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Assembly and repair of eye-to-brain connections

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Vision is the sense humans rely on most to navigate the world and survive. A tremendous amount of research has focused on understanding the neural circuits for vision and the developmental mechanisms that establish them. The eye-to-brain, or ‘retinofugal’ pathway remains a particularly important model in these contexts because it is essential for sight, its overt anatomical features relate to distinct functional attributes and those features develop in a tractable sequence. Much progress has been made in understanding the growth of retinal axons out of the eye, their selection of targets in the brain, the development of laminar and cell type-specific connectivity within those targets, and also dendritic connectivity within the retina itself. Moreover, because the retinofugal pathway is prone to degeneration in many common blinding diseases, understanding the cellular and molecular mechanisms that establish connectivity early in life stands to provide valuable insights into approaches that re-wire this pathway after damage or loss. Here we review recent progress in understanding the development of retinofugal pathways and how this information is important for improving visual circuit regeneration.

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Current Opinion in Neurobiology 2018, 53:198–209

This review comes from a themed issue on **Developmental neuroscience**

Edited by **Alex Kolodkin** and **Guillermina López-Bendito**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 16th October 2018

<https://doi.org/10.1016/j.conb.2018.10.001>

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Introduction

Understanding how the nervous system ‘wires up’ is one of the central quests of biology. More than 100 years ago, Cajal initiated work to understand how nerve cells grow out their processes and connect with each other- in an effort to understand how to ‘generate’ the nervous system

[1]. Cajal also proposed that, in order to understand how to regenerate the nervous system after injury, one should look to the normal course of developmental events that established them in the first place [2]. Here we review recent progress exploring how a particular pathway — the connections linking the eyes to the brain — initially grow out their axons and target appropriate brain areas, topographic locations, and laminar depths within their targets — all of which are necessary for light — driven percepts and behaviors. We also discuss recent work showing how developmental mechanisms can be leveraged towards regeneration of visual connections and visual function.

The eye-to-brain pathway: basic features and developmental emergence

The eye-to-brain (or ‘retinofugal’) pathway consists of the axons from retinal ganglion cells (RGCs), the output neurons of the eye. There are ~30 RGC types, each of which responds best to a particular feature in the visual environment by virtue of the cell-type specific connections it collects on its dendrites within the retina [3^{••},4^{••}], and its connections to 1, or as many as 4, of the ~40 subcortical retinorecipient nuclei in the brain [5[•]]. These specific axonal connections in turn drive conscious perception of visual scenes (‘sight’) and also non-image-forming visual functions that support sight such as eye movements and pupil reflexes. Some RGC connections also drive other non-image-forming visual functions that influence the brain and body over long time scales, including entrainment of circadian rhythms and hormone secretion [6^{••}].

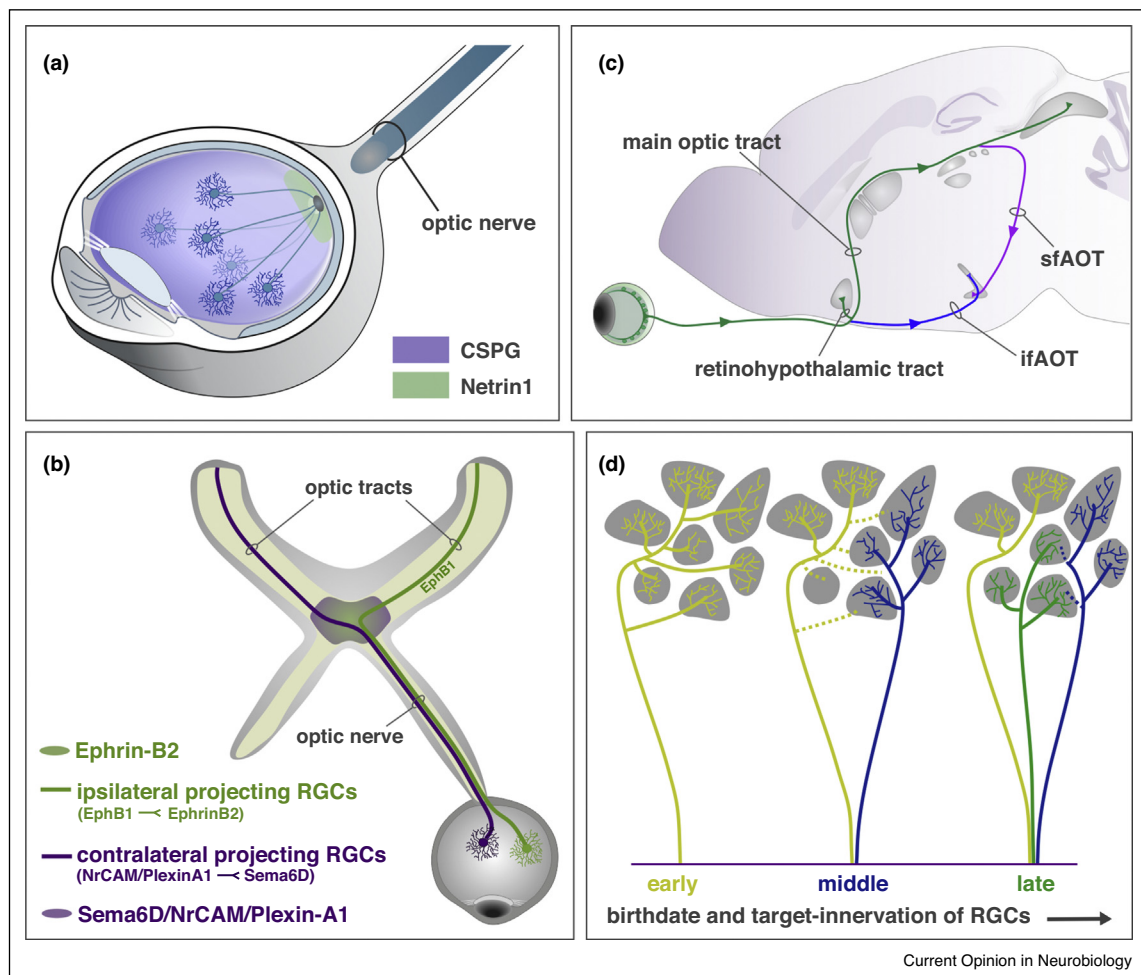
The retinofugal pathway, in addition to being experimentally accessible, has all of the anatomical and functional features one could wish for in a system of study where the goal is to understand the mechanisms of neural circuit development and regeneration. It has distinguishable cell types that require specification and whose dendritic targeting patterns are both visible and meaningful. It exhibits long range and short range axon targeting specificity, and also within-target specificity such as topographic and eye-specific organization crucial for accurate representation of the outside world. Therefore, this pathway has remained a prominent focus of developmental neurobiologists for >50 years. Moreover, the retinofugal pathway is vulnerable to many common neurodegenerative disease such as glaucoma [7], stroke, and head trauma [8]. Together, all of these features continue to provide a platform for addressing how to generate and regenerate eye-to-brain connections after injury, both for the sake of reversing blindness and as a general model for CNS injury.

RGC axon growth out of the eye and down the optic nerve

RGCs are born in the ventricular zone of the nascent eye-cup, where they are endowed with the expression of different transcription factors that segregate them into distinct functional types [9^{*},10^{**},11,12]. RGCs extend axons as they migrate into the inner retina [13–15]. The trajectory of these axons away from the periphery and

toward the optic nerve head, where they can exit the eye, is governed both by the inhibitory influence of chondroitin-sulfate proteoglycans (CSPGs) expressed around the border of the eye-cup and by the attractive influence of Netrin-1 (Figure 1a) [16,17]. Netrin-1 is expressed at the center of the eye and attracts RGC axons through interactions with the Netrin-1 receptor Deleted in colorectal cancer (Dcc) expressed by RGC axons [17].

Figure 1



RGC axon pathfinding and target selection.

(a) Retinal ganglion cells extend axons away from the periphery due to repulsive influences from chondroitin sulfate proteoglycans (CSPG, purple) and grow towards the optic disc. Netrin 1 expressed by glial cells at the optic disc (green) provides local attractive cues and enables axons to exit the eye, into the optic nerve.

(b) Schematic of the optic nerve reaching the optic chiasm, a midline choice point for RGC axons. EphrinB2 expressed at the midline repels EphB1 expressing RGC axons to form ipsilateral projections (green), while Sema6D/NrCAM/PlexinA1 complex directs other RGCs to cross at the chiasm and form the contralateral projections (magenta).

(c) Schematic showing the anatomical position of the eye, optic nerve, and optic tracts projecting to targets in the brain. After crossing the chiasm, the contralateral projection ascends into the brain to form the main optic tract (green). A smaller bundle projects into the SCN at the base of the hypothalamus, forming the retinohypothalamic tract. Two bundles deviate from the tract however: the inferior fasciculus of the accessory optic tract (ifAOT, blue), extends at the base of the brain to project to the MTN, while another bundle continues from the main optic tract and dives down to form the superior fasciculus of the AOT (sfAOT, pink).

(d) Birth order of RGCs determines their target-selection and exploration. Early-born RGCs extend axon branches into many targets (yellow lines). Axons born shortly after (blue) extend to a few different targets while the early born axons retract some connections (yellow dotted lines). The later-born axons project directly to their targets without much exploration (green).

The neurogenic birthdates of different RGC types vary in time [18*,19], as do the birthdates of RGCs programmed to extend their axons to the contralateral versus the ipsilateral side of the brain [20*,21]. However, in general RGC axons obey a common growth program as they extend out of each eye and form the developing optic nerves. Once they exit, individual RGC axon mingles with axons of other RGC types from the same eye and course toward the optic chiasm in a tight bundle at the base of the brain (Figure 1b–d).

RGC axon tract segregation and pathfinding

When RGC axons reach the optic chiasm at the base of the brain, they orient to the midline and either cross ('decussate') or remain ipsilateral, a binary decision that is crucial for establishment of visuotopic and binocular visual maps in downstream central targets [21]. Decussation of SoxC expressing RGCs [22**] is mediated in large part by the expression of a Sema6D/Nr-CAM/Plexin-A1 receptor complex that promotes growth in NrCAM-PlexinA1-expressing contralateral projecting RGCs [23]. The RGC axons that project ipsilaterally do so because they express EphB1, which transduces a repellent signal from ephrinB2 expressed by midline radial glial cells [24*]. The EphB1 expression in ipsilateral-projecting cells is driven by the transcription factor Zic2 (Figure 1b) [25]. Interestingly, species with eyes located in more frontal positions express Zic2 in larger populations of RGCs [26]. Moreover, cell autonomous factors mediate fasciculation of axons within the optic tract such that ipsilateral and contralateral projections remain segregated in the optic nerve and tract [27*].

After they navigate the chiasm, RGC axons select 1 of 3 tracts to reach their targets (Figure 1c). First, a limited number of RGC axons (originating from M1 and M2 type intrinsically photosensitive RGCs—so-called because they act as photoreceptors [28]) project into the hypothalamus to innervate the master circadian pacemaker: the 'suprachiasmatic nucleus' (SCN). In doing so, they form the 'retinohypothalamic tract' (Figure 1c). This connection is crucial for a myriad of non-image-forming functions such as linking of endogenous arousal and hormone secretion rhythms with the environmental light-dark cycle [reviewed in Ref. [6**]]. A second population of RGC axons, upward-selective on-type direction selective RGCs that express Spig1 [29*], depart the posterior optic chiasm and travel in a tight fascicle along the base of the brain in what is termed the 'inferior fasciculus of the accessory optic tract' (ifAOT) (Figure 1c). This tract innervates the dorsal aspect of the medial terminal nucleus (MTNd), a structure made up of a narrow column of neurons residing at the base of the midbrain-hindbrain border. While the molecular factors that enable RGC axons to innervate the MTN have been identified (see below), the signals that direct upward-sensing On-

DSGCs to leave the chiasm and embark on their trajectory along the base of the brain remain unknown.

The third trajectory for RGC axons to reach their targets is the major one. It includes ~30 functionally distinct subtypes, and by sheer numbers, represents ~90% of eye-to-brain connections. After navigating the chiasm, these RGC axons ascend dorso-caudally from the base of the diencephalon toward the dorsal thalamus and midbrain to form the main optic tract (Figure 1c). RGC axons emerging from the main optic tract are actively repelled from entering the diencephalon and non-visual nuclei by Slit/Robo interactions [30]. Technically, there is also a fourth optic tract in which downward sensing On-DSGCs depart the main optic tract just anterior to the SC and dive ventrally, forming the 'superior fasciculus of the AOT' (sfAOT) and innervating the ventral division of the medial terminal nucleus (vMTN) at the base of the brainstem (Figure 1c).

Different RGC types destined to innervate different brain targets are born and send out axons at different developmental stages, such that early-born axons are able to pioneer and sample many targets, whereas later deployed axons have fewer options for targets to innervate and therefore exhibit correspondingly less target-sampling and refinement (Figure 1d) [18*].

Retinal ganglion cell axon-target matching

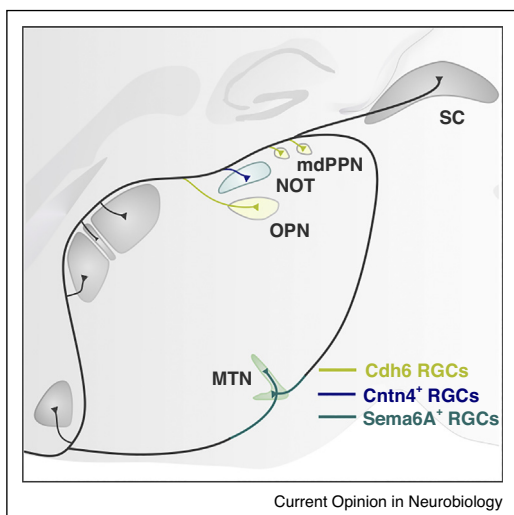
The process of axon-target matching reflects the mechanisms by which different RGC types that encode functionally distinct features in the visual world, such as directional motion or overall luminance, connect to appropriate brain targets in order to process those features into the correct perceptual or behavioral events. For example, RGCs that sense directional motion project to targets involved in conscious sight, such as the dorsal lateral geniculate nucleus (dLGN), whereas RGCs that detect overall levels of ambient luminance project to targets such as the olivary pretectal nucleus (OPN), which is involved in generating pupil reflexes (reviewed in Refs. [6**,31*]). Axon-target matching is a process involving dynamic interactions between presynaptic and postsynaptic components. The various retinorecipient targets along the optic tract undergo maturation during the same time when RGC axons arrive in their vicinity. In general, retinofugal maturation proceeds in a caudal-to-rostral manner — the most distal RGC target, the midbrain superior colliculus (SC), matures before the dLGN, and other targets follow suit. In fact, many RGC axons first grow all the way to SC, bypassing the >20 retinorecipient targets that reside between the chiasm and SC (Figure 1c, d), before innervating more proximal targets [18*,32], perhaps because those targets do not yet harbor the full array of cell types and signals required for accurate wiring. It is also worth pointing out that, in the mouse, most (~90%) of RGCs connect to the SC and therefore all

RGC inputs to targets such as the dLGN reflect the elaboration of RGC axon collaterals that also project to the SC [33^{••}]. Thus, individual axons target multiple brain structures separated by other RGC targets, mainly through a process of highly regulated collateralization and not primary growth cone termination [18[•],32].

In recent years, the mechanisms underlying the process of target-selective collateralization have started to become clear. Osterhout *et al.* [34^{••}] discovered that target selection by subsets of RGCs is facilitated by adhesive interactions: the classical cadherins mediate homophilic interactions between RGCs and target cells that express Cdh6 [34^{••}], whereas contactin-4 (CNTN4) is involved in the assembly of a parallel pathway consisting of ‘forward sensing On-DSGCs’ that project to the nucleus of the optic tract (NOT) — a circuit element essential for horizontal image stabilizing eye movements [35^{••}] (Figure 2).

In a related set of pathways, the On-DSGCs that target the MTNd and MTNv also rely on adhesive interactions between Sema6A (acting as a receptor) expressed by On-DSGC axons and plexinA2/4 (acting as a ligand) expressed by MTNd/v neurons (Figure 2) [36^{••}]. In the dorsal thalamus, reelin, an extracellular glycoprotein present as a gradient in target tissues, promotes target-specific innervation via LRP8 and VLDLR [37^{••},38[•],39]. Thus, multiple parallel retinofugal pathways rely on adhesive interactions to achieve axon-target specificity.

Figure 2



Molecular mechanisms regulate axon projections to specific targets. Cadherin 6 (Cdh6) RGCs (yellow) grow to Cdh6 expressing target cells in the OPN. Contactin-4 (CNTN4⁺) expressing RGCs (blue) act with their co-receptor amyloid precursor protein (APP) to regulate branch formation of direction-selective ganglion cells (DSGC) in the NOT. Sema6A expressing DSGCs (green) enter the MTN by interacting with Plexin A2/A4 expressed by cells in the MTN.

Recently, Seabrook *et al.* showed that early genetic removal of the RGCs that target the OPN did not result in NOT-projecting RGCs arborizing in the OPN, even though NOT-projecting RGC axons extend past the OPN en route to their target [40[•]]. Collectively, the model of RGC axon-target selection that has emerged is one in which axon growth and exploration of targets is the default mode early in development, with RGC targeting being tightly regulated by specific ligand-receptor pairs and unaltered by axon competition.

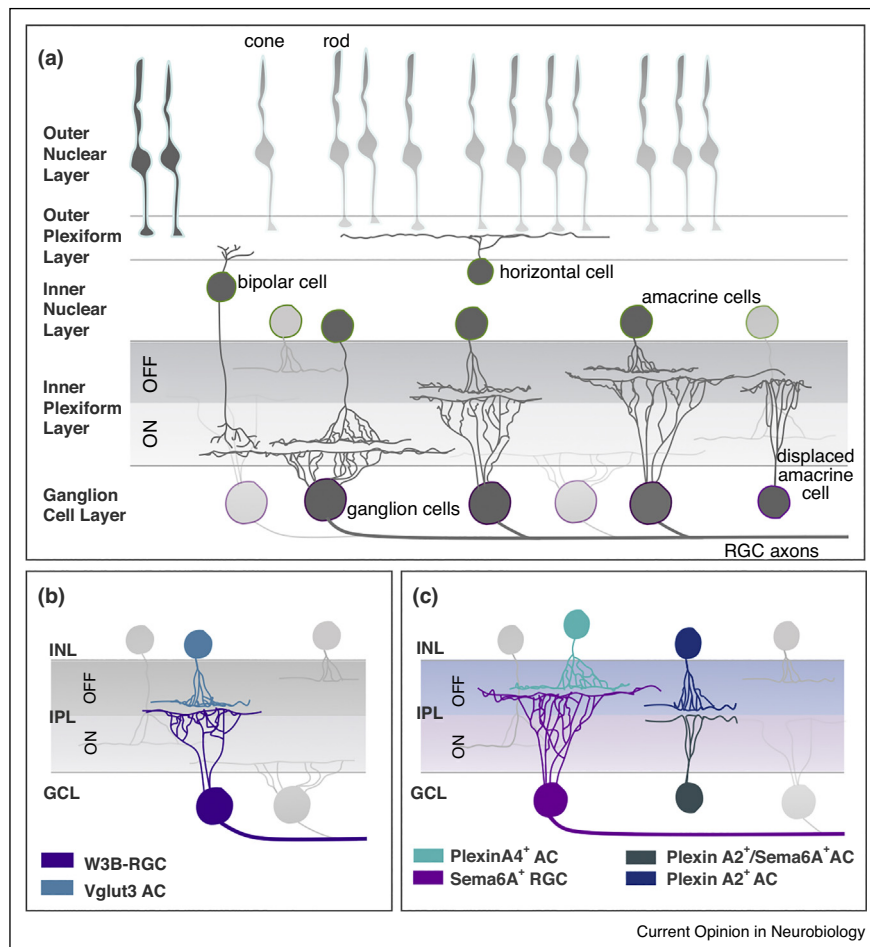
Targeting the correct topographic zone

Upon arriving at their targets, RGCs map to the location within the target appropriate for their topographic address in the retina. The basic rule is that RGCs that are neighbors in the retina project to neighboring regions in the target [41]. This process is mediated largely by repellent interactions between Eph receptors expressed by RGCs that transduce signals from ephrin ligands, although reverse signaling occurs as well [41]. Members of the ephrinA-EphA family mediate mapping along the nasal-temporal axis [42], and there is some evidence that ephrinB-EphB signaling mediates mapping along the dorsal-ventral axis [e.g. 43]. Other signals for mapping the medial-lateral axis include Wnt-Ryk [44]. It should be noted that not all topographic sorting of RGC axons relies on axon-target interactions; as they approach their targets, RGCs located in the dorsal versus ventral location of the retina sort from one another within the optic tract [45[•]]. Also, neural activity in the form of spontaneous retinal waves [46] drives refinement of topographic mapping. If these waves are quieted, or their patterns altered [42,47,48], RGC axons still map to the correct general area but the arbor termination zone becomes diffuse [49]. The interplay between guidance molecules and activity in this system is complementary; when all ephrin/Eph interactions are eliminated by genetic knockouts, RGC axons map to the wrong locations but still form focal termination zones, whereas removal of both ephrin/Eph's and activity causes complete disruption of topographic targeting and diffuse arborizations [48,49].

Directing RGC dendrites and axons to appropriate laminar depth

The functional integrity of retinofugal connections is also constrained by intra-retinal wiring events. In the retina, the location and architecture that neuronal dendrites adopt dictates the type and pattern of pre-synaptic inputs from amacrine and bipolar cells that are available to them. The specificity of these inputs essentially determines to which features in the outside world a given RGC (and therefore its target neuron in the brain) will respond. Intra-retinal cell-type-specific connectivity is, in large part, determined by the RGC dendritic laminar depth in the inner plexiform layer (Figure 3a) [3^{••},4^{••}]. RGC axons, too, must select the correct laminar depth within their targets, and in doing so, bias the number, type and

Figure 3



Laminar specification of RGC dendrites in the inner plexiform layer.

(a) Schematic showing the layers of the mouse retina and the different types of cells present in each layer.

(b) A particular type of RGC, the W3B-RGC (blue) dendrites receive inputs from Vglut3 (vesicular glutamate transporter 3) amacrine cells (purple). Both W3B-RGCs and VG3-AC express sidekick 2 (Sdk2), thus binding via homophilic interactions.

(c) Sema6A expressing RGCs (pink) receive dendritic inputs from PlexinA4 expressing dopaminergic amacrine cells (light blue) in the OFF sublamina of the inner plexiform layer. OFF starburst amacrine cells (SAC) expressing Plexin A2 (dark blue) are repelled by Sema6A-PlexinA2 expressing ON SACs (teal) thus specifying laminar depth in SACs.

location of postsynaptic neurons to which they connect [50]. The process of laminar depth selection appears to be independent of topographic mapping, since altering spontaneous neural activity or ephrin signaling disrupts precise topographic fidelity but not the depth to which RGCs of a given type projects [33^{••},51]. In fish, elegant work from Baier and co-workers shows that slit1 interactions with type IV collagen expressed at the pial surface of the tectum is essential for laminar RGC axon targeting [52^{••},53[•]]. The molecular signals that promote RGC axon laminar-specific targeting in mammals remain unknown — a crucial gap that needs to be resolved.

The signals directing RGC dendrites to their correct layers, on the other hand, have been extensively described and include repellant interactions between

specific semaphorins and plexins that restrict RGC dendrites to particular depths in the IPL [54^{••},55] (Figure 3b, c) [56]. There is still much work to do, however, in order to figure out how the incredible degree of target and within-target specificity is achieved, and how inputs from different RGC types are combined and transformed to yield coherent behavioral and perceptual outputs.

Eye-specific segregation

Contralateral versus ipsilateral-projecting RGC axons are segregated from one another in every retinofugal target in which they converge, except in the SCN where they overlap, at least at the overt scale (they may be segregated onto individual cells). This segregation emerges during development from a state in which axons from the two eyes initially overlap [57]. The segregation process

requires spontaneous waves of neural activity in the retina [58,59] driven by acetylcholine and ephrin-A/EphA interactions to define where the eye-specific zones, which reflect retinotopically-matched positions from the two eyes, will reside within each target [60,61] [reviewed in Ref. [47]].

Key cell types and mechanisms in the segregation process include astroglial and microglial ‘engulfment’ of weaker synapses that encroach upon the opposite eye-specific zone, and recruitment of immune system proteins such as complement [62**] and MEGF10 [63**] [reviewed in Ref. [64]].

Regeneration of eye-to-brain connections

As bona fide central nervous system (CNS) neurons, mammalian RGCs lack the capacity to regenerate [2,65**,66*]. Most traumatic injuries and diseases that damage the retina or the optic nerve eventually lead to RGC degeneration; the axons wither and eventually the entire cell dies. Similarly, degenerative diseases that cause RGC damage either directly or indirectly, such as glaucoma, result in irreversible vision loss [7].

RGCs in cold-blooded vertebrates such as fish and lizards readily regenerate and even re-establish accurately mapped connections [67]. To understand the barriers to mammalian RGC regeneration, the field has looked to both cold-blooded vertebrates and developmental mechanisms in mammals. Generally speaking, barriers to RGC regeneration fall into two categories: extrinsic and intrinsic factors [68*]. In terms of extrinsic factors, after injury, scarring accumulates at the lesion site, and while some scar-related factors can aid repair [69*], scarring is generally restrictive for regeneration due to the inflammatory cytokines and physical barriers to axon passage that it creates [69*,70*]. In addition, myelin present in most nerve tracts (including the optic nerve and tract) maintains factors that prohibits RGC axon growth. Intrinsic barriers include the slowing of RGC axon growth as a function of age, injury-induced death and lack of RGC replenishment. This last point is essential. Whereas fish naturally produce more RGCs as they age and their eyes grow [71], after development the number of mammalian RGCs is fixed and injury reduces those numbers. Thus, maintaining RGC viability after injury is a time-pressured limitation on post-injury regeneration and reformation of synapses with brain targets.

Maintaining cell survival and capacity to regenerate

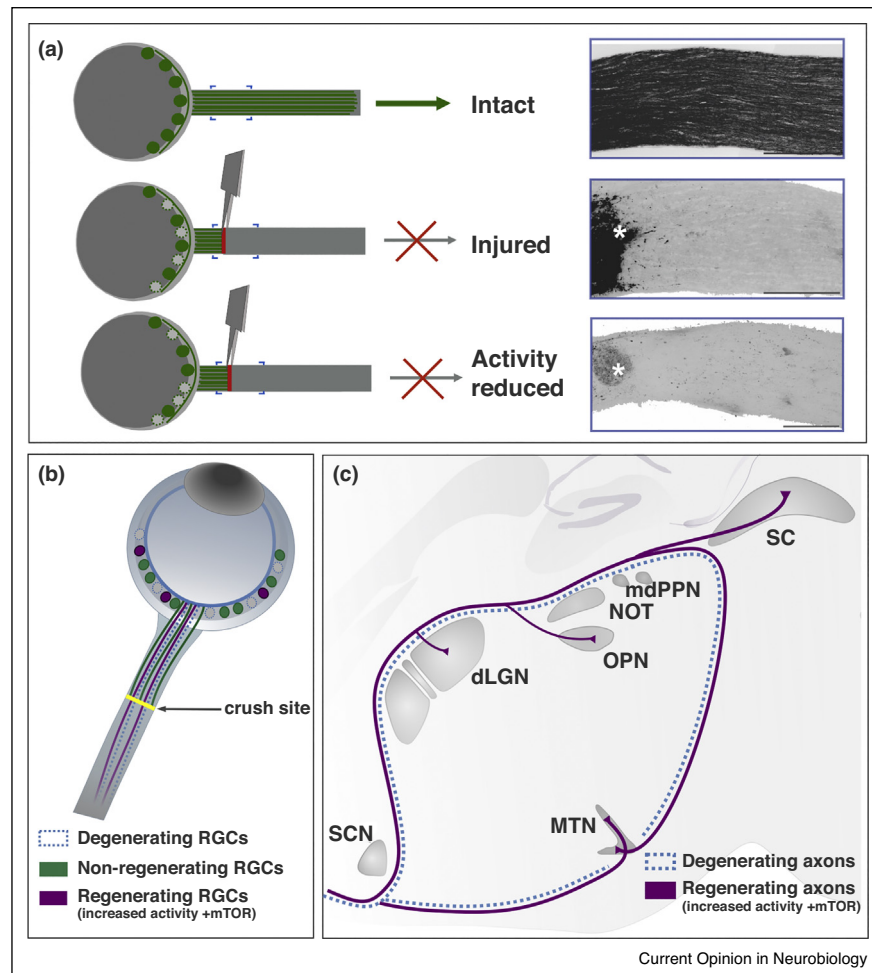
A prerequisite for axon regeneration by endogenous RGCs is that they remain viable long enough following injury to allow for a regeneration-inducing intervention to work. However, very quickly after axon crush or transection more than half of RGCs die, just as in development [72], and eventually lead to death of all RGCs. Why are

RGCs so susceptible to axotomy-induced death, and what can be done to increase RGC viability? Possible answers come from the fact that during neural development activity is crucial for RGC sustenance [72,73**]. In their exploration of the role played by neural activity in optic nerve regeneration, Lim *et al.* showed that suppressing electrical activity after nerve crush adversely impacts RGC survival [74**] (Figure 4a). Others showed that subsets of RGC types, the melanopsin-expressing ipRGCs, as well as the alpha RGCs that express osteopontin, insulin-like-growth factor1 (IGF1) and high levels of mTOR [75**], together can account for almost all the RGCs that survive axotomy and remain viable 2 weeks after nerve crush [76–78]. However, new findings demonstrate that other RGC subsets are endowed with factors that promote survival capabilities; overexpression of the transcription factor Sox11 promotes non-alpha-RGC survival and regeneration following optic nerve injury while, somewhat paradoxically, it preferentially kills alpha-RGCs [79*]. This suggests that threshold levels of Sox11 may be crucial for RGC survival and raises the possibility that alpha-RGCs typically survive axotomy because they have already elevated endogenous Sox11 levels.

The success with which RGCs re-extend and connect axons to any one target after injury plays an important role in determining sustenance of the cell itself; if an RGC is unable to re-form connections, the lack of target-derived trophic factors signals cell death [80]. Several groups have tested different strategies to promote re-extension of axons after injury [81*,82]. Interestingly, most of these hinge on re-activating developmental growth pathways and/or suppressing the growth-inhibiting pathways that characterize RGCs. Manipulations that have proved successful in this regard include increasing inflammatory factors such as oncomodulin [83*], increasing insulin signaling [84], or inhibiting negative regulators of growth such as PTEN (phosphatase/tensin homolog) [65**]. Other inhibitors of axon growth include the transcription factors SOCS3 (suppressor of cytokine signaling 3) [85,86*], Klf 4 and Klf 9 [87]. Increasing positive regulators of cell growth, including mammalian target of rapamycin (mTOR), ciliary neurotrophic factor (CNTF), doublecortin-like kinases (DCLK2) [88] or the transcription factors Klf 6 and Klf7 can also shift RGCs into a growth mode [87].

Further, increasing RGC electrical activity using chemogenetic techniques [74**], or a combination of increasing activity and growth promoting pathways, have been shown to have synergistic effects on RGC axon growth. And while no single manipulation has proven to be a ‘magic bullet’ for regeneration, combining enhancement of developmental growth programs while at the same time inhibiting growth suppression pathways leads to modest long-distance RGC axon re-extension

Figure 4



Developmentally informed regenerative strategies.

(a) Schematic to show how injury impacts the optic nerve. Injured RGCs start dying within two weeks and cannot regenerate their axons without therapeutic intervention. Silencing neural activity using chemogenetic approaches, reduces the survival of RGCs. Images from Lim *et al.* [74**].

(b, c) Combinatorial approaches that increase neural activity and mTOR signaling in RGCs (pink circles, **b**) promote regenerated axons to grow past the injury site (yellow line, **b**) and reinnervate visual targets in the brain (pink lines, **c**).

[74**,86*,89**]. One thing to note is that, even in studies where successful regeneration is induced, RGCs continue dying, further emphasizing that if RGCs are to be induced to regenerate in large numbers, approaches to sustain RGC survival must be introduced as well.

Re-targeting the correct brain nuclei, reforming myelination and synapses after injury

A longstanding question has been: if RGCs can regenerate, will they navigate to visual targets, and if so, to the correct ones? Apparently the answer is yes; much as it is in lizards and fish [71,90*]. Two papers, De Lima *et al.* [89**] and Lim *et al.* [74**], show that when RGCs are triggered to regenerate after injury, their axons innervate visual targets in the brain and still actively avoid non-visual targets, as they did during development (Figure 4b).

Others showed that when lesions are more distal to the optic nerve [86*], target-specific regeneration still occurs, but in the absence of neural activity, myelination of the regenerated fibers does not. This inadequate functional transmission from the regenerated axons/synapses could explain why some, but not all, visual functions were restored in these mice.

One approach that has yet to gain traction in the field of visual regeneration is the use of guidance cues to promote axon re-extension. Axon guidance cues provide a particularly important regenerative mechanism that couples intrinsic and extrinsic promoters of growth during development. Perhaps a strategy that utilizes neural activity and guidance cues will be key in promoting sufficient and accurate regeneration of axons in order to achieve complete functional restoration of vision. However,

comparison between developmental and regenerative roles of neural activity, indicates that although the same genes and mechanistic features may be reactivated in adulthood, the outcomes could vary. Thus, a caveat is that the context in which a guidance cue is activated is relevant if it is to be reused in regeneration.

Conclusions

The assembly of eye-to-brain connections is now a fairly well understood process in terms of the overall sequence of events. However, given the diversity of RGC types, targets and their functional roles, quite a bit more work remains to be done. Retinal cell-type-specific RNA profiling [91,92] is likely to unveil new candidate molecules on both the RGC and target sides that regulate retinofugal connectivity, and also new cell types and patterns of connectivity. Advanced labeling, ultrastructural and functional microscopy methods [93] will no doubt advance this pursuit even further. The field of retinofugal regeneration is gaining ground thanks to progress in understanding developmental mechanisms and the development of genetic and viral approaches used to label and manipulate-specific RGCs subsets [e.g. 94,40*]. Using these approaches to provide factors that stimulate survival and re-extension of specific RGC axons will allow for higher resolution views of the regeneration process, compared to labeling RGCs *en mass*.

Given the key roles of target derived cues and overall developmental events that direct retinofugal connectivity, the role of target neurons in the regeneration process also deserves study; one wonders, for example, if increasing neural activity in distal targets retrogradely increases responses to trophic support, as it does during development, thus promoting regeneration. Therefore, we view the implications of developmentally informed regenerative strategies for therapeutic interventions as an extremely exciting area likely to yield major progress in the near future.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank Onkar Dhande and Tania Seabrook for helpful comments. Support was provided by NIH/NEI R01 EY026100, NIH/NEI R01 EY027713, NIH U01 EY27261 and The Glaucoma Research Foundation to ADH.

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This paper describes another important role for astrocytes in synapse elimination that is dependent on neural activity. The results implicate two phagocytic pathways MEGF10 and MERTK in the engulfment of unwanted synapses, especially during the refinement of retinogeniculate projections.

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