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A Unique Class of Neural Progenitors in the *Drosophila* Optic Lobe Generates Both Migrating Neurons and Glia

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SUMMARY

How neuronal and glial fates are specified from neural precursor cells is an important question for developmental neurobiologists. We address this question in the *Drosophila* optic lobe, composed of the lamina, medulla, and lobula complex. We show that two gliogenic regions posterior to the prospective lamina also produce lamina wide-field (Lawf) neurons, which share common progenitors with lamina glia. These progenitors express neither canonical neuroblast nor lamina precursor cell markers. They bifurcate into two sub-lineages in response to Notch signaling, generating lamina glia or Lawf neurons, respectively. The newly born glia and Lawfs then migrate tangentially over substantial distances to reach their target tissue. Thus, Lawf neurogenesis, which includes a common origin with glia, as well as neuronal migration, resembles several aspects of vertebrate neurogenesis.

Graphical abstract

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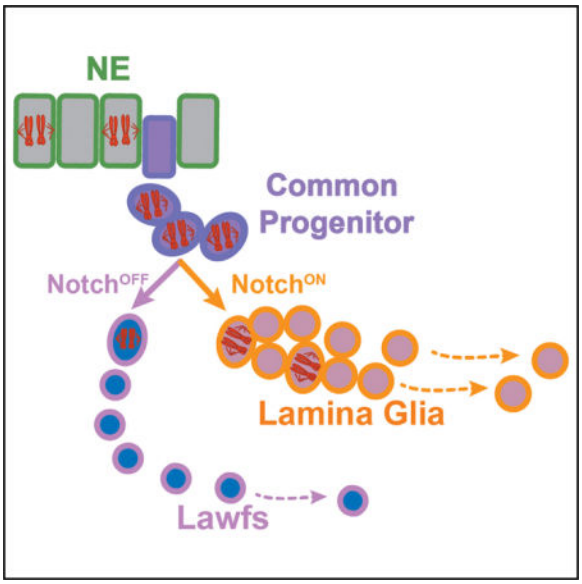
SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.03.061.

AUTHOR CONTRIBUTIONS

Z.C., V.M.F., X.L., and C.D. designed the experiments. Z.C., A.D.V., V.M.F., T.E., and X.L. performed the experiments. Z.C., V.M.F., X.L., and C.D. wrote the paper, and all authors commented on the manuscript.



INTRODUCTION

Building a functional nervous system is staggeringly complex and depends critically on the coordinated proliferation, differentiation, and survival of diverse neuronal and glial cell types. Interactions within and across these cell types regulate a wide range of processes, from cell migration to axon guidance, necessary for establishing neural circuitry. How neuronal and glial diversity arises from precursor cells and how they participate in circuit formation remain central questions in developmental neurobiology.

We used the complex but spatially ordered visual system of *Drosophila* to investigate neurogenesis and gliogenesis fate decisions during development. Glial cells comprise 10% of the *Drosophila* nervous system and can arise from dedicated precursors (glioblasts) or from precursors with mixed neuronal and glial potential. For the latter case, a neuroblast (NB) generates either intermingled neurons and glia or a glioblast and an additional NB that form bifurcating lineages of glia and neurons (Bernardoni et al., 1999; Bossing et al., 1996; Schmid et al., 1999; Schmidt et al., 1997; Udolph et al., 2001).

Three types of NBs have been described in *Drosophila*. Type I NBs undergo multiple asymmetric divisions to self-renew and produce ganglion mother cells (GMCs). Each GMC terminally divides to produce two differentiated cells, two neurons or a neuron and a glia (reviewed in Doe, 2008). Type II NBs generate transit-amplifying intermediate progenitor cells (INPs) that produce multiple GMCs, leading to larger lineages (Bayraktar and Doe, 2013; Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008). Type 0 NBs divide asymmetrically multiple times to self-renew and generate a single differentiated daughter neuron (Ulvklo et al., 2012; Bertet et al., 2014). Recently, a new category of migrating neural precursors that are not neuroblasts was described in the optic lobes (Apitz and Salecker, 2015).

The *Drosophila* optic lobe has four neuropils: lamina, medulla, lobula, and lobula plate, containing over one hundred neural cell types. In this study, we focus on the developmental origin of lamina wide-field (Lawf) neurons, multicolumnar neurons with processes in the medulla and lamina neuropils and cell bodies in the medulla cortex (Figure 1A). There are two types of Lawf neurons, Lawf1 (Fischbach and Dittrich, 1989; Morante and Desplan, 2008; Rivera-Alba et al., 2011) and Lawf2 (Hasegawa et al., 2011), with distinctive arborization patterns (Figure 1A). Although there is some insight into the function of Lawf neurons in regulating motion detection (Tuthill et al., 2013, 2014), their developmental origins have not been characterized, but they are assumed to be derived from the medulla neuroepithelium due to their cell body position.

Both the lamina and the medulla are derived from a crescent of neuroepithelial cells at the surface of the larval optic lobe, called the outer proliferation center (OPC). Medulla neurons are generated when a wave of neurogenesis that travels through the OPC crescent sequentially transforms neuroepithelial cells into type I NBs (Yasugi et al., 2010; Yasugi et al., 2008). As medulla NBs divide, they progress through different temporal stages and sequentially express different temporal transcription factors (tTFs) (Li et al., 2013; Suzuki et al., 2013; Bertet et al., 2014). In addition to patterning in the temporal axis, medulla NBs are also patterned spatially: the OPC neuroepithelium is subdivided into several different regions that express specific TFs—visual system homeobox (Vsx), Optix (Gold and Brand, 2014), or retinal homeobox (Rx)—or signaling molecules—Decapentaplegic (Dpp), Wingless (Wg), and Hedgehog (Hh) (Figure S2A). The combinatorial input of temporal and spatial cues specifies progeny fate (Bertet et al., 2014; T.E., unpublished data).

The lamina is involved in the first steps of motion vision. Neurons comprising the lamina neuropil are generated from lamina precursor cells (LPCs) on the lateral side of the OPC crescent, which is innervated by photoreceptor axons. Their development is strictly coupled to the ingrowth of developing photoreceptor axons. Photoreceptor axons secrete Hh, which controls LPC proliferation, and Spitz, which promotes their differentiation into lamina neurons through epidermal growth factor receptor signaling (Selleck et al., 1992; Huang and Kunes, 1996, 1998; Huang et al., 1998). The lamina is also populated by several types of glia that originate outside of the lamina precursor region. The dorsal and ventral tips of the OPC crescent express Rx; these Rx^+ regions can be subdivided into Wg- and Dpp-expressing regions (Kaphingst and Kunes, 1994). The lateral (lamina) side of the Rx regions does not receive photoreceptor innervation and produces epithelial and marginal glia (eg/mg) from glial precursors cells (GPCs). These differentiating glia then migrate from the GPC areas into the developing lamina (Chotard and Salecker, 2007; Chotard et al., 2005; Perez and Steller, 1996; Poeck et al., 2001; Yoshida et al., 2005).

Here, we report that Lawf neurons, previously assumed to be medulla derived, are instead derived from the GPC areas that produce eg/mg. We show that Lawfs and eg/mg share the same common progenitor cells. This represents a neuro-gliogenesis model in which the progenitors do not express typical NB markers but instead molecularly resemble GMCs of type I NBs. However, unlike GMCs, the common progenitors have high mitotic potential and divide to generate two groups of precursors of restricted gliogenic or neurogenic potential. We show that this binary fate choice is regulated by Notch activity such that the

gliogenic lineage is Notch^{ON} and the neuronal lineage is Notch^{OFF}. The gliogenic precursors generate eg/mg that migrate to the lamina. Because of their origin from the GPC areas, Lawf neurons also need to migrate tangentially over substantial distances from their dorsal (Lawf2) or ventral (Lawf1) origins to evenly populate the medulla cortex. This migratory neuronal behavior, which is widespread in mammals (Rakic, 1988; Noctor et al., 2001), is rare in *Drosophila*. Along with the common progenitors of glia and neurons that resemble precursors in mammals (Gaiano and Fishell, 2002; Park and Appel, 2003; Park et al., 2004; Namihira et al., 2009; Petryniak et al., 2007; Rowitch and Kriegstein, 2010; Matsumoto et al., 2011), this suggests that formation of neural circuits from distant progenitors share multiple common features between vertebrates and invertebrates.

RESULTS

Specific Expression of Transcription Factors in Lawf Neurons

To study the developmental origin of Lawf neurons, we performed an antibody screen at larval and adult stages to identify Lawf-specific markers throughout development. Hth was previously identified as a marker of Lawf1 and Lawf2 neurons (Hasegawa et al., 2011) (Figures 1C and 1E). However, as a temporal transcription factor, Hth is also expressed in many other neurons in the medulla cortex (Li et al., 2013; Suzuki et al., 2013). We identified two Lawf-specific TFs: Lim1, a LIM homeodomain protein (Lilly et al., 1999; Roignant et al., 2010), and Eyes absent (Eya), a member of an evolutionarily conserved set of nuclear transcription cofactors involved in retinogenesis (Bonini et al., 1993). Eya is expressed early in both larval Lawf1 and Lawf2 neurons and is maintained in most of these neurons in the adult (Figures 1B, 1D, 1G, S1A, and S1B), but it is absent from a subpopulation of adult Lawf2 neurons (arrowheads in Figures 1D′ and S1B′). Lim1 is only expressed in Lawf2 neurons (Figures 1D and S1). These Lawf-specific TFs are expressed early in the larval brain and allowed us to track the development of Lawf cells. Additionally, we used two specific Gal4 lines to mark adult Lawf neurons: 1118-Gal4 for Lawf1 (Morante and Desplan, 2008) and 11D03-Gal4 for Lawf2 (Tuthill et al., 2013).

During the third larval instar (L3), Hth^{+} neurons are generated from newly born medulla NBs and are pushed deep inside the medulla as additional neurons are born from older NBs. Thus a crescent of Hth^{+} neurons (Figures 1F and 1G) occupies the deepest layer of the medulla (Li et al., 2013; Hasegawa et al., 2011; Suzuki et al., 2013). Both Lawf1 and Lawf2 neurons are found in the deepest layer of the Hth^{+} crescent (Figures 1F and 1G), with Lawf1 neurons (Hth^{+} Eya^{+}) in the ventral arm and Lawf2 neurons (Hth^{+} Eya^{+} Lim1^{+}) in the dorsal arm (Figures 1F and 1G), below the Optix regions of the neuroepithelium.

Lawf Neurons Migrate to Populate the Medulla Cortex during Pupation

In contrast to their restricted ventral or dorsal location in the larval brain, Lawf1 and Lawf2 neurons are found throughout the medulla cortex in the adult. This redistribution occurs at early pupal stages (see below). We used 17C11-Gal4 (Li et al., 2014; Tuthill et al., 2013), which is expressed in both Lawf1 and Lawf2 from larval to adult stages (Figures 1H–1L), to track Lawf migration during pupation. At prepupal stages, Lawfs start moving in opposite directions toward each other from their ventral or dorsal locations (Figure 1I). One day after

pupation, Lawf1 and Lawf2 populations are already intermingled in the medulla cortex (Figure 1J). A number of Lawf2s lose Eya expression at this stage (Figures 1J and J′, arrows). Two days after pupation, Lawf1 and Lawf2 have already established their distinctive arborizations throughout the medulla neuropil and in the lamina (Figure 1K).

Lawf Neurons Are Not Generated by Medulla Neuroblasts

Hth is expressed in the OPC neuroepithelium and in the youngest medulla NBs (Li et al., 2013). Early-born medulla neurons inherit Hth expression. Thus, based on their position in the larval medulla, and their expression of Hth, it was tempting to hypothesize that Lawfs are the first-born neurons from NBs in the Optix medulla region. Accordingly, Lawfs should share the same lineage as all later-born medulla neurons from the same NBs. However, mosaic analysis with a repressible cell marker (MARCM) clones labeled by a ubiquitous Gal4 driver line (*tubP*-Gal4) in adult brains gave homogenous clones containing multiple Lawf1s (Figure 2A) or multiple Lawf2s (Figure 2B) and no other medulla neurons. This is not consistent with a medulla origin of Lawfs but instead argues that Lawfs arise from distinct dedicated precursors.

Lawf Neurons Are Generated from the Lamina Side of the OPC

To determine the developmental origin of Lawf neurons, we induced MARCM clones in L3 brains. Lawf neurons were never recovered in medulla NB clones in larval brains (Figures 2C and 2D), further arguing against the possibility of a medulla origin for these neurons. When Lawfs were recovered in clones, they mostly contained other Lawfs, consistent with what we observed in adult clones. These Lawf clones traced back to a few Hth^+ presumptive progenitor cells from the dorsal and ventral posterior tips of the lamina side of the OPC neuroepithelium (Figures 2E and 2F). To confirm these results, we performed G-trace experiments with *gcm*-Gal4 (Chotard et al., 2005), an enhancer trap line that recapitulates the expression pattern of the *glial cells missing* (*gcm*) gene required for general gliogenesis. *gcm* is expressed in the cells on the lamina side of the OPC neuroepithelium, in the main lamina region, and in the lateral side of the posterior tip regions (Figures 2H, 2J, and S2B). It is required for lamina neurogenesis (Chotard et al., 2005). *gcm-Gal4* expression overlapped with Eya, which is also strongly expressed in cells in this region (Piñeiro et al., 2014) (Figure S2B). *gcm-Gal4* G-trace marked Hth^+ Eya^+ cells that could be observed moving away from the lateral neuroepithelium and toward the region of the medulla where Lawfs are located at larval stages (Figure 2G). In agreement with these results, *gcm*-Gal4 memory trace in adult brains labeled the cell bodies and neuronal projections of both Lawf1 and Lawf2 neurons (Figures S2D and S2E). Thus, Lawf neurons are derived from the lamina side of the neuroepithelium. This was an unexpected finding as it indicates that, like the glia produced from the Gcm^+ GPC areas, which migrate to assume their positions in the lamina, Lawf neurons also arise from the same domain and then migrate substantial distances. To address this, we turned to live imaging of L3 eye-brain complex explants in which nuclear-GFP expression was driven by *gcm-Gal4* (Movie S1). In addition to lamina glia and Lawfs, *gcm-Gal4* also drives expression in lamina precursors, lamina neurons, and a subset of medulla glia (Chotard et al., 2005). Strikingly, we observed Lawfs and lamina glia emerging from regions posterior and adjacent to the lamina, the presumptive GPC areas, and migrating along two separate paths, one for lamina glia and one for Lawfs (Movie S1).

Next, we set out to identify which regions along the dorsoventral axis of the lamina neuroepithelium the Lawfs come from. The tips of the OPC, which are characterized by the expression of Rx (T.E., unpublished data), are composed of a domain of Dpp expression that lies adjacent to the posterior-most domain that expresses Wg (Dearborn and Kunes, 2004; Kaphingst and Kunes, 1994). Since *gcm-Gal4*, which marks all Lawfs and lamina glia, widely overlaps with the Dpp region but only slightly with the Wg domain (which does not express the Lawf marker Hth) (Figure 2H), we used *dpp-Gal4* and *dpp-lacZ* lines to test the origin of Lawf neurons. *dpp-lacZ* marks Eya^+ cells that delaminate from the Dpp regions of the lamina neuroepithelium (Figure 2K). Consistent with this observation, *dpp-Gal4* G-trace marked most Lawf cells (Figure 2L). Finally, to confirm that Lawfs are not produced by medulla NBs, we performed G-trace experiments with *MzVum-Gal4*, which is expressed in the Vsx and Optix neuroepithelial regions in early L3 brains, but these did not label any Lawf cells (Figure S2C). Together these results indicate that Lawf1 and Lawf2 neurons do not originate from medulla NBs. Instead, they emerge from the lamina side (inner rim) of each of the two Dpp regions at the tips of the OPC neuroepithelium (Figure 2M).

We posited that one tip gives rise to Lawf1s and the other to Lawf2s. We performed G-trace experiments with *hh-Gal4*, which is expressed early in the ventral half of the OPC neuroepithelium (T.E., unpublished data; Evans et al., 2009) (Figure S2A). This only labeled Lawf1 cells, and not the $Lim1^+$ Lawf2 cells, indicating that Lawf1 cells originate from the ventral OPC and Lawf2 cells originate from the dorsal OPC (Figure 2I).

Together, these results indicate that Lawfs have surprising origins: Lawf1 neurons are born from the lamina side of the neuroepithelium in the ventral Dpp region, while Lawf2s are born from the equivalent dorsal region. They undergo two steps of migration: during L3, they migrate tangentially over long distances from their posterior places of origin along the deepest layers of the medulla toward the anterior. However, they stop below the Optix regions without intermingling. Later at pupal stages, both groups of Lawfs cells move to the medulla cortex and intermingle to assume their uniform broad distribution throughout the medulla.

Lawf and Marginal/Epithelial Glia Are Generated by a Common Progenitor

The *gcm* gene is required for gliogenesis in *Drosophila* and is a very early marker for lamina glial specification under the control of the Dpp pathway (Dearborn and Kunes, 2004; Yoshida et al., 2005). Since Lawfs originate from the same regions as the lamina glia and express the glial marker *gcm-Gal4*, we wondered whether Lawfs and lamina glia could share the same lineage. To test this hypothesis, we used specific glial and neuronal markers, proliferation markers, and live imaging to analyze the *10C12-Gal4* line (Li et al., 2014), which exhibits strong expression during L3 in Lawf neurons and in lamina neuropil glia, eg, and mg (Figures 3B–3D). Strikingly, *10C12-Gal4* also marked a population of progenitor cells that delaminate from the lamina neuroepithelium to form a small aggregate under it (Figures 3E and 3D). From this aggregate of cells, Lawfs and lamina glia developed and migrated as two independent streams of cells (Figures 3D and 4B–4D; Movie S2). The Lawf cells first migrated proximally a short distance from the progenitor cell region and then tangentially toward the anterior, along the deepest layer of medulla neurons to reach their

positions in the medulla cortex (Figures 4C and 4D; Movie S1). Glial cells migrated directly anteriorly into the lamina (Figure 4D; Movie S2). During migration, the glial population gradually turned off Eya expression and activated the expression of Optix, a marker for lamina eg and mg (Figures 3D–3D″ and S3A).

We named the progenitor cells that generate both Lawf neurons and lamina glia (both eg and mg) "common progenitor cells." They did not express Deadpan (Dpn), a universal NB marker (Figure 3F). Instead, Prospero (Pros) was turned on while cells were still in the neuroepithelium and Asense (Ase) was activated when cells delaminated (Figure 3G). Ase was later turned off in Lawf and glial progeny, while Pros was maintained (Figure 3G). The lack of Dpn expression suggests that these cells are not canonical NBs. Instead, Ase and Pros expression suggests that these common progenitor cells resemble GMCs that could represent a distinctive example of neuroepithelial cells that delaminate, bypass the NB stage, and transform directly into GMC-like cells.

To determine whether the common progenitor cells could instead resemble LPCs, we examined the expression of several lamina-specific markers. The common progenitor cells emerge in the Rx/Dpp region of the neuroepithelium while LPCs are only produced in the Vsx1 or Optix regions of the neuroepithelium innervated by photoreceptors. Tll and Dachshund (Dac), which mark LPCs (Figures S3B and S3E), were not expressed in the common progenitors of Lawfs and lamina glia (Figures S3C–S3E). In contrast, the common progenitor cells expressed Ase and Pros, while LPCs did not (Figure S3F). Thus, Lawf/eg/mg common progenitor cells do not resemble LPCs.

Two Subgroups of Mitotically Active Precursors Generate Lawfs and Lamina Glia

The common progenitor cells together generate a large number of Lawf1s and Lawf2s (~140 per optic lobe for Lawf2) (Tuthill et al., 2014) and even more lamina glia (~800 per optic lobe; Edwards et al., 2012). To gain insight into the lineage, we examined cell division patterns using the mitotic marker phospho-histone 3 (PH3) as well as live imaging. Whereas neuroepithelial cells actively divided (Figure 3H, arrowheads), delaminating $Pros^{+}$ cells usually did not divide (Figure 3H, open arrowhead). However, after delamination, $Pros^{+}$ cells regained high mitotic activity (Figure 3H, arrows). Indeed, several rapid mitotic events were observed in the common progenitor region in time-lapse images (Movie S2; Figure 4A, arrowhead and dashed circle). Mitoses were also observed in the two bifurcating groups of cells that become lamina glia and Lawfs, respectively. Consistent with previous observations (Perez and Steller, 1996; Winberg et al., 1992; Chotard et al., 2005), our PH3 labeling showed active division of migrating and of settled lamina glia (Figure 3I). These cells expressed the glia-specific marker Repo, suggesting that they are dedicated glial precursor cells that maintain mitotic activity after glial fate specification to amplify the lamina glial population. In contrast to the widespread mitoses in the glial sub-lineage, mitoses in the Lawf sub-lineage only occurred adjacent to the common progenitor region (Figures 3I and 4A; Movie S2). Note that while the terminal mitosis of Lawf precursors could be detected in time-lapse images, the mitoses in the glia sub-lineage could not be accurately tracked through time, due to cell crowding in the lamina glial layers. Once the Lawf neurons started

migrating in the medulla, the postmitotic neuronal marker Elav (Robinow and White, 1991) was turned on (Figure 1G).

We propose that there are at least two phases of cell division in the development of Lawfs and lamina glia (Figure 3K). First, delaminated common progenitors divide and generate two classes of more restricted precursor cells for either Lawf or glia. Later, these restricted precursors divide again to generate Lawf neurons or lamina glia. The mitotic activity of the Lawf sub-lineage is lower than the glial sub-lineage, explaining the difference in their population sizes.

The Notch Pathway Instructs the Fate Choice of Restricted Precursors

How are the two types of precursors that are generated by the common progenitor cell restricted to either gliogenic or neurogenic potential? The Notch pathway is often implicated in binary fate choices, especially during neurogenesis (Bertet et al., 2014; Li et al., 2013; reviewed in Cau and Blader, 2009). We induced MARCM clones (labeled with 10C12-Gal4) to mark cells that lack Notch activity because of a $Su(H)^{\Delta 47}$ (*Suppressor of Hairless*) mutation (Morel and Schweisguth, 2000) or cells with elevated Notch pathway activity due to the $numb^{15}$ mutation (Berdnik et al., 2002). In the larva, wild-type control clones in the dorsal or ventral progenitor regions included both gliogenic and neuronal precursors (Figure 5A); clones examined in the target area of migration contained both mature Lawf neurons and lamina glia (Figure 5D). However, the lamina glia marker Optix was absent in $Su(H)^{\Delta 47}$ clones, and only Lawf neurons were recovered (Figures 5B and 5E). In contrast, elevated Notch activity in $numb^{15}$ mutant clones resulted in the loss of Lawf neuronal fate, while glia were still recovered (Figures 5C and 5F). Together, these data suggest a Notch-dependent binary fate choice where gliogenic precursors are $Notch^{ON}$ and neuronal precursors are $Notch^{OFF}$ (Figure 5L).

The Two Sub-lineages Have Different Requirements for Photoreceptor Input

We have shown that Lawf neurons and lamina eg/mg share common progenitors and both exhibit migratory behavior. Although photoreceptors do not innervate the regions where they are born, the proliferation, differentiation, and migration of lamina glial precursors require an as-yet-unidentified long-range cue from photoreceptors that involves the *Drosophila* homolog of *Jun-activation-domain binding protein 1 (Jab1)* (Dearborn and Kunes, 2004; Huang and Kunes, 1998; Perez and Steller, 1996; Suh et al., 2002). Therefore, we asked whether the common progenitor cells and their subsequent development and migration as Lawfs also depended on photoreceptor input.

To test this, we used $Lobe^{si}$ mutants that lack the ventral portion of the eye (Chern and Choi, 2002). In $Lobe^{si}$ mutants, the ventral third of the lamina lacks photoreceptor innervation and LPCs fail to divide (Figure 5G). Lamina eg/mg glia are also absent in the ventral third of the lamina (Figures 5H–5J). Instead, a small number of cells that express both Optix and Eya remain very close to the ventral Dpp neuroepithelial region, where lamina eg/mg glia originate (Figures 5H–5J). The expression of Optix suggests that these cells have features of lamina glia precursor cells. However, Eya expression (which should be turned off quickly in glial lineage) indicates that they still retain characters of common progenitors. Although

photoreceptor input to the main part of the lamina is required for the migration and proliferation of lamina glia (Perez and Steller, 1996), our results show that gliogenesis is also impaired in newborn glial precursor cells after they are specified by Notch. In contrast, the Lawf lineage was not affected by loss of photoreceptor axons, as the number of ventral Lawf neurons and their migration to the medulla appeared unaffected in *Lobe*^{si} mutants (Figure 5K).

DISCUSSION

A Unique Class of Neural Progenitors

Two distinct modes of neurogenesis have been described for the OPC. On the medulla side, neuroepithelial cells are transformed into NBs that express Dpn and Ase (Egger et al., 2007, 2010; Li et al., 2013). On the lamina side of the Optix and Vsx regions, neuroepithelial cells generate post-mitotic LPCs that express Dac (Huang and Kunes, 1996) (Figure S3E) and Tll (Kurusu et al., 2009) in response to signals from ingrowing photoreceptor axons, which also trigger LPC differentiation into lamina neurons (Huang and Kunes, 1996; Huang et al., 1998). Lawf1 and Lawf2 multi-columnar neurons express Hth and have their cell bodies in the medulla and were thought to originate from medulla NBs in the Hth tTF window (Hasegawa et al., 2011). However, here we show that Lawf neurons are not produced by medulla NBs but instead arise from a unique class of progenitors with both gliogenic and neurogenic potential from the lamina (lateral) side of the Dpp regions. These common progenitors give rise to eg/mg, which populate the lamina, as well as Lawfs. Because Lawfs originate from regions far from their final location, they need to migrate anteriorly to first reach the deepest neuronal layer in the dorsal and ventral Optix regions of the medulla. Later in development, both Lawf1 and Lawf2 neurons intermingle and evenly populate the whole medulla cortex. Our observation of distinct and non-medullary origins for Lawf1 and Lawf2 neurons is also supported by clones containing either multiple Lawf1s or multiple Lawf2s in a recent publication (Nern et al., 2015).

The common progenitor cells described here are unlike NBs or neuroglioblasts previously described in *Drosophila*. Like canonical NBs, ventral nerve cord neuroglioblasts (e.g., NB6-4T) express Dpn, but they do so only in the neuroblast hemi-lineage and not in the glioblast hemi-lineage (Bernardoni et al., 1999). In contrast, the common progenitor cells we describe here never express Dpn but instead express the GMC markers Ase and Pros. Whereas GMCs commonly have very restricted mitotic potential, the glial/Lawf common progenitor cells exhibit extensive mitotic activity and generate two populations of precursors with restricted gliogenic or neurogenic potential. These two populations of restricted precursors exhibit different levels of mitotic activity and generate progeny of different fates. Thus the common progenitors share some resemblance with the transit-amplifying INPs of type II NB lineages of the central brain (Bayraktar et al., 2010; Boone and Doe, 2008). However, mature INPs express Dpn together with Ase and Pros. Common progenitor cells also do not resemble LPCs, as they lack LPC-specific Dac and Tll marker expression. Thus, the Lawf and lamina glia common progenitors represent a unique class of progenitor cells in the *Drosophila* optic lobe.

Notch-Mediated Gliogenesis

In *Drosophila*, glia can be generated by dedicated glioblasts, by glial precursor cells as part of a larger neuro-gliogenesis lineage (reviewed in Jones, 2001), by terminal GMC divisions into a glial cell and a neuron, or at the terminal division of NBs at the end of their life (Li et al., 2013). The involvement of the Notch pathway in promoting gliogenesis is well documented in *Drosophila* (reviewed in Gaiano and Fishell, 2002), for example, in the GMC divisions of the ventral nerve cord NBs 1-1A (Udolph et al., 2001), which produce neuron-glia siblings, and in the embryonic CNS progenitors, which produce midline glia (Wheeler et al., 2008). In contrast, during sensory organ precursor development, Notch plays different roles depending on the context. For example, Notch represses glial precursor fate in pIIB sibling progeny (Van De Bor and Giangrande, 2001; Gho et al., 1999) while promoting glia fate in the dorsal bipolar dendritic (dbd) sensory lineage in the embryonic peripheral nervous system (PNS) (Umesono et al., 2002).

In vertebrates, neural progenitors often generate neurons before switching to producing glia. The Notch pathway is also involved in the neuron-to-glia fate determination to regulate neurogenesis versus gliogenesis potential (Gaiano and Fishell, 2002; Rowitch and Kriegstein, 2010; Taylor et al., 2007). This regulation of gliogenesis by Notch has important medical implications since Notch-induced premature gliogenesis may underlie Hirschsprung disease, the most common neurocristopathy in humans (Ngan et al., 2011).

In the zebrafish spinal cord, oligodendrocyte progenitor cells and motor neurons are generated from the same precursor cells in ventral domain of motor neuron precursors (Park et al., 2004), where Notch positively regulates $Olig2^{+}$ oligodendrocyte progenitor cell specification (Park and Appel, 2003; Park et al., 2004; Snyder et al., 2012). Similarly, in the mouse brain, oligodendrocytes and GABAergic interneurons appear to share a common pool of ventral progenitor cells. These progenitor cells have mutually exclusive fate restriction to become either neurogenic precursors or gliogenic precursor cells (Petryniak et al., 2007). Increased Notch activity reduces neuron numbers and increases the oligodendrocyte population (Hoeck et al., 2010; Matsumoto et al., 2011). Thus, the Notch-dependent binary fate choice for precursors of gliogenic potential over neurogenic potential identified here resembles modes of gliogenesis in vertebrates.

Neuronal Migration in the Fly Brain

To date, no instance of postmitotic neuronal migration has been documented in the *Drosophila* brain. In contrast, neuronal migration has been studied extensively in vertebrates. Cortical neurons migrate radially from proliferative areas to produce neocortical layers (Rakic, 1988; Noctor et al., 2001). Interneurons migrate tangentially to distinct target tissues in the brain where they adopt different cell fates (Harwell et al., 2015; Mayer et al., 2015).

Here we show that Lawf neurons born in dorsal (Lawf2) or ventral (Lawf1) regions from the lamina side of the Dpp neuroepithelium, i.e., far from their final position, need to migrate tangentially for relatively long distances to reach their destination in the medulla cortex and contribute to medulla and lamina neural circuits. In the lamina, neurons and glia work in tight coordination for early steps of motion detection: photoreceptors send visual signals to

lamina neurons that relay them to the medulla (Behnia et al., 2014; Rister et al., 2007); Lawf neurons collect feedback from the medulla to modulate lamina neuron activity (Tuthill et al., 2014). Lamina epithelial and marginal glial cells tightly wrap photoreceptor synapses and lamina neuron projection in the lamina neuropils and serve to support neuronal function (Chaturvedi et al., 2014). Despite their close functional relationships, these cells have very different developmental origins and lineages. We also recently reported that medulla NBs from the Wg-expressing region of the OPC neuroepithelium contribute different neuron types to layers of several neuropils (Bertet et al., 2014). However, their migration was not documented. Thus, as is the case for vertebrate nervous systems, *Drosophila* neuronal cells contributing to a specific circuit need not to be related by lineage. Neural circuit formation strategies in vertebrate and insect brains might share more similarities than previously thought.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Common progenitor cells generate Lawf neurons and lamina glia in bifurcating lineages
- Common progenitor cells differ from typical *Drosophila* neuroblasts
- Notch regulates the decision between lamina glial precursors and Lawf neuronal precursors
- Lawf neurons migrate over long distances to assume their final positions in the medulla

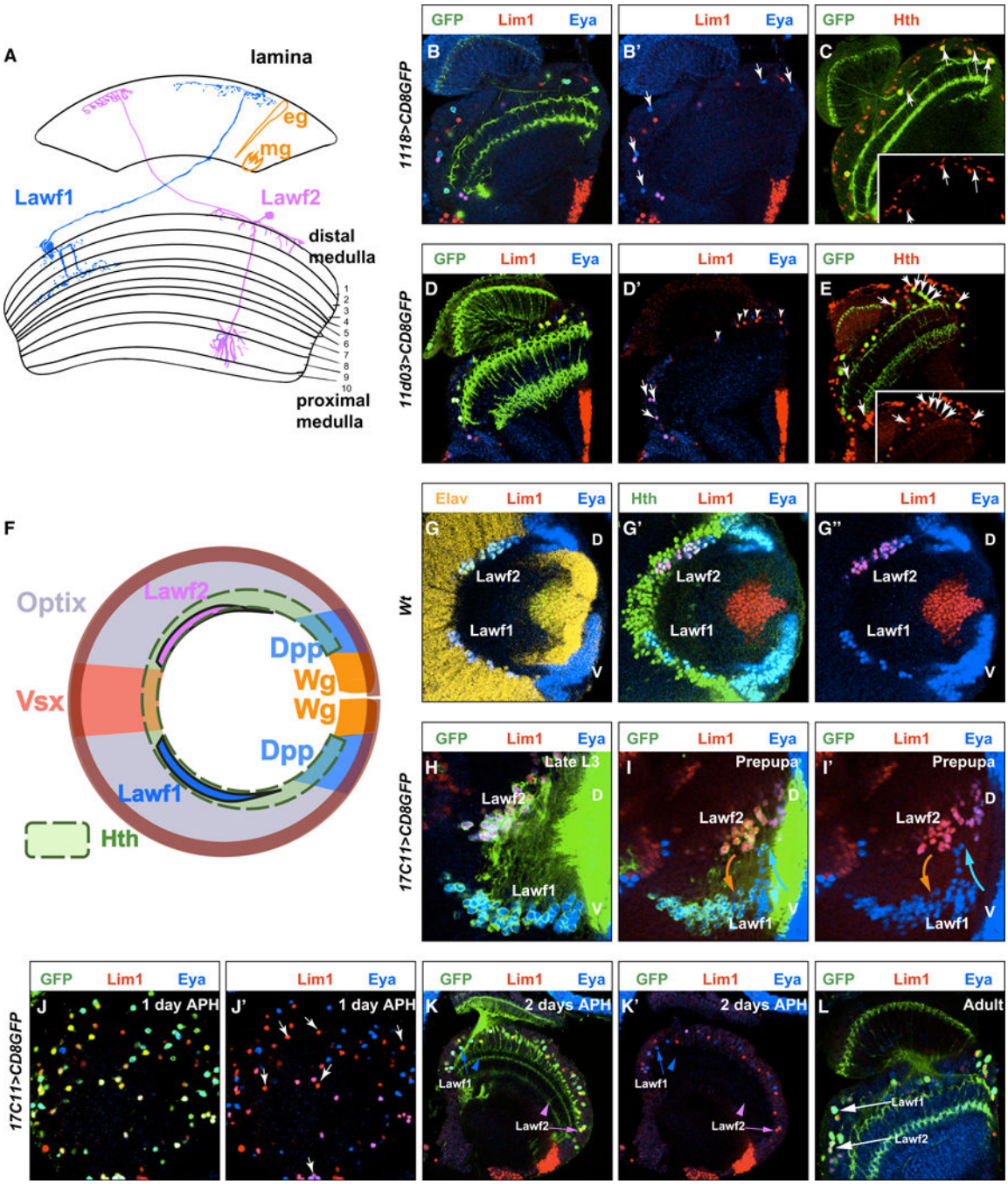

Figure 1. Lamina Wide-Field Neurons Express Specific Transcription Factors

(A) Lamina wide-field neurons have cell bodies in the medulla cortex and arborizations in both the lamina and medulla neuropils. Lawf1 cells (blue) project to layers M1 and M3 of the medulla, whereas Lawf2 cells (magenta) project to M1 and M9. The locations of adult epithelial glia (eg) and marginal glia (mg) are also shown. Adapted from Tuthill et al. (2013) and Edwards et al. (2012).

(B and C) 1118-Gal4 drives expression in Lawf1 neurons in adults. Lawf1s express Eya (blue in B) and Hth (red in C) but not Lim1 (red in B).

(D–E) 11D03-Gal4 drives expression in Lawf2s in adults (green). Lawf2 neurons express Lim1 (red in D) and Hth (red in E). While most Lawf2 neurons express Eya (arrows, magenta cells in D), some do not (arrowheads in D and D′).
(F) Schematic drawing of larval optic lobe and Lawf positions (sagittal cross section).
(G) In larval brains, Lawf1 neurons express Hth (green) and Eya (blue); Lawf2 neurons express Lim1, Hth, and Eya.
(H–K′) 17C11-Gal4 drives expression in both Lawf1s and Lawf2s. (H) In a later larval brain, all Lawf neurons are Eya^{+}. (I) At the prepupal stage, the two Lawf populations move toward each other (curved arrows). (J) One day into pupation, $Lim1^{+}$ Lawf2 and $Lim1^{-}$ Lawf1 populations migrate to populate the entire medulla cortex and intermingle with each other. Eya is lost in some Lawf2 neurons (arrows). (K) Two days into pupation, Lawf1 and Lawf2 populations form their arborization patterns in the lamina and medulla neuropils. 17C11-Gal4 expression is reduced in Lawf2s. Blue arrow points to a Lawf1 cell body. Blue arrowhead points to Lawf1 projections in the M3 layer. Magenta arrow points to a Lawf2 cell body. Magenta arrowhead points to Lawf2 projections in the M3 layer.
(L) In the adult, 17C11-Gal4 labels Lawf1 strongly and Lawf2 weakly.
See also Figure S1.

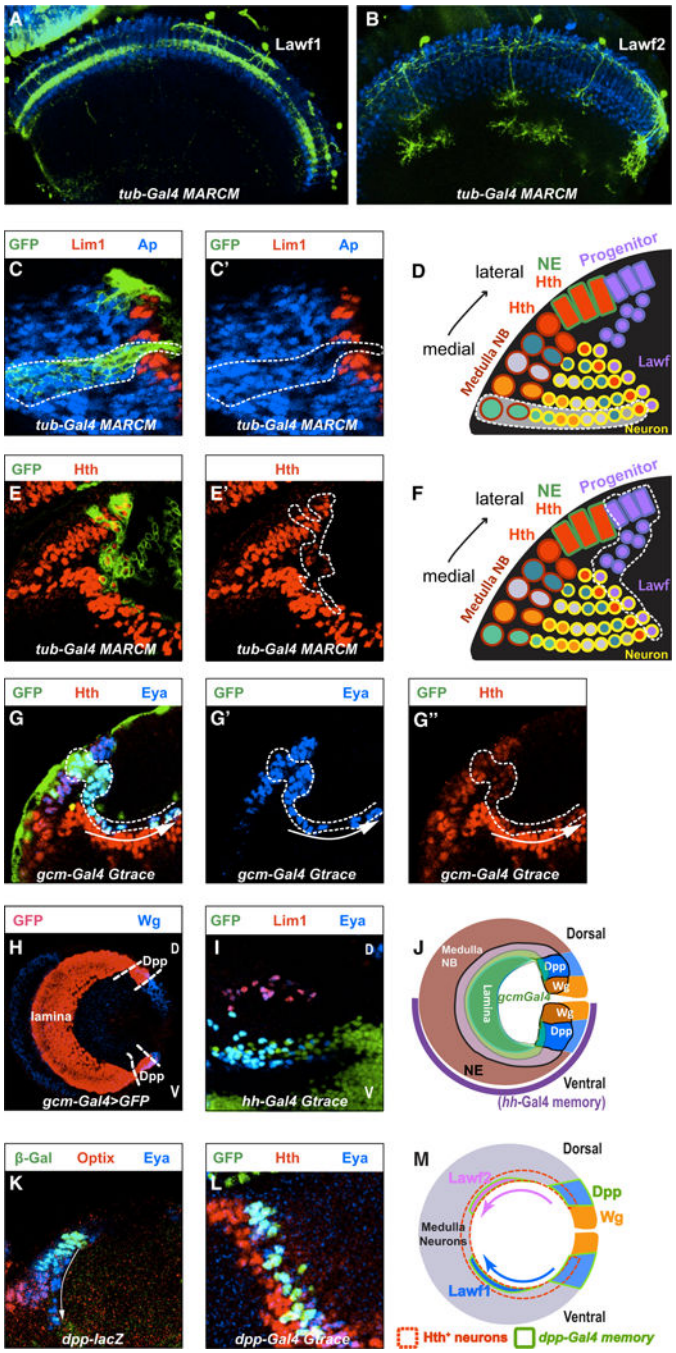

Figure 2. Lawf Neurons Are Generated in the Lamina Side of the OPC Neuroepithelium in Dpp Regions

(A and B) Randomly induced MARCM clones marked by *tub*-Gal4 label homogeneous groups of Lawf1 (A) or Lawf2 (B) neurons in adults.

(C) Apterous (Ap, blue), expressed in half of medulla neurons (Li et al., 2013), is not expressed in Lawf2 Lim1^{+} neurons (Lim1, red) in a medulla NB clone.

(D) Schematic representation of medulla and Lawf neurogenesis. Medulla NBs are generated from the medulla side of the neuroepithelium and progress through a tTF series

beginning with Hth. Hth is also expressed in neuroepithelium cells before they become NBs. Lawf neurons are not generated by medulla NBs (dashed line outlines a medulla NB clone).
(E) Clone from the lamina side of the OPC neuroepithelium generates progeny into the medulla neuron region. Progeny maintain Hth and Eya expression, confirming their Lawf identity, and migrate into the medulla neuropil.
(F) Schematic representation as in (D) showing a neuroepithelial clone generating Lawf progeny (dashed line).
(G) *gcm-Gal4* G-trace where GFP marks Gal4 history. Lawf neurons (Eya^+ and Hth^+) originate from precursor cells in the lamina side of the neuroepithelium and migrate to the medulla (arrow shows direction of migration).
(H) *gcm-Gal4* drives expression on the lamina side of the OPC neuroepithelium of the main lamina region and *dpp* regions in the dorsal and ventral tips. The expression of gcm-Gal4 overlaps slightly with Wg regions.
(I) Lawf1 cells are marked by the ventral-specific *hh*-Gal4 driving G-trace. Lawf2 cells (magenta) from the dorsal OPC are not marked by *hh*-Gal4 > G-trace.
(J) Schematic representation of (H) and (I).
(K) Eya^+ Lawf precursor cells delaminate from the neuroepithelium region marked by *dpp*-lacZ (arrow shows the direction of delamination).
(L) Lawf neurons in medulla marked by *dpp-Gal4* driven G-trace.
(M) Schematic representation of Lawf1 origin from the ventral Dpp (Gcm) region and Lawf2 origin from the dorsal Dpp (Gcm) region.
See also Figure S2 and Movie S1.

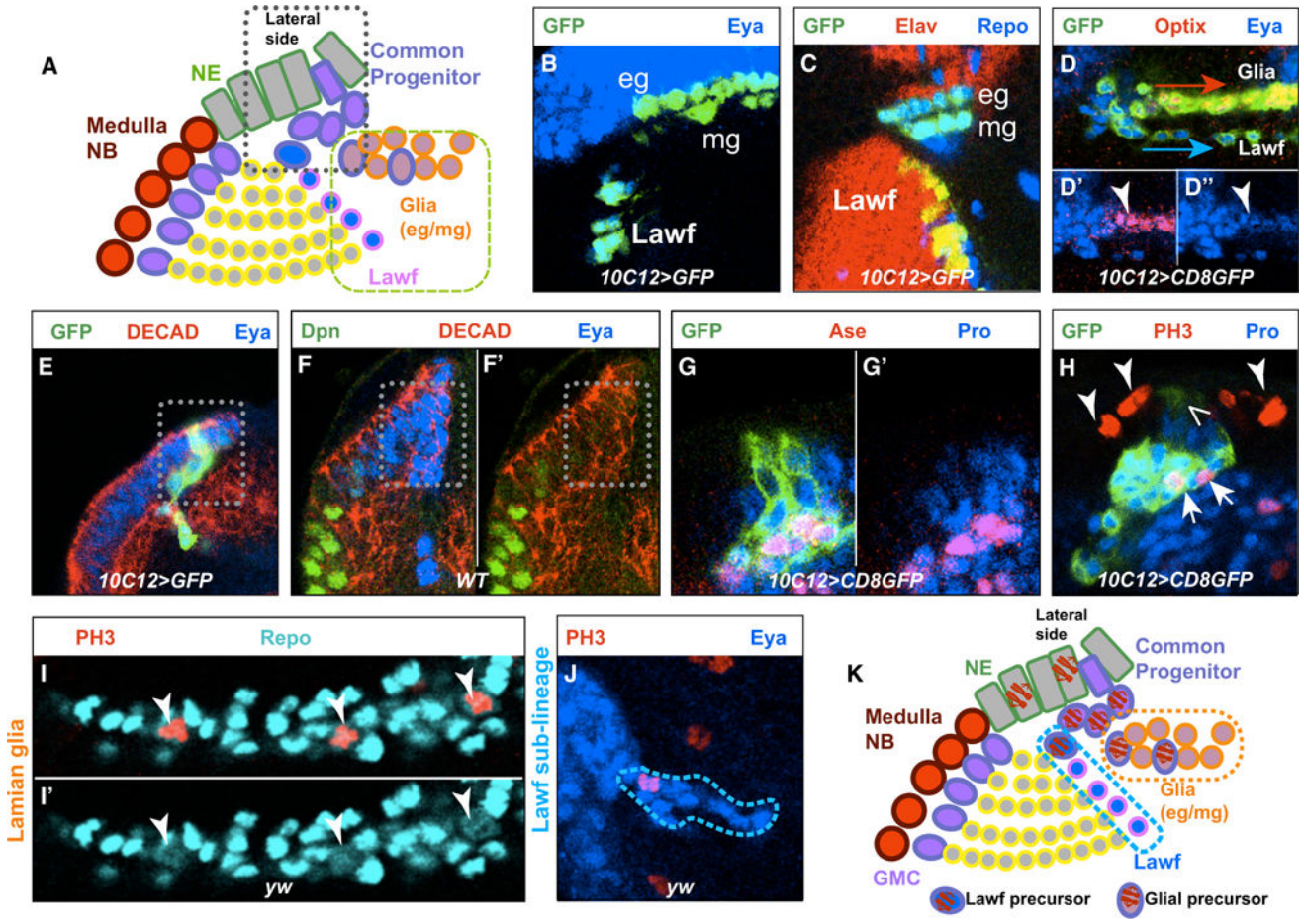

Figure 3. Lawf Neurons, Lamina Epithelial, and Marginal Glia Share Common Progenitor Cells

(A) Schematic showing delaminating neuroepithelial cells in the GPC region giving rise to common progenitors (gray dashed line) that generate Lawf neurons and epithelial (eg) and marginal glia (mg; green dashed line)

(B and C) Lawf neurons (Eya^+, $Elav^+$) and lamina eg and mg cells ($Repo^+$) are labeled by *10C12-Gal4* driving UAS-GFP.

(D) Progenitors marked by 10C12-Gal4-driven GFP generate both Lawfs (blue arrow) and Lamina glia (eg/mg; red arrow). Arrows indicate migratory routes. In the glia sub-lineage, Optix expression is turned on (D′), while Eya expression is reduced (D″). Arrowheads mark cells that coexpress Eya and Optix.

(E) 10C12-Gal4 drives GFP in Eya^+ progenitor cells delaminating from the neuroepithelium stained with anti-DE-cadherin (DECAD). Gray dashed line shows the location of the common progenitor cells.

(F) Staining of the NB marker Dpn and neuropil marker DE-cadherin (DECAD) in the progenitor delamination region of neuroepithelium. Eya marks delaminating common progenitor cells. Gray dashed line shows the location of the common progenitor cells.

(G) Ase and Pros staining in common progenitor cells (10C12-Gal4) indicate resemblance with GMCs. Pros is expressed when cells are still in the neuroepithelium, whereas Ase is expressed in common precursor cells after delamination.

(H–J) Mitotic activity (PH3) in Lawf and glia sub-lineages. (H) Proliferating cells marked by PH3 staining in the neuroepithelium (arrowheads) and common progenitor cells (arrow). (I and I′) A number of Repo^{+} glial cells still maintain mitotic activity during migration. (J) Rare mitotic events are detected in the Lawf sub-lineage.

(K) Model of Lawf and lamina glia (eg/mg) origin from common progenitor cells with their mitotic potential indicated.

See also Figure S3 and Movies S1 and S2.

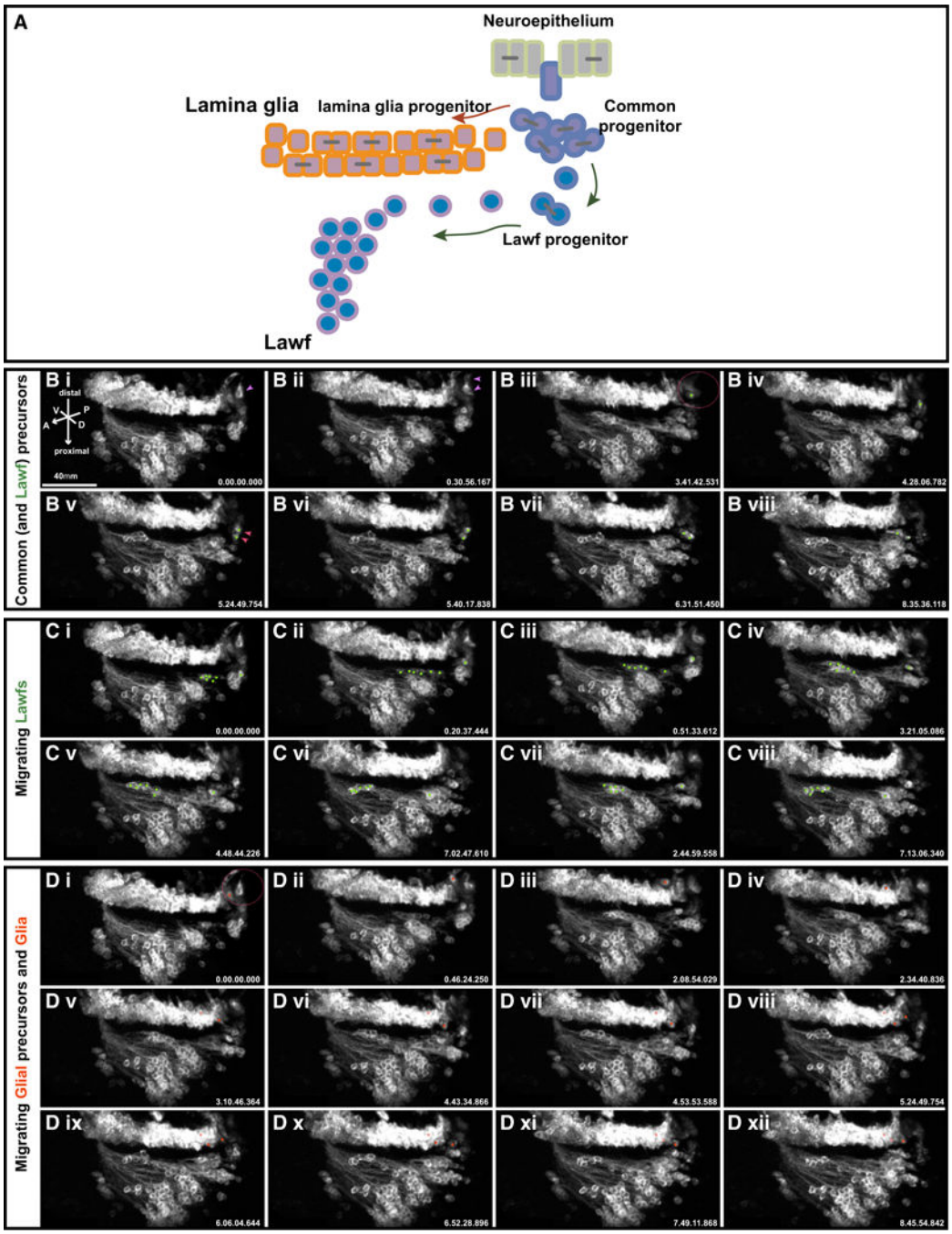

Figure 4. Live Imaging of *10C12>CD8GFP* Reveals the Origin and Mitotic Potential of Lawf Neurons

(A) Diagram of L3 brains showing common progenitor cells (on the dorsal side), Lawf progenitors, and committed Lawfs and lamina glial cells along with their mitotic potential. Lawf and glial lineage migratory routes are indicated by arrows.

(B–D) Still images captured from time-lapse two-photon imaging of the same late L3 *10C12>CD8GFP* eye-brain complex explant. 10C12-Gal4 drives GFP expression in all epithelial and marginal glia and in all Lawfs. The complete dorsal side of the lamina glial layers and Lawf cells are visible along with a small portion of the ventral region. The

orientation of the optic lobe is labeled in (B.i) and matches (A). Lawfs and their precursors are marked by green dots and glial and their precursors are marked by red dots. Time is displayed in hours: minutes: seconds: milliseconds. Scale bar, 40 μm. (B.i and B.ii) A presumptive precursor in the most posterior dorsal region undergoes mitosis (arrowhead). This region is defined as the common progenitor region (dashed circle) and is populated by mitotically active cells (refer to Movie S2).

(B.iii and B.iv) A restricted neuronal precursor destined to take on Lawf fate (green dot) migrates proximally from the common precursor region and (B.v) undergoes mitosis, (B.vi–B.viii) resulting in two Lawf cells, which begin to migrate tangentially.

(C) A population of committed Lawfs (green dots) migrate tangentially over substantial distances from a starting point proximal to the common precursor pool. These cells appear to grow their axons during the course of migration but more prominently once they reach more anterior positions.

(D.i–D.iv) A cell (red dot) migrates to join the lamina glial layers from the precursor region (dashed circle) along a route distinct from the Lawfs to enter the epithelial glial layer from the distal side.

(D.v–D.xii) Two additional glia/glial precursors (D.v and D.viii) migrate shorter distances to join the presumptive marginal glial layer from the proximal side. Note that cells that are lost during the tracking process are changed to a lighter color and kept stationary.

See also Movie S2.

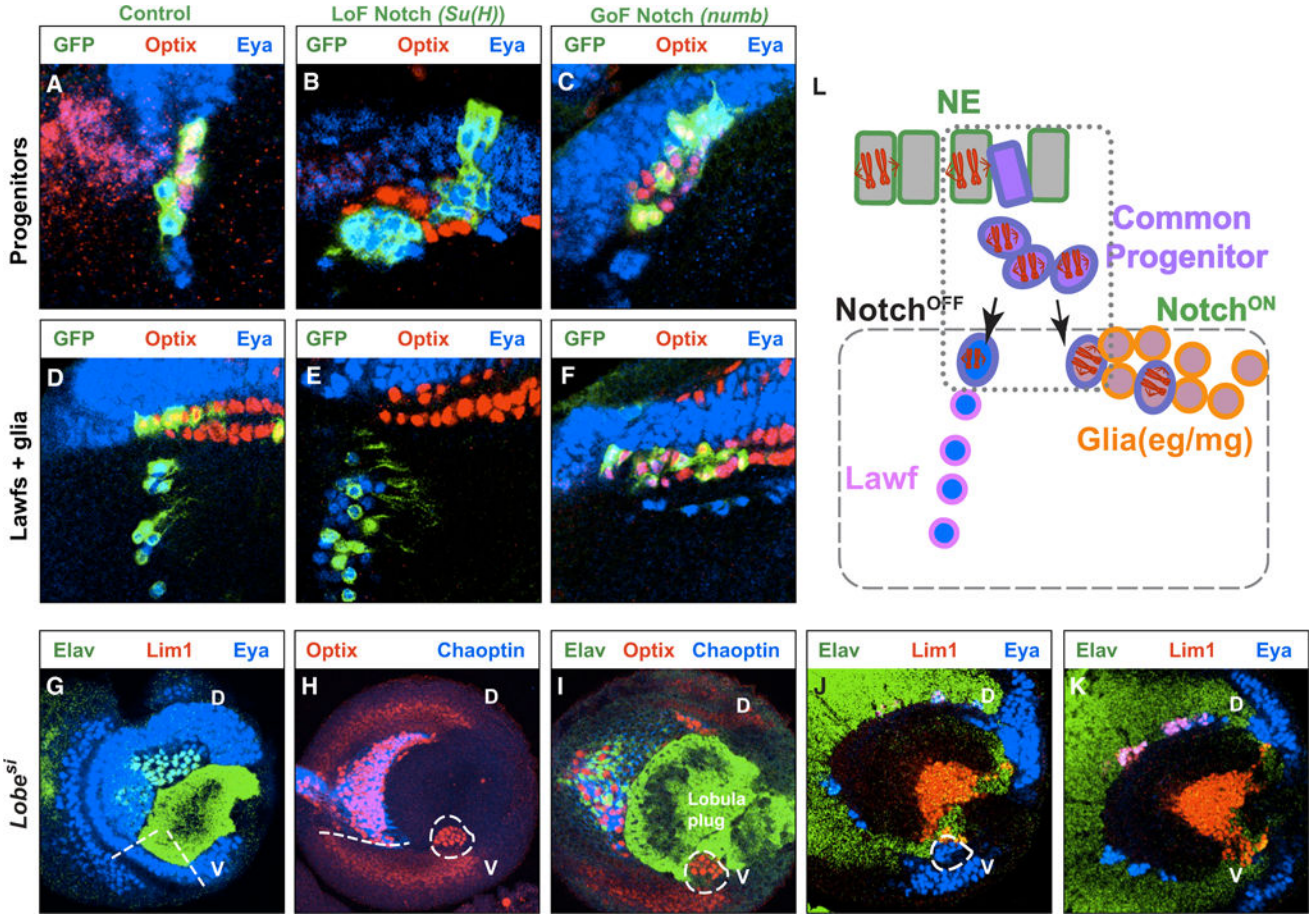

Figure 5. Notch-Dependent Binary Fate Choice between Lawf and Lamina Glial Precursors

(A–C) 10C12-Gal4 marked MARCM clones (control and mutant) in the common progenitor cells close to the neuroepithelium, and in committed Lawf and glial lineages (D–F). (A) A control 10C12 Gal4 clone contains both Lawf (Eya$^+$, blue) and Glia (Optix$^+$, red) sub-lineages. (B) In a *Su(H)* mutant clone, which contains Lawfs (Eya$^+$, blue), glial cells (Optix$^+$, red) are not recovered. (C) In a *numb* mutant clone, glial cells (Optix$^+$, red), but not Lawfs (Eya$^+$), are recovered.

(D) Control clone in a more anterior region of the optic lobe contains both Lawf and lamina eg/mg cells.

(E) *Su(H)* mutant cells develop into Lawf neurons as they mature but never include glia.

(F) Mature *numb* mutant cells develop into lamina glial cells.

(G–K) Brains of *Lobe^{si}* mutants (lateral view). Ventral photoreceptors do not develop in *Lobe^{si}*. (G) Ventral lamina precursor cells (region between the dashed lines) fail to proliferate and generate lamina. (H) Maximum projection. In *Lobe^{si}* mutants, the ventral lamina is absent and shows impaired ventral gliogenesis. Photoreceptor axons (Chaoptin, blue) and lamina glia (Optix, red) are only in the central and dorsal parts of the lamina (above the dashed line). (I) Ventral glia precursor cells form a small cluster of Optix$^+$ cells squeezed into the lobula plug region (dashed circle). (J) These clustered glial precursor cells are also Eya$^+$. (K) Lawf-specific markers are unaffected in *Lobe^{si}* mutant.

(L) Model for Notch dependent fate decision between Lawfs neurons and lamina glia (eg and mg).