CHAPTER EIGHT

Milestones and Mechanisms for Generating Specific Synaptic Connections between the Eyes and the Brain

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Abstract
All information about the visual world is conveyed to the brain by a single type of neurons at the back of the eye called retinal ganglion cells (RGCs). Understanding how RGC axons locate and wire up with their targets is therefore critical to understanding visual development. In recent years, several important technological and conceptual advances have been made in this area, and yet, many fundamental questions remain unanswered. Indeed, while much is now known about how RGC axons pathfind at the optic chiasm and form retinotopic...
maps within their targets, how RGCs select their overall targets in the first place is poorly understood. Moreover, the signals that direct mammalian RGC axons to their appropriate layer within those targets remain unknown. The recent advent of genetic tools to selectively label and manipulate defined groups of RGCs is starting to provide a way to resolve these and other important questions about RGC wiring specificity. This field is therefore positioned to reveal new principles of visual circuit development that no doubt will extend to other regions of the CNS.

1. Introduction

Retinal ganglion cells (RGCs) encode different features of the visual environment and send that information to the brain where it is processed into perceptions and behaviors. RGC connections are exquisitely precise to ensure accurate visual processing. For example, neighboring RGCs project to neighboring portions of their targets and thereby convey information about the spatial position of objects in the environment. Moreover, functionally distinct RGCs project to different depths or “layers” within their targets and thereby establish parallel circuits for analyzing different features of the visual scene such as motion, color, or brightness. From a developmental perspective, each component of eye-to-brain connectivity translates into a different requirement for axon growth, pathfinding, and target recognition during development. Thus, understanding the complete sequence of events that enable RGCs to wire up with their targets is not only critical for understanding the genesis of vision but it also provides a comprehensive model for exploring how complex neural circuits are built. Here, we review studies focused on how mammalian RGCs establish precise synaptic connections in the brain. In doing so, we often mention experiments that were carried out on lower vertebrates and Drosophila, because they provide a conceptual framework for thinking about the cellular and molecular mechanisms that generate circuit specificity. Throughout this review, we also emphasize important aspects of mammalian eye-to-brain development that remain poorly understood, in hopes that our readers will be inspired to design and implement experiments to elucidate them.

2. Connecting the Eyes to the Brain

2.1. Preliminary steps for connecting the eyes to the brain

Connecting the eyes to the brain is a multistep process that begins as RGCs migrate to their correct layer of the retina and extend their axons. At this stage, RGCs must achieve three important milestones before they can begin
to search for their targets: First, they have to grow their axons toward the exit point of the eye, the optic disk. Second, RGC axons have to pass through the optic disk and into the optic nerve. Third, when RGC axons reach the ventral chiasm midline, they have to decide whether to cross to the opposite (contralateral) side of the brain or, alternatively, to remain in the same (ipsilateral) hemisphere (Fig. 8.1).

Some of the cellular and molecular mechanisms that regulate these early milestones are known. For example, RGCs extend their axons toward the optic disk in response to axon repellants expressed at the retinal periphery or over the retinal surface (Birgbauer et al., 2000; Brittis et al., 1992). Once RGC axon growth cones arrive at the optic disk, local expression of the chemoattractant, netrin-1, causes them to pass through the disk, leave the eye, and form the optic nerve (Deiner et al., 1997). The extension of RGC axons down the optic nerve likely reflects their robust capacity for growth at embryonic ages (Goldberg et al., 2002a). How do RGCs decide which hemisphere of the brain to project to? Work from Mason and coworkers revealed a molecular pathway that endows a subset of RGCs with strong sensitivity to chemorepulsion at the optic chiasm. Consequently, this subset of RGCs steers away from the midline to remain on the ipsilateral side of the brain (Herrera et al., 2003; Williams et al., 2003). There also appears to be a molecular genetic program related to chiasm crossing (e.g., Pak et al., 2004; Williams et al., 2006). Given that several excellent and thorough reviews were recently published on the topics of pathfinding out the eye, into the nerve, and at the chiasm (see Oster et al., 2004; Oster et al., 2004).

Figure 8.1  Critical early choice points for retinal ganglion cell (RGC) axons to reach the brain. In order for retinal ganglion cells to connect to the brain, they must first extend their axons toward the optic nerve head (ONH), down the optic nerve, and through the optic chiasm. Posterior to the chiasm, RGC axons travel to their retinorecipient targets via the optic tracts. A small subset of RGC axons travel to their targets via the accessory optic tracts (AOT).
Petros et al., 2008), we do not discuss them in further detail here. We mention them because (i) they are necessary for RGCs to eventually reach their correct targets in the brain and (ii) RGC axon–axon interactions within the eye, nerve, or chiasm could impact the targeting of those axons at more distal locations along the visual pathway. Indeed, the degree to which the total RGC population is divided into decussating or nondecussating fractions impacts the targeting of those axons in downstream targets (see below). So with the importance of these early pathfinding events in mind, we now consider the choices RGC axons face as they enter the brain and grow toward their targets.

2.2. Choosing the correct retinorecipient target/s

What we refer to as “the connections between the eyes and the brain” actually consists of axons arising from ~20 functionally distinct subtypes of RGCs (Dacey et al., 2003; reviewed in Berson, 2008). Each subtype carries information about a different feature of the visual environment such as edges, motion, or color (Dacey et al., 2003; Roska and Werblin, 2001) and sends that information to a restricted number of retinorecipient targets, where it is processed into perceptions or behaviors (Callaway, 2005; Nassi and Callaway, 2009). For example, the RGCs that encode luminance project to the intergeniculate leaflet (IGL) and ventral lateral geniculate nucleus (vLGN), thalamic structures that mediate nonphotic entrainment of circadian rhythms (Hattar et al., 2002; Muscat and Morin, 2006; reviewed in Harrington, 1997), whereas the RGCs that encode directional object motion project to the dorsal lateral geniculate nucleus (dLGN), a thalamic structure that relays visual information to the cortex for conscious image perception (Huberman et al., 2009; Stewart et al., 1971; for a comprehensive review of mammalian RGC subtypes and their central projections, see Berson, 2008). In order for the visual system to function properly, it is critical that each RGC subtype innervate the correct retinorecipient targets. How RGCs accomplish that task is not well understood, but the organization of the mature visual system displays several features that constrain how this could occur. The dominant constraint on RGC target selection is the existence of ~24 retinorecipient targets but only two ways for RGC axons to reach them (Figs. 8.1 and 8.2). All RGC axons travel to their targets via the main or accessory optic tracts—dense bundles that course next to (and past) each retinorecipient target (Fig. 8.2). The decision made by a RGC to innervate a particular retinorecipient target therefore reflects the decision to defasciculate from and exit the optic tract. It is also worth noting that, at maturity, many RGCs project to multiple retinorecipient targets (Bowling and Michael, 1980; Crook et al., 2008; Huberman et al., 2008a; Tamamaki et al., 1995). Moreover, individual RGC axons often select one of several targets that reside adjacent to one another (e.g., Huberman et al., 2008a,
Thus, stringent axon–target recognition systems must exist to allow RGCs to distinguish among closely positioned nuclei along the visual pathway.

Very little is known about the cellular and molecular mechanisms that control overall target recognition in the mammalian visual system. Lesion studies have shown if their visual targets are ablated, RGCs can innervate nonvisual targets such as the auditory or somatosensory thalamus (Frost and Metin, 1985; Sur et al., 1988), but how different subtypes of RGCs distinguish among the various retinorecipient targets during normal development is not known. In large part, this gap in knowledge arose because of a lack of tools to label specific and defined subtypes of RGCs across development. In other words, without a means to visualize and unequivocally identify RGCs that are destined to innervate certain targets and not others, it is virtually impossible to study how target selection develops or changes in response to experimental manipulation.

A small number of studies examined how RGCs pick their correct targets by combining retrograde labeling and morphology-based classification of RGC subtype. Notably, Shatz and coworkers carried out a study in
which they retrogradely labeled RGCs from different retinorecipient targets across development. Their analysis of the back-filled RGCs showed that one particular RGC type—the so-called “X” RGCs—initially project to the dLGN and to the midbrain superior colliculus (SC), but then withdraw their connections to the SC (Ramoa et al., 1989). Similar results were later obtained in ferrets (Wingate and Thompson, 1995). Those findings indicate that specificity of RGC axon–target connections can arise through removal of inappropriate connections, but whether this is a general rule for all RGC subtypes is not known. The recent discovery of transgenic mice that selectively express fluorescent proteins in defined subtypes of RGCs (Hattar et al., 2002; Huberman et al., 2008a, 2009; Kim et al., 2008; Siegert et al., 2009; Yonehara et al., 2009) now make it possible to systematically study RGC target selection.

Studies of cold-blooded vertebrates reveal that RGC axons often follow one another to their targets. Chien and coworkers found that when early born RGCs are deleted from zebra fish, the axons of later born RGCs make large-scale targeting errors when they eventually reach the brain (Pittman et al., 2008). This suggests that some RGC axons act as “pioneers” to guide later growing axons. The concept of pioneer pathfinding has longstanding support from studies of insects (e.g., Taghert et al., 1982), but has received less experimental attention in mammals. It will therefore be exciting to see whether RGCs with similar functional characteristics (e.g., “luminance detecting” RGCs) are led to their targets by pioneers of the same or similar subtype. Alternatively, the axons of early born RGCs may pioneer the way for later born RGCs, irrespective of their functional or subtype identity. Again, these questions can now finally be addressed using the abovementioned transgenic mice that distinguish functionally unique RGC subtypes in the mature and developing brain (Hattar et al., 2002; Huberman et al., 2008a, 2009; Kim et al., 2008; Siegert et al., 2009; Yonehara et al., 2009).

Studies in cold-blooded vertebrates revealed one of the molecular pathways that mediate target selection. Holt and coworkers discovered that in Xenopus, fibroblast growth factor (FGF) induces RGC axon extension (McFarlane et al., 1995). Interestingly, levels of FGF are low within the target tectum (the structure homologous to the SC)—which could explain why RGCs slow down and enter this target. Indeed, if FGF is ectopically expressed at the tectal border, RGCs grow past the tectum (McFarlane et al., 1996). These findings suggest such that growth factors may play a role in mammalian target recognition. As this field moves forward and the factors that promote mammalian target recognition are identified, it will be interesting to see if they generally operate by promoting axon–target adhesion or rather by promoting repulsion. Of course both mechanisms could collaborate to enforce target choice specificity. With many genetic tools now available for monitoring neurons in fixed tissue and in vivo, our understanding of how RGCs pick their targets is sure to expand rapidly in the next decade.
2.3. Finding the correct retinotopic termination zone

After RGC axons locate and enter their appropriate targets, they have to navigate to the correct retinotopic location. “Correct” in this context means that RGCs must conserve their spatial relationships by projecting to neighboring locations within the target and thereby establish an orderly representation of the visual field. Retinotopic mapping has been studied mainly by a technique called focal tracing in which the lipophilic dye, DiI, is injected into a restricted location on the retina and the precision of the labeled termination zone (TZ) is visualized in the brain. Classic work from O’Leary and coworkers used focal tracing to demonstrate that early in development, RGC axons overshoot their correct TZ by a large distance (Simon and O’Leary, 1992). In the rodent SC, this overshoot is particularly dramatic: RGCs axons from the temporal retina initially extend across the full anterior–posterior extent of the SC, bypassing their correct TZ by several millimeters. Once the overshoot is maximal, an interstitial branch forms at the correct retinotopic location and the mistargeted portion of the axon is pruned back through a process that may involve Wallerian–like degeneration (Hoopfer et al., 2006; Simon and O’Leary, 1992; Fig. 8.3).

The degree of axonal overshoot varies depending on the location in the retina from which the RGC axon arises, but all RGCs nonetheless appear to undergo these cellular changes before arriving at the appropriate TZ (see McLaughlin and O’Leary, 2005 for review).

It is worth mentioning that retinotopic maps are present in virtually all retinorecipient targets. Given that many RGC subtypes project to more

Figure 8.3 Retinotopic mapping in normal, ephrin-A, and retinal wave-deficient mice. The blue box includes the normal developmental sequence that RGC axons (red) undergo to find their correct retinotopic termination zone (TZ) in their targets. (See text for detailed description of these events.) The dashed red line indicates axonal degeneration. The blue gradient reflects a typical distribution of ephrin-A ligands across the target. The gray box encompasses the phenotypes seen after knockout (KO) of ephrin-A2/5, when early retinal waves are altered, or when ephrin-As and waves are both disrupted (see text for full description).
than one of these targets (Bowling and Michael, 1980; Crook et al., 2008; Tamamaki et al., 1995), RGC axons must form retinotopically correct TZs in multiple locations along the visual pathway. The two major forces by which RGC axons establish retinotopic maps are (i) spontaneous waves of neural activity and (ii) gradients of molecular guidance cues. We now consider how these forces work.

2.3.1. Correlated RGC firing drives retinotopic refinement

In mammals, retinotopic map formation occurs prior to vision but during the time when spontaneous “waves” propagate across the retina, causing neighboring RGCs to fire action potentials (reviewed in Huberman et al., 2008b; Torborg and Feller, 2005a; Wong, 1999). This correlated RGC firing is hypothesized to drive retinotopic map refinement by engaging Hebbian-type plasticity at central synapses (Butts et al., 2007; Katz and Shatz, 1996). The first direct test of this hypothesis was done by comparing the retinotopic maps in the brain of wild-type mice and mice lacking the beta2 subunit of the nicotinic acetylcholine receptor. Early experiments showed that beta2 mice lack retinal waves and instead exhibit noncorrelated RGC firing during the period of retinotopic refinement (McLaughlin et al., 2003; Torborg and Feller, 2005a,b). More recent experiments suggest, however, that retinas from beta2 mice can support waves under certain conditions, but those waves are much larger and much faster than normal, and they lack a characteristic directional bias found in wild-type retinas (Stafford et al., 2009; Sun et al., 2008). Using focal DiI tracing to evaluate the precision of retinotopic mapping, McLaughlin et al. (2003) and Grubb et al. (2003) found that RGC axons fail to refine into a focal TZs in the dLGN or SC of beta2 mice (Fig. 8.3). Extracellular recordings (Cang et al., 2008; Chandrasekaran et al., 2005; Grubb et al., 2003) and optical imaging studies (Mrsic-Flogel et al., 2005) also showed that the spatial organization of dLGN and SC receptive fields are expanded in beta2 knockouts—essentially “smearing” the representation of the visual space. Collectively, these studies show that normally patterned retinal waves are essential for retinotopic mapping in the mammalian visual system.

After retinotopic maps are established, spontaneous retinal waves continue to drive the removal of excess RGC synapses onto target neurons. These refinements occur on a scale much too fine to detect with focal DiI tracing, but electrophysiological recordings have shown that the number of RGCs connecting to each dLGN neurons reduces from ~12 to 1–3 during this period (Chen and Regehr, 2000; Jaubert-Miazza et al., 2005; reviewed in Huberman, 2007). Waves are necessary for this pruning to occur because intraocular injection of the sodium channel tetrodotoxin (TTX) prevent fine-scale pruning (Hooks and Chen, 2006). Thus, even before vision, retinal waves drive refinement of RGC axons into progressively sharper and sharper TZs.
2.3.2. Guidance molecules and the polarity of retinotopic maps

In beta2 mutant mice, RGC terminations are abnormally diffuse (Chandrasekaran et al., 2005; Grubb et al., 2003; McLaughlin et al., 2003) but they still project to roughly the correct area of the target (e.g., the temporal retina still maps to anterior SC, and the nasal retina to posterior SC). Thus, the basic polarity and global structure of retinotopic maps is likely to be controlled by activity-independent factors. In past two decades, studies in chicks and mice showed that the ephrins and their receptors (Ephs) are the molecular cues that set the basic structure of retinotopic maps—not just in the SC, but in multiple retinorecipient targets along the subcortical visual pathway (Feldheim et al., 1998; and reviewed in McLaughlin and O’Leary, 2005). Here, we highlight the basic principles by which ephrins perform this role and we describe some recently published experiments that expand on those principles.

Ephrins establish retinotopic maps through “gradient matching.” Several different ephrin-As (in mice, ephrins-A2/3/5) are expressed in high-posterior, low-anterior gradients across the SC. At the same time particular Eph-A receptors (EphA3 in chick, EphA5 in mouse) are expressed in high temporal, low nasal gradients across the ganglion cell layer of the retina (reviewed in Huberman et al., 2008b; McLaughlin and O’Leary, 2005). Because ephrin-As generally act as repellants for axons expressing high levels of Eph-As, two logical predictions emerge from these expression patterns: first, RGCs in the temporal retina will avoid the ephrin-A-rich posterior SC and instead map to the anterior SC. Second, RGCs in the nasal retina will be able to map further “up” the ephrin-A5 gradient, into the posterior SC (Huberman et al., 2008b; McLaughlin and O’Leary, 2005). Overexpression and genetic knockout data nicely support these predictions; in mice lacking ephrins-A2/5, RGC axons form ectopic terminations along the A–P axis of the target (Feldheim et al., 2000; Frisen et al., 1998; Fig. 8.3). Similar results are seen in mice lacking EphA5 receptors (Feldheim et al., 2004). It should be noted, however, that multiple ephrin-As and Eph-As are expressed both in the retina and in the SC (Hornberger et al., 1999). Indeed, removal of target-derived EphA7 alters RGC targeting (Rashid et al., 2005). A simple model based on Eph-A receptors in the retina and ephrin-A ligands in the target therefore is not sufficient to explain the development of N–T retinotopic maps. Conditional, region-specific knockouts of ephrin-As and Eph-As are urgently needed to resolve precisely where and how these molecules influence visual map development. In the meantime, one can generally conclude that ephrin-A:Eph-A interactions are essential for delivering RGC axons to their correct retinotopic termination sites in the brain.

A hallmark principle of retinotopic mapping is that RGCs define their correct TZ according to the relative levels of ephrin-A:Eph-A signaling in neighboring RGC axons (McLaughlin and O’Leary, 2005; Reber et al., 2004). This principle was elegantly demonstrated by Brown et al. (2000),
who made a knockin (ki/ki) mouse with EphA3 expressed in approximately every other RGC. The retinas of ki/ki mice thus have a gradient of EphA3 superimposed onto the normal endogenous gradient of EphA5. The striking consequence of this arrangement is that the EphA3/5 expressing RGCs establish a retinotopic map that is distinct from the retinotopic map formed by the EphA5-only expressing RGCs. Indeed, in ki/ki mice there are two complete, orderly retinotopic maps in the SC, each arising from RGCs in the same eye. Those results provide strong evidence that RGC axons map not according to the absolute amount of ephrin-A they encounter in their targets but rather according to the relative amount of Eph-A–ephrin-A signaling in neighboring RGC axons. The results of Brown et al. (2000) are consistent with classic studies in which half of the SC was ablated; in those experiments, a complete (albeit compressed) retinotopic map still formed (e.g., Marotte and Mark, 1987)—demonstrating there is no strict addressing of RGCs to specific retinotopic coordinates. At the same time, recent work in zebra fish argues that RGC axons project to specific locations in the tectum irrespective of other RGC axons (Gosse et al., 2008). These contrasting results could relate to differences in the precision of retinotopic maps across species. Nevertheless, the double SC maps in ki/ki mice (Brown et al., 2000; Triplett et al., 2009) provide strong evidence that relative signaling between Eph receptors and ephrin ligands is an important factor for establishing orderly retinotopic maps in the mammalian brain. An important goal now is to understand how RGC axons “read out” the relative levels of ephrin-As. Recent studies suggest these interactions are mediated at least in part by the p75 and/or TrkB neurotrophin receptors (Lim et al., 2008; Marler et al., 2008) but how those receptors drive the axonal changes required for TZ formation remain unknown.

2.3.3. The dorsal–ventral map

Thus far we have only discussed how RGCs establish maps along the N–T axis. Less is known about the formation of the dorsal–ventral (D–V) retinotopic map. Based on their complementary expression in the retina and target, gradient matching of Eph–Bs and ephrin–Bs have been implicated in D–V mapping. Indeed, when multiple Eph–Bs are knocked out, RGCs exhibit retinotopic D–V targeting errors in the SC (Hindges et al., 2002). However, molecules other than Eph/ephrin–Bs also contribute to D–V mapping. In chicks, wingless (Wnt) signaling acting through Ryk receptors is crucial for D–V mapping (Schmitt et al., 2006). Whether Wnts play a role in retinotopic mapping in mammals is yet to be tested. Another consideration is that axons from RGCs situated along the D–V axis of the retina are “preordered” in the optic tract before they reach their targets (Plas et al., 2005). Whether this order is due to axon–axon recognition cues or whether it reflects differences in the timing of outgrowth for dorsal- versus ventral-RGCs, is not known. Clearly, more work is needed to understand
the mechanisms that establish D-V maps and some of those mechanisms appear to engage before RGC axons even reach their targets.

2.3.4. Activity and ephrins: Separate paths toward the same goal
We have described how neural activity and molecular guidance cues contribute to retinotopic map development. A key question therefore is: do activity and ephrins operate in the same pathway or do they operate in parallel? *In vitro* studies show that cAMP oscillations can influence RGC responsiveness to ephrin-As (Nicol *et al.*, 2007). Given that retinal waves are strongly dependent on cAMP levels (Stellwagen *et al.*, 1999), crosstalk between waves and ephrin-As could impact retinotopic mapping. However, *in vivo* evidence indicates that altering waves does not impact Eph-A expression in RGCs, nor does altering ephrin-As perturb wave activity (Huberman *et al.*, 2005; Pfeifferberger *et al.*, 2005). Indeed, the influence of activity, ephrin-As, and D–V mapping are strikingly separable by different experimental manipulations. As mentioned previously, altering retinal waves disrupts the precision of retinotopic TZs, but the approximate position of the TZ is normal (Chandrasekaran *et al.*, 2005; Grubb *et al.*, 2003; McLaughlin *et al.*, 2003; Fig. 8.3). Conversely, genetic removal of Eph-As or ephrin-As alters retinotopic mapping, but the ectopic TZs that form are normal in size (Feldheim *et al.*, 2000; Frisen *et al.*, 1998; Fig. 8.3). Finally, if both wave activity and ephrin-As are disrupted, the N–T retinotopic map is completely abolished, but the D–V map is spared (Cang *et al.*, 2008; Fig. 8.3). Thus, the current model of retinotopic mapping is based in the idea that patterned activity and mapping molecules operate in parallel: RGC axons are delivered to their grossly appropriate location by ephrin-As, ephrin-Bs, and Wnts and then neural activity drives those axons to cluster into a focal TZ (Feldheim and O’Leary, 2010; Huberman *et al.*, 2008b; McLaughlin and O’Leary, 2005). A major emphasis in the field now is to understand the signaling pathways that act downstream of activity and ephrins to mediate RGC axon–axon remodeling.

2.4. Segregating into eye-specific territories
After they establish retinotopic maps, mammalian RGCs are faced with a unique challenge. Because mammals have eyes positioned toward the front of their skulls, the visual fields viewed by each of the two eyes will overlap to some degree. As a result, some RGCs in the two eyes (i) view the same portion in visual space, (ii) project to the same side of the brain, and (iii) innervate the same retinorecipient targets. Ephrins in turn direct a subset of RGC axons from the two eyes to identical locations in their targets. Indeed, in developing carnivores, axons from the two eyes are retinotopically aligned and they overlap within their targets (Jeffery, 1985). This overlap does not persist, however; right and left eye axons
Figure 8.4  Eye-specific segregation in normal, ephrin-A, and wave-deficient mice. RGC axons from the contralateral (red) and ipsilateral (green) eyes and the regions where they overlap (yellow) are shown in the visual thalamus of the mouse (see Fig. 8.1 for description of these targets). (Top row) Axons from the two eyes overlap early in development. (Middle row) If normal retinal waves are present, over time axons from
always refine into contralateral and ipsilateral domains—a process referred
to as eye-specific segregation (Fig. 8.4). How RGC axons progress from an
overlapping to a segregated state has been a major focus of visual neurosci-
ence for more than three decades (Godement et al., 1984; Linden et al.,
1981; Rakic, 1976; Shatz, 1983), and remains a premiere model system for
studying CNS circuit refinement (reviewed in Huberman et al., 2008b).

2.4.1. Cellular changes that drive eye-specific segregation
Eye-specific segregation has mostly been studied in the dLGN where axons
from the right and left eyes occupy territories of relatively stereotyped
shape, size, and position. In an elegant series of now-classic studies, Shatz
and coworkers explored the cellular rearrangements that RGC axons
undergo as they progress from an intermingled to eye-specific state. They
labeled individual RGC axons in fetal cats of different ages and analyzed the
morphology of the labeled terminals in the dLGN (Sretavan and Shatz,
1984, 1986). Overall, they observed that RGC axons undergo dramatic
growth and remodeling to achieve eye-specific segregation. During the
overlap stage, RGC axons extend across the full width of the dLGN and
display multiple “side branches” along their length. EM studies later showed
those side branches are the substrate by which right and left eye axons form
synapses onto the same dLGN neurons (Campbell and Shatz, 1992). The
progression from an overlapping to an eye-specific state occurs as all the side
branches are removed—except one—which in turn expands to form dense
terminal arborizations in the correct eye-specific territory (Fig. 8.4;
reviewed in Shatz, 1996). Although the precise cellular rearrangements
that occur may somewhat vary across species (e.g., Snider et al., 1999), the
classic studies of Shatz and co-workers provided the basis for understanding
how RGC axons remodel in order to achieve an eye-specific state.

2.4.2. Spontaneous activity is essential for eye-specific targeting
What forces drive eye-specific segregation in the dLGN? One thing is
certain: it is not visual experience, because this process is completed before
photoreceptors are capable of responding to light (reviewed in Huberman
et al., 2008b). Rakic (1976, 1977) showed that if one eye is removed during
the two eyes segregate into nonoverlapping, “eye-specific” domains. If early retinal
waves are altered, RGC axons remain overlapping. In ephrin-A2/5 KO mice, axons
from the two eyes segregate but into retinotopically misplaced patches. (Bottom row)
Blocking waves after eye-specific segregation is completed causes desegregation. Con-
versely, if waves are blocked but then wave activity is allowed to recover, eye-specific
patches form, but at retinotopically misplaced locations in the target. In ephrin-A2/5
KO mice, the ectopic eye-specific patches are stable over time.
the early overlap stage, axons from the intact eye remain throughout the
target. Thus, eye-specific segregation is dependent on competitive interac-
tions between axons from the two eyes.

Sretavan et al. (1988) proposed that spontaneously generated activity
mediates binocular competition leading to ocular segregation in the dLGN.
They tested that hypothesis by infusing TTX into the brain of fetal cats,
starting at the time when right and left eye axons overlap. TTX prevented
eye-specific segregation by inducing dramatic growth of RGC axons. That
eventually led to the idea that retinal waves are the source of activity that
drives binocular competition in the dLGN (Shatz, 1996). The retinal waves
that occur during eye-specific segregation are driven by acetylcholine
(Feller et al., 1996; Torborg and Feller, 2005a), so Penn et al. (1998) used
the cholinergic drug, epibatidine, to perturb retinal waves in neonatal
ferrets. The results of that manipulation were clear: when spontaneous
wave activity was reduced in one eye, axons from the contralateral eye
expanded within the dLGN and axons from the activity-manipulated eye
shrank their overall TZ. By contrast, when wave activity was reduced
activity in both eyes, RGC axons failed to segregate and remained diffuse
throughout the target (Penn et al., 1998). Those results have now been
confirmed many times over in ferrets and mice (Fig. 8.4; reviewed in
Huberman et al., 2008b; also see Koch and Ullian, 2010). The evidence is
therefore strong that spontaneous retinal activity is necessary for eye-specific
segregation in the dLGN. Indeed, the need for retinal waves is ongoing
throughout early development; if the retinal waves are first eliminated or
altered starting after eye-specific segregation is complete, axons from the two
eyes desegregate in the dLGN (Chapman, 2000; Demas et al., 2006;
Fig. 8.4). Together these studies show that retinal waves play a fundamental
role in both establishing and consolidating the basic architecture of eye-
specific connections in the mammalian visual system.

2.4.3. What forms of activity drive segregation?
Retinal waves induce correlated firing in RGCs. A key question is whether
those correlations are the parameter underlying eye-specific segregation or
whether waves play a more “permissive” role in shaping visual connections.
One could imagine, for example, that correlated RGC firing induced by
retinal waves directly mediates Hebbian refinements at retino-dLGN
synapses in a manner similar to how visual experience influences OD
plasticity during the critical period (reviewed in Feller, 2009; Hensch,
2005; Smith et al., 2009). Computational modeling based on the spatial
temporal properties of retinal waves supports that idea (Butts et al., 2007;
Feller, 2009). Alternatively, eye-specific segregation may be dictated by
guidance molecules that are only capable of exerting their effects on RGCs
that have normal levels and patterns of activity (Chalupa, 2009; Crowley
and Katz, 2000). Indeed, there is evidence that neural activity can modulate
RGC axon outgrowth, branching, and guidance (Goldberg et al., 2002b; Nicol et al., 2007). To distinguish among these possibilities, it is necessary to alter waves without silencing RGC firing altogether and then evaluate the consequences of that on eye-specific refinement. Stellwagen and Shatz (2002) were the first to accomplish that feat. They used cAMP-augmenting drugs to increase wave size and frequency in one or both eyes of developing ferrets. When they increased waves in one eye, they saw that axons from the normal unmanipulated eye lost territory in the dLGN. By contrast, when they increased waves in both eyes, binocular connections formed normally (Stellwagen and Shatz, 2002). Those results show that the relative level of activity in the two eyes is critically important for eye-specific segregation and that normal activity levels do not necessarily lead to normal patterns of visual connections. Together those data challenge the idea that activity is merely “permissive” for RGC axon growth and targeting.

The correlated firing of neighboring RGCs is the property of retinal waves that most models consider important for retinotopic and eye-specific refinement (Butts et al., 2007). That makes sense because RGC firing is what actually drives the spiking and synaptic plasticity of dLGN and SC target neurons (Mooney et al., 1996; Shah and Crair, 2008). However, to test if RGC firing patterns are in fact crucial for eye-specific segregation, it is necessary to somehow eliminate the “correlated” component of spontaneous retinal waves while retaining overall levels of activity. The first study to accomplish this used an immunotoxin directed against the interneurons that generate early retinal waves. That reduced the correlated firing of neighboring RGCs but did not change the overall levels of spiking activity in the retina. Surprisingly, eye-specific segregation proceeded normally under these conditions (Huberman et al., 2003). Those findings, as well as a study showing that intraocular TTX injections do not prevent eye-specific segregation (Cook et al., 1999), supported the idea that activity plays a permissive role in shaping binocular visual circuits. However, it is important to note that calcium wave activity persisted for some time in the immunotoxin-treated retinas and that TTX does not prevent calcium waves (Cook et al., 1999; Huberman et al., 2003; Stellwagen et al., 1999). It therefore remains possible that broader scale correlations (i.e., larger waves) and/or calcium waves were present at levels sufficient to drive eye-specific segregation. More rapid and complete ablations of correlated activity, combined with large-scale recordings of retinal neurons (e.g., Stafford et al., 2009), are needed to better understand if wave-like patterns of activity are necessary for eye-specific segregation.

A large number of studies make one thing certain: something in the pattern of spontaneous retinal activity is critically important for eye-specific segregation. As mentioned above, beta2 knockout mice can, under some recording conditions, exhibit waves that are abnormally large and fast and that exhibit altered directionality (Sun et al., 2008; Stafford et al., 2009).
Beta2 mice also exhibit defects in eye-specific segregation (Huberman et al., 2008b; Muir-Robinson, 2002; Pfieffenberger et al., 2005; Rossi et al., 2001; Fig. 8.4). Still, it remains unclear exactly which parameters of retinal waves directly relate to eye-specific refinement. The ultimate experiment would be to systematically control the patterns of RGC spiking in the two eyes and thereby isolate which patterns of RGC activity drive eye-specific segregation—something that is now possible with the advent of optogenetic tools to modulate neural activity (e.g., Boyden et al., 2005; Zhang et al., 2007).

### 2.4.4. A potential role for guidance molecules in eye-specific targeting

Any review of eye-specific targeting would be remiss if the concept of activity-independent factors was not addressed. In a purely activity-dependent model, right and left eye axons should sort into salt and pepper like patterns. In reality, however, the basic pattern of eye-specific projections is essentially invariant for a given species. This stereotypy argues that factors other than neural activity help shape eye-specific connections. Differences in the timing of ingrowth for contralateral versus ipsilateral eye axons were hypothesized to ensure the stereotyped ordering of eye-specific territories (Shatz, 1996). Unfortunately, timing of axon growth is a difficult variable to manipulate in vivo and therefore has never been tested. Another hypothesis is that molecular cues pattern the regular spatial layout of eye-specific domains (Chalupa, 2009; Crowley and Katz, 2000). A naturally occurring experiment that indirectly supports that idea is the Belgian achiasmatic sheepdog—a spontaneously occurring mutant in which all RGCs project the ipsilateral side of the brain. In this remarkable dog, axons from the temporal retina form a domain in the dLGN that is separate from the domain formed by the axons arising from the nasal retina—even though both sets of axons arise from the same eye (Williams et al., 1994). Binocular competition cannot underlie this segregation because axons from the two eyes never had the chance to interact. Similar observations have been made in ferrets, cats, and mice with altered RGC pathfinding at the optic chiasm (Guillery, 1969a,b; Rebsam et al., 2009). In those animals, axons from RGCs in different parts of the retina segregate from one another, even though they originate from the same eye.

What sort of molecules might contribute to eye-specific patterning? In considering this question, it is worthwhile to note that contralateral versus ipsilateral eye-specific domains correspond to inputs from RGCs in the nasal versus temporal retina, respectively. Since Eph-A levels distinguish RGCs along the N–T retinal axis, they are good candidates to test in the context of eye-specific mapping. In mice, the story is very straightforward: ephrin-A2/5 are expressed in gradients suitable for a role in retinotopic mapping along the N–T axis of the dLGN (Feldheim et al., 2000). If ephrin-A2/5 are knocked out, axons from the two eyes form patches scattered throughout the dLGN. However, ipsi- and contra-axons still segregate...
from one another in ephrin-A2/5 mutants (Fig. 8.4; Pfieffenberger et al., 2005). In ferrets, the role of ephrins is slightly more complicated and suggests they play a more direct role in segregating axons from the two eyes. If Eph-As are misexpressed in the retinas of neonatal ferrets, many axons from the two eyes fail to segregate from one another. Indeed, altering Eph-As in newborn ferret causes right and left eye RGC axons to overlap almost as much as they do following epibatidine-induced activity blockade (Huberman et al., 2005). It is not entirely clear why ephrins mediate eye-specific segregation in ferrets but not in mice. This discrepancy may relate to the fact that ferrets have eye-specific projections that are mirrored by distinct cellular layers (Linden et al., 1981), whereas mice do not (Godement et al., 1984; but also see Reese, 1988).

Recent experiments show that contra- versus ipsi-projecting RGCs are molecularly distinct in a way that is independent of their different trajectories at the optic chiasm because in albino or ephrin mutants, eye-specific zones cluster into mini-islands of purely contra- or purely ipsi-eye axons (Pfieffenberger et al., 2005; Rebsam et al., 2009). Screens for molecules that are differentially expressed in contralateral versus ipsilateral domains of the dLGN have not yet revealed any candidate eye-specific patterning molecules (Kawasaki et al., 2004), but as the sensitivity of genomic and proteomic screens improve, such cues may eventually be identified. In the meantime, the dominant model of eye-specific segregation is that spontaneous retinal activity helps cluster inputs from the same eye, and ephrin-As position those eye-specific projections into stereotyped retinotopically appropriate locations in the dLGN.

2.5. Picking a depth: Laminar-specific targeting

At this point in development, RGC axons have found their overall targets, arrived at the correct retinotopic zone and segregated into the appropriate eye-specific domain. Next they face the task of finding and forming synapses with the correct target neurons. One way that nature has simplified this task is by positioning different types (or portions) of postsynaptic neurons at different depths within their targets, creating parallel “layers” for different aspects of visual processing. By directing RGC axons to particular layers, functionally specific synaptic connections are maintained (Nassi and Callaway, 2009; Sanes and Yamagata, 1999).

One of the more salient examples of laminar specificity in the eye-to-brain pathway is the division of magnocellular (M), parvocellular (P), or koniocellular (K) layers in the primate dLGN (reviewed in Callaway, 2005; Nassir and Callaway, 2005). Generally speaking, the three different types of dLGN laminae receive axons from RGCs that encode motion (M), color and form (P), or yellow/blue color opponency (K). It should be noted, however, that
the number of different RGC subtypes that are known to project to the dLGN is ever-expanding (Crook et al., 2008; Dacey et al., 2003).

Despite the fact that laminar specificity is a salient and functionally relevant aspect of visual circuit organization, how it develops is not well understood. The main reason for this gap in understanding is that, until recently, there was no way to distinguish axons rising from functionally distinct RGCs until they achieved their adult patterns of connectivity. Work in hamsters suggested that RGC axons initially project broadly across the depth of the retinorecipient SC before refining to their correct lamina (Sachs et al., 1986). By contrast, a study in the macaque showed that magnocellular and parvocellular projecting RGCs diverge early on to target separate dLGN regions (Meissirel et al., 1997). The interpretation of those studies is complicated, however, by the fact that without markers to distinguish RGC axons destined to project to particular SC or dLGN laminae, it is impossible to know if a given axon is making correct versus incorrect targeting choices.

Recently, the discovery of a RGC subtype-specific marker was used to study the development of laminar specificity. In calbindin2 (CB2)–green fluorescent protein (GFP) mice, Off-alpha RGCs selectively express GFP and project specifically to the deeper layer of the retinorecipient SC (Huberman et al., 2008a; Fig. 8.5). By monitoring the GFP-labeled alpha axons across development, it was observed that laminar-specific targeting

![Image of Figure 8.5](image-url)

**Figure 8.5** Laminar specificity of Off-alpha projections to the superior colliculus. (A, B) Off-alpha RGCs (outlined dashed circles) selectively express GFP (green fluorescent protein) in CB2–GFP mice. Amacrine cells (smaller cell bodies) also express GFP in these mice. (B) The axons of Off-alpha RGCs terminate at a specific laminar depth of the retinorecipient SC (superior colliculus). The axons from all RGCs are shown in red, whereas Off-alpha axons are shown in green. The green axons also form patches or “columns” within their layer of the SC. (D) Schematic diagram of Off-alpha RGC axons during the stage they pick their correct laminar depth in the SC (see main text and Huberman et al., 2008a for details).
occurs through broad-scale axonal refinement; Off-alpha axons initially project across the entire depth of the retinorecipient SC before removing their input from the superficial lamina (Fig. 8.5; Huberman et al., 2008a). A subsequent study replicated that finding for a different RGC subtype, and showed that some other RGCs target their correct lamina from the outset (Kim et al., 2010). Ultimately, however, labeling of specific RGC subtypes and their postsynaptic neurons is needed to determine if laminar-specific refinements reflect axonal retraction, synapse loss, or degeneration. In the meantime, one can conclude that laminar specificity often emerges from initially imprecise connections.

As with the other aspect of eye-to-brain connectivity described above, laminar specificity occurs prior to vision and during the period when waves propagate across the retina. Whether retinal waves help establish laminar-specific connections was tested by crossing CB2–GFP mice with beta2 knockout mice. The phenotype of those mice was highly consistent; laminar specificity of Off-alpha RGC axons developed normally in both the SC and dLGN (Fig. 8.6; Huberman et al., 2008a). That result is consistent with studies in fish and chickens that completely silenced RGCs or tectal cells and no impact on laminar-specific targeting of RGC axons (Inoue and Sanes, 1997; Nevin et al., 2008). No study has yet examined the impact of removing all activity on laminar specificity in mammals, but the bulk of evidence points to the idea that RGCs establish layered axonal connections on the basis activity-independent cues.

2.5.1. Molecular cues that direct laminar specificity in mammals:

The search continues

Despite numerous lines of evidence pointing to the idea that adhesion molecules promote laminar specificity of RGC dendrites and axons in mammals, the identity of those molecules has remained elusive. In chickens, the

![Diagram](image-url)
sidekicks and the DSCAMs (down syndrome cell adhesion molecules) are expressed in subsets of RGCs and amacrine cells where they regulate dendritic targeting (Yamagata and Sanes, 2008). A recent study from the Burgess lab (Fuerst et al., 2009) asked if DSCAMs perform a similar function in mammals. They generated DSCAM and DSCAM-like (DSCAML1) knock-out mice. Surprisingly, removal of one or both DSCAMs caused dramatic alterations in RGC soma spacing (a feature called “mosaicism”) but it did not prevent laminar-specific targeting of RGC dendrites. Indeed, even functional specificity of synaptic connections was preserved in DSCAM mutant retinas (Fuerst et al., 2009). Whether DSCAM mutant axons maintain laminar specificity of their connections is still unknown, but overall, the results of Fuerst et al. suggest that DSCAMs are unlikely to mediate laminar synaptic specificity in the mammalian visual system.

If DSCAMs are dispensable for laminar specificity in mammals, then what sorts of molecules might perform this role? Cadherins are a large class of cell adhesion molecules that typically promote homophilic attraction. Given their established role in promoting laminar specificity of the fly and chick visual systems (Inoue and Sanes, 1997; reviewed in Clandinin and Zipursky, 2002), the cadherins are exciting candidates to mediate RGC laminar specificity in mammals. At the same time, axon repellants could establish laminar-specific connectivity through graded expression across the depth of the target (reviewed in Huberman et al., 2010). Given that not a single molecule has yet been identified as critical for establishing laminar-specific RGC connections in the mammalian brain, the search for laminar cues is going to be an intense and exciting area of research in the next few years.

2.6. Dividing into columns: Functional modules

The arrival of an RGC axon to its appropriate layer in the brain is analogous to the arrival at a particular “zip code”—it represents a specific and defined territory but not necessarily a final destination. After RGC axons arrive at the appropriate layer, they still have to distinguish among the various cell types that reside there. This aspect of RGC wiring specificity relates to different aspects of visual circuit function. As such, it is not revealed unless the entire population of one RGC subtype is selectively labeled—something that has only recently become possible as genetic tools for labeling specific RGC subtypes have become available. For example, in the above-mentioned CB2–GFP mice, the entire mosaic of Off-alpha RGCs not only projects to a specific layer in the dLGN and SC, but within the SC, those connections are also arranged into regular alternating patches or “columns” of synaptic terminals (Huberman et al., 2008a; Fig. 8.5). These columns do not correspond to right versus left eye connections so one idea is that they are modules representing specific aspects of the visual scene. In this sense, RGCs that view the same location in visual space and encode the same
quality of visual information will send that information to specific target neurons. Indeed, closer inspection of the columns formed by Off-alpha RGC axons in the SC revealed that (i) $\sim 3$–4 Off-alpha RGCs project to the same column and (ii) individual RGC axons often branch to innervate multiple columns (Huberman et al., 2008a). Other RGC subtypes also can form columns in the SC (Huberman, unpublished data). Synaptic columns arising from the retina therefore represent an intriguing case of microcircuitry that elevates the demands for developmental mechanisms that can distinguish among RGC subtypes.

How do RGC axonal columns develop? Analysis of postnatal CB2–GFP mice revealed that Off-alpha columns emerge from an initially imprecise state; shortly after birth, there is a crude semblance of columnar specificity but only once RGCs begin to elaborate their arbors and form synapses do these columns become readily apparent (Huberman et al., 2008a). Thus, like many of the other forms of eye-to-brain connectivity (Simon and O’Leary, 1992; Sretavan and Shatz, 1986), RGC subtype-specific columns emerge through axonal refinements and directed synapse formation (Fig. 8.5).

The columns formed by Off-alpha RGCs emerge during the early postnatal period when retinal waves occur. CB2–GFP:beta2 knockout mice thus provide a direct test of whether waves play an important role in establishing axonal columns. As we noted above, Off-alpha RGCs still refine into the correct synaptic layer in the dLGN and SC in the presence of altered waves. However, those abnormal waves completely prevent Off-alpha axons from achieving columnar specificity (Fig. 8.6; Huberman et al., 2008a). This cannot be due to loss of beta2 nicotinic AChRs in target SC neurons because the phenotype is mimicked by injecting a cholinergic blocker into the eyes of otherwise wild-type CB2–GFP mice. Collectively these results tell us that waves heavily influence columnar-specific axon targeting, presumably by inducing distinct firing patterns in different RGC subtypes (Kerschensteiner and Wong, 2008).

2.7. Subcellular targeting and microcircuitry

As one looks closer and closer at RGC wiring specificity, it becomes increasingly clear that there will be additional developmental programs to ensure precision of RGC axonal connections. For example, fine-scale anatomical studies revealed that RGC synapses are selectively concentrated on the soma and proximal dendrites of dLGN neurons, whereas cortical input to dLGN cells resides elsewhere on the dendritic tree (Bickford et al., 2010; Sherman, 2004). How RGCs achieve this immensely precise synaptic targeting is not known. It could be that RGC axons are the first afferents to arrive in the dLGN and therefore synapse onto dLGN neurons whose dendrites are still small and immature. Alternatively, portions of the dLGN neuron’s dendritic tree may be molecularly distinct and thus reserved
for retinal versus nonretinal sources. The formation of perisomatic connections onto cortical neurons is regulated by adhesion molecules belonging to the IgG superfamily (Ango et al., 2004), so there is a prescient for this idea. Given that where synapses reside on the dendritic tree is critical for local circuit computations and output (Poirazi et al., 2003; Sherman, 2004), probing how subcellular targeting develops in the visual system is an important and relatively untapped area of study.

2.8. Forming and eliminating a synapse

As we have reviewed the various stages of visual circuit wiring, we have periodically mentioned “synapses”—which of course are the basis by which RGCs communicate with the brain. With the exception of RGC pathfinding out of the eye and through the chiasm, essentially all the wiring milestones we have discussed involve synapse formation and/or elimination to some degree or another. Thus, the critical question remains—how do RGCs establish actual synaptic connections with their target neurons?

Several of the molecules that promote glutamatergic synapse formation have been identified including neuroligins, SynCAMs, thrombospondins, FGfs, and SynDigs (for review, see McAllister, 2007; Waites et al., 2005; also see Kalashnikova et al., 2010). Indeed, the thrombospondins were isolated as astrocyte-derived factors capable of promoting synapse formation in mammalian RGCs in vitro (Christopherson et al., 2005; Ullian et al., 2001). Most synaptogenic molecules are not thought capable of distinguishing among different neuronal subtypes to promote synaptic specificity. However, there is evidence that different neuroligin family members control excitatory versus inhibitory synaptogenesis in hippocampal neurons (Chih et al., 2005; Chubykin et al., 2007; Graf et al., 2004), so the possibility cannot be ruled out that different RGC subtypes employ different synaptogenic molecules to connect with their specific targets, layers and postsynaptic cells in the brain.

Throughout this review, we also described RGC axonal “refinement”—some of which (e.g., eye-specific refinement) involve the elimination of functional synapses (Campbell and Shatz, 1992; Chen and Regehr, 2000; Jaubert-Miazza et al., 2005). Recent experiments have greatly enhanced our understanding of the molecular signals that promote synapse elimination. Indeed, many of these were discovered for their impact on the stability of RGC synapses. For example, in order to understand how TTX prevents eye-specific refinement (Sretavan et al., 1988), Shatz and co-workers screened for molecules whose expression is regulated by spontaneous activity and is altered by TTX. They discovered that the immune family of major histocompatibility complex (MHC) I proteins are strongly regulated by spontaneous activity (Corriveau et al., 1998). They went on to show that the MHC receptors are expressed in the developing visual system where they are required for eyespecific segregation (Huh et al., 2000). Recently, experiments from the Shatz
lab also identified the ligands that mediate MHC-dependent synapse elimination in the dLGN (Datwani et al., 2009). The MHCs belong to the adaptive immune system but molecules of the innate immune system are also important for removal of excessive RGC synapses leading to circuit refinements. Stevens et al. (2007) discovered that the complement protein C1q is expressed by RGCs during development, and is required for eye-specific segregation and fine-scale elimination of retino-dLGN synapses (Stevens et al., 2007). Recent studies also point to the neuronal pentraxins as crucial for translating activity into structural refinements at developing RGC synapses by affecting the conversion of synapses from a “silent” to an “active” state (Bjartmar et al., 2006; Koch and Ullian, 2010). Others have proposed that immune proteins act upstream of activity by regulating glutamate transmission and dendritic dynamics (Xu et al., 2010). Regardless of mechanism, the emerging theme is that immune genes are important regulators of RGC synapse elimination during development. Given their widespread expression throughout the developing CNS, these genes are likely to regulate developmental refinement of diverse CNS circuits.

The discovery of new molecules that influence RGC targeting is ongoing. Culican et al. (2009) also recently identified a ubiquitin-ligase related molecule that is capable of modifying RGC synapses that is independent of activity or ephrin-As and that does not appear directly linked to the immune system. This underscores the idea that diverse molecular pathways will converge to direct proper synapse formation and refinement in the developing visual system.

2.9. Modifying synapses in response to experience

By the time of eye opening, RGC axonal connections are essentially adultlike. A hallmark principle of critical period visual plasticity is that RGC projection patterns are not strongly impacted by visual experience. This appears true for eye-specific projections in the dLGN (Wiesel and Hubel, 1963). However, other aspects of RGC axonal connectivity appear susceptible to visual experience. For instance, Hooks and Chen (2006) showed that visual deprivation alters the fine-scale retinotopic mapping in the dLGN by causing RGC inputs to revert to a poly-innervated state. Other features of RGC connectivity could be malleable in response to experience as well, but surprisingly few studies have examined this.

3. Conclusions and Future Directions

We have now reviewed the complete journey that an RGC axon takes in order to achieve its precise circuit connections. In doing so, we hope to have made apparent that each milestone—exiting the eye,
recognizing a target, selecting a layer, etc.—further constrains the number and type of synapses that a RGC can make and ultimately leads to highly precise circuitry. Two broad themes were intended to emerge from our sequential description of eye-to-brain wiring. First, guidance molecules demarcate correct versus incorrect territories at progressively finer scales over time. Second, neural activity plays an ongoing and critical role in honing the precise location and size of RGC arbors. We hope to have also made clear that many fundamental discoveries remain to be made in this area. Indeed, huge gaps remain in our understanding of how RGCs achieve overall target, laminar, and subcellular wiring specificity. The recent advent of genetic markers for functionally distinct RGC subtypes, combined with the rich set of tools to manipulate neural activity and gene expression, make this a truly unprecedented and exciting time for probing how the eyes wire up with the brain and ultimately, how that wiring influences visual perception and behavior.

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