SAVING THE OPTIC NERVE


Studying neuroregeneration and neuroprotection.

BY MELISSA L. COOPER, BS, AND DAVID J. CALKINS, PHD

OPTIC NERVE REGENERATION IN MAMMALS: REGENERATED OR SPARED AXONS?

Fischer D, Harvey AR, Pernet V, et al[1]

ABSTRACT SUMMARY

In this review article, Fischer and colleagues examined controversies regarding the interpretation of the results of studies of optic nerve regeneration that use common crush models. They discussed typical outcomes in crush studies that serve as controls for validating actual regeneration of retinal ganglion cell (RGC) axons and reinnervation of central targets in studies of therapeutic interventions. The review compared morphologic and neurochemical differences between potentially regenerated axons and those that were spared in the putative total crush. Finally, the reviewers made several recommendations on how to promote more rigorous empirical practices and to facilitate the correct interpretation of results.

DISCUSSION

Why is the interpretation of axonal state difficult?

As Fischer and colleagues pointed out, owing to its simplicity, intraorbital optic nerve crush is widely used to study factors that inhibit spontaneous RGC axonal regeneration and potential interventions to promote regrowth and reinnervation of brain targets. Nearly 1,000 PubMed results cite the keywords *optic nerve crush*. In these studies, however, even so-called *complete* or *total* crush can spare some RGC axons, thereby confounding the interpretation of results.

Unless actually severed, axons have a certain pliability that allows them to become functionally quiescent for weeks after being crushed. Because of this property, intact axons can persist alongside those that were more severely damaged and may or may not show signs of regeneration. Even in the brain, functional quiescence of a few stressed—but not degenerated—RGC axonal projections can masquerade as newly reformed arborizations with appropriate innervation. These functional projections unpredictably influence the regenerative properties of nearby degenerated axons. Moreover, interventions designed to promote regeneration may influence injured but not completely severed axons in ways that differ from normal axons.

How could investigations of optic nerve regeneration be improved?

Fischer and colleagues noted that evidence interpreted as indicative of RGC axonal regeneration is often circumstantial, and they proposed new standards for the field. Following the lead of spinal cord injury investigations, one option for reducing or eliminating ambiguity would be to abandon crush in favor of transection. Although this improvement seems obvious, this is the age of murine transgenics, and total nerve

STUDY IN BRIEF

▶ Intraorbital optic nerve crush is widely used to study factors that inhibit spontaneous retinal ganglion cell (RGC) axonal regeneration and potential interventions to promote regrowth and reinnervation of brain targets. Even so-called *complete* or *total* crush can spare some RGC axons, however, thereby confounding interpretation of the results.

WHY IT MATTERS

In addition to orbital and head trauma, glaucoma and other optic neuropathies assault RGC axons in the optic nerve. Because these axons are part of the central nervous system, once injured, their capacity for recovery is limited. Increasingly, investigators test therapeutic interventions for their ability to promote regeneration of lost axons in the optic nerve. As researchers' understanding of the signals that inhibit neuronal growth increases, so do possibilities for leveraging this information clinically.

transection is an extremely difficult surgery to perform in the small postorbital space of the mouse.

To gauge the extent of initial damage, Fischer and colleagues recommended the use of neurochemical surrogates, particularly markers for astroglia processes, which can reach across the injury site. Identifying specific markers for a newly generated axon would also distinguish regenerating from spared axons.

Finally, improved in vivo optical imaging to track recovery of damaged optic nerve axons might eliminate some of the post hoc inference necessary in current acute approaches to regeneration.

ASTROCYTE-DERIVED LIPOXINS A_4 AND B_4 PROMOTE NEUROPROTECTION FROM ACUTE AND CHRONIC INJURY

Livne-Bar I, Wei J, Liu HH, et al[2]

ABSTRACT SUMMARY

This laboratory investigation explored a prosurvival relationship between retinal astrocyte glia and RGCs challenged by disease-relevant stressors in experimental models. Astrocytes distribute densely across the retinal nerve fiber layer, forming intimate connections with both RGC axons and retinal vasculature. Astrocytes also provide metabolic and other support to RGC axons in the optic nerve. Livne-Bar and colleagues probed how small lipid mediators called *lipoxins* (LXA_4 and LXB_4) derived from isolated retinal astrocyte secretions influence the survival of RGCs challenged by acute injury (kainic acid injection to the eye) and by chronic glaucomatous insult (elevated IOP via circumlimbal suture). Lipoxins are members of a superfamily of small polyunsaturated fatty acid-derived metabolites with known anti-inflammatory properties.

The investigators found that LXA_4 and LXB_4 delivered via intravitreal injection or via a combination of intraperitoneal and topical application provided structural and functional neuroprotection to RGCs in their injury models.

DISCUSSION

Are astrocytes important mediators of neuronal survival?

In the canonical view, astrocyte signals in eye disease have a proinflammatory, proapoptotic, late role in pathogenesis known collectively as *reactive gliosis*. By maintaining glutamate homeostasis, supplying antioxidants, and providing metabolic support for neuronal signaling between the retina and brain, however, astrocytes support normal axonal function. A high degree of astrocyte remodeling prior to frank degeneration in glaucoma suggests that the cells play a more complex role in pathogenesis, one likely involving modulation of these key physiologic elements to preserve axonal function for as long as possible.

As Livne-Bar and colleagues noted, neurodegeneration involves a combination of the induction of neuroinflammatory signals and a concordant loss of homeostatic prosurvival cues. Importantly, the investigators demonstrated diminished LXA_4 and LXB_4 activity in the retina in response to injury. Because treatment improves RGC survival, this finding indicates an important role for astrocyte-derived lipoxin signaling in normal function.

What are the implications for clinical care?

Livne-Bar and colleagues emphasized that dysregulation of lipid mediators is implicated in a variety of age-related diseases, including Alzheimer disease, stroke, and age-related macular degeneration. Their study presented the first evidence, however, that astrocyte-derived lipoxins have a directly neuroprotective role. Furthermore, the researchers proposed a plausible mechanism of action involving modulation of signaling via immune-derived tumor necrosis factor α, a proinflammatory cytokine implicated in neurodegenerative injury. Although there is general consensus that astrocytes in the

STUDY IN BRIEF

- Investigators studied how small lipid mediators derived from isolated retinal astrocyte secretions influence the survival of retinal ganglion cells challenged by acute injury and by chronic glaucomatous insult. They found that lipoxins delivered via intravitreal injection or via a combination of intraperitoneal and topical application provided structural and functional neuroprotection to retinal ganglion cells in injury models.

WHY IT MATTERS

An important goal in glaucoma research is the development of therapeutic strategies to abate vision loss independent of IOP. This study offers the first evidence that astrocyte-derived lipoxins have a directly neuroprotective role.

optic nerve head influence the early axonopathy that characterizes glaucoma, this study suggested that retinal astrocytes may be equally important in regulating RGC survival. If that idea proves to be accurate, the pursuit of a topical treatment that protects RGCs independent of IOP may become both mechanistically and clinically feasible. ■

1. Fischer D, Harvey AR, Pernet V, et al. Optic nerve regeneration in mammals: regenerated or spared axons? *Exp Neurol*. 2017;296:83-88.
2. Livne-Bar I, Wei J, Liu HH, et al. Astrocyte-derived lipoxins A_4 and B_4 promote neuroprotection from acute and chronic injury. *J Clin Invest*. 2017;127(12):4403-4414.

JAMES C. TSAI, MD, MBA | SECTION EDITOR
- President, New York Eye and Ear Infirmary of Mount Sinai, and Chair of Ophthalmology, Mount Sinai Health System, New York, New York
- Member, *Glaucoma Today* Editorial Board
- jtsai@nyee.edu

DAVID J. CALKINS, PhD
- The Denis M. O'Day Professor of Ophthalmology and Visual Sciences; Vice-Chairman and Director for Research, The Vanderbilt Eye Institute; and Director, Vanderbilt Vision Research Center, Vanderbilt University Medical Center, Nashville, Tennessee
- david.j.calkins@vanderbilt.edu
- Financial disclosure: None

MELISSA L. COOPER, BS
- PhD Candidate, Neurosciences Graduate Program, The Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, Tennessee
- Financial disclosure: None