Cellular Requirements
for Building a Retinal Neuropil

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SUMMARY

How synaptic neuropil is formed within the CNS is poorly understood. The retinal inner plexiform layer (IPL) is positioned between the cell bodies of amacrine cells (ACs) and retinal ganglion cells (RGCs). It consists of bipolar cell (BC) axon terminals that synapse on the dendrites of ACs and RGCs intermingled with projections from Müller glia (MG). We examined whether any of these cellular processes are specifically required for the formation of the IPL. Using genetic and pharmacological strategies, we eliminated RGCs, ACs, and MG individually or in combination. Even in the absence of all of these partner cells, an IPL-like neuropil consisting of only BC axon terminals still forms, complete with presynaptic specializations and sublaminar organization. Previous studies have shown that an IPL can form in the complete absence of BCs; therefore, we conclude that neither presynaptic nor postsynaptic processes are individually essential for the formation of this synaptic neuropil.

INTRODUCTION

Synaptic neuropil is a major component of nervous systems, yet how it forms in specific places is not understood. It consists of intermingled axonal, dendritic, and glial processes, which form a dense array of specialized cellular connections. In many brain regions, neuropils form discrete layers beside or between collections of highly connected neurons. In the vertebrate retina (Figure 1A) there are two major layers of neuropil: the inner plexiform layer (IPL) and the outer plexiform layer (OPL). These neuropils, which consist of sets of cell-type-specific synapses, are completely devoid of cell bodies and thus are ideal models for studying the formation of synaptic neuropil. The IPL is where bipolar cell (BC) axons synapse onto the dendritic processes of retinal ganglion cells (RGCs) and the neurites of amacrine cells (ACs). The retina contains a single type of intrinsic glial cell, called Müller glia (MG). MG span the retina and send extensive processes into the plexiform layers (Figure 1A). Within the IPL,

dendritic and axonal processes stratify within up to ten discrete sublaminae. This segregation relies on adhesive and repulsive guidance cues present in the IPL to guide them to their correct partner neurons (Matsuoka et al., 2011a, 2011b; Yamagata and Sanes, 2008, 2012; Yamagata et al., 2002). It is thought that for such interactions to occur, cues must be expressed either by synaptic partners or by other neurons or glia projecting within the neuropil (Timofeev et al., 2012; Matsuoka et al., 2011a). Therefore, it is important to identify the cell types that are critical for controlling the development of the neuropil layers.

One might hypothesize that the earliest-born cells, the RGCs, could organize a pre-IPL scaffold via their apically emerging dendrites. However, in *ath5 (atoh7)* mutants, RGCs are absent, yet the IPL still forms (Kay et al., 2004). Similarly, BCs appear to be unnecessary for IPL formation, as the IPL still forms in *Chx10; p27* (Green et al., 2003) and *Math3; Mash1* (Tomita et al., 2000) double-mutant mice, which completely lack BCs. It has been suggested that ACs establish the IPL (Huberman et al., 2010; Kay et al., 2004). This suggestion was bolstered by a recent study that showed that extra misplaced IPLs formed when ACs failed to polarize their dendritic processes properly due to a loss of the protocadherin Fat3 (Deans et al., 2011). This work established the sufficiency of ACs for IPL formation, but whether they are necessary for such formation has not been yet tested. MG are also strong candidates for establishing the IPL, because retinal reaggregates form recognizable cell and plexiform layers when grown in the presence of a monolayer of MG (Willbold et al., 2000), but are disorganized and structurally inverted in the absence of MG (Layer et al., 1998).

To determine whether any particular component cells are essential for IPL-like neuropil formation, we selectively removed ACs, MG, and RGCs as individual cell types or in combination using mutants, morpholinos, and pharmacological inhibitors. Surprisingly, an IPL-like neuropil still formed in cellularly simplified retinas consisting of only BCs and photoreceptors (PRs). Remarkably, in this presynaptic-only neuropil, BC axons could still make presynaptic structures and display sublaminar organization of their axonal terminals. Together with previous findings, our results indicate that no single retinal cell type is critical for the formation of an IPL-like neuropil, and suggest that neuropil formation in the vertebrate CNS may result from the coordinated action of multiple autonomously stratifying cell types.

RESULTS

BC Basal Processes Retract from the Basal Surface of the Retina to Stratify Early within the Nascent IPL

To identify when BC axons first begin to enter the IPL, we labeled BCs either by *vsx1:GFP* or individually by the MAZe transgene (Collins et al., 2010), and imaged them by time-lapse confocal microscopy. BCs at early stages of their stratification usually have a thin distal basal process that extends to the basal lamina (Figures 1B and S1A; Movie S1). Later these distal processes retract, and BC axons branch within the IPL (arrows). This retraction is similar to that previously described for mouse BCs (Morgan et al., 2006), although this process happens much faster in zebrafish, taking 01:48 ± 00:13 (hr:min, mean ± SEM, n = 10 cells from four retinas) compared with 1 week in mice. The Kif5c560-based axon reporter (Distel et al., 2010; Jacobson et al., 2006; Randlett et al., 2011) labels these BC processes during the retraction and branching phase (Figure S1B, arrowheads) suggesting that these are indeed axonal processes.

If AC dendrites are critical for IPL formation, one would expect them to arborize into the nascent IPL before the BC axons do. To test this, we transplanted cells from transgenic donors containing the *vsx1:GFP* (to label BCs) and *ptf1a:DsRed* (to visualize all ACs and horizontal cells [HCs]) transgenes into unlabeled hosts (Godinho et al., 2005; Jusuf and Harris, 2009; Vitorino et al., 2009). These studies showed that BC axons began to collect among the cell bodies of differentiating ACs (arrows in Figure 1C; Movie S2) and appeared to part the displaced ACs (dACs) and normal ACs. This result supports the idea that BC axons arrive relatively early within the forming IPL. A similar imaging strategy, using the *ath5:GAP-RFP* transgenic to label RGCs and many ACs, demonstrated that BC axon terminals did not follow the emergence of an RFP-labeled plexus. Instead, the first BC axon branches and the RFP-labeled plexus became visible in the prospective IPL at approximately the same time (arrows in Figure 1D; Movie S3). Finally, we imaged the developing IPL using a fluorescent membrane marker expressed by all retinal cells. This allowed us to visualize BC axonal branches in an environment where all membranes were labeled (Figure S1C; Movie S4). Again, BC axons were visible within the earliest signs of IPL structure (arrows), indicating that BC axons are among the earliest colonizers of the IPL.

ACs Are Not Required for IPL Formation

Because AC dendrites do not obviously lead BC axons with respect to the time of arborization in the IPL, we wondered whether ACs are necessary for IPL formation. Ptf1a is a transcription factor that is expressed by all ACs and HCs in the zebrafish retina, and its disruption causes the respecification of these inhibitory neurons into excitatory ones (Jusuf et al., 2011; Jusuf and Harris, 2009). Ptf1a morpholinos alone do not completely remove all ACs (Jusuf et al., 2011), so we made use of a tilling mutant from the Zebrafish Mutation Project (*ptf1a^{sa126}*). The *ptf1a^{sa126}* mutant allele is a nonsense mutation that results in a truncation within the loop domain (Figure S2A). The *ptf1a^{-/-}* embryos did not have an obvious morphological phenotype. Although they were markedly reduced in numbers, a substantial number of 5E11- and HuC/D-positive ACs remained in the

ptf1a^{-/-} embryos, indicating that the *ptf1a^{sa126}* allele is not a null mutation (Figures S2B and S2C). However, when two translation-blocking morpholino oligonucleotides (MO; Jusuf et al., 2011) were coinjected into the *ptf1a^{-/-}* mutants with the *p53* MO to reduce nonspecific apoptotic effects (Robu et al., 2007), nearly all ACs were eliminated. Less than 1% of the HuC/D-positive cells in the AC layer remained throughout the retina (mean ± SEM for wild-type [WT] = 3482 ± 614 ACs, *ptf1a^{-/-}; ptf1aMOs* = 33 ± 15.7 cells, n = 10 retinas at 72 hr post-fertilization [hpf]), and there were large stretches of retina with no HuC/D-positive cell bodies or 5E11 staining (Figures S2i and S2ii). Despite the lack of ACs, phalloidin staining indicated that a robust F-actin-rich, IPL-like neuropil was still able to form in these retinas, although it was clearly thinner than the normal IPL (Figures 2A and 2B).

BC Partner Neurons Are Not Required for Neuropil Formation

Because neither of the two postsynaptic partners of BCs on their own appeared to be needed to form an IPL, we wondered whether they might act redundantly in this regard. If so, eliminating both RGCs and ACs simultaneously might lead to a failure of IPL formation. The *ptf1a^{-/-};ptf1aMOs* treatment, along with the *ath5/lakritz* mutant (in which RGCs are absent; Kay et al., 2001), provided the necessary tools to answer this question. *vsx1:GFP;ath5^{-/-};ptf1a^{-/-};ptf1aMOs* embryos were fixed at 72 hpf and stained with HuC/D to determine the extent of AC/RGC loss (Figures S2D and S2E, arrowheads). Despite the loss of all neuronal postsynaptic partners, the BC axons still appeared to organize into an actin-rich neuropil, or IPL-like neuropil, positioned along the basal surface of the retina (Figures 2C and 2D). The simultaneous elimination of RGCs and ACs did not reduce the width of the IPL appreciably compared with the elimination of ACs alone (Figure 2E). Interestingly, the IPL was significantly thicker than WT after the elimination of RGCs alone. Because ACs are overproduced in this context (Kay et al., 2001), the thickness of the IPL in zebrafish may largely reflect the contribution of AC processes.

The sublaminar organization of the IPL is proposed to result from homophilic adhesion and guidance cue and receptor interactions between pre- and postsynaptic cell types (Matsuoka et al., 2011a, 2011b; Yamagata and Sanes, 2008, 2012; Yamagata et al., 2002). Therefore, we expected that IPL-like neuropil in the absence of ACs and RGCs might be completely devoid of any sublaminar organization. To test this, we used two transgenics that label different populations of BCs: *Q16* and *Q19*. *Q16 (nyx::mYFP)* labels a population of BCs that stratify in the basal half of the IPL (Schroeter et al., 2006). We generated a transgenic based on the *vsx1* promoter (*Q19*), which labels BCs that stratify in the apical half of the IPL. In WT retinas, imaging of the *Q16;Q19* double transgenic revealed two bands of nonoverlapping terminals in the IPL-like neuropil (Figures 2F and 2H). These double transgenics were then injected with the *ptf1aMOs* and *ath5MOs* to eliminate RGCs and many ACs. Although the sublamination of the IPL was clearly less organized than in the WT, in this very thin IPL-like neuropil the typical apical/basal pattern was still obvious (Figures 2G and 2I) and highly significant (p = 3.0 × 10^{-144}; Figure 2J). Sublamination of BC populations was also observed
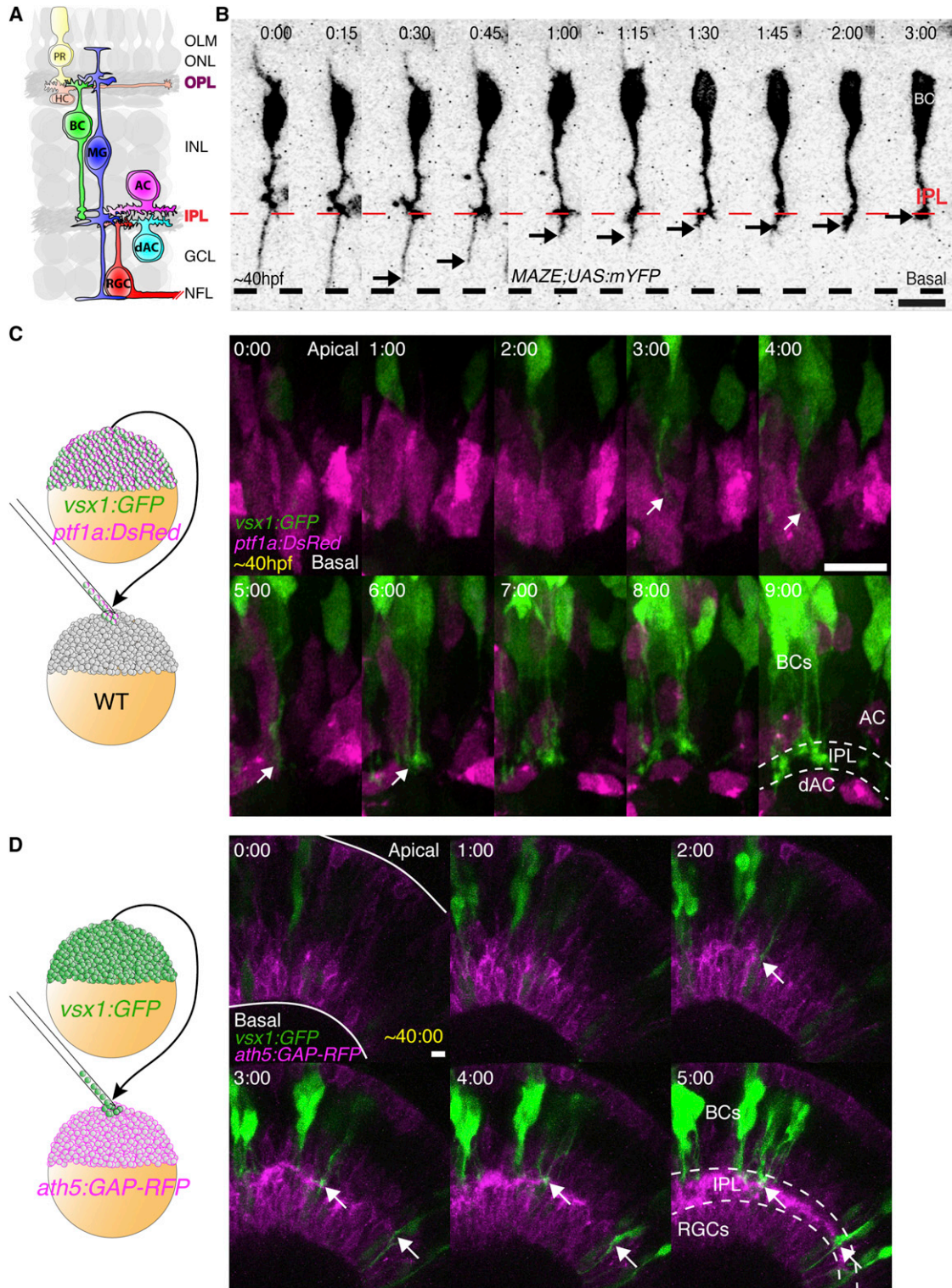
Figure 1. BC Axons Overshoot and Retract to Colonize the Nascent IPL

(A) Schematic showing the general organization of the vertebrate retina, including the neuropil layers, the IPL and OPL, and the retinal neurons and glia that will synapse within them.

(B) An individual BC labeled by *MAZe;UAS:mYFP* transgenes. The distal process extends to the basal surface (dashed line) of the retina. Branching into the IPL region can be seen, and the distal portion of the axon retracts to this point (arrow). At the same time, the apical process is also retracted from the apical surface of the retina.

(legend continued on next page)

using the markers *ath5:GAP-RFP* and protein kinase C (PKC) in *ath5−/−;ptf1a−/−;ptf1aMOs* embryos lacking ACs and RGCs (Figure S3).

We wondered whether BC axon terminals would differentiate properly in a neuropil lacking their normal postsynaptic partners. In the WT retina, the RibeyeA antibody labeled puncta in the IPL (Figure 3A), reflecting its accumulation in BC ribbon synapses. A similar pattern was seen in the AC/RGC-free neuropil (Figure 3B), indicating that presynaptic structures might still be forming. This was confirmed by electron microscopy (Figure 3C), which revealed vesicle-filled structures resembling BC axonal boutons in the neuropil. Surprisingly, ribbon structures with tethered vesicles in these BC boutons were sometimes localized at appositions with other BC boutons, almost as if they were trying to make synaptic contacts with each other (arrowhead, Figure 3C [inset] and s1–s3). However, because we did not find any postsynaptic densities at these membrane contact sites (arrowhead, Figures 3D and 3E), it is unlikely that these are functional synapses.

MG Are Not Required for IPL Formation

If BCs, ACs, and RGCs (i.e., the full neuronal complement of the IPL) are each unnecessary for the formation of synaptic neuropil, there is only one other cellular component of the IPL that could be essential: the MG. Indeed, in the zebrafish *mind bomb* (*mib*) mutant, which lacks MG, retinal layering fails (Bernardos et al., 2005). The early interference with Notch signaling in this mutant, however, appears to compromise the differentiation of many other retinal cell types. To remove MG more selectively, we took a pharmacological approach. We administered the Notch pathway-blocking gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (DAPT) at 30–33 hpf, after neurogenesis had begun. Using this treatment regime, we found that retinal layering was intact, the IPL and OPL formed, and all of the neuronal cell types were still present and correctly positioned. *vsx1:GFP*-labeled BCs and *ath5:GAP-RFP*-labeled RGCs, ACs, HCs, and PRs were all visible (Figure 4A). However, MG were completely absent throughout early development until at least 5 days postfertilization (dpf). This was confirmed by three separate immunohistochemical markers: anti-glutamine synthetase (anti-GS), anti-glial fibrillary acidic protein (anti-GFAP), and anti-cellular retinaldehyde-binding protein (anti-Cralbp; Figures 4B and 4C), as well as the transgenic marker *gfap:GFP* (Figure 4D). To determine whether the IPL was still properly organized into sublaminar compartments, we again made use of the *Q16* and *Q19* transgenes. After the MG were removed, the BC axon terminals still separated into their proper distinct layers (arrow, Figure 4D). We also assayed for the presence of synaptic proteins by staining for the presynaptic vesicle marker SV2, the ribbon synapse component RibeyeA, and the postsynaptic density protein Maguk. All were present after the removal of MG at 72 hpf, suggesting that synapses were still able to form (Figure 4E).

In the Absence of All Other Component Cells, BC Axons Still Make an IPL-Like Neuropil

We have shown that a presynaptic IPL-like neuropil still forms after the removal of ACs, RGCs, and MG. However, again considering the potential for redundancy in this system, it is possible that any one of these cell types is sufficient on its own, and only the simultaneous removal of all three cell types will prevent IPL formation. Therefore, we removed all three of these cell types simultaneously by treating *lak−/−;ptf1a−/−;ptf1aMO* embryos in DAPT beginning at 33 hpf. Although ACs, RGCs, and MG were all absent from these retinas (Figure 5A), the BC axons still formed an actin-rich, neuropil-like layer along the basal surface of the retina (arrowhead, Figure 5B), and contained the presynaptic protein RibeyeA (arrowhead, Figure 5C). Remarkably, this BC-axon-only IPL-like layer also maintained overall sublaminar structure between two BC populations (Figure 5D).

DISCUSSION

Previous work has shown that the IPL forms in the absence of either BCs or RGCs, pointing to either ACs or MG as being essential for retinal layering and IPL formation (Bernardos et al., 2005; Huberman et al., 2010; Kay et al., 2004; Willbold et al., 2000). Indeed, it was suggested that BCs are passive players in IPL formation and are recruited to the preformed IPL through interactions with partner neurons (Huberman et al., 2010; Kay et al., 2004). In contrast to these models, we find that BC axons stratify within the nascent IPL, and that neither ACs nor MG are essential for IPL-like neuropil formation. Not only are each of the individual cells discussed above unessential for IPL formation, but BCs are capable of forming a neuropil in a remarkably autonomous fashion when RGCs, ACs, and MG are simultaneously eliminated. Although BC axons are capable of forming a neuropil autonomously and are present within the nascent IPL, they are themselves dispensable for IPL formation in mice (Green et al., 2003; Tomita et al., 2000). It is also interesting to note that in the absence of RGCs and ACs, this IPL-like neuropil forms along the basal surface of the retina (rather than at its normal, more apical position), indicating that although these cells' partner cells may not be necessary for IPL formation, they may play a role in the positioning of this neuropil layer.

(C) Transplantation scheme to create mosaic retinas with clones of *vsx1:GFP*-expressing BCs and *ptf1a:DsRed*-expressing ACs in an unlabeled host retina. At the onset of imaging, *ptf1a:DsRed*-expressing ACs and HCs have migrated to the AC layer. However, a separation between the dACs and ACs is not apparent. Over time, the BC axons appear (arrows) and begin to stratify in between the *ptf1a:DsRed*-expressing cells. As these BC axons elaborate, the ACs are separated into displaced and nondisplaced populations that are parted by the expanding IPL. Images are confocal reconstructions.
(D) Transplantation scheme to create mosaic embryos with *vsx1:GFP*-expressing BCs in a host retina where RGCs and ACs are labeled by *ath5:GAP-RFP*. BC axons (arrows) accumulate coincidentally with the appearance of the IPL, as shown by *ath5:GAP-RFP*-labeled RGCs and ACs (dashed line). Images represent maximum intensity projections of nine confocal slices. Time shown in hr:min. Imaging begins at ∼40 hpf. Scale bars = 10 μm. GCL, ganglion cell layer; INL, inner nuclear layer; NFL, neurofiber layer; OLM, outer limiting membrane; ONL, outer nuclear layer.
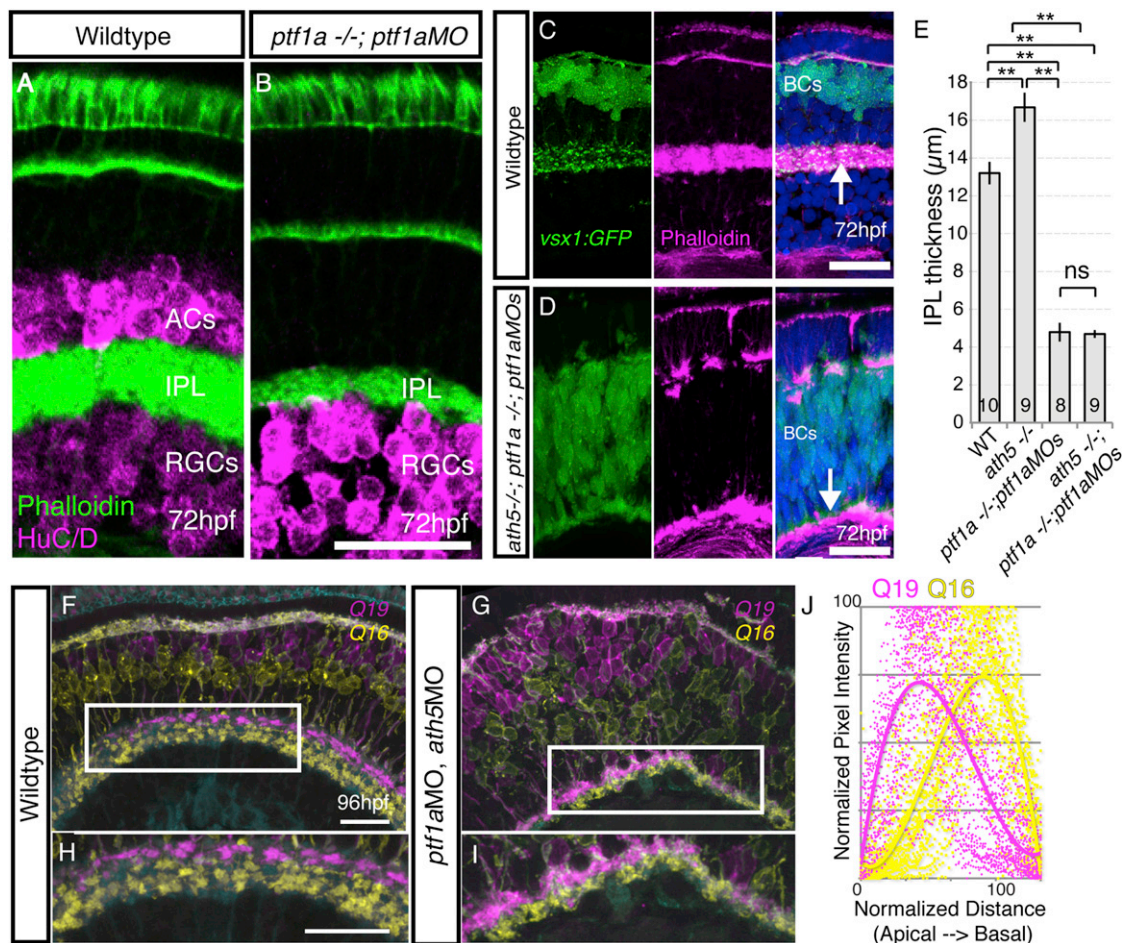See also Figure S1 and Movies S1, S2, and S3.

Figure 2. Simultaneous Removal of BC Partner Neurons (RGCs and ACs) Does Not Prevent BC Axons from Forming a Sublaminated Neuropil

(A and B) Despite the loss of ACs, a phalloidin-rich IPL forms in the $ptf1a^{-/-};ptf1aMO$ retina.

(C) WT retina with the actin-rich IPL shown by phalloidin (arrow) and BC terminals labeled with *vsx1:GFP*.

(D) Phalloidin staining demonstrates that in the AC/RGC-free retina ($ath5^{-/-};ptf1a^{-/-};ptf1aMO$), the IPL is actin rich and positioned along the basal surface of the retina (arrow).

(E) The thickness of the IPL was measured for each genotype at 5 dpf, demonstrating it is significantly thinner after AC removal ($ptf1a^{-/-};ptf1aMOs$), and thicker after RGC removal ($ath5^{-/-}$). One-way ANOVA and Tukey post hoc tests; **$p < 0.01$; error bars ± 1 SEM. The number of retinas measured is given within the bars.

(F) In the WT retina, *Q16*-labeled BCs stratify basally to *Q19*-labeled BCs in the IPL.

(G) After the removal of many ACs and RGCs by *ptf1aMO* and *ath5MO* injections (G), the typical *Q16*-basal, *Q19*-apical pattern is apparent in many areas.

(H) Inset in (F).

(I) Inset in (G).

(J) Line intensity profile measurements from 45 regions (three different retinas/animals) demonstrates the basal enrichment of *Q16* signal and apical enrichment of *Q19* signal in *ath5;ptf1a* morphants. Scale bars, 20 μm.

See also Figures S2 and S3.

It is important to note that our treatment using mutants, morpholinos, and/or pharmacological inhibitors does not result in a loss of these cells from the retina; rather, the cells are respecified to other available fates. Although it is unlikely, we cannot rule out the possibility that respecified cells retain properties reminiscent of their original fate (e.g., ACs with some RGC properties in $ath5^{-/-}$) that may subsequently affect lamination and/or BC axon positioning. Similarly, we were not able to completely rid the retina of ACs, but it seems very unlikely that the <1% of ACs that remain can drive the stratification of all other neurons across the retina.

Interestingly, in the absence of normal postsynaptic partners, BCs still appear to form presynaptic terminals replete with ribbons and synaptic vesicles abutting the processes of other BCs. This is reminiscent of previous reports that proper postsynaptic muscle targets are not necessary for presynaptic specializations in the *Drosophila* neuromuscular junction (Prokop et al., 1996), and that cultured spinal cord axons will form presynaptic specializations when in contact with a polyornithine-coated bead (Peng et al., 1987). More surprisingly, the BC-only neuropil exhibited clear vestiges of sublaminar organization. BC axons that normally laminate in the apical half of the IPL still laminated
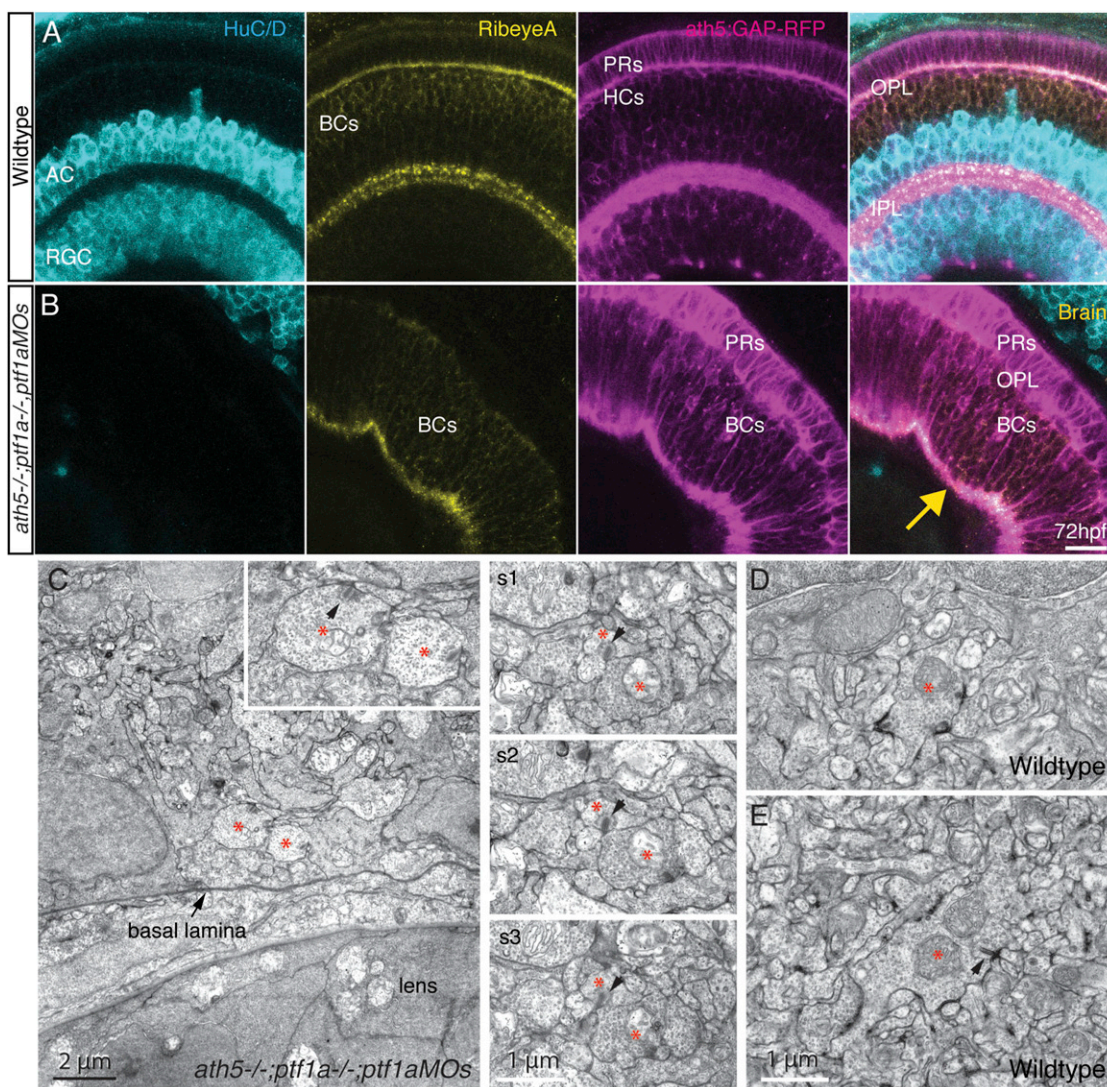
Figure 3. BC Axons Form Presynaptic Structures without Postsynaptic Neurons

(A) WT *ath5:GAP-RFP* retinas stained with anti-HuC/D (to label ACs/RGCs) and anti-RibeyeA (to label ribbon synapses). Obvious punctate RibeyeA staining is visible in the IPL, whereas weak staining is seen in the BC cell bodies in the apical half of the INL and the OPL.

(B) When ACs and RGCs are absent in the *ath5:GAP-RFP;ath5$^{-/-}$;ptf1a$^{-/-}$;ptf1aMO* embryos, the weakly RibeyeA-staining BC cell bodies span the entire INL, and punctate RibeyeA staining is visible in the BC-only IPL. Scale bar in (A) and (B), 10 μm.

(C) Examples of vesicle-filled bouton-like structures (asterisks) containing ribbons near the retinal basal lamina of animals with severely reduced numbers of ACs and RGCs. Left: Higher magnification of the two such structures, presumed to be BC axonal boutons (arrowhead) shown in the inset. Right: Three consecutive sections (s1–s3) of processes in basally located neuropil in another animal. Arrows indicate ribbons juxtaposed to appositions with other processes, one of which can be another BC terminal (s1–s3).

(D and E) WT BC synapses exhibit readily apparent postsynaptic densities (thickenings, arrow) that are not seen in the mutant/morphant plexus (s1–s3).

apically to BC axons, which normally laminate basally. This observation highlights the importance of interactions among classes of presynaptic neurons, rather than simply among different types of neurons and glia, in setting up these segregated layers.

Because no single intrinsic retinal cell type is absolutely essential for retinal neuropil formation, and BCs can form a rudimentary neuropil autonomously, it seems reasonable to suspect that each of the three major neuronal types that contribute neu-

rites to the IPL may also be able to do so autonomously. This is in line with studies in the embryonic *Drosophila* nerve cord, wherein it has been suggested that growing axons and dendrites are independently delivered to appropriate volumes of the developing neuropil by position-dependent guidance cues (Zlatic et al., 2009). If this explanation is correct, it will be important to discover which guidance cues are used to establish and position the retinal neuropil layers and how these cues are regulated.
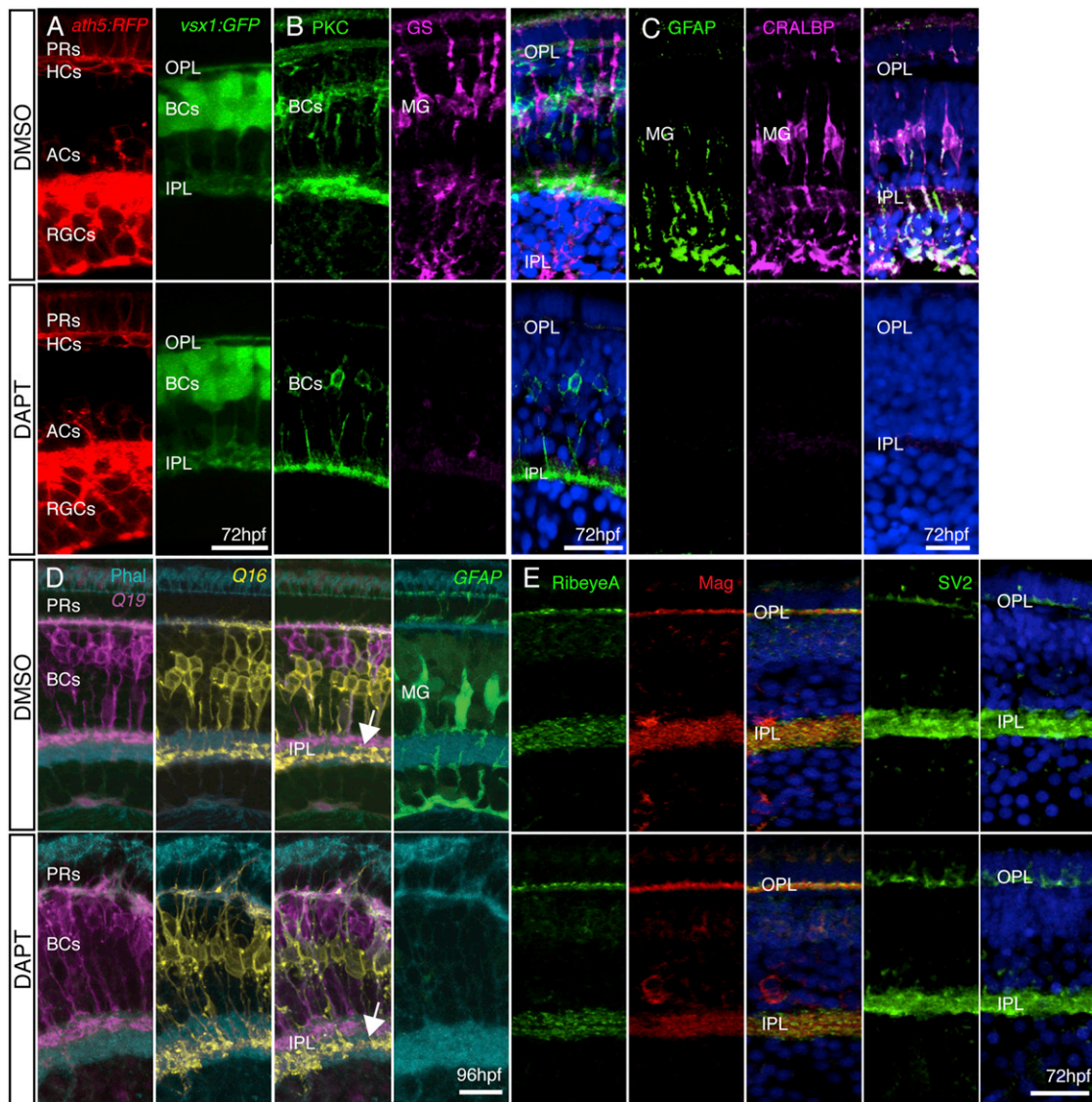
Figure 4. MG Are Not Required for Retinal Organization or Plexiform Layer Development

(A) *ath5:GAP-RFP* and *vsx1:GFP* show that all neural retinal cell types are present and correctly positioned in DMSO-treated control embryos and after DAPT treatment.

(B and C) Treatment with DAPT at 33 hpf completely removed MG in the retina. PKC staining is unperturbed by DAPT treatment, indicating BC differentiation is unaffected. However, DAPT-treated embryos show a complete loss of MG when stained with specific markers for GS, CRALBP, and GFAP when compared with DMSO controls.

(D) The IPL forms and sublaminates properly in the absence of MG. *nyx:Gal4; UAS:MYFP*Q16, and *vsx1:MCerulean*Q19 fish show labeling of specific subsets of BCs that stratify in the apical (OFF) or basal (ON) domains of the IPL. Imaging of triple transgenics *Q16;Q19;gfap:GFP* treated with DAPT shows that the IPL still forms and BC axons sublaminate properly (arrow). Lack of *gfap:GFP* signal confirms that there are no MG in the areas of proper sublamination.

(E) Pre- and postsynaptic markers remain in the plexiform layers lacking MG. Staining for the presynaptic markers RibeyeA and SV2 and the postsynaptic density protein Maguk (Mag) is unperturbed in embryos lacking MG at 72 hpf. Scale bars, 10 μm (A and D) and 20 μm (B, C, and E).

EXPERIMENTAL PROCEDURES

Transgenic Lines and Constructs

Transgenic lines Tg*(atoh7:gap43-mRFP1)cu2*, Tg*(vsx1:GFP)nns5*, Tg*(MAZe)*, Tg*(Ptf1a:DsRed)*, and Tg*(nyx:Gal4-VP16)q16a;Tg(UAS:gap43-YFP)q16b* have been described previously (Collins et al., 2010; Kimura et al., 2008; Schroeter et al., 2006; Vitorino et al., 2009; Zolessi et al., 2006) and are abbreviated here as *ath5:GAP-RFP*, *vsx1:GFP*, *MAZe*, *ptf:DsRed*, and

Q16, respectively. The *Vsx1:MCerulean* (Q19) line was created using the upstream region of the Vsx1 gene (see Extended Experimental Procedures).

DAPT Treatment

Zebrafish embryos were treated with DAPT (50 μM) in 0.5% DMSO solution from 33 hpf to 3–4 dpf, and then fixed with 4% paraformaldehyde and cryoprotected in 30% sucrose in 1X PBS overnight prior to cryosectioning.
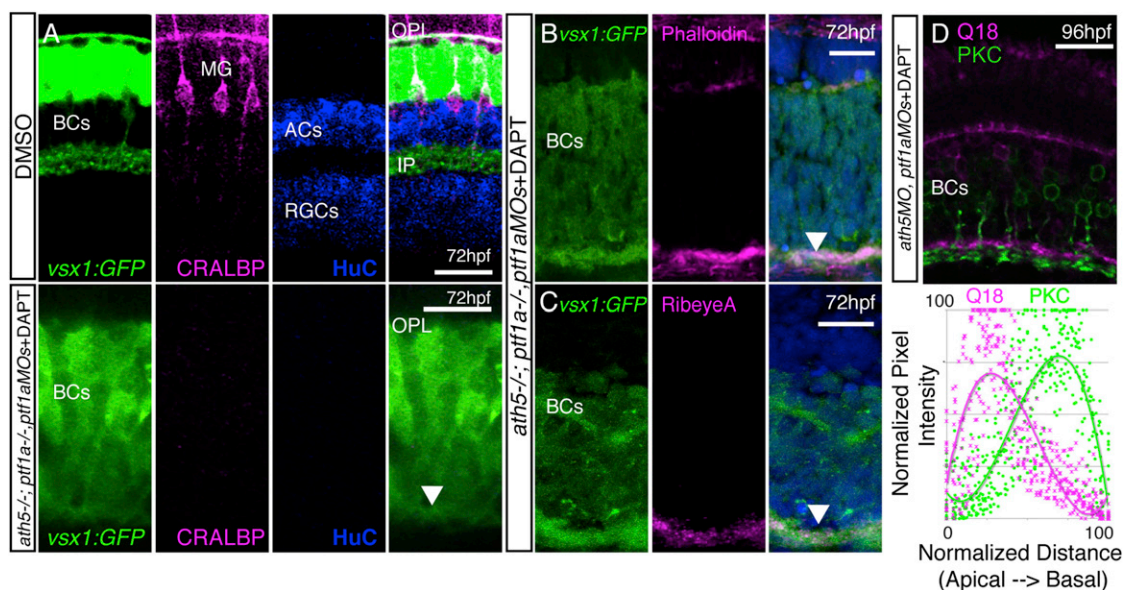
Figure 5. BC Axons Can Form a Sublaminated Neuropil in the Absence of Partner Neurons and Glia

(A–C) A neuropil layer (arrowhead) forms at the basal surface of a cellularly simplified retina $ath5^{-/-};ptf1a^{-/-};ptf1aMOs$ treated with DAPT, lacking ACs, HCs, RGCs, and MG (A). The neuropil layer is actin rich (B) and contains RibeyeA positive staining at 72 hpf (C).

(D) Gross sublaminar structure is maintained in cellularly simplified retinas, with Q19 apical to the PKC staining in the IPL-like layer. This sublamination was statistically significant (p = 6.22 × 10^{-44}, n = 3 sections). Scale bars, 10 μm (A, D, and E) and 20 μm (B, C, F, G, H, and I).

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, four movies, and Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.01.020.

LICENSING INFORMATION

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