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Four Steps to Optic Nerve Regeneration

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Abstract: The failure of the optic nerve to regenerate after injury or in neurodegenerative disease remains a major clinical and scientific problem. Retinal ganglion cell (RGC) axons course through the optic nerve and carry all the visual information to the brain, but after injury, they fail to regrow through the optic nerve and RGC cell bodies typically die, leading to permanent loss of vision. There are at least 4 hurdles to overcome in preserving RGCs and regenerating their axons: 1) increase RGC survival, 2) overcome the inhibitory environment of the optic nerve, 3) enhance RGC intrinsic axon growth potential, and 4) optimize the mapping of RGC connections back into their targets in the brain.

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Many disorders insult retinal ganglion cell (RGC) axons in the optic nerve (Fig. 1), including traumatic optic neuropathy (1), ischemic optic neuropathy (2), optic neuritis (3,4), and glaucoma (5). The underlying causes of these diverse disorders vary. In some diseases, like Leber hereditary optic neuropathy, the damage is thought to begin within the RGCs themselves; in others, such as in optic neuritis, damage to RGC axons is secondary to dysfunction or loss of the surrounding optic nerve glial cells. While there are multiple well-characterized animal models for these conditions (Table 1), much is still unknown about their initial causes and progression. In none of these diseases, however, can RGC axon fibers regenerate back to their targets, and in most of these, RGCs die (6). We review critical advances in the understanding of why regenerating optic nerves is such a daunting task.

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SURVIVAL OF RGCS AFTER OPTIC NERVE INJURY

One of the effects following optic nerve axon injury is RGC death, which can be seen in histopathological samples from human optic neuropathies (7) and can be studied in greater detail in animal models. For example, in adult rats, 85%–90% of RGCs die by 2 weeks after crushing or cutting the optic nerve (Fig. 2) (8). The more distant the injury from the eye, the less the severity of initial RGC death (8–10), possibly due to the support of optic nerve glial cells (see below, Fig. 1) or persistence of collateral axon branches to other supportive targets (11). The majority of dying RGCs undergo apoptosis, or programmed cell death (8,9,12). After RGC injury, there is an upregulation of proapoptotic proteins (8,13,14); conversely, overexpression of anti-apoptotic proteins, such as Bcl-2, results in an increased survival of injured RGCs (15,16). Alternatively, some RGCs undergo necrotic death or secondary degeneration after optic nerve injury but in minimal numbers (17). Depending on the location of the injury, at the cell body or along the axon, activation of distinct RGC death pathways occurs (18).

What Causes RGCs to Die After Optic Nerve Injury?

Axon injury disrupts the connections of RGCs to their target, resulting in a loss of target-derived neurotrophic support. Target-derived signals are retrogradely transported to the cell body and are hypothesized to be required for neuronal survival (19–23). Removal of this support leads to apoptosis, and addition of exogenous neurotrophic factors increases survival and regeneration (24). One of the neurotrophins shown to regulate RGC survival is brain-derived neurotrophic factor (BDNF), expressed in both the retina and the superior colliculus, which is targeted by

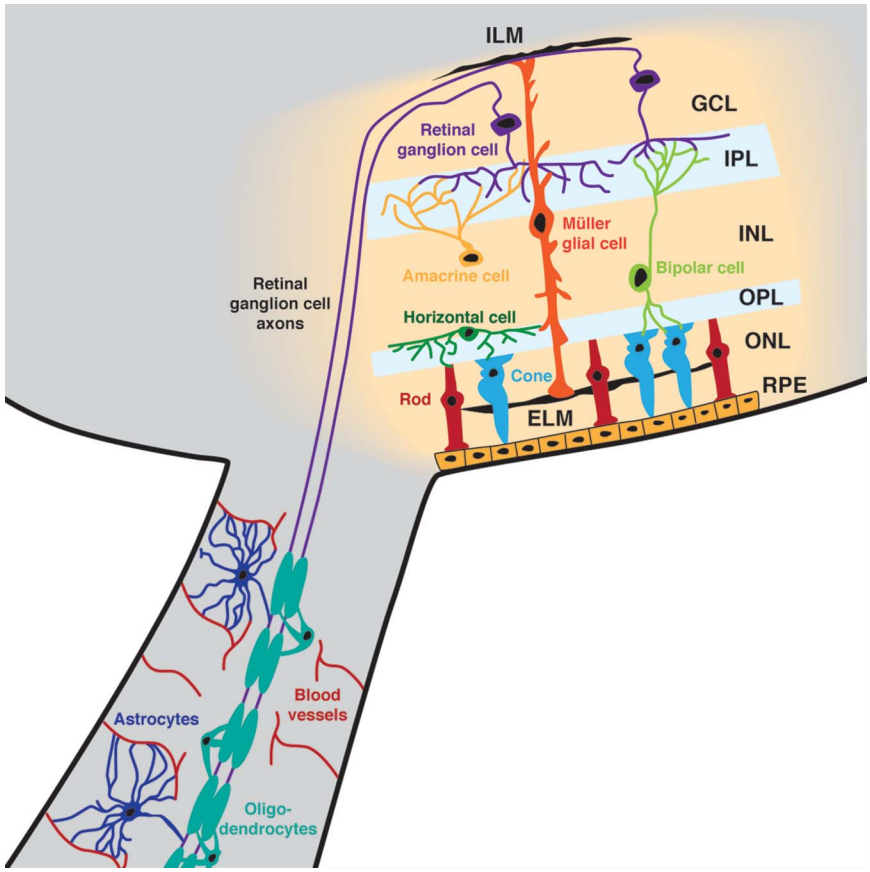

FIG. 1. The retina contains at least 6 major classes of neurons plus Muller glial cells and other astrocytes, but retinal ganglion cells (RGCs) are the only output neurons of the retina. Most of the processing of light begins with rod and cone photoreceptors in the outer nuclear layer (ONL), which transduce photons into a chemical code passed onto neurons of the inner nuclear layer (INL). The signal is modified and enhanced by these interneurons before providing input onto RGCs, which transmit both visual and non–image-forming information to the brain. In the optic nerve, RGC axons are myelinated by oligodendrocytes, increasing the speed and probably the fidelity of electrical signaling to the brain. Astrocytes also play critical roles throughout the optic nerve, communicating with both blood vessels and RGC axons in the spaces between the ensheathing myelin. The RGC axons in turn propagate an electrical signal down their length, arriving at their target structures in the brain. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RPE, retinal pigmented epithelium; ILM, inner limiting membrane; ELM, external limiting membrane.

approximately 100% of RGC axons in the rodent and about 30% of RGC axons in humans. BDNF supports RGC survival in vitro (25,26) and in vivo (27) by binding to its receptor, tropomyosin receptor kinase B (trkB), resulting in activation of downstream effectors, including the Ras-MAPK and PI3K-Akt pathways. The increased survival after axotomy following BDNF application appears to be due to a combination of these 2 pathways (28).

Would trophic factor injection alone be enough to save RGCs? After optic nerve injury, intraocular injection of BDNF neurotrophin 4/5, nerve growth factor, or insulin-like growth factor 1 leads to a temporary increase in the RGC survival (8,13,27,29–33). For example, with application of BDNF or ciliary neurotrophic factor (CNTF), 25% of RGCs are alive at 3 weeks, when nearly all RGCs without treatment would be dead, but by 7 weeks, 95% are dead with or without treatment (27). Sustained overexpression of trophic factors does not solve this problem. For example, transducing other retinal cells, such as Muller glial cells, to overexpress BDNF is neuroprotective for RGCs, but these effects do not persist (29). Neurotrophic factors, such as glial-derived neurotrophic factor (34–36) and CNTF (27,37,38), have also been found to increase RGC survival after injury.

Why Is the Response to Trophic Factors So Limited?

Receptors for these factors are expressed in RGCs (39–42), but after injury, these cells only transiently upregulate their neurotrophin receptors, followed by a long-term decrease in their expression (40,43). Interestingly, recent studies demonstrate that exogenous application of BDNF can lead to

TABLE 1. Animal models of optic nerve disease

Disease	Animal Model	References
Glaucoma	DBA/2J mouse	(187–190)
	Decreasing aqueous outflow	(191,192); for review, see (193)
Ischemic optic neuropathy	Photochemically induced ischemic optic neuropathy	(194,195)
Optic neuritis	2D2 myelin oligodendrocyte glycoprotein–specific T-cell receptor mice	(196,197)
Traumatic optic neuropathy	Optic nerve crush	(198)
	Optic nerve transection	(199)
Leber hereditary optic neuropathy	Mutated human ND4 gene targeted to mitochondria	(200)

For an additional review see Levkovitch-Verbin (201).

a decrease in regeneration (44), and application of neurotrophic factors in general can lead to downregulation of the receptors, creating a longer-term reduced responsiveness to these factors (45), at least in animal models. It is not known whether this limited responsiveness would also be seen in humans. Neurotrophic factors for retinal neuroprotection are in the early phases of testing in humans (46,47).

The failure of neurotrophins to support sustained RGC survival may also be due to a decreased ability of RGCs to respond to neurotrophins following optic nerve axon injury (48). After optic nerve injury in animal models, RGCs lose their trophic responsiveness, such that they are unable to respond to neurotrophic factors or activate their downstream intracellular signaling components, even in the presence of BDNF, for example (26,49). This trophic responsiveness can be restored by increasing the number of trkB receptors present on the plasma membrane, either by directly overexpressing them (50) or by enhancing their recruitment to the surface. Surface recruitment can be elicited by elevating RGCs' intracellular cyclic adenosine monophosphate (cAMP) levels by pharmacologic treatment or depolarization (26,49). RGCs exhibit a decrease in cAMP levels after injury, possibly due to decreased electrical activity (49). These findings suggest a therapeutic approach, that is, to increase RGCs' cAMP levels after injury, although cAMP injections alone do not increase survival (51) and may need to be accompanied by neurotrophic factors.

Trophic responsiveness and neuroprotection of RGCs after axon injury can also be enhanced by electrical stimulation (52). RGCs are less electrically active after optic

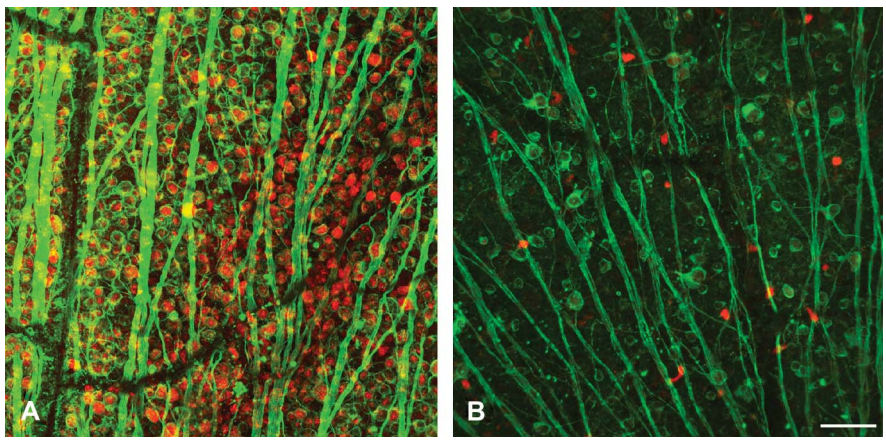

FIG. 2. Retinal ganglion cell (RGC) survival is dramatically decreased following optic nerve crush in adult mice. **A**. RGCs can be labeled by injections of a fluorescent dye into the superior colliculus, the primary target for RGC axons in the rodent. The dye (in this example, Fluorogold, shown in red) is retrogradely transported along the length of the axons back to the cell bodies in the retina, allowing for specific labeling of RGCs. The dissected retina can be viewed en face in flatmount as above and can also be immunostained for RGC markers like beta-III tubulin (shown in green), which label RGC bodies as well as their bundled axons in the nerve fiber layer (NFL). **B**. When one optic nerve is crushed after such retrograde labeling, by 2 weeks, a severe loss of retrogradely labeled or tubulin-positive RGCs is noted, along with atrophy of the axon bundles in the NFL. Scale bar, 50 μm.

nerve injury (53). Increasing activity through electrical stimulation increases cAMP levels in RGCs (49) and greatly potentiates the neuroprotective effects of neurotrophic factor treatment (54), possibly by increasing the number of neurotrophin receptors on the neuronal surface (26). In the peripheral nervous system (PNS), electrical stimulation after injury results in an accelerated expression of regeneration-associated genes (55,56), suggesting that electrical activity may also positively influence axon growth. Recent studies have shown that transcorneal stimulation after optic nerve crush increases not only the number of surviving RGCs but also the number of axons projecting past the lesion (57,58). This experimental result suggests that electrical stimulation may be used as a therapeutic strategy to increase RGC survival and growth in future studies.

In addition to the loss of positive signals such as trophic support and electrical activity, there is an increase in negative prodeath signals after optic nerve injury. For example, there is an increase in superoxide levels in RGCs from the mitochondrial electron transport chain; blocking this increase leads to a reduction in RGC death (59). Providing extra neurotrophins cannot block this increase in superoxides (59), suggesting that the cell death pathway for trophic factor withdrawal is separate from that of free radical–induced cell death. Treating RGCs with novel reducing agents to block this rise in superoxides is neuroprotective in RGCs at low doses (60).

These studies suggest that there are multiple strategies for increasing RGC survival after injury, including elevating cAMP, providing multiple exogenous trophic factors, electrically stimulating RGCs to increase their activity, and reducing superoxide levels. It is likely that combinations of these strategies will be required to optimally increase RGC survival and regeneration. Some of the same signals that increase survival also increase the RGC ability to regenerate (48).

THE INHIBITORY ENVIRONMENT OF THE OPTIC NERVE

Why Do RGC Axons Fail to Regenerate in the Injured or Diseased Optic Nerve?

After injury anywhere in the adult central nervous system (CNS), the ability of axons to regenerate is actively inhibited by the mature CNS environment and the cellular response to injury (Fig. 3). The response by meningeal cells, microglia, oligodendrocytes, and astrocytes can include migration to the site of injury, proliferation, and changes in cellular morphology and protein expression. The expression and secretion of inhibitory molecules and proteins, and the presence of myelin debris, create an unfavorable environment for axon regeneration in the CNS. These inhibitory phenomena do not occur in the PNS, where axons regenerate after injury. One difference between the CNS and PNS is the makeup of the glial cells. Whereas peripheral nerve Schwann cells are supportive of axon growth due to their secretion of neurotrophins and lack of associated inhibitory factors, optic nerve oligodendrocytes and reactive astrocytes are inhibitory to axon growth, expressing many inhibitory proteins, as has also been demonstrated throughout the brain and spinal cord (61).

Damaged oligodendrocytes degenerate after injury, leaving myelin debris containing inhibitory proteins such as Nogo-A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (Omgp) at the site of injury. Astrocytes, however, respond to injury by becoming hypertrophic and proliferating, forming a ''glial scar'' at the optic nerve injury site. Astrocytes release inhibitory extracellular matrix molecules, such as chondroitin sulfate proteoglycans (CSPGs), creating a molecular barrier to regeneration (61). In the spinal cord, treatment with a bacterial enzyme chondroitinase ABC degrades the sulfated glycosaminoglycan side chains and can partially interfere with this inhibition (62). In addition to actively inhibiting axon growth, CSPGs may also mask growth-promoting proteins such as laminin (63) or may change normally growth-attracting proteins like semaphorin 5A into repulsive cues (64). Other semaphorins secreted from infiltrating meningeal cells and expressed by oligodendrocytes and neuroepithelial cells also inhibit axon regeneration (65,66). Some of these inhibitory molecules are used as guidance cues during development and may be reexpressed after injury. For example, semaphorin 3A acts as a repulsive guidance cue during development (67) but is also upregulated after injury, inhibiting axon regeneration (68). The expression of netrin-1 at the optic nerve head, which normally attracts RGC axons to exit the retina and grow into the optic nerve in early development, becomes an inhibitory signal in the later life due to the low levels of cAMP in adult RGCs (69). The complexity with which these inhibitory proteins interact with RGCs needs to be further defined.

Can We Simply Turn Off the RGCs' Response to Such Negative Cues?

Semaphorin receptors such as neuropilin-1 are upregulated following injury (70). Nogo-A, MAG, and Omgp activate neuronal Nogo receptor (NgR) protein complexes that may be constitutively expressed. Downstream signaling molecules such as Rho and Rho kinase (ROCK) are normally found in RGC axons. Research involving these proteins has led to varied results, limiting the ability to draw complete conclusions as to their exact mechanisms. After optic nerve crush in the chick, inactivation of MAG increased axon regeneration (71); however, optic nerve crush in MAG-knockout mice did not lead to RGC regeneration (72). CNS injuries performed on the multiple different Nogo and NgR knockouts have revealed varied results in regeneration, from none to modest regeneration and sprouting, although more positive results have been seen using dominant-negative and pharmacoinhibition strategies against these

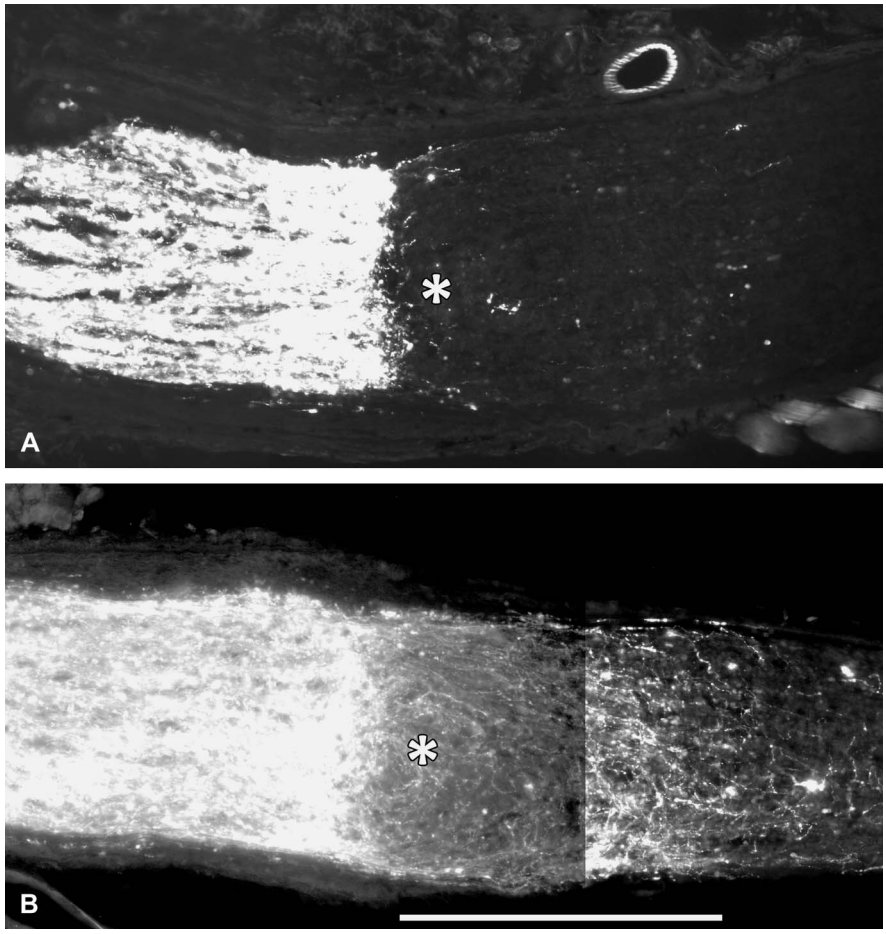

FIG. 3. Optic nerve crush experiments allow researchers to test approaches to increase optic nerve regeneration. Rodent optic nerves can be crushed a short distance behind the eyeball using fine forceps and left for 2 or more weeks to assess for any regeneration to occur. One day before collecting the nerves for processing, a fluorescent anterograde tracer is injected into the vitreous and is taken up by retinal cells including retinal ganglion cells (RGCs), which transport the dye down the length of their axons. **A**. In animals with no therapeutic treatment (control, *top*), the axons typically stop at the crush site (*asterisk*) and very few will regenerate any further into the distal optic nerve. **B**. In contrast, animals treated with multiple injections of ciliary neurotrophic factor plus cyclic adenosine monophosphate (cAMP) demonstrate increased RGC axon regeneration past the crush site (*bottom*). Scale bar, 200 μm.

same molecules (73). Treatment with the IN-1 antibody, for example, which neutralizes Nogo-A, resulted in greater regeneration than Nogo-A gene knockouts (74,75). Treatment of spinal cord injury with anti-Nogo antibodies has entered clinical trials in Europe and, if successful, is almost certain to be followed by optic nerve regeneration clinical trials (http://www.axregen.eu/team/university-of-zurich/).

Pretreating neurons with neurotrophins prior to exposure to MAG and myelin decreases their ability to inhibit axon growth. Neurotrophin pretreatment increases levels of intracellular cAMP (76), and a developmental decrease in cAMP correlates with a developmental increase in the negative response to MAG/myelin (77). These phenomena identify cAMP as an important modulator of axon growth after injury.

Many of these inhibitory environmental signals converge on a downstream target called Rho, a small GTPase (78). Targeting a convergent downstream signal relieves the need to block each glial-associated inhibitor separately. Rho signaling leads to actin cytoskeleton remodeling (79) and growth cone collapse (80). Experiments inactivating Rho with C3 transferase, which ribosylates Rho proteins, showed increased regeneration of CNS fibers (81–84) when Rho was inactivated soon after injury (85). Multiple injections of this inhibitor after injury also increased RGC survival (85). The combination of Rho inactivation, overexpression of CNTF, cAMP treatment, and peripheral nerve grafting after axotomy enhanced viability as well as increased regeneration of those surviving fibers (86). Recently, collapsin response mediator protein 4b (CRMP4) was identified as interacting with Rho to carry out its inhibitory functions. Knockdown of CRMP4 or blocking of CRMP4 and Rho interaction resulted in attenuation of inhibition from myelin substrates, identifying an even

more specific therapeutic target (87). Inhibition of ROCK, a downstream effector of Rho, has also shown promising results in overcoming environmental inhibition and promoting neurite outgrowth both in vitro and in vivo (38,88–93).

Besides activation of RhoA by these inhibitory environmental signals, there is an increase in intracellular calcium that may be involved in the downstream activation of the epidermal growth factor receptor (EGFR) and protein kinase C (PKC), although whether these 2 are interrelated has yet to be determined. Inhibition of PKC activity by CSPG and myelin-based activation increases regeneration in dorsal column axons (94). Inhibiting EGFR pharmacologically after optic nerve crush blocks myelin and CSPG inhibition on neurite growth and promotes regeneration of RGCs (95), although this EGFR inhibitor may be acting through other mechanisms (96,97). A number of drugs that block the responses of RGCs and other CNS neurons to inhibition are now in clinical trials for spinal cord injury, and identification of further downstream targets of inhibitory signaling will create more specific therapeutic targeting strategies for future studies (98).

Not all the cellular responses to injury or disease are negative or inhibitory. For example, macrophages associated with inflammation can potentially be neuroprotective and induce axon outgrowth (99). Macrophages are recruited with lens injury and elicit an 8-fold increase in RGC survival and a 100-fold increase in regeneration of RGC axons past the site of an optic nerve crush (100–102). Similar macrophage activation can be elicited by injection of zymosan, a yeast cell wall preparation that activates macrophages (101,103,104). How does lens injury create these effects? Macrophages migrating into the retina express oncomodulin, which causes extensive outgrowth of RGCs (with concurrent elevation of cAMP) after optic nerve crush in the adult optic nerve (105). However, activated macrophages and oncomodulin may not be the primary effectors of increased regeneration after injury (106). Combinatorial approaches may further enhance regenerative response, as by using macrophage-derived factors to ''sensitize'' neurons prior to dominant-negative suppression of the activity of NgR (107) or in combination with Rho inactivation (108).

The immune system has recently been targeted for clinical trials in optic nerve neuropathies and spinal cord injuries. Immunization with a peptide derived from Nogo-A, an inhibitory protein present on myelin, can increase recovery after spinal cord injury (109). In addition, vaccination with copolymer 1 (Cop-1), a synthetic chain of amino acids that cross-reacts with myelin basic protein, could activate the immune system and decrease secondary degeneration of surviving fibers after optic nerve injury. Cop-1 is a drug presently used to treat multiple sclerosis and is not known to create any additional immunogenic effects, making it a good candidate for clinical testing (110). Clinical trials were started for Cop-1 treatment in progressive optic nerve degeneration and started but suspended for transplantation of autologous activated macrophages into the injured spinal cord (111). Further studies to find additional proteins released following lens injury or macrophage activation may reveal potential candidates involved in increasing CNS survival and regeneration.

Bypassing the inhibitory optic nerve environment entirely is the oldest approach. As early as 1911, Tello (112) used peripheral nerve grafts attached to cut optic nerve to demonstrate that RGCs could regenerate a short distance if given a permissive substrate. Such experiments were rejuvenated by Aguayo et al (113) in the 1980s, using sciatic nerve transplants to connect the retina to the superior colliculus. Although the majority of RGCs died as a result of the optic nerve injury, replacement of a portion of the injured optic nerve with a piece of peripheral nerve enabled about 20% of the surviving RGCs to regrow long axons back to their targets. This process took approximately 2 months (114–116).

Why Are Peripheral Nerve Grafts Able to Support CNS Regeneration?

The less inhibitory environment of the peripheral nerve, as well as the trophic environment secreted by Schwann cells, creates a very permissive substrate for growth. In addition, the peripheral nerve graft may act to change the role of cells already present in the injured optic nerve. Whereas astrocytes typically respond to CNS injury by hypertrophy, proliferation, expression of inhibitory proteins such as CSPGs, and creation of a glial scar in peripheral nerve grafts, astrocytes were found to encircle axonal bundles and act in conjunction with the Schwann cells of the peripheral nerve to guide those axons, which regenerated through the peripheral graft, ultimately changing the environmental response at the injury site (117).

In addition to peripheral nerve grafts, other substrates have been grafted in experiments to increase RGC outgrowth and regeneration. Transplanted perinatal optic nerves (118), RGC target tissue from fetal brain (119–122), cell transplants (123–125), various bridge matrices containing Schwann cells (126–130), exogenously delivered neurotrophic factors (131–134), olfactory ensheathing cells (135,136), and peripheral nerve transplants into the vitreous (137,138) have shown varied results. The graft and transplantation studies have shown that, at a minimum, some CNS neurons can regenerate to their targets. It is now important to determine if these techniques can be translated into therapeutic treatments for patients.

THE DEVELOPMENTAL LOSS OF RGC INTRINSIC AXON GROWTH ABILITY

In nearly all the experiments described above, only a small percentage of RGCs have regenerated and typically very slowly, even when some of the inhibitory signals have been neutralized. Could it be that the adult neurons have lost

their capacity to regrow axons? In spinal cord injury studies in cats, the neonatal nervous system retains its ability to regenerate, but this ability is lost as the animals develop (139). This finding was confirmed in rats whose spinal cords were injured at birth or in adulthood, demonstrating that the neonatal CNS retains the ability to regenerate, whereas the adult CNS does not (140). Is this simply because the adult glia turned on their expression of inhibitory molecules? This question has been addressed by using "heterochronic cultures," in which tissues from younger and older animals are cocultured. For example, embryonic retinal explants can extend axons into embryonic or adult brain explants, but adult retinal explants cannot extend axons into either, suggesting that the problem is with the retina or RGCs (141). Similarly, coculturing mature postnatal explants with young environmentally permissive explants have demonstrated a differential effect of tissue age on regenerative ability in hippocampal (142), cerebellar (143,144), and hindbrain tissues (145). Such studies have suggested that intrinsic changes within the neurons themselves limit their regenerative ability.

Pure RGC cultures were finally used to demonstrate definitively that RGCs turn off their intrinsic capacity for rapid axon growth during early development. To remove all influence of any potential extrinsic inhibitory environment, RGCs from embryonic or postnatal ages were purified away from all other cell types, allowing the study of their intrinsic axon growth capacity. Whether cultured in a strongly trophic environment or even transplanted back in vivo, purified embryonic RGCs extended their axons up to 10-fold faster than postnatal or adult RGCs (Fig. 4) (146). Altering the extrinsic environment did not change this fundamental observation, pointing to intrinsic limitations in RGC axon growth ability. Additionally, it was found that this developmental decrease can be initiated by a membrane-associated signal on presynaptic amacrine cells (Fig. 1) (146). These findings confirm the in vivo and in situ experiments, suggesting that a developmental program in RGCs is involved in their inability to regenerate.

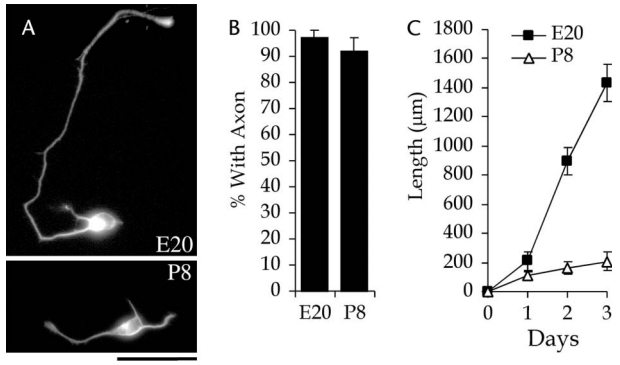

FIG. 4. Retinal ganglion cells (RGCs) lose their intrinsic axon growth ability during development. Purified RGCs from embryonic day 20 (E20) and postnatal day 8 (P8) were cultured and immunostained against beta-III tubulin to visualize neurites. **A**. E20 RGCs (*top*) extended longer axons than P8 RGCs (*bottom*) after 18 hours in culture. **B**. At 3 days in vitro (DIV), the percentage of neurons that extended an axon were quantified at each age, demonstrating that there was no difference in the ability to extend an axon between E20 and P8 RGCs. **C**. The growth of E20 and P8 RGCs were measured over 3 days, revealing that E20 RGCs grew their axons approximately 10-fold faster than P8 RGCs. Taken together, these results suggest that postnatal RGCs lose their ability to rapidly extend their axons during development. Reprinted with permission from Goldberg et al (146).

Can the Intrinsic Capacity of RGCs to Regenerate in Adulthood Be Increased to Embryonic Levels?

One method of increasing intrinsic axon growth ability may be to alter the expression of specific genes that are upregulated or downregulated during development or after injury or in disease. For example, overexpression of Bcl-2, an anti-apoptotic gene whose expression is decreased developmentally, increases RGC survival and slightly increases the regenerative capacity of RGCs in tissue explants (147). In vivo, Bcl-2 overexpression increases regeneration of RGC axons after injury in early postnatal rodents but not in later postnatal rodents, even when the negative influence of astrocytes is minimized (148). Using lithium to induce Bcl-2 expression and removing astrocytes at the injury site by means of an astrocyte toxin resulted in an increase in optic nerve regenerations but did not increase RGC survival (149). Bcl-2 may yet be a promising target to increase RGC survival and possibly regeneration in vivo despite these mixed results.

The levels of cAMP within neurons also appear to be developmentally regulated such that embryonic neurons have high cAMP levels that drop sharply postnatally and remain low throughout adulthood (77). The response of these neurons to MAG/myelin is also dependent on the neuron's stage of development and the neuron's intrinsic level of cAMP, as described previously (77). Embryonic axons, possessing endogenously high cAMP levels, are promoted by MAG/myelin, and this effect requires the activation of the transcription factor cAMP response element–binding protein (CREB) (150). CREB's upregulation of arginase I (Arg I), an enzyme that synthesizes polyamines, may be a part of the molecular pathway involved in overcoming myelin inhibitors (150–152). The expression profile of Arg I parallels that of cAMP levels during development, and its overexpression is sufficient to block the switch from promotion to inhibition by MAG/myelin (150,151). Therefore, the change in cAMP levels and downstream effectors during development is a switch that allows neurons to respond differently to extrinsic inhibitory environments (77,151).

Other developmentally regulated signaling pathways could be important in axon regeneration. For example, the

activation of the MAP kinase pathway was recently found to be important in the regeneration of motor neurons in *Caenorhabditis elegans* (153). The Dlk-1/MKK-4/PMK-3 MAP kinase pathway was found to be required for axon regeneration and also for normal growth cone formation and morphology. This pathway was not necessary during development, suggesting that there are specific signaling pathways activated during regeneration that are separate from those activated during development (153). Dlk has also been shown to be important for the degeneration of severed axons, a necessary component in successful regenerative systems, clearing the way for regeneration following injury (154). Thus, the effect of Dlk seen in regeneration could be due, at least in part, to its effect on degeneration.

Another signaling pathway that is developmentally regulated centers on the mammalian target of rapamycin (mTOR) protein. Deleting the phosphatase and tensin homolog protein or the tuberous sclerosis complex 1, both of which normally inhibit mTOR, leads to a dramatic increase in the number of regenerating axons after optic nerve injury, as well as to an increase in RGC survival (155).

Could the Activation or Inactivation of Transcriptional Programs Be Important in the Loss of Intrinsic Axon Growth Ability?

In the cerebellar granule neurons (another type of CNS neuron), the ubiquitin ligase Cdh1-anaphase promoting complex (Cdh1-APC) and its downstream targets have been identified as important players in the intrinsic regenerative ability of CNS neurons. Cdh1-APC was first identified to be a cell cycle ubiquitin ligase; however, a new role for this complex has been discovered in regulating axonal growth, in particular for axon growth inhibition (156–160). Cdh1-APC targets the inhibitor of differentiation 2 (Id2) for degradation, releasing a basic helix-loop-helix transcription factor (E47) to upregulate genes involved in axon growth inhibition, including NgR (157). Cdh1 also targets the transcription factor SnoN, whose degradation results in reduced axonal growth (158). These results suggest that the loss of intrinsic axon growth ability could be induced by critical transcriptional changes. It is not known, however, if these pathways function similarly in RGCs.

Our lab has recently found that a family of transcription factors, called Krüppel-like factors (KLFs), may affect axon growth ability during development and regeneration. There are 17 members of the KLF family; 15 are expressed in RGCs, and many of these are developmentally regulated (161). For example, the expression of KLF4 and KLF9 increases during postnatal development. When overexpressed in RGCs, these KLFs decrease neurite growth significantly. Using a gene knockout of KLF4 in RGCs, we have found that removal of KLF4 increases neurite growth of RGCs in vitro, and more importantly, increases axon regeneration after optic nerve injury. Taken together, these results suggest that the KLF family of transcription factors may be involved in the loss of intrinsic axon growth ability seen during development in RGCs.

The presence of such a large number of signaling pathways, proteins, and transcription factors suggests that not one single target will be adequate to increase optic nerve regeneration. Coaxing RGC axons through an injured or degenerating optic nerve may take a multifactorial approach, addressing RGC survival, glial inhibitors, and RGCs' intrinsic capacity for rapid axon regeneration.

TARGET REINNERVATION AFTER OPTIC NERVE INJURY

Once We Are Able to Enhance RGC Survival After Injury, Overcome the Inhibitory Molecules Present at the Lesion, and Re-establish an Embryonic Growth Phenotype, Will the Axons Be Guided Back to Their Developmental Targets and Create Functional Maps of Visual Space?

Work on regeneration is in too early a stage to permit predictions on what will happen "postregeneration." However, much is known about developmental mapping from which assumptions can be drawn. For example, during embryonic development, RGC axons are attracted to the optic nerve head by glial cells expressing netrin-1 (162). Half of these axons are guided by ephrin B2 ligands to remain ipsilateral at the optic chiasm (163) and are funneled within the optic nerve by semaphorin 5A, expressed by neuroepithelial cells (164), and Slit (165–167). To keep spatial orientation intact, RGC axons must topographically map onto the superior colliculus, their target in the midbrain. This mapping occurs through gradients of ephrin ligands, which create the specificity of the visual map, allowing RGCs to communicate their positional relevance (168).

Are Any of These Molecules Available for Regenerating Axons Finding Their Way to Their Target Brain Regions in the Adult?

Experiments have shown that after deafferentation of the superior colliculus (the major target for RGCs in rodents), there is reexpression of some of the same guidance cues, creating a crude topographic map (169–172). After optic nerve injury, ephrin A2 expression in the superior colliculus is upregulated (173–175). If RGC survival is enhanced, RGCs express the appropriate Eph A5 receptors in a gradient that mimics development (176). Other developmental guidance molecules such as ephrin B1, however, are only minimally expressed in the adult and deafferented superior colliculus (175). In experiments in which a small percentage of regenerating axons were able to reach their target through peripheral nerve grafts, functional synapses were identified at

the target tissue (177–183), although the axonal arbors made by these neurons were much smaller than normal (184). The fibers that reinnervated the superior colliculus created a rough topographic map, suggesting that developmental cues may be reexpressed in the target tissue to allow for appropriate mapping (172). Additional grafting studies connecting a severed optic nerve with the pretectum (important for pupillary reflexes) revealed a restoration of a functional pupillary reflex after 2–4 months (182,185,186), supporting the notion that regenerating axons can functionally reinnervate their target tissues. Taken together, these findings suggest that if RGCs can be promoted to regenerate, there are some developmental cues available to guide axons back to their targets and possibly re-create topographic maps of visual space.

CONCLUSIONS

Promoting optic nerve regeneration may seem an insurmountable task. Research in the field of CNS regeneration suggests that we must simultaneously address RGC death after optic nerve injury, the inhibitory glial environment, and changes in the RGCs' intrinsic potential for axon regeneration. We must ultimately think about how regenerating axons may innervate their targets in the brain. With a number of these approaches entering into human clinical trials in the optic nerve or the spinal cord, and with many new technologies and strategies, we are getting closer to offering real hope to those with optic nerve disease.

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REFERENCES

1. **Wu N,** Yin ZQ, Wang Y. Traumatic optic neuropathy therapy: an update of clinical and experimental studies. J Int Med Res. 2008;36:883–889.
2. **Hayreh SS.** Ischemic optic neuropathy. Prog Retin Eye Res. 2009;28:34–62.
3. **Meyer R,** Weissert R, Diem R, Storch MK, de Graaf KL, Kramer B, Bahr M. Acute neuronal apoptosis in a rat model of multiple sclerosis. J Neurosci. 2001;21:6214–6220.
4. **Guy J.** Optic nerve degeneration in experimental autoimmune encephalomyelitis. Ophthalmic Res. 2008; 40:212–216.
5. **Lebrun-Julien F,** Di Polo A. Molecular and cell-based approaches for neuroprotection in glaucoma. Optom Vis Sci. 2008;85:417–424.
6. **Levin LA.** Axonal loss and neuroprotection in optic neuropathies. Can J Ophthalmol. 2007;42:403–408.
7. **Spencer WH.** Ophthalmic Pathology: An Atlas and Textbook, 1st edition. Philadelphia, PA: W.B. Saunders Co., 1996.
8. **Berkelaar M,** Clarke DB, Wang YC, Bray GM, Aguayo AJ. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci. 1994;14: 4368–4374.
9. **Villegas-Pérez MP,** Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ. Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats. J Neurobiol. 1993;24:23–36.
10. **Hull M,** Bahr M. Differential regulation of c-JUN expression in rat retinal ganglion cells after proximal and distal optic nerve transection. Neurosci Lett. 1994;178:39–42.
11. **Bernstein-Goral H,** Bregman BS. Axotomized rubrospinal neurons rescued by fetal spinal cord transplants maintain axon collaterals to rostral CNS targets. Exp Neurol. 1997; 148:13–25.
12. **Lingor P,** Koeberle P, Kügler S, Bähr M. Down-regulation of apoptosis mediators by RNAi inhibits axotomy-induced retinal ganglion cell death in vivo. Brain. 2005;128: 550–558.
13. **Homma K,** Koriyama Y, Mawatari K, Higuchi Y, Kosaka J, Kato S. Early downregulation of IGF-I decides the fate of rat retinal ganglion cells after optic nerve injury. Neurochem Int. 2007;50:741–748.
14. **Isenmann S,** Engel S, Gillardon F, Bähr M. Bax antisense oligonucleotides reduce axotomy-induced retinal ganglion cell death in vivo by reduction of Bax protein expression. Cell Death Differ. 1999;6:673–682.
15. **Bonfanti L,** Strettoi E, Chierzi S, Cenni MC, Liu XH, Martinou J-C, Maffei L, Rabacchi SA. Protection of retinal ganglion cells from natural and axotomy-induced cell death in neonatal transgenic mice overexpressing bcl-2. J Neurosci. 1996;16:4186–4194.
16. **Cenni MC,** Bonfanti L, Martinou JC, Ratto GM, Strettoi E, Maffei L. Long-term survival of retinal ganglion cells following optic nerve section in adult bcl-2 transgenic mice. Eur J Neurosci. 1996;8:1735–1745.
17. **Bien A,** Seidenbecher CI, Böckers TM, Sabel BA, Kreutz MR. Apoptotic versus necrotic characteristics of retinal ganglion cell death after partial optic nerve injury. J Neurotrauma. 1999;16:153–163.
18. **Whitmore AV,** Libby RT, John SW. Glaucoma: thinking in new ways-a role for autonomous axonal self-destruction and other compartmentalised processes? Prog Retin Eye Res. 2005;24:639–662.
19. **Riccio A,** Pierchala BA, Ciarallo CL, Ginty DD. An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science. 1997;277: 1097–1100.
20. **Bhattacharyya A,** Watson FL, Bradlee TA, Pomeroy SL, Stiles CD, Segal RA. Trk receptors function as rapid retrograde signal carriers in the adult nervous system. J Neurosci. 1997;17:7007–7016.
21. **Grimes ML,** Beattie E, Mobley WC. A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc Natl Acad Sci U S A. 1997;94: 9909–9914.
22. **Senger DL,** Campenot RB. Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures. J Cell Biol. 1997;138:411–421.
23. **Ure DR,** Campenot RB. Retrograde transport and steady-state distribution of 125I-nerve growth factor in rat sympathetic neurons in compartmented cultures. J Neurosci. 1997;17:1282–1290.
24. **Yip HK,** So KF. Axonal regeneration of retinal ganglion cells: effect of trophic factors. Prog Retin Eye Res. 2000; 19:559–575.
25. **Johnson JE,** Barde YA, Schwab M, Thoenen H. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. J Neurosci. 1986;6: 3031–3038.
26. **Meyer-Franke A,** Kaplan MR, Pfrieger FW, Barres BA. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron. 1995;15:805–819.
27. **Mey J,** Thanos S. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res. 1993;602: 304–317.

28. **Nakazawa T,** Tamai M, Mori N. Brain-derived neurotrophic factor prevents axotomized retinal ganglion cell death through MAPK and PI3K signaling pathways. Invest Ophthalmol Vis Sci. 2002;43:3319–3326.
29. **Di Polo A,** Aigner LJ, Dunn RJ, Bray GM, Aguayo AJ. Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells. Proc Natl Acad Sci U S A. 1998;95:3978–3983.
30. **Mansour-Robaey S,** Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A. 1994;91:1632–1636.
31. **Peinado-Ramón P,** Salvador M, Villegas-Pérez MP, Vidal-Sanz M. Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. Invest Ophthalmol Vis Sci. 1996;37:489–500.
32. **Zhang CW,** Lu Q, You SW, Zhi Y, Yip HK, Wu W, So KF, Cui Q. CNTF and BDNF have similar effects on retinal ganglion cell survival but differential effects on nitric oxide synthase expression soon after optic nerve injury. Invest Ophthalmol Vis Sci. 2005;46:1497–1503.
33. **Zhi Y,** Lu Q, Zhang CW, Yip HK, So KF, Cui Q. Different optic nerve injury sites result in different responses of retinal ganglion cells to brain-derived neurotrophic factor but not neurotrophin-4/5. Brain Res. 2005;1047: 224–232.
34. **Klöcker N,** Bräunling F, Isenmann S, Bähr M. In vivo neurotrophic effects of GDNF on axotomized retinal ganglion cells. Neuroreport. 1997;8:3439–3442.
35. **Koeberle PD,** Ball AK. Effects of GDNF on retinal ganglion cell survival following axotomy. Vision Res. 1998;38: 1505–1515.
36. **Yan Q,** Wang J, Matheson CR, Urich JL. Glial cell line-derived neurotrophic factor (GDNF) promotes the survival of axotomized retinal ganglion cells in adult rats: comparison to and combination with brain-derived neurotrophic factor (BDNF). J Neurobiol. 1999;38: 382–390.
37. **Cui Q,** Yip HK, Zhao RC, So KF, Harvey AR. Intraocular elevation of cyclic AMP potentiates ciliary neurotrophic factor-induced regeneration of adult rat retinal ganglion cell axons. Mol Cell Neurosci. 2003;22:49–61.
38. **Lingor P,** Tönges L, Pieper N, Bermel C, Barski E, Planchamp V, Bähr M. ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially regulate survival and regeneration in retinal ganglion cells. Brain. 2008;131:250–263.
39. **Jelsma TN,** Friedman HH, Berkelaar M, Bray GM, Aguayo AJ. Different forms of the neurotrophin receptor trkB mRNA predominate in rat retina and optic nerve. J Neurobiol. 1993;24:1207–1214.
40. **Ju WK,** Kim KY, Lee MY, Hofmann HD, Kirsch M, Cha JH, Oh SJ, Chun MH. Up-regulated CNTF plays a protective role for retrograde degeneration in the axotomized rat retina. Neuroreport. 2000;11:3893–3896.
41. **Pachnis V,** Mankoo B, Costantini F. Expression of the c-ret proto-oncogene during mouse embryogenesis. Development. 1993;119:1005–1017.
42. **Suzuki A,** Nomura S, Morii E, Fukuda Y, Kosaka J. Localization of mRNAs for trkB isoforms and p75 in rat retinal ganglion cells. J Neurosci Res. 1998;54: 27–37.
43. **Cui Q,** Tang LS, Hu B, So KF, Yip HK. Expression of trkA, trkB, and trkC in injured and regenerating retinal ganglion cells of adult rats. Invest Ophthalmol Vis Sci. 2002;43: 1954–1964.
44. **Pernet V,** Di Polo A. Synergistic action of brain-derived neurotrophic factor and lens injury promotes retinal ganglion cell survival, but leads to optic nerve dystrophy in vivo. Brain. 2006;129:1014–1026.
45. **Spalding KL,** Cui Q, Harvey AR. Retinal ganglion cell neurotrophin receptor levels and trophic requirements following target ablation in the neonatal rat. Neuroscience. 2005;131:387–395.
46. **Tao W.** Application of encapsulated cell technology for retinal degenerative diseases. Expert Opin Biol Ther. 2006;6:717–726.
47. **Lambiase A,** Aloe L, Centofanti M, Parisi V, Mantelli F, Colafrancesco V, Manni GL, Bucci MG, Bonini S, Levi-Montalcini R. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: Implications for glaucoma. Proc Natl Acad Sci U S A. 2009; 106:13469–13474.
48. **Goldberg JL,** Barres BA. The relationship between neuronal survival and regeneration. Annu Rev Neurosci. 2000;23: 579–612.
49. **Shen S,** Wiemelt AP, McMorris FA, Barres BA. Retinal ganglion cells lose trophic responsiveness after axotomy. Neuron. 1999;23:285–295.
50. **Cheng L,** Sapieha P, Kittlerova P, Hauswirth WW, Di Polo A. TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. J Neurosci. 2002;22: 3977–3986.
51. **Monsul NT,** Geisendorfer AR, Han PJ, Banik R, Pease ME, Skolasky RL Jr, Hoffman PN. Intraocular injection of dibutyryl cyclic AMP promotes axon regeneration in rat optic nerve. Exp Neurol. 2004;186:124–133.
52. **Morimoto T,** Miyoshi T, Fujikado T, Tano Y, Fukuda Y. Electrical stimulation enhances the survival of axotomized retinal ganglion cells in vivo. Neuroreport. 2002;13:227–230.
53. **Duan Y,** Kong W, Benny Klimek M, Goldberg JL. Loss of retinal ganglion cell trophic responsiveness is correlated with reduced electrical activity. Presented at: ARVO; 2009: Fort Lauderdale, FL. Abstract 127/A171.
54. **Goldberg JL,** Espinosa JS, Xu Y, Davidson N, Kovacs GT, Barres BA. Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. Neuron. 2002;33:689–702.
55. **Al-Majed AA,** Tam SL, Gordon T. Electrical stimulation accelerates and enhances expression of regeneration-associated genes in regenerating rat femoral motoneurons. Cell Mol Neurobiol. 2004;24:379–402.
56. **Geremia NM,** Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. Exp Neurol. 2007;205:347–359.
57. **Miyake K,** Yoshida M, Inoue Y, Hata Y. Neuroprotective effect of transcorneal electrical stimulation on the acute phase of optic nerve injury. Invest Ophthalmol Vis Sci. 2007;48:2356–2361.
58. **Tagami Y,** Kurimoto T, Miyoshi T, Morimoto T, Sawai H, Mimura O. Axonal regeneration induced by repetitive electrical stimulation of crushed optic nerve in adult rats. Jpn J Ophthalmol. 2009;53:257–266.
59. **Lieven CJ,** Hoegger MJ, Schlieve CR, Levin LA. Retinal ganglion cell axotomy induces an increase in intracellular superoxide anion. Invest Ophthalmol Vis Sci. 2006;47: 1477–1485.
60. **Schlieve CR,** Tam A, Nilsson BL, Lieven CJ, Raines RT, Levin LA. Synthesis and characterization of a novel class of reducing agents that are highly neuroprotective for retinal ganglion cells. Exp Eye Res. 2006;83:1252–1259.
61. **Yiu G,** He Z. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci. 2006;7:617–627.
62. **Rhodes KE,** Fawcett JW. Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? J Anat. 2004;204:33–48.
63. **Bovolenta P,** Fernaud-Espinosa I. Nervous system proteoglycans as modulators of neurite outgrowth. Prog Neurobiol. 2000;61:113–132.
64. **Kantor DB,** Chivatakarn O, Peer KL, Oster SF, Inatani M, Hansen MJ, Flanagan JG, Yamaguchi Y, Sretavan DW, Giger RJ, Kolodkin AL. Semaphorin 5A is a bifunctional

axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. Neuron. 2004;44:961–975.
65. **Goldberg JL,** Vargas ME, Wang JT, Mandemakers W, Oster SF, Sretavan DW, Barres BA. An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci. 2004;24:4989–4999.
66. **Kaneko S,** Iwanami A, Nakamura M, Kishino A, Kikuchi K, Shibata S, Okano HJ, Ikegami T, Moriya A, Konishi O, Nakayama C, Kumagai K, Kimura T, Sato Y, Goshima Y, Taniguchi M, Ito M, He Z, Toyama Y, Okano H. A selective Sema3A inhibitor enhances regenerative responses and functional recovery of the injured spinal cord. Nat Med. 2006;12:1380–1389.
67. **Luo Y,** Raible D, Raper JA. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. Cell. 1993;75:217–227.
68. **Pasterkamp RJ,** Giger RJ, Ruitenberg MJ, Holtmaat AJ, De Wit J, De Winter F, Verhaagen J. Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. Mol Cell Neurosci. 1999;13:143–166.
69. **Shewan D,** Dwivedy A, Anderson R, Holt CE. Age-related changes underlie switch in netrin-1 responsiveness as growth cones advance along visual pathway. Nat Neurosci. 2002;5:955–962.
70. **Nitzan A,** Kermer P, Shirvan A, Bähr M, Barzilai A, Solomon AS. Examination of cellular and molecular events associated with optic nerve axotomy. Glia. 2006;54:545–556.
71. **Wong EV,** David S, Jacob MH, Jay DG. Inactivation of myelin-associated glycoprotein enhances optic nerve regeneration. J Neurosci. 2003;23:3112–3117.
72. **Bartsch U,** Bandtlow CE, Schnell L, Bartsch S, Spillmann AA, Rubin BP, Hillenbrand R, Montag D, Schwab ME, Schachner M. Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron. 1995;15:1375–1381.
73. **Chaudhry N,** Filbin MT. Myelin-associated inhibitory signaling and strategies to overcome inhibition. J Cereb Blood Flow Metab. 2007;27:1096–1107.
74. **Buchli AD,** Schwab ME. Inhibition of Nogo: a key strategy to increase regeneration, plasticity and functional recovery of the lesioned central nervous system. Ann Med. 2005;37:556–567.
75. **Teng FY,** Tang BL. Why do Nogo/Nogo-66 receptor gene knockouts result in inferior regeneration compared to treatment with neutralizing agents? J Neurochem. 2005; 94:865–874.
76. **Cai D,** Shen Y, De Bellard M, Tang S, Filbin MT. Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron. 1999;22:89–101.
77. **Cai D,** Qiu J, Cao Z, McAtee M, Bregman BS, Filbin MT. Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. J Neurosci. 2001;21:4731–4739.
78. **Gross RE,** Mei Q, Gutekunst CA, Torre E. The pivotal role of RhoA GTPase in the molecular signaling of axon growth inhibition after CNS injury and targeted therapeutic strategies. Cell Transplant. 2007;16:245–262.
79. **Maekawa M,** Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, Narumiya S. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science. 1999; 285:895–898.
80. **Fournier AE,** Kalb RG, Strittmatter SM. Rho GTPases and axonal growth cone collapse. Methods Enzymol. 2000; 325:473–482.
81. **Bertrand J,** Winton MJ, Rodriguez-Hernandez N, Campenot RB, McKerracher L. Application of Rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and regeneration of retinal ganglion cell axons in the optic nerve of adult rats. J Neurosci. 2005;25: 1113–1121.
82. **Dergham P,** Ellezam B, Essagian C, Avedissian H, Lubell WD, McKerracher L. Rho signaling pathway targeted to promote spinal cord repair. J Neurosci. 2002; 22:6570–6577.
83. **Lehmann M,** Fournier A, Selles-Navarro I, Dergham P, Sebok A, Leclerc N, Tigyi G, McKerracher L. Inactivation of Rho signaling pathway promotes CNS axon regeneration. J Neurosci. 1999;19:7537–7547.
84. **Winton MJ,** Dubreuil CI, Lasko D, Leclerc N, McKerracher L. Characterization of new cell permeable C3-like proteins that inactivate Rho and stimulate neurite outgrowth on inhibitory substrates. J Biol Chem. 2002;277: 32820–32829.
85. **Bertrand J,** Di Polo A, McKerracher L. Enhanced survival and regeneration of axotomized retinal neurons by repeated delivery of cell-permeable C3-like Rho antagonists. Neurobiol Dis. 2007;25:65–72.
86. **Hu Y,** Cui Q, Harvey AR. Interactive effects of C3, cyclic AMP and ciliary neurotrophic factor on adult retinal ganglion cell survival and axonal regeneration. Mol Cell Neurosci. 2007;34:88–98.
87. **Alabed YZ,** Pool M, Ong Tone S, Fournier AE. Identification of CRMP4 as a convergent regulator of axon outgrowth inhibition. J Neurosci. 2007;27:1702–1711.
88. **Borisoff JF,** Chan CC, Hiebert GW, Oschipok L, Robertson GS, Zamboni R, Steeves JD, Tetzlaff W. Suppression of Rho-kinase activity promotes axonal growth on inhibitory CNS substrates. Mol Cell Neurosci. 2003;22:405–416.
89. **Chan CC,** Khodarahmi K, Liu J, Sutherland D, Oschipok LW, Steeves JD, Tetzlaff W. Dose-dependent beneficial and detrimental effects of ROCK inhibitor Y27632 on axonal sprouting and functional recovery after rat spinal cord injury. Exp Neurol. 2005;196:352–364.
90. **Fournier AE,** Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. J Neurosci. 2003;23:1416–1423.
91. **Sagawa H,** Terasaki H, Nakamura M, Ichikawa M, Yata T, Tokita Y, Watanabe M. A novel ROCK inhibitor, Y-39983, promotes regeneration of crushed axons of retinal ganglion cells into the optic nerve of adult cats. Exp Neurol. 2007;205:230–240.
92. **Lingor P,** Teusch N, Schwarz K, Mueller R, Mack H, Bähr M, Mueller BK. Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan in vitro and axonal regeneration in the adult optic nerve in vivo. J Neurochem. 2007;103:181–189.
93. **Ichikawa M,** Yoshida J, Saito K, Sagawa H, Tokita Y, Watanabe M. Differential effects of two ROCK inhibitors, Fasudil and Y-27632, on optic nerve regeneration in adult cats. Brain Res. 2008;1201:23–33.
94. **Sivasankaran R,** Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, He Z. PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. Nat Neurosci. 2004;7:261–268.
95. **Koprivica V,** Cho KS, Park JB, Yiu G, Atwal J, Gore B, Kim JA, Lin E, Tessier-Lavigne M, Chen DF, He Z. EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans. Science. 2005;310:106–110.
96. **Ahmed Z,** Jacques SJ, Berry M, Logan A. Epidermal growth factor receptor inhibitors promote CNS axon growth through off-target effects on glia. Neurobiol Dis. 2009;36:142–150.
97. **Douglas MR,** Morrison KC, Jacques SJ, Leadbeater WE, Gonzalez AM, Berry M, Logan A, Ahmed Z. Off-target effects of epidermal growth factor receptor antagonists mediate retinal ganglion cell disinhibited axon growth. Brain. 2009;132:3102–3121.
98. **Thuret S,** Moon LD, Gage FH. Therapeutic interventions after spinal cord injury. Nat Rev Neurosci. 2006;7: 628–643.
99. **Filbin MT.** How inflammation promotes regeneration. Nat Neurosci. 2006;9:715–717.

100. **Fischer D,** Pavlidis M, Thanos S. Cataractogenic lens injury prevents traumatic ganglion cell death and promotes axonal regeneration both in vivo and in culture. Invest Ophthalmol Vis Sci. 2000;41:3943–3954.
101. **Leon S,** Yin Y, Nguyen J, Irwin N, Benowitz LI. Lens injury stimulates axon regeneration in the mature rat optic nerve. J Neurosci. 2000;20:4615–4626.
102. **Fischer D,** Heiduschka P, Thanos S. Lens-injury-stimulated axonal regeneration throughout the optic pathway of adult rats. Exp Neurol. 2001;172:257–272.
103. **Lorber B,** Berry M, Logan A. Lens injury stimulates adult mouse retinal ganglion cell axon regeneration via both macrophage- and lens-derived factors. Eur J Neurosci. 2005;21:2029–2034.
104. **Yin Y,** Cui Q, Li Y, Irwin N, Fischer D, Harvey AR, Benowitz LI. Macrophage-derived factors stimulate optic nerve regeneration. J Neurosci. 2003;23:2284–2293.
105. **Yin Y,** Henzl MT, Lorber B, Nakazawa T, Thomas TT, Jiang F, Langer R, Benowitz LI. Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. Nat Neurosci. 2006;9:843–852.
106. **Leibinger M,** Müller A, Andreadaki A, Hauk TG, Kirsch M, Fischer D. Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J Neurosci. 2009;29:14334–14341.
107. **Fischer D,** He Z, Benowitz LI. Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J Neurosci. 2004;24:1646–1651.
108. **Fischer D,** Petkova V, Thanos S, Benowitz LI. Switching mature retinal ganglion cells to a robust growth state in vivo: gene expression and synergy with RhoA inactivation. J Neurosci. 2004;24:8726–8740.
109. **Hauben E,** Ibarra A, Mizrahi T, Barouch R, Agranov E, Schwartz M. Vaccination with a Nogo-A-derived peptide after incomplete spinal-cord injury promotes recovery via a T-cell-mediated neuroprotective response: comparison with other myelin antigens. Proc Natl Acad Sci U S A. 2001;98:15173–15178.
110. **Kipnis J,** Yoles E, Porat Z, Cohen A, Mor F, Sela M, Cohen IR, Schwartz M. T cell immunity to copolymer 1 confers neuroprotection on the damaged optic nerve: possible therapy for optic neuropathies. Proc Natl Acad Sci U S A. 2000;97:7446–7451.
111. **Knoller N,** Auerbach G, Fulga V, Zelig G, Attias J, Bakimer R, Marder JB, Yoles E, Belkin M, Schwartz M, Hadani M. Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. J Neurosurg Spine. 2005;3: 173–181.
112. **Tello F.** La influencia del neurotropismo en la regeneración de los centros nerviosos. Trab Lab Invest Biol Univ Madrid. 1911;9:123–128.
113. **Aguayo AJ,** Vidal-Sanz M, Villegas-Pérez MP, Bray GM. Growth and connectivity of axotomized retinal neurons in adult rats with optic nerves substituted by PNS grafts linking the eye and the midbrain. Ann N Y Acad Sci. 1987; 495:1–9.
114. **Aguayo AJ,** Bray GM, Carter DA, Villegas-Perez MP, Vidal-Sanz M, Rasminsky M. Regrowth and connectivity of injured central nervous system axons in adult rodents. Acta Neurobiol Exp (Wars). 1990;50:381–389.
115. **Bray GM,** Villegas-Pérez MP, Vidal-Sanz M, Aguayo AJ. The use of peripheral nerve grafts to enhance neuronal survival, promote growth and permit terminal reconnections in the central nervous system of adult rats. J Exp Biol. 1987;132:5–19.
116. **Vidal-Sanz M,** Bray GM, Villegas-Pérez MP, Thanos S, Aguayo AJ. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. J Neurosci. 1987;7:2894–2909.
117. **Dezawa M,** Kawana K, Negishi H, Adachi-Usami E. Glial cells in degenerating and regenerating optic nerve of the adult rat. Brain Res Bull. 1999;48:573–579.
118. **Sievers J,** Bamberger C, Debus OM, Lucius R. Regeneration in the optic nerve of adult rats: influences of cultured astrocytes and optic nerve grafts of different ontogenetic stages. J Neurocytol. 1995;24:783–793.
119. **Harvey AR,** Tan MM. Spontaneous regeneration of adult rat retinal ganglion cell axons in vivo. Neuroreport. 1992; 3:239–242.
120. **Hausmann B,** Sievers J, Hermanns J, Berry M. Regeneration of axons from the adult rat optic nerve: influence of fetal brain grafts, laminin, and artificial basement membrane. J Comp Neurol. 1989;281: 447–466.
121. **Sievers J,** Hausmann B, Berry M. Fetal brain grafts rescue adult retinal ganglion cells from axotomy-induced cell death. J Comp Neurol. 1989;281:467–478.
122. **Harvey AR,** Gan SK, Pauken JM. Fetal tectal or cortical tissue transplanted into brachial lesion cavities in rats: influence on the regrowth of host retinal axons. J Comp Neurol. 1987;263:126–136.
123. **Girard C,** Bemelmans AP, Dufour N, Mallet J, Bachelin C, Nait-Oumesmar B, Baron-Van Evercooren A, Lachapelle F. Grafts of brain-derived neurotrophic factor and neurotrophin 3-transduced primate Schwann cells lead to functional recovery of the demyelinated mouse spinal cord. J Neurosci. 2005;25:7924–7933.
124. **Lu P,** Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp Neurol. 2003;181:115–129.
125. **Lu P,** Jones LL, Tuszynski MH. BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. Exp Neurol. 2005;191:344–360.
126. **Berry M,** Hall S, Follows R, Rees L, Gregson N, Sievers J. Response of axons and glia at the site of anastomosis between the optic nerve and cellular or acellular sciatic nerve grafts. J Neurocytol. 1988;17:727–744.
127. **Dezawa M,** Kawana K, Adachi-Usami E. The role of Schwann cells during retinal ganglion cell regeneration induced by peripheral nerve transplantation. Invest Ophthalmol Vis Sci. 1997;38:1401–1410.
128. **Negishi H,** Dezawa M, Oshitari T, Adachi-Usami E. Optic nerve regeneration within artificial Schwann cell graft in the adult rat. Brain Res Bull. 2001;55:409–419.
129. **Plant GW,** Harvey AR. A new type of biocompatible bridging structure supports axon regrowth after implantation into the lesioned rat optic tract. Cell Transplant. 2000;9:759–772.
130. **Xu XM,** Guénard V, Kleitman N, Bunge MB. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. J Comp Neurol. 1995;351:145–160.
131. **Hagg T,** Gulati AK, Behzadian MA, Vahlsing HL, Varon S, Manthorpe M. Nerve growth factor promotes CNS cholinergic axonal regeneration into acellular peripheral nerve grafts. Exp Neurol. 1991;112:79–88.
132. **Xu XM,** Guénard V, Kleitman N, Aebischer P, Bunge MB. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp Neurol. 1995;134: 261–272.
133. **Iannotti C,** Li H, Yan P, Lu X, Wirthlin L, Xu XM. Glial cell line-derived neurotrophic factor-enriched bridging transplants promote propriospinal axonal regeneration and enhance myelination after spinal cord injury. Exp Neurol. 2003;183:379–393.
134. **Yick LW,** Wu W, So KF, Yip HK. Peripheral nerve graft and neurotrophic factors enhance neuronal survival and expression of nitric oxide synthase in Clarke's nucleus after hemisection of the spinal cord in adult rat. Exp Neurol. 1999;159:131–138.

135. **Smale KA,** Doucette R, Kawaja MD. Implantation of olfactory ensheathing cells in the adult rat brain following fimbria-fornix transection. Exp Neurol. 1996;137: 225–233.
136. **Cui Q,** Pollett MA, Symons NA, Plant GW, Harvey AR. A new approach to CNS repair using chimeric peripheral nerve grafts. J Neurotrauma. 2003;20:17–31.
137. **Berry M,** Carlile J, Hunter A. Peripheral nerve explants grafted into the vitreous body of the eye promote the regeneration of retinal ganglion cell axons severed in the optic nerve. J Neurocytol. 1996;25:147–170.
138. **Berry M,** Carlile J, Hunter A, Tsang W, Rosenstiel P, Sievers J. Optic nerve regeneration after intravitreal peripheral nerve implants: trajectories of axons regrowing through the optic chiasm into the optic tracts. J Neurocytol. 1999;28:721–741.
139. **Bregman BS,** Goldberger ME. Anatomical plasticity and sparing of function after spinal cord damage in neonatal cats. Science. 1982;217:553–555.
140. **Kunkel-Bagden E,** Dai HN, Bregman BS. Recovery of function after spinal cord hemisection in newborn and adult rats: differential effects on reflex and locomotor function. Exp Neurol. 1992;116:40–51.
141. **Chen DF,** Jhaveri S, Schneider GE. Intrinsic changes in developing retinal neurons result in regenerative failure of their axons. Proc Natl Acad Sci U S A. 1995;92: 7287–7291.
142. **Li D,** Field PM, Raisman G. Failure of axon regeneration in postnatal rat entorhinohippocampal slice coculture is due to maturation of the axon, not that of the pathway or target. Eur J Neurosci. 1995;7:1164–1171.
143. **Bouslama-Oueghlani L,** Wehrlé R, Sotelo C, Dusart I. The developmental loss of the ability of Purkinje cells to regenerate their axons occurs in the absence of myelin: an in vitro model to prevent myelination. J Neurosci. 2003; 23:8318–8329.
144. **Dusart I,** Airaksinen MS, Sotelo C. Purkinje cell survival and axonal regeneration are age dependent: an in vitro study. J Neurosci. 1997;17:3710–3726.
145. **Blackmore M,** Letourneau PC. Changes within maturing neurons limit axonal regeneration in the developing spinal cord. J Neurobiol. 2006;66:348–360.
146. **Goldberg JL,** Klassen MP, Hua Y, Barres BA. Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. Science. 2002;296:1860–1864.
147. **C Chen DF,** Schneider GE, Martinou JC, Tonegawa S. Bcl-2 promotes regeneration of severed axons in mammalian CNS. Nature. 1997;385:434–439.
148. **Cho KS,** Yang L, Lu B, Feng Ma H, Huang X, Pekny M, Chen DF. Re-establishing the regenerative potential of central nervous system axons in postnatal mice. J Cell Sci. 2005;118:863–872.
149. **Cho KS,** Chen DF. Promoting optic nerve regeneration in adult mice with pharmaceutical approach. Neurochem Res. 2008;33:2126–2133.
150. **Gao Y,** Deng K, Hou J, Bryson JB, Barco A, Nikulina E, Spencer T, Mellado W, Kandel ER, Filbin MT. Activated CREB is sufficient to overcome inhibitors in myelin and promote spinal axon regeneration in vivo. Neuron. 2004; 44:609–621.
151. **Cai D,** Deng K, Mellado W, Lee J, Ratan RR, Filbin MT. Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron. 2002;35:711–719.
152. **Deng K,** He H, Qiu J, Lorber B, Bryson JB, Filbin MT. Increased synthesis of spermidine as a result of upregulation of arginase I promotes axonal regeneration in culture and in vivo. J Neurosci. 2009;29:9545–9552.
153. **Hammarlund M,** Nix P, Hauth L, Jorgensen EM, Bastiani M. Axon regeneration requires a conserved MAP kinase pathway. Science. 2009;323:802–806.
154. **Miller BR,** Press C, Daniels RW, Sasaki Y, Milbrandt J, DiAntonio A. A dual leucine kinase-dependent axon self-destruction program promotes Wallerian degeneration. Nat Neurosci. 2009;12:387–389.
155. **Park KK,** Liu K, Hu Y, Smith PD, Wang C, Cai B, Xu B, Connolly L, Kramvis I, Sahin M, He Z. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science. 2008;322:963–966.
156. **Konishi Y,** Stegmüller J, Matsuda T, Bonni S, Bonni A. Cdh1-APC controls axonal growth and patterning in the mammalian brain. Science. 2004;303:1026–1030.
157. **Lasorella A,** Stegmüller J, Guardavaccaro D, Liu G, Carro MS, Rothschild G, de la Torre-Ubieta L, Pagano M, Bonni A, Iavarone A. Degradation of Id2 by the anaphase-promoting complex couples cell cycle exit and axonal growth. Nature. 2006;442:471–474.
158. **Stegmüller J,** Konishi Y, Huynh MA, Yuan Z, Dibacco S, Bonni A. Cell-intrinsic regulation of axonal morphogenesis by the Cdh1-APC target SnoN. Neuron. 2006;50:389–400.
159. **Huynh MA,** Stegmüller J, Litterman N, Bonni A. Regulation of Cdh1-APC function in axon growth by Cdh1 phosphorylation. J Neurosci. 2009;29:4322–4327.
160. **Stegmüller J,** Huynh MA, Yuan Z, Konishi Y, Bonni A. TGFbeta-Smad2 signaling regulates the Cdh1-APC/SnoN pathway of axonal morphogenesis. J Neurosci. 2008;28: 1961–1969.
161. **Moore DL,** Blackmore MG, Goldberg JL, Transcriptional control of the intrinsic loss of axon growth ability in retinal ganglion cells. In: Axonal Connections: Molecular Cues for Development and Regeneration. Keystone, Colorado: Keystone Symposia; 2009. Abstract 215.
162. **Deiner MS,** Kennedy TE, Fazeli A, Serafini T, Tessier-Lavigne M, Sretavan DW. Netrin-1 and DCC mediate axon guidance locally at the optic disc: loss of function leads to optic nerve hypoplasia. Neuron. 1997;19:575–589.
163. **Williams SE,** Mann F, Erskine L, Sakurai T, Wei S, Rossi DJ, Gale NW, Holt CE, Mason CA, Henkemeyer M. Ephrin-B2 and EphB1 mediate retinal axon divergence at the optic chiasm. Neuron. 2003;39:919–935.
164. **Oster SF,** Bodeker MO, He F, Sretavan DW. Invariant Sema5A inhibition serves an ensheathing function during optic nerve development. Development. 2003;130: 775–784.
165. **Erskine L,** Williams SE, Brose K, Kidd T, Rachel RA, Goodman CS, Tessier-Lavigne M, Mason CA. Retinal ganglion cell axon guidance in the mouse optic chiasm: expression and function of robos and slits. J Neurosci. 2000;20:4975–4982.
166. **Niclou SP,** Jia L, Raper JA. Slit2 is a repellent for retinal ganglion cell axons. J Neurosci. 2000;20:4962–4974.
167. **Plump AS,** Erskine L, Sabatier C, Brose K, Epstein CJ, Goodman CS, Mason CA, Tessier-Lavigne M. Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. Neuron. 2002; 33:219–232.
168. **Inatani M.** Molecular mechanisms of optic axon guidance. Naturwissenschaften. 2005;92:549–561.
169. **Bahr M,** Eschweiler GW. Regenerating adult rat retinal axons reconnect with target neurons in-vitro. Neuroreport. 1991;2:581–584.
170. **Bahr M,** Eschweiler GW. Formation of functional synapses by regenerating adult rat retinal ganglion cell axons in midbrain target regions in vitro. J Neurobiol. 1993;24: 456–473.
171. **Wizenmann A,** Thies E, Klostermann S, Bonhoeffer F, Bähr M. Appearance of target-specific guidance information for regenerating axons after CNS lesions. Neuron. 1993;11: 975–983.
172. **Sauve Y,** Sawai H, Rasminsky M. Topological specificity in reinnervation of the superior colliculus by regenerated retinal ganglion cell axons in adult hamsters. J Neurosci. 2001;21:951–960.
173. **Rodger J,** Lindsey KA, Leaver SG, King CE, Dunlop SA, Beazley LD. Expression of ephrin-A2 in the superior colliculus and EphA5 in the retina following optic

nerve section in adult rat. Eur J Neurosci. 2001;14: 1929–1936.
174. **Rodger J,** Symonds AC, Springbett J, Shen WY, Bartlett CA, Rakoczy PE, Beazley LD, Dunlop SA. Eph/ephrin expression in the adult rat visual system following localized retinal lesions: localized and transneuronal up-regulation in the retina and superior colliculus. Eur J Neurosci. 2005;22:1840–1852.
175. **Knöll B,** Isenmann S, Kilic E, Walkenhorst J, Engel S, Wehinger J, Bähr M, Drescher U. Graded expression patterns of ephrin-As in the superior colliculus after lesion of the adult mouse optic nerve. Mech Dev. 2001;106: 119–127.
176. **Symonds AC,** King CE, Bartlett CA, Sauvé Y, Lund RD, Beazley LD, Dunlop SA, Rodger J. EphA5 and ephrin-A2 expression during optic nerve regeneration: a 'two-edged sword'. Eur J Neurosci. 2007;25:744–752.
177. **Carter DA,** Bray GM, Aguayo AJ. Regenerated retinal ganglion cell axons can form well-differentiated synapses in the superior colliculus of adult hamsters. J Neurosci. 1989;9:4042–4050.
178. **Keirstead SA,** Rasminsky M, Fukuda Y, Carter DA, Aguayo AJ, Vidal-Sanz M. Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons. Science. 1989;246:255–257.
179. **Sauve Y,** Sawai H, Rasminsky M. Functional synaptic connections made by regenerated retinal ganglion cell axons in the superior colliculus of adult hamsters. J Neurosci. 1995;15:665–675.
180. **Thanos S.** Neurobiology of the regenerating retina and its functional reconnection with the brain by means of peripheral nerve transplants in adult rats. Surv Ophthalmol. 1997;42(Suppl 1):S5–S26.
181. **Thanos S,** Naskar R, Heiduschka P. Regenerating ganglion cell axons in the adult rat establish retinofugal topography and restore visual function. Exp Brain Res. 1997;114: 483–491.
182. **Vidal-Sanz M,** Avilés-Trigueros M, Whiteley SJ, Sauvé Y, Lund RD. Reinnervation of the pretectum in adult rats by regenerated retinal ganglion cell axons: anatomical and functional studies. Prog Brain Res. 2002;137:443–452.
183. **Vidal-Sanz M,** Bray GM and Aguayo AJ. Regenerated synapses persist in the superior colliculus after the regrowth of retinal ganglion cell axons. J Neurocytol. 1991;20:940–952.
184. **Carter DA,** Bray GM, Aguayo AJ. Regenerated retinal ganglion cell axons form normal numbers of boutons but fail to expand their arbors in the superior colliculus. J Neurocytol. 1998;27:187–196.
185. **Thanos S.** Adult retinofugal axons regenerating through peripheral nerve grafts can restore the light-induced pupilloconstriction reflex. Eur J Neurosci. 1992;4:691–699.
186. **Whiteley SJ,** Sauvé Y, Avilés-Trigueros M, Vidal-Sanz M, Lund RD. Extent and duration of recovered pupillary light reflex following retinal ganglion cell axon regeneration through peripheral nerve grafts directed to the pretectum in adult rats. Exp Neurol. 1998;154:560–572.
187. **Howell GR,** Libby RT, Jakobs TC, Smith RS, Phalan FC, Barter JW, Barbay JM, Marchant JK, Mahesh N, Porciatti V, Whitmore AV, Masland RH, John SW. Axons of retinal ganglion cells are insulted in the optic nerve early in DBA/2J glaucoma. J Cell Biol. 2007;179:1523–1537.
188. **John SW,** Smith RS, Savinova OV, Hawes NL, Chang B, Turnbull D, Davisson M, Roderick TH, Heckenlively JR. Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. Invest Ophthalmol Vis Sci. 1998;39: 951–962.
189. **Chang B,** Smith RS, Hawes NL, Anderson MG, Zabaleta A, Savinova O, Roderick TH, Heckenlively JR, Davisson MT, John SW. Interacting loci cause severe iris atrophy and glaucoma in DBA/2J mice. Nat Genet. 1999;21:405–409.
190. **Libby RT,** Anderson MG, Pang IH, Robinson ZH, Savinova OV, Cosma IM, Snow A, Wilson LA, Smith RS, Clark AF, John SW. Inherited glaucoma in DBA/2J mice: pertinent disease features for studying the neurodegeneration. Vis Neurosci. 2005;22:637–648.
191. **Morrison JC,** Moore CG, Deppmeier LM, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. Exp Eye Res. 1997;64:85–96.
192. **Morrison JC.** Elevated intraocular pressure and optic nerve injury models in the rat. J Glaucoma. 2005;14:315–317.
193. **Levin LA.** Animal and culture models of glaucoma for studying neuroprotection. Eur J Ophthalmol. 2001; 11(Suppl 2):S23–S29.
194. **Bernstein SL,** Guo Y, Kelman SE, Flower RW, Johnson MA. Functional and cellular responses in a novel rodent model of anterior ischemic optic neuropathy. Invest Ophthalmol Vis Sci. 2003;44:4153–4162.
195. **Goldberg J,** Duan Y, Kong W, Watson B. Characterization of a novel photochemically induced ischemic optic neuropathy model. Presented at: ARVO; 2009. Abstract 3198/D727.
196. **Bettelli E.** Building different mouse models for human MS. Ann N Y Acad Sci. 2007;1103:11–18.
197. **Bettelli E,** Pagany M, Weiner HL, Linington C, Sobel RA, Kuchroo VK. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. J Exp Med. 2003;197: 1073–1081.
198. **Duvdevani, R,** Rosner, M, Belkin, M, Sautter, J, Sabel, BA, Schwartz, M. Graded crush of the rat optic nerve as a brain injury model: combining electrophysiological and behavioral outcome. Restor Neurol Neurosci. 1990;2: 31–38.
199. **Solomon AS,** Lavie V, Hauben U, Monsonego A, Yoles E, Schwartz M. Complete transection of rat optic nerve while sparing the meninges and the vasculature: an experimental model for optic nerve neuropathy and trauma. J Neurosci Methods. 1996;70:21–25.
200. **Qi X,** Sun L, Lewin AS, Hauswirth WW, Guy J. The mutant human ND4 subunit of complex I induces optic neuropathy in the mouse. Invest Ophthalmol Vis Sci. 2007;48:1–10.
201. **Levkovitch-Verbin H.** Animal models of optic nerve diseases. Eye. 2004;18:1066–1074.