Glaucoma, a leading cause of irreversible blindness, is characterized by loss of retinal ganglion cells (RGCs). In glaucoma, RGCs are thought to sustain axonal injury at the glial lamina [1]. This injury triggers molecularly distinct cell death pathways governing degeneration of the RGC soma and the distal axon. Much work has elucidated the mechanisms controlling degenerative processes in both RGC compartments [2]. In ocular hypertensive DBA/2J mice and after acute mechanical RGC axonal injury (controlled optic nerve crush, CONC), the apoptotic molecule BAX was shown to be required for degeneration of the soma, but not distal Wallerian degeneration of the axon [3]. In contrast, manipulation of molecules important for axonal degeneration (e.g. expression of WldS) lessened death of the entire RGC in DBA/2J glaucoma [1]. Of note, after CONC (which allows independent analysis of the RGC somal and axonal compartments), WldS expression significantly delayed axonal degeneration but did not lessen RGC somal degeneration [4]—suggesting WLDLS’s activity is restricted to the RGC axon. Taken together, these data suggest axon-localized degenerative pathways ultimately drive degeneration of both RGC compartments in glaucoma. In contrast, there is evidence that effectors originating from the soma are important in initiating axonal degeneration after neurodegenerative injury [5], suggesting that the factor(s) governing both somal and axonal degeneration in glaucoma may be initially triggered in the soma. Elucidating the inciting mechanism(s) driving both somal and axonal degeneration after glaucoma-relevant injury will be important in the development of neuroprotective therapies.

Recently, it was shown that overexpression of BclXl protected the entire RGC in DBA/2J glaucoma [6]. BCLXl inhibits BAX induction and is the principal pro-survival family member of the Bcl2 gene family expressed in RGCs [7]. BclXl deletion significantly increases RGC death after CONC, suggesting BCLXl activity protects RGCs after glaucoma-relevant injury [8]. BCLXl was shown to localize to both somas and axons in dorsal root ganglion neurons [5]. Given this, it is possible that loss of BCLXl activity from the RGC soma, axon, or from both compartments, drives RGC degeneration after glaucoma-relevant injury. Locating BCLXl’s protective effect will aid in understanding the role of somal and axonal contributions to RGC degeneration in glaucoma. Here, we utilize CONC to investigate the protective effect of BclXl overexpression in the RGC soma and axon compartments independently.

To study the compartment-specific effects of BclXl overexpression after CONC, BclXl was overexpressed (BclXlAAV) in the retinas of C57BL/6J mice (aged 3–7 months) by bilateral intravitreal delivery of AAV2.2-Pkg-mCherry-BclXl vector, performed as previously described [6]. Control animals (WT) were bilaterally intravitreally injected with volume-matched PBS. Mice were randomly selected to receive intravitreal AAV2.2-Pkg-mCherry-BclXl or PBS. Mice were fed chow and water ad libitum and housed on a 12-hour light-to-dark cycle. All experiments were conducted in adherence with the Association for Research in Vision and Ophthalmology’s statement on the use of animals in opthalmic and vision research and were approved by the University of Rochester’s University Committee on Animal Resources. A priori exclusionary criteria included abnormal eye phenotypes (e.g. shrunken eye, cataracts, displaced pupil, lens damage). CONC (performed as previously described [9]) was done no earlier than 28 days after intravitreal injection to allow for sufficient transduction. To determine gross physiological function of RGC somas, pattern electroretinography (PERG) was performed using the Celeris Diagnosys system according to manufacturer’s instructions. To assess physiological function of RGC axons, compound action potentials (CAPs) were recorded as previously described [4, 9] with peak amplitudes measured at 37 °C. Immunohistochemistry and imaging for retinal flat mounts and optic nerve longitudinal sections were performed as previously described [9] using antibodies against RBPMS (Genetex, GTX118619, 1:250), RFP (Chromotek, 5f8-100, 1:1000), cCASP3 (R&D, AF835, 1:1000), and Neurofilament (Millipore, AB5539SP, 1:1000). RBPMS+ cell counts and soma size measurements were performed using Image J. In all cases, experimenters were masked to experimental group and condition. Experimental groups had roughly equal numbers of males and females, were sex- and age-matched, and littermates were used wherever possible. Power analyses were performed a priori to determine appropriate sample sizes. Data are reported as mean ± standard error of the mean, and in all cases, data sets being compared had similar variances and met the assumptions of each statistical test used.

To determine the compartment-specific effect of BclXl overexpression after mechanical axonal injury, CONC was performed on BclXlAAV and WT control mice. Of note, as assessed by the percentage of mCherry+ RBPMS+ cells, AAV2.2-Pkg-mCherry-BclXl transduced ~76% of RGCs (Fig. 1A), consistent with previously

**COMMENT**

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**BclXl (Bcl2I1) gene therapy lessens retinal ganglion cell soma loss but not axonal degeneration after acute axonal injury**

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published results [6]. Five days post-CONC, BclXL<sup>AAV</sup> retinas had significantly fewer dying (cCASP3+) RGCs (Fig. 1B), and 14 days post-CONC, had significantly improved RGC survival compared to WT controls (Fig. 1C). Therefore, consistent with previous reports [6, 10], BclXL overexpression improved RGC somal survival after axonal injury. These data suggest loss of BCLXL activity in the soma contributes to RGC somal degeneration in glaucoma and could also possibly contribute to degeneration of the axonal compartment.

Strikingly, despite improved somal survival in BclXL<sup>AAV</sup> retinas, surviving BclXL<sup>AAV</sup> RGC somas were significantly shrunken 14 days post-CONC as compared to WT (Fig. 1D). This finding suggests that BCLXL overexpression may promote somal survival without adequately restoring axonal function, potentially contributing to the persistent neurodegeneration observed in glaucoma.

**Figure 1:**
- **A:** Immunofluorescence images showing AAV-mediated expression of mCherry and RBPMS in BclXL<sup>AAV</sup> retina (top) and quantification of cells/mm<sup>2</sup> (bottom) with a significant increase in cCASP3+ cells in the WT compared to BclXL<sup>AAV</sup> retinas 5 days post-CONC.
- **B:** Quantification of cCASP3+ cells/mm<sup>2</sup> 5 days post-CONC showing a significant decrease in WT compared to BclXL<sup>AAV</sup> retinas.
- **C:** Immunofluorescence images of WT and BclXL<sup>AAV</sup> retinas 14 days post-CONC comparing sham and CONC conditions.
- **D:** Graph showing RGC soma size (mm<sup>2</sup>) with a significant decrease in WT compared to BclXL<sup>AAV</sup> retinas.
- **E:** Graph showing % PERG amplitude with a significant decrease in WT compared to BclXL<sup>AAV</sup> retinas.
- **F:** Immunofluorescence images of sham and CONC conditions in WT and BclXL<sup>AAV</sup> retinas.
- **G:** Graph showing CAP amplitude (mV) with a significant decrease in WT compared to BclXL<sup>AAV</sup> retinas.
post-CONC compared to Sham controls (Fig. 1D), suggesting injury or metabolic stress [11, 12]. This somal shrinkage was also observed in Bax deficient RGCs after CONC [13]. In addition, BclX<sub>L</sub> overexpression was not sufficient to prevent a decrease in PERG amplitude (which is thought to be reflective of RGC activity [14]) 14 days after CONC (Fig. 1E). Thus, while BclX<sub>L</sub> overexpression improved RGC soma survival after CONC, RGC somas did not appear to retain normal function. These data imply the separable nature of the mechanisms governing RGC somal survival and retention of physiological function.

Given that BclX<sub>L</sub> overexpression protected RGC axons and somas in a model of ocular hypertension [6], it remained important to distinguish whether somal BCLXL confers protection to the RGC axon, or if axonal BCLXL affords this protection. To investigate this, axonal degeneration of BclX<sub>L</sub><sup>AAV</sup> and WT optic nerves was assessed after CONC. Of note, the BCLXL fusion protein (mCherry) prominently co-localized to RGC axons in the optic nerve (Fig. 1F), as was shown previously [6]. Axonal health was assessed histologically (labeling for neurofilament-H) and electrophysiologically by measuring CAPs. BclX<sub>L</sub> overexpression did not lessen histological hallmarks of RGC axonal degeneration (Fig. 1G), nor prevent CAP amplitude decline after CONC (Fig. 1H). Thus, BclX<sub>L</sub> overexpression did not appear to elicit proteolytic effects by acting in the RGC axon after glaucoma-relevant injury. Taken together, these data suggest that the detrimental effect of BCLXL loss may be localized to the soma in the context of glaucomatous injury. This implicates the importance of degenerative mechanisms initiated in the RGC soma in ultimately driving the loss of the entire RGC. Future work should elucidate the mechanisms by which loss of somal BCLXL activity initiates axonal degenerative activity to further uncover the earliest drivers of glaucomatous neurodegeneration.

DATA AVAILABILITY

The datasets used in the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

OJM, RWN, and RTL designed the experiments. OJM, SERY, and PGS performed the experiments and analyzed the data. OJM prepared the figure and wrote the manuscript. OJM, SERY, RWN, PGS, and RTL reviewed and edited the manuscript. All authors read and approved the final version.
COMPETING INTERESTS
The authors declare no competing interests.

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