

Wiring visual systems: common and divergent mechanisms and principles

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The study of visual systems has a rich history, leading to the discovery and understanding of basic principles underlying the elaboration of neuronal connectivity. Recent work in model organisms such as fly, fish and mouse has yielded a wealth of new insights into visual system wiring. Here, we consider how axonal and dendritic patterning in columns and laminae influence synaptic partner selection in these model organisms. We highlight similarities and differences among disparate visual systems with the goal of identifying common and divergent principles for visual system wiring.

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Introduction: pre- and post-specification of visual system synapses during development

Invertebrate and vertebrate visual systems map color, motion, and feature information onto retinotopic visual maps in the brain. However, the actual anatomical structures are quite different. Fly photoreceptors (R cells) are the primary retinal output neurons that carry visual information to the first and second visual system relay stations (Figure 1A). In contrast, visual information from photoreceptors in the vertebrate eye is extensively processed within the retina. Like R cells in flies, vertebrate retinal ganglion cells (RGCs) convey information to the first visual system relay stations in the brain, including the optic tectum/superior colliculus (OT/SC), lateral geniculate nucleus (LGN), and numerous other retinorecipient nuclei. Hence, with respect to retina output, fly R cells and vertebrate RGCs are comparable (Figure 1A). In contrast, at the level of connectivity and visual information processing, the two synaptic plexiform layers

upstream of RGCs in the vertebrate retina are comparable to brain neuropils downstream of R cells in the fly optic lobe (Figure 1B): the vertebrate retina outer plexiform layer (OPL) to the fly lamina, and the inner plexiform layer (IPL) to the fly distal medulla [1]. These comparisons make sense in terms of circuit connectivity and function, but the actual structures and cell types are not analogous. For example, a subset of RGCs that are intrinsically photosensitive reveal that RGCs may share evolutionary origins with invertebrate photoreceptor neurons [2,3]; vertebrates may have evolved modern retinal connectivity subsequent to development of the first photosensitive cells, while connectivity in the fly lamina and medulla may have evolved independently and downstream of retinal output (Figure 1A,B).

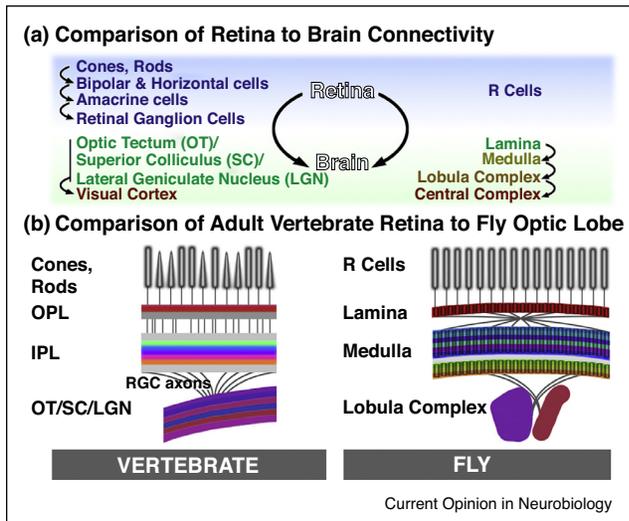
Similar design principles among disparate visual circuit ensembles may be best appreciated in the context of shared developmental processes that orchestrate iterative patterns of synaptic connectivity [4]. Synaptic specification is determined by two core processes: (1) precision of wiring before initial synapse formation (*pre-specification*); and (2) pruning and fine-tuning of connections (*post-specification*). In vertebrates, activity-dependent fine-tuning of synaptic specificity plays an important role in visual system connectivity, showcasing the importance of post-specification (Figure 2) [5,6,7^{*}]. In contrast, visual system wiring in *Drosophila* appears to be predominantly determined by a genetic program, highlighting pre-specification (Figure 2) [4,8,9]. However, in both systems pre- and post-specification likely work hand in hand: initial axonal and dendritic targeting to distinct columnar or laminar structures provides important milestones along the road to mature synaptic specificity [1,4,10^{**},11].

In fly and vertebrate visual systems, processing of parallel information streams is morphologically preserved in repetitive columns or mosaics of similar cell types. Orthogonal to this lateral organization is the prevalent subdivision of visual system components into layers, or laminae; these elements provide anatomically restricted regions where presumptive synaptic partners are in close proximity and facilitate synaptic partner identification, revealing common and divergent developmental principles across visual systems.

Columns and mosaics in synaptic specification

During development, vertebrate cones and rods extend short axon terminals that contact horizontal cell dendrites

Figure 1



Adult vertebrate and fly visual system wiring. **(A)** Comparison of retina-to-brain connectivity based on retina output neurons and possible evolutionary relationships between vertebrate RGCs and fly R cells. **(B)** Comparison of vertebrate retina to the fly optic lobe-based on similarities of functional connectivity.

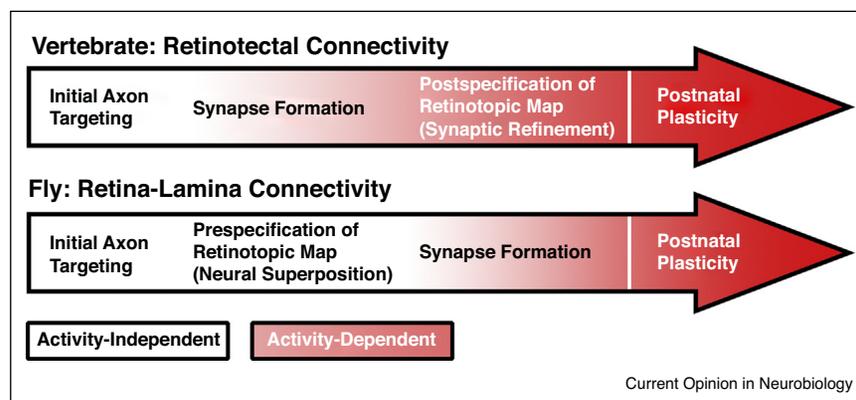
and axons, respectively, and also cone and rod bipolar cell dendrites. Since photoreceptor projections to these interneurons are short and anatomically parallel, retinotopy is maintained in both the OPL and IPL (Figure 3A). In contrast, during larval development fly photoreceptors extend long axons that project from the developing eye disc into the brain (Figure 3B). Vertebrate retina output neurons, RGCs, also maintain retinotopy in their central projections to certain retinorecipient regions. Topographic mapping of RGC axons onto the tectum/superior colliculus is facilitated by orthogonal EphA/ephrin-A and EphB/ephrin-B gradients [5]. These gradients establish topographic mapping through relative, not absolute,

levels of ephrin signaling to RGC axons [5,12]. *Drosophila* has a single Eph gene that is expressed in a gradient in the early developing medulla, so fly R cells may also respond to relative, and not absolute, levels of Eph receptor activity [13]. Therefore, Eph/ephrin signaling may contribute to synaptic pre-specification without providing an absolute synaptic address system.

In flies, adjacent columns that process information from neighboring visual fields are called 'cartridges' in the lamina and 'columns' in the medulla. Lamina cartridges exhibit an intricate wiring pattern that reflects the optical organization of the retina according to the principle of neural superposition [1,8,9]. Neural superposition is an interesting case of pre-specification. Owing to the optics of the overlying retina ommatidia, each lamina cartridge receives input from six R cells that each project from a different ommatidium. Though this creates an intricate wiring problem, a few simple pattern formation rules can generate correct axon sorting [14,15]. This sorting step is genetically separable from synapse formation, and in large part pre-specifies synaptic partners since the correct number of synapses form between incorrect partners when sorting is aberrant [8]. These simple rules must be executed by molecular mechanisms that ensure patterning and such mechanisms have been identified, providing support for the idea that 2-dimensional differential adhesion is achieved through the action of cell adhesion molecules such as N-cadherin and the proto-cadherin Flamingo [9,16]. The sorting process synchronously organizes each column without the need for a large number of different cues to selectively label neighboring columns, demonstrating the utilization of cell adhesion molecules to establish overall patterning of connections as opposed to synapse-specific targeting cues.

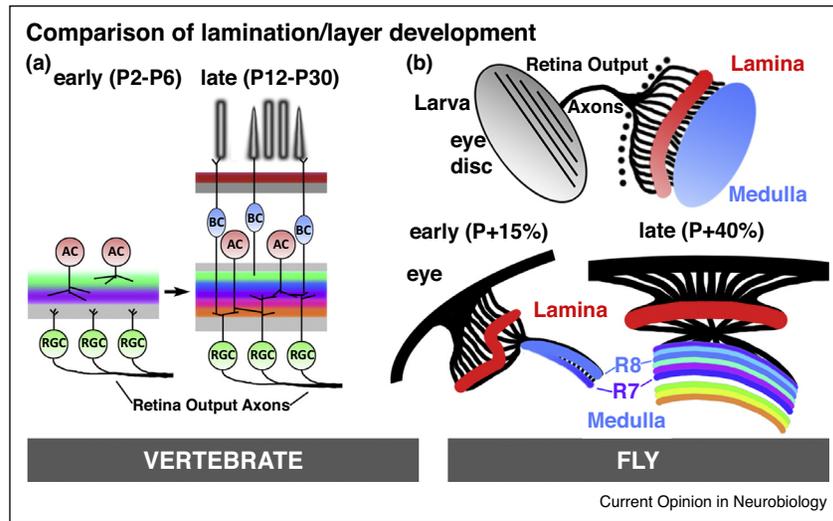
In the vertebrate retina no clear columnar organization develops that maps, point-to-point, neighboring regions of the visual field to synaptic ensembles. However, in the

Figure 2



Pre-specification and post-specification of synapses in vertebrate retinotectal connectivity versus fly retina-lamina connectivity.

Figure 3



Development of layers/lamination in the vertebrate retina compared to the fly optic lobe. **(A)** Early during vertebrate retina development, ACs start forming laminae, while RGC axons remain in a waiting layer. Between P12–P30 RGCs and BCs target pre-existing layers to form contacts. **(B)** During *Drosophila* visual system development, R1–R8 grow through the optic stalk into the lamina (R1–R6) and medulla (R7, R8) in a temporal wave. Early during puparium formation (P + 15%) medulla layer development still exhibits a temporal gradient, but layer development synchronizes shortly thereafter.

SC columnar organization has been shown to arise in tOFF- α -RGCs through spontaneous activity-dependent axon segregation [17], and in the visual cortex columnar organization is morphologically well-characterized [18,19]. In contrast to flies, columnar organization in part emerges through activity-dependent mechanisms in vertebrate brain regions, whereas in the retina a stochastic distribution and mosaic spacing of retinal neurons prevails [1,20,21]. Non-random mosaic spacing is achieved through molecular cues including MEGF10 and MEGF11 [20]. Photoreceptors, postsynaptic bipolar cells (BCs) and horizontal cells (HCs) in the outer retina create cellular mosaics that obey distribution and spacing rules. Amacrine cells (ACs) in the inner nuclear layer, and displaced ACs and RGCs in the ganglion cell layer, also display a mosaic distribution [17,22]. Self-avoidance is a key principle that contributes to the elaboration of non-overlapping dendritic processes from the same neuron [23], and is most famously executed by DSCAM in flies [24] and protocadherins in vertebrates [21,25]. Tiling among neuronal process arborizations from individual neurons of the same subtype, as observed in certain RGC dendrites and bipolar cell axons and dendrites [22,26], prevents interneuronal process overlap. Together, neuronal mosaic spacing, self-avoidance, and tiling assure maximal receptive field coverage and preservation of retinotopy.

Similarities between fly and vertebrate retina development are apparent in the context of lateral cell–cell interactions. Notch-dependent lateral inhibition can

create evenly distributed neuronal architecture during neuronal branch elaboration in the fly medulla [27]. Restriction of axon branches to single columns is a process akin to tiling and is observed for several columnar neurons, including L1 in medulla columns [28]. Similarly, dendritic fields of the postsynaptic Tm20 cells observe columnar restriction in the medulla. Different molecular mechanisms execute underlying non-self avoidance/repulsion: the cell-surface molecule Dscam2 autonomously in L1 [28], and R cell-derived activin non-autonomously in Tm20 [29]. In addition, intrinsic transcriptional regulation contributes to R7 axon columnar restriction [30].

In contrast to processes that obey columnar restriction, Dm8 dendrites, the main R7 postsynaptic partners, span 10–16 medulla columns [31]. Individual Dm8s exhibit variable, non-deterministic coverage patterns, raising questions about synaptic specificity [29,32–34]. Recent findings on Ig superfamily cell adhesion molecules (21 Dprs and 9 DIPs) show that different Dm8 subtypes and their specific presynaptic R7 partners express interacting molecular pairs [35,36]. Dpr11 exhibits remarkably specific expression in R7 cells of the ‘yellow’ type (yR7), and it is unclear if an identical number of Dm8s express the presumptive matching interaction partner DIP- γ . If the synaptic matchmaking code is strict, Dpr11-positive yR7 cells must either always reside in columns with a matching Dm8, or form no synapses with a resident non-matching Dm8. EM reconstruction of seven medulla columns only showed Dm8 cells that form

synapses with both R7s in the home column, as well as several neighboring columns [33,37] (S Takemura, personal communication). A strict code would require that these synapses in neighboring columns are selective only for matching types. If the code were less strict, however, proximity of presynaptic and postsynaptic processes within a column could provide a simple explanation for the observed synapse distribution.

In the vertebrate visual system, synapses between ACs and RGCs in the IPL were thought to be a simple function of the degree of lateral overlap among their dendritic processes within the same lamina, a principle called Peter's rule [38]. However, a 10-fold increase in synaptic specificity between W3B RGCs and VG3 ACs is dependent upon the homophilic Ig superfamily adhesion molecule *sidekick2* [10^{**}]. Therefore, a strict application of Peter's rule is insufficient in this case, and *sidekick2* likely adds specificity to the establishment of precise connections in this crowded region of the retina. The same may apply to Dm8s in the fly medulla: Peter's rule may facilitate synapse formation between Dm8 and R7s within its home column, while molecular interactions may further specify which synapses form or are stabilized.

In summary, the following common principles, none of which on their own are sufficient to determine synaptic specificity, underlie the development of retinotopic organization in both flies and vertebrates:

1. Synchronous lateral sorting and restricting neuronal processes that will subsequently define synaptic partners within a 2D plane (*'stay out of 3D'*) facilitates pattern formation and limited pre-specification of synaptic partners during development.
2. Intrinsic intra-neuronal spacing involving process self-avoidance, cell-autonomous control of branching, and neuronal outgrowth promotion or restriction sets receptive field parameters.
3. Extrinsically controlled inter-neuronal spacing of cell bodies to achieve mosaics, and of process overlap to achieve tiling, facilitates columnar restriction and stochastic spacing of neurons and their processes.
4. Pattern formation mechanisms bring correct presynaptic and postsynaptic processes into the same local vicinity (Peter's rule), with molecular cues being required subsequently to increase specificity in crowded CNS regions.

The role of layers and laminae for synaptic specification

Most of the principles discussed above are implemented synchronously in a defined, two-dimensional layer. The development of layers is a process of temporal succession; new layers emerge through relative positioning based on sequential addition of neuronal processes [39–41]. During

vertebrate retina development, transitional stages of layer formation prefigure complex IPL laminar organization (Figure 3A). Developmental and live imaging studies establish that RGCs are born early, however ACs are the first to extend neurites into the nascent IPL [41]. Very early in the establishment of mouse IPL stratification, initial domains that influence the targeting of select ACs are defined by complementary expression of the repellent semaphorin 6A (Sema6A) and its receptor plexin A4 (PlexA4) [42]. At this time only a few transitional layers are present and neural processes position themselves relative to each other. Similarly, in the fly medulla early R7 growth cones stop relative to R8 growth cones just after passing them (Figure 3B). R7 growth cones never actively extend thereafter, forming early contacts with their main postsynaptic partners, Dm8; new layers form through intercalations between these early R8 and R7 layers [32,43^{*}] (Figure 3B). Hence, an R7 layer is stabilized very early in what will become adult layer M6, but neither adult M6 morphology nor its molecular markers adequately predict the transitional stage.

In contrast to lateral pattern formation mechanisms (mosaic spacing, self-avoidance, and tiling), clearly defined repulsive and attractive cues for transitional target regions play critical roles in elaborating IPL lamination. In the absence of the transmembrane repellents semaphorin 5A and Sema5B, RGCs, ACs, and BCs all extend numerous processes from the IPL to ectopic locations in the outer retina, suggesting that Sema5A/5B together contribute to retinal organization and maintain the overall OPL and the IPL separation [39]. Sema6A/PlexA4-dependent development of transitional lamination generates a non-permissive zone in the inner regions of the IPL from the earliest times of IPL development, maintaining the positioning of a limited number of ACs that normally project in the outermost S1 IPL region [42]. Sema6A also regulates laminar stratification of starburst amacrine cells (SACs), interneurons that project in either lamina S2 (Off SACs) or lamina S4 (On SACs) of the IPL, and Sema6A-mediated repulsion prevents SAC dendrite mis-stratification [44]. Though many retinal neurons that project dendrites within the ON region of the IPL express Sema6A, the select expression of the Sema6A receptors PlexA4 and A2 imparts specificity to subtype-specific Sema6A-mediated laminar targeting.

Select adhesive interactions are also critical for targeting specific classes of retinal neurons to the lamina. Immunoglobulin (Ig) super family homophilic adhesion molecules with unique IPL distribution patterns identified in the chick retina play important roles in directing IPL laminar organization [45,46]. Adhesion molecules contribute to the assembly of functional retinal circuitry, as exemplified by type II cadherins and their role in wiring up direction selective circuits in the IPL [47^{**}]. 'Type

2 Off' BCs express cadherin 8 (Cdh8), and 'Type 5 On' BCs express Cdh9. Cell-type specific Cdh8 and Cdh9 loss- and gain-of-function experiments demonstrate that these cadherins are required to direct these two BC types to their distinct laminar strata. Swapping the two cadherins between these BCs is sufficient to redirect type 2 Off BC axons to the strata normally occupied by type 5 Off BCs, and *visa versa*. Loss of Cdh8 compromises Off direction selective responses, whereas loss of Cdh9 affects On direction selectivity. BC axons stratify after IPL laminae are already formed by ACs and RGC dendrites. Therefore, BC axons achieve their specific laminar positioning in the context of pre-existing laminae by utilizing adhesive cues to select appropriate laminar targets.

In flies, some cell types also initially target temporary layers and later actively extend to layers that are formed by different cells. For example, L3 interneuron growth cones reach their correct medulla target layer through a two-step process, suggesting similarities to vertebrate retina IPL development [39]. A combination of repulsive signaling mediated by semaphorin-1a functioning in L3 neurons and adhesive functions among L neurons mediated by more broadly expressed N-cadherin mediates initial axon targeting in the fly medulla. The role of N-cadherin is often interpreted as 'broadly adhesive' and functioning redundantly with other factors, in part because mutant neurons often target correctly during early development [1,9,39]. However, R7 live imaging revealed that *cadN* mutant fly R7 axons actively jump between correct and incorrect medulla layers, arguing against a strict role for CadN in preferential adhesion within the correct layer [43*]. Subsequent to its own targeting, L3 produces the secreted attractant netrin, which influences R8 growth cones as they actively extend to this same layer [48]. Recent live imaging results show that netrin signaling stabilizes R8 terminals in the target area, but does not target R8 axons *per se*, similar to the stabilizing function of CadN for R7 [49*]. Interestingly, R cell growth cones in turn produce a ligand (jelly belly) that is required for L3 survival, and therefore maintenance of R8 targeting [50]. These mechanisms highlight a cellular feedback loop whereby temporal succession of neuronal process extension remobilizes and then stabilizes growth cones during layer formation.

Adhesive interactions are also crucial for assembly of OPL connections, where rod and cone photoreceptors form synapses with On-rod BCs and both On- and Off-cone BCs, respectively, in stereotyped positions. On-rod BC dendrites express the mGluR6 glutamate receptor, and the transmembrane cell adhesion protein ELFN1, which is selectively expressed in rod photoreceptor axons, trans-synaptically interacts with mGluR6 [51**]. Loss of ELFN1 in rods leads to a failure of rod/On-rod BC synapse formation and a loss of low light rod pathway-mediated detection. Future work will reveal additional

mechanisms that establish specific connections between the ~13 BC types and rods and cones.

Select RGC axons project to specific layers in retinorecipient targets, including in the SC and the dLGN [52,53]. How important are these layers for development and adult function? *Astray* mutants in zebrafish lack the slit1 receptor Robo2, causing an absence of clear RGC axon lamination in the tectum; although direction selective tuning of *astray* RGCs is perturbed at earlier developmental stages, at late developmental stages directional RGC tuning (and underlying synaptic connectivity) is similar to wild-type, even in the absence of wild-type laminar organization [54*]. These results show that lamination is not an absolute requirement for functional assembly of this circuit. Similarly, in the visual cortex of *reeler* mice sensory maps in barrel cortex are unaffected despite dramatic disorganization of cortical laminar organization [55,56]. The first EM 'connectome' of the adult mouse LGN surprisingly identified no clear anatomical subdivisions that reflect the processing of parallel visual information streams, but rather a 'fuzzy logic' of connectivity [57]. These studies suggest that lamination may facilitate neural development but is not absolutely required for many mature circuit functions.

Does layer-specific targeting pre-specify synaptic partners? Similar to development of lateral retinotopic organization, axons and dendrites that end up in the wrong location may form synapses there. Fly R8s that mistarget to the M6 layer in the medulla form aberrant synapses with Dm8 [58], and ectopic photoreceptors created genetically outside of the visual system synapse in brain regions far from their normal environment [59]. However, synapses in the medulla are not strictly layer-specific [37]; consequently, layer-specific targeting is unlikely to be a sufficient determinant of synaptic specificity. In the mouse retina, lamination prevents synaptogenesis with incorrect partners: some RGCs make ectopic connections with OFF bipolar cells when the correct ON bipolar cells are not present during development [7*]. In contrast, mouse M1 intrinsically photosensitive RGCs (ipRGCs) maintain their association with specific ACs when both M1 RGCs and their partner ACs are misdirected to incorrect laminae [42]. Here, a short-range cell type-specific cue distinct from Sema6A is more important for synaptic connectivity than layer-specific localization. These examples highlight the importance and limitations of layers for synaptic specificity by facilitating connectivity in 2D rather than 3D. This principle includes Peter's rule with limitations, as exemplified by the function of *sidekick2* in the IPL [10**].

Finally, synapse formation itself may be linked to growth cone stabilization in specific layers. In flies, the RhoGAP Syd-1 and the adapter protein liprin-alpha serve as early determinants of synapse formation [60]. Mutations

affecting either gene, or the upstream cell adhesion molecule Dlar, cause R7 layer-specific targeting defects [61,62]. This may be analogous to the stabilization of mGluR6-containing synapses between rod photoreceptors and rod-Off BC cells through trans-synaptic interactions [40,51^{••}]. How synapse formation stabilizes growth cones, and how growth cone dynamics influence synapse formation, remain exciting questions for the future.

In summary, the following common principles underlie the development of layered organization in both flies and vertebrates:

1. Temporal succession and relative positioning (e.g., through intercalation) establish new layers.
2. Some cell types form and define layers, while axons and dendrites of others initially wait for lamination to be established and subsequently target-based on layer-specific cues.
3. Lamination facilitates development, but may not be an absolute requirement for connectivity and mature circuit function.

Conclusions

Taken together, this brief consideration of common and divergent principles in visual system wiring highlights how neurons of different origins adopt common mechanisms to connect circuits for the processing of parallel information streams. Both columnar and laminar organization play crucial roles in patterning neural connectivity to connect correct presynaptic and postsynaptic partners. In the case of columnar/lateral retinotopy development, flies and vertebrates exhibit greater anatomical divergence since there are no columns in the vertebrate retina. However, pattern formation mechanisms including mosaic spacing, self-avoidance, self/non-self recognition and tiling play strikingly similar roles in disparate visual systems, ensuring retinotopic wiring of large numbers of neurons to allow for parallel information processing. In contrast, during the development of layers/laminae, neurons utilize specific molecular cues, most notably when axons or dendrites choose among layers that have already formed. A key question remains: to what extent are synaptic partnerships pre-specified by columnar and layer patterning? Continued study of visual system development will provide critical insights into how complex neuronal organization is achieved through a balanced utilization of pattern formation rules, differential molecular adhesion/repulsion and synaptic specification molecules.

Conflict of interest statement

Nothing declared.

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