 20μm (A, E); 0.5μm (C); 0.1μm (D)

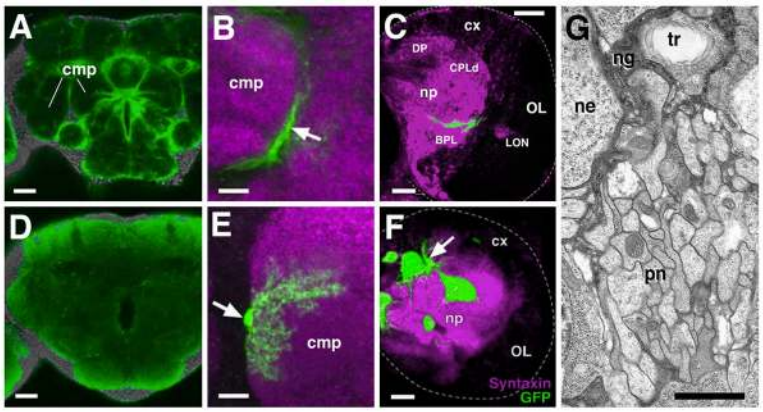

Fig. 5.

Neuropile glia. A–C: Ensheathing glia labeled in toto in adult brain by the specific driver line NP1243 (A; from Awasaki et al., 2008), as a clone in adult brain (B; from Awasaki et al., 2008), and as a clone in larval brain (C; from Pereanu et al., 2005). Note that ensheathing glial cell bodies (arrow in B) surround the surface of neuropile compartments (cmp) without penetrating into the interior of the compartments. D–F: Reticular (astrocyte-like) glia, labeled in adult brain hemisphere by specific driver line NP6520 (from Awasaki et al., 2008), as a clone in adult (E; from Awasaki et al., 2008), and as a larval clone (F; from Pereanu et al., 2005). In reticular glia, cell bodies lie at the neuropile surface, but numerous processes invade the neuropile (arrow in E and F). G: TEM photograph of neuropile of early larval brain, showing interface of cortex with neuronal cell bodies (ne) and neuropile, formed by the neurites of primary neurons (pn). Note electron-dense glial layer (ng) along cortex-neuropile boundary, and surrounding neurites and trachea (tr) in depth of neuropile. Bars: 20μm (A, C, D, F); 10μm (C, E); 1μm (D)

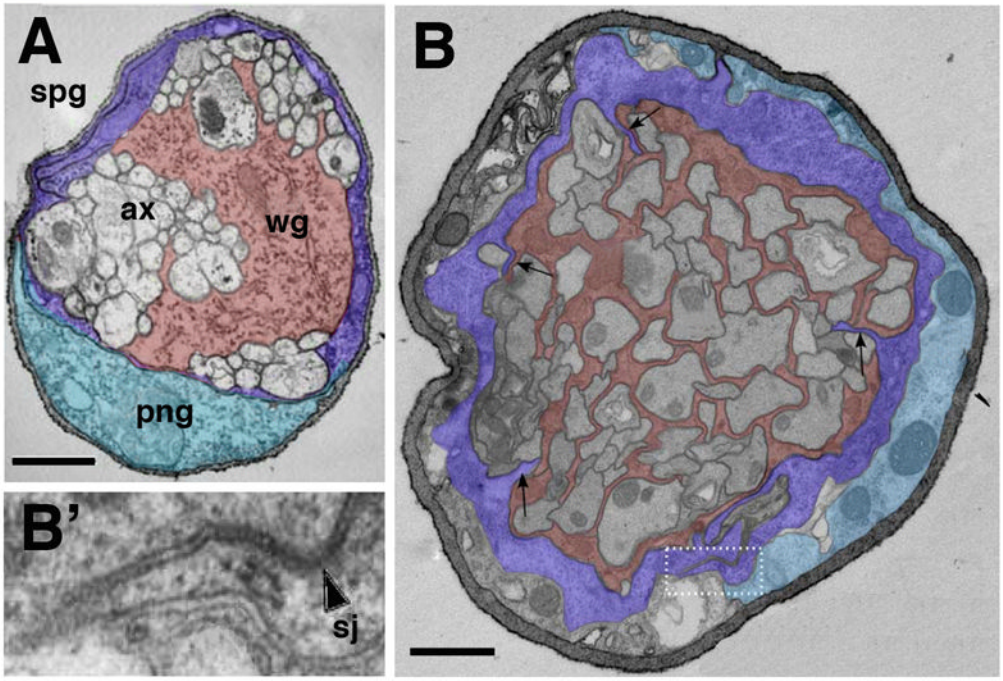

Fig. 6.

Peripheral glia. TEM cross section of peripheral nerve of first instar (A) and late third instar larva (B, B′). Surface glia comprises an outer perineurial glial (png; light green) layer and an inner subperineurial layer (spg; dark green). Axons (ax) are surrounded by wrapping glia (red). Note that perineurial glia has not yet expanded around the entire nerve in early larva, as opposed to late larva where it completely surrounds the nerve (B); similarly, wrapping glia forms processes around individual axons only in late larva. B′ shows magnified view of boxed area in B, illustrating auto-septate junction (sj) of subperineurial glia. Arows in (B) point at protrusions of subperineurial glia contacting axons, a phenomenon not observed in subperineurial glia of CNS. Modified, from Stork et al., 2008.
Bar: 1μm

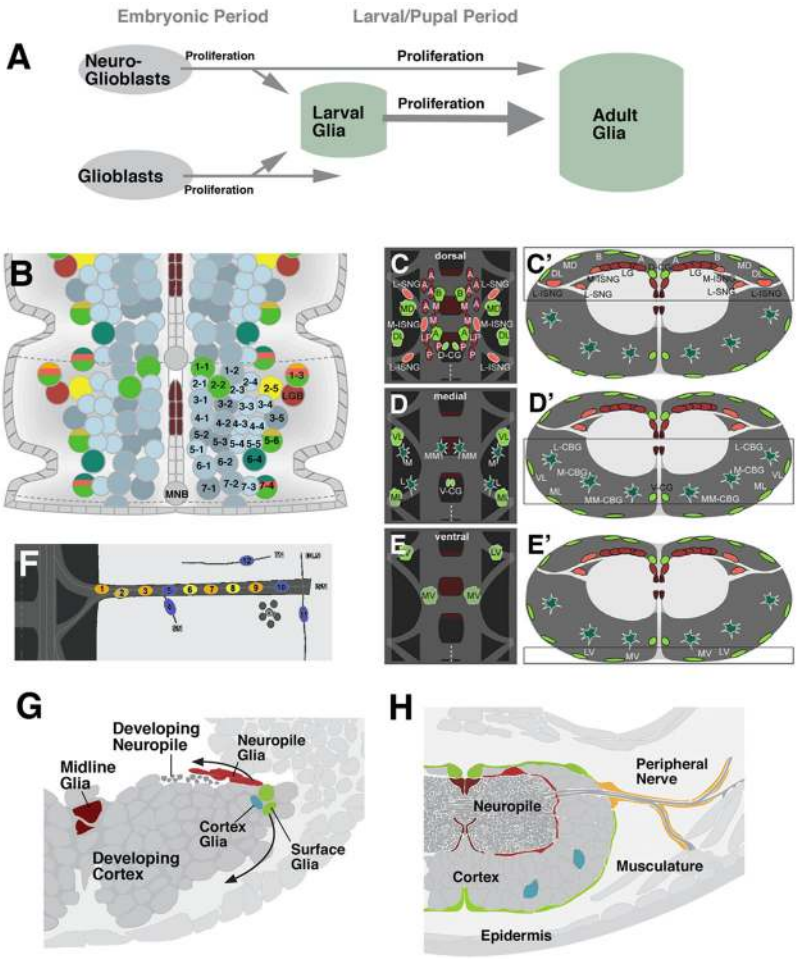

Fig. 7.

Embryonic origin of ventral nerve cord glia. A: Overview of glial development. B: Neuroblast map of the ventral nerve cord with glioblasts and neuro-glioblasts indicated (after Goodman and Doe, 1993; Campos-Ortega and Hartenstein, 1997; Ragone et al., 2003). Neuroblasts/neuroglioblasts of one hemineuromere are identified alphanumerically. C–E′: schematic horizontal sections (C–E) and cross sections (C′–E′) of late embryonic ventral nerve cord, showing location of glial cells relative to the boundaries of neuromere, neuropile, and peripheral nerves (modified, from Beckervordersandforth et al., 2008). Boxed areas in C′-E′ indicate dorso-ventral levels of corresponding horizontal sections shown in C–E. Color code used in B (neuroblasts) and C–E′ (differentiated glial cells) allows to discern the type of glial cell produced by a given neuroblast (light green: surface/subperineurial glia; turquoise: cortex glia; dark red: longitudinal neuropile glia; light red: nerve glia; bown: midline neuropile glia; yellow/orange: peripheral glia). F: Schematic rendering of peripheral nerve of one hemisegment (modified, from von Hilchen et al., 2008). Location of peripheral glial cells, in colors allowing to distinguish the neuro-glioblasts (panel B) they are derived from. G, H: Schematic cross sections of ventral nerve cord at embryonic stage 13 (G) and 16 (H), showing location and migration pathways of glial cells.

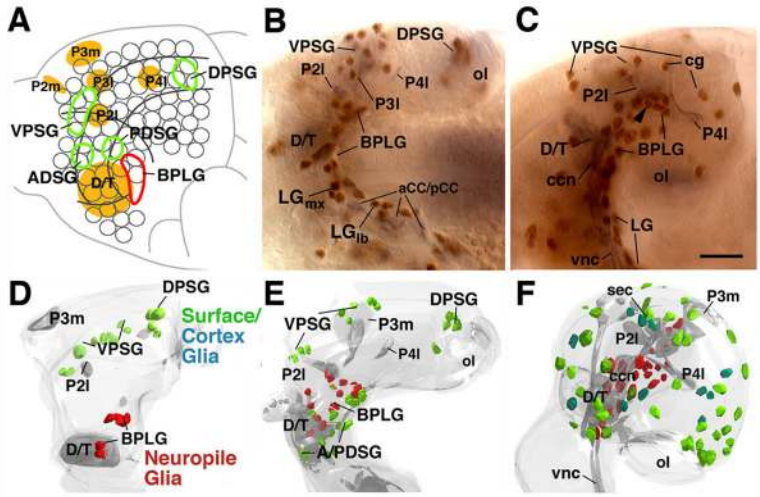

Fig. 8.

Embryonic origin of brain glia. A: Approximate location of the clusters of glial progenitors (outlined in green and red) in relation to the neuropile founder clusters (orange) and the brain neuroblast map (Younossi-Hartenstein et al., 1996; Urbach and Technau, 2003). Neuropile founder (or pioneer) clusters are groups of early differentiating neurons that express the Fasciclin II adhesion molecule, and form an early scaffold of axons prefiguring the brain neuropile (Nassif et al., 1998). The P2l/P2m clusters pioneer the brain commissure; D/T pioneers the longitudinal fiber tracts of the tritocerebrum and deutocerebrum that connect the ventral nerve cord with the brain commissure. P3m/P3l/P4l prefigure the neuropile of the protocerebrum. B, C: lateral view of stage 12 embryo (B) and late stage 14 embryo (C) labeled with an antibody against Repo (brown) and against Fas II protein (neuropile founder clusters; purple; anterior to the left). D–F: Digital models of brain hemispheres of stage 11 (D), late stage 12 (E) and late stage 14 (F) embryos, illustrating the pattern of different populations of glia cell precursors (see color key in D) in lateral view. At stage 11 (D) the BPLG cluster of Repo-positive cells makes its appearance adjacent to the D/T neuropile founder cluster. BPLG comprises the progenitors of the neuropile associated glia cells of the brain. Two clusters located in the ventral (VPSG) and dorsal (DPSG) part of the protocerebrum include precursors of subperineurial glia. During stage 12 (B, E) neuropile glia precursors (BPLG) have increased in number and migrated dorsally, reaching the P2l neuropile founder cluster that pioneers the brain commissure. Ventrally, cells of the BPLG have linked up with longitudinal glia cells of the ventral nerve cord (vnc; LG_{lb}, LG_{mx}: longitudinal glial precursors of labial and maxillary neuromere, forming the anterior part of the ventral nerve cord). In addition to VPSG and DPSG, two additional clusters of surface glia precursors are present: ADSG and PDSG. In late embryo (C, F), neuropile glia derived from the BPLG cluster forms a continuous covering of the brain commissure (sec), and the cervical connective (ccn; axon tracts of the deutocerebrum and tritocerebrum in between brain commissure and ventral nerve cord). Surface glia precursors (ADSG, PDSR, VPSG, DPSG) have spread over the entire lateral and dorsal brain hemisphere. Cortex glial cells, probably descendants of the VPSG and DPSG clusters, can be distinguished by their position within the depth of the brain cortex. Other abbreviations: *aCC/pCC* anterior and posterior corner cells (neuropile founder clusters of anterior neuromeres of ventral nerve cord); *ol* optic lobe

Bar: 20μm

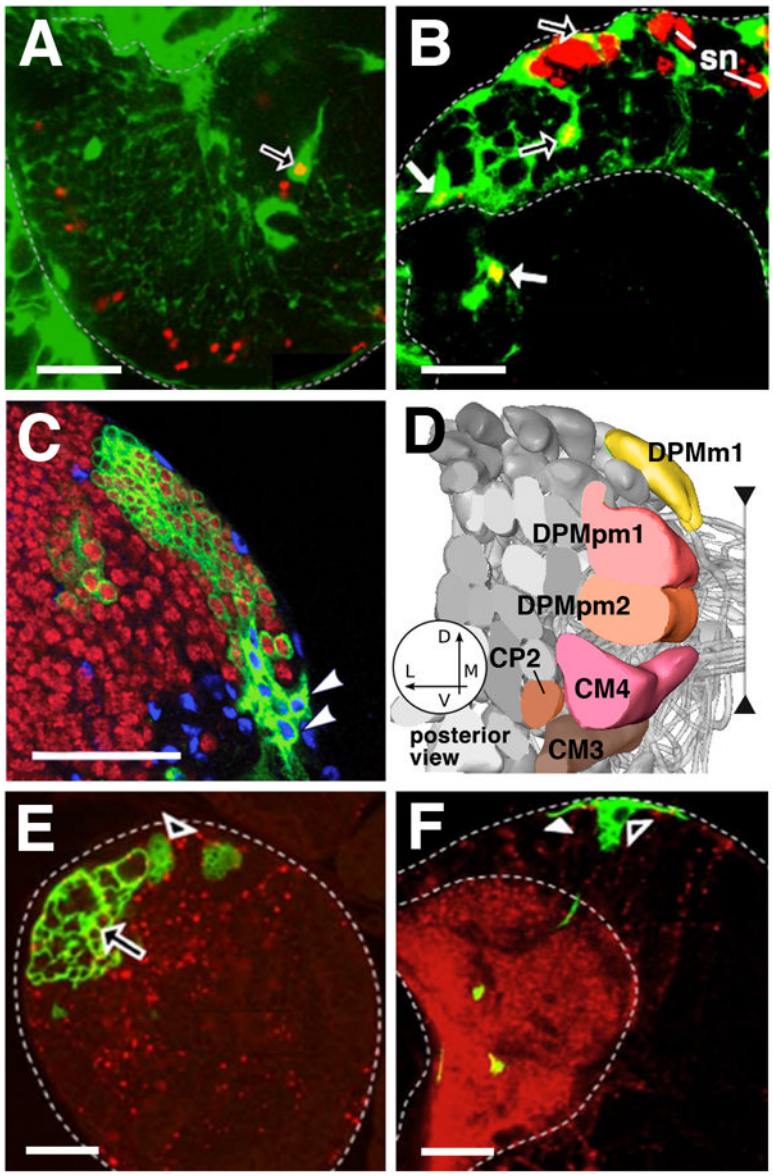

Fig. 9.

Glial proliferation during the larval period. A, B: Confocal sections of third larval instar brains. In A, mitotic cells are labeled by anti-phospho-histone H3 (red), and glial cells by Nrv2-Gal4 > UAS-GFP (green). Arrow indicates mitotic glial cell. In B, larva had been fed BrdU for 12h prior to dissection. BrdU label appears in secondary neural lineages (sn), and in all three classes of glial cells (arrowheads: surface glia; open arrows: cortex glia; solid arrows: neuropile glia). C: GFP labeled clone of type II DPMm1 lineage (green) containing Gcm-positive (blue) glial precursors (from Izergina et al., 2009). D: 3D digital model of lineages of larval brain hemisphere; posterior view; medial to the right. Arrowheads indicate midline. Type II lineages are rendered in color and are identified alphanumerically (after Pereanu and Hartenstein, 2006; Fung et al., 2009). E, F: GFP labeled clones of secondary lineages with adjacent glial cells in third larval instar brains (from Pereanu et al., 2006). E: Cortex glia (open arrow), located directly adjacent to neuroblast (open arrowhead). F: Surface glia (solid arrowhead) forming part of secondary lineage (open arrowhead).

Scale bars: 20μm

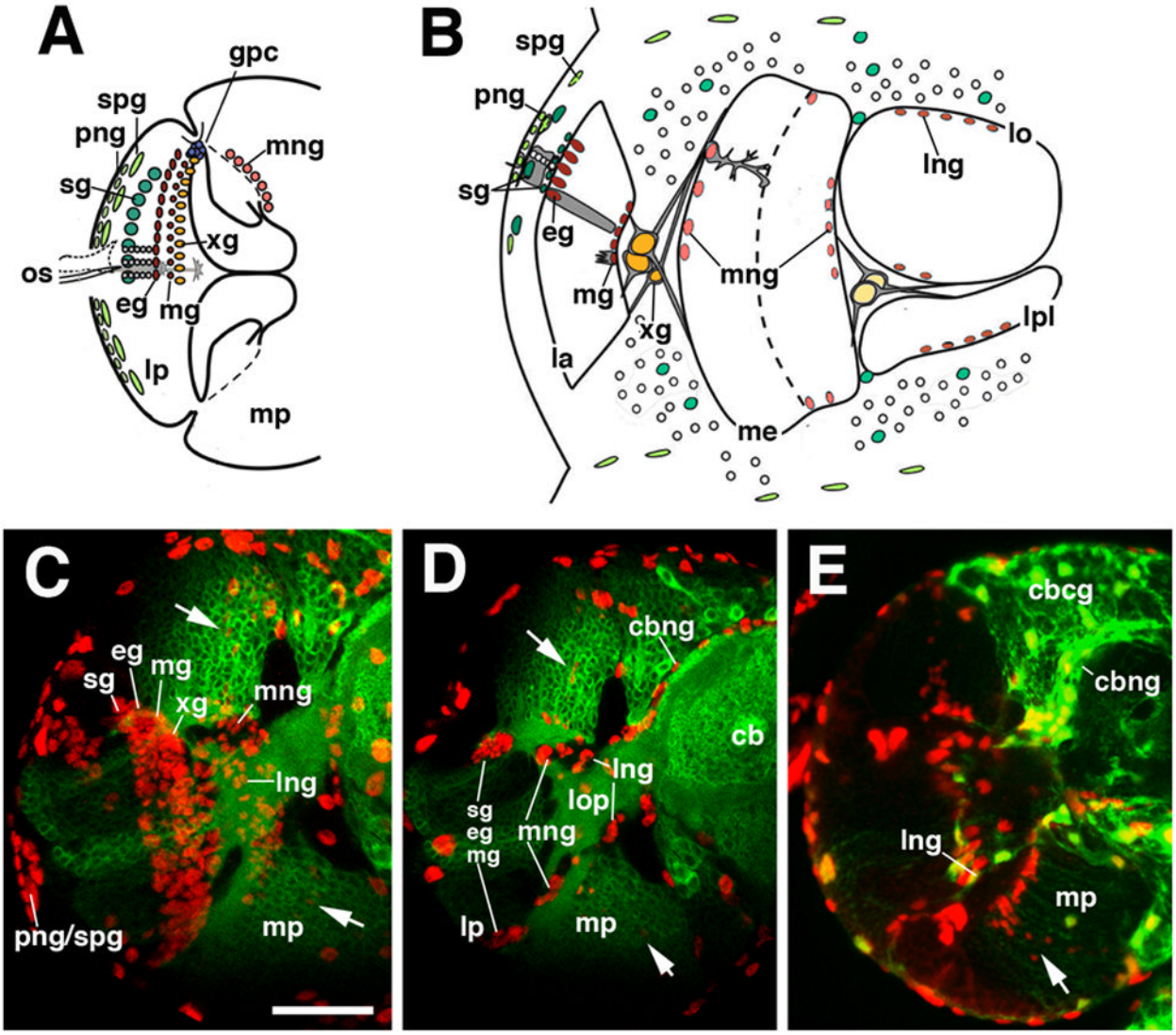

Fig. 10.

Glia of the optic lobe. A, B: Schematic sections of the larval (A) and adult (B) optic lobe (la lamina; lp lamina primordium; me medulla; mp medulla primordium; lo lobula; lp lobula plate; lop lobula/lobula plate primordium). Glia and glial progenitors are depicted, following color code of previous figures. C–E: Frontal confocal sections of late larval brain hemisphere, labeled with glial marker anti-Repo (red). In C and D, neurons are labeled by Elav-Gal4>UAS-GFP (green). In E, glial membranes are labeled by Nrv2-Gal4>UAS-GFP (green). In C, red channel (Repo-positive glial cells) is shown as Z-projection. Here, rows of glial progenitors associated with the lamina primordium and medulla primordium appear as continuous bands, similar as in schematic (A) above. In the sections shown in D and E, rows of glial progenitors appear twice, at a dorsal level and a ventral level. Note in E lack of differentiation of optic lobe glia (Nrv2-Gal4 driven GFP is faint or absent), with the exception of several cells associated with the lobula neuropile glia (lng). White arrows point at scattered, small Repo-positive cells which appear among the columns of freshly produced medulla neurons (mp).

Abbreviations: cb central brain; cbcg cortex glia of central brain; cbng neuropile glia of central brain; eg epithelial glia (neuropile glia of lamina); gpc glial progenitor center; la

lamina; lng neuropile glia of lobula/lobula plate; lo lobula; lop lobula/lobula plate primordium; lp lamina primordium; lpl lobula plate; mg marginal glia (neuropile glia of lamina); mng medulla neuropile glia; mp medulla primordium; os optic stalk; png perineurial glia; sg sattelite glia (cortex glia of lamina); spg subperineurial glia; xg glia of outer chiasma.