Effects of Chronic and Acute Intraocular Pressure Elevation on Scotopic and Photopic Contrast Sensitivity in Mice

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Glaucoma is a leading cause of blindness worldwide and a major public health concern.1,2 The disease is characterized by loss of the output cells of the retina, the retinal ganglion cells (RGCs), which leads to worsening of vision over time. At this time, the only conclusively identified modifiable risk factor for glaucoma is intraocular pressure (IOP), and essentially all this time, the only conclusively identified modifiable risk factor for glaucoma is intraocular pressure (IOP), and essentially all pharmacologic, laser, and surgical—treatments for glaucoma—pharmacologic, laser, and surgical—are based on the reduction of IOP.3,4 While IOP-lowering pharmacologic, laser, and surgical—medications are the mainstay of therapy, there have been only a small number of prospective studies to demonstrate that these medications are the mainstay of therapy, there have been only a small number of prospective studies to demonstrate that these drugs preserve visual function in glaucoma patients. Furthermore, several recently developed agents have gained acceptance into clinical practice based on the assumption that the reduction of IOP by any method leads to the preservation of vision. However, one recent randomized controlled trial called this assumption into question, showing that while two common IOP-lowering medications were equally effective at IOP lowering, their impact on visual preservation was quite different.5 These results hint at a more complex relationship between IOP and visual function than previously assumed.

Other clinical data suggest that contrast sensitivity may be among the earliest aspects of visual function to be impacted in patients with glaucoma.6,7 In addition, scotopic (dark, or rod-based) visual function has been shown to be disrupted in clinical and experimental settings. First, patients with glaucoma are known to have poor vision in dark conditions and during transitions between dark and light conditions.8–11 Second, evidence from animal models of glaucoma suggests that scotopic visual function, on both the retinal and cellular level, is rapidly impacted by elevations in IOP.12–15 Since the scotopic and photopic (light, or cone-based) visual pathways incorporate distinct retinal circuitry, it is possible that IOP elevation may affect scotopic and photopic contrast sensitivity differently, and that loss of scotopic contrast sensitivity might be an early indication of disease.

In this manuscript, we report the first direct comparisons between scotopic and photopic visual function under conditions of experimental glaucoma in mice. To do so, we assessed contrast sensitivity using optokinetic reflexes (OKRs) under both lighting conditions in animals exposed to elevated IOP induced by one of two experimental glaucoma models. To generate mild, chronic increases in IOP, we used a variation of the well-established “microbead occlusion” model, which involves the injection of micron-range diameter beads into the
Contrast Sensitivity and IOP

weekly for four cycles. For subsequent IOP elevations, the injected eyes. The procedure was repeated on the same eye
ensure an elevation to at least 35 mm Hg. After the procedure,
injected to fill the anterior chamber and the IOP measured to
Sodium hyaluronate was
pette attached to a tube of sodium hyaluronate was inserted
30-gauge needle, and a 75-
midperipheral cornea of one eye was punctured with a fresh
anesthesia was provided with a drop of 0.5% proparacaine. The
additional topical
injected to fill the anterior chamber and the IOP measured to

sensitivity, is preferentially impacted by IOP elevation. These
comparisons highlight potential changes to scotopic-specific
sensitivity, rather than photopic contrast
sensitivity, that was administered through a nose cone. Additional topical anesthesia was
provided with a drop of 0.5% proparacaine. Anterior chamber
injection of polystyrene beads into the dilated eye was
described previously.12,13 Following bead injection, a drop of 0.5% moxifloxacin was placed on the cornea.
The other eye was not injected and served as an intra-animal
control. Mice that were treated with IOP-lowering medications
received a single drop of either brimonidine tartrate 0.1%
(Alphagan P; Allergan, Inc., Dublin, Ireland) or brinzolamide 1%
(Azopt; Alcon, Fort Worth, TX, USA) once per day for the
duration of the experiment, including weekends. This drop was administered within a strict time window: between 10 AM
and 2 PM.

For the acute elevation of IOP, we performed an injection of
highly cohesive sodium hyaluronate (Healon 5, Abbott Medical
Optics, Abbott Park, IL, USA) into the anterior chamber of one
eye, while the other eye served as an un.injected, intra-animal
control. Animals were anesthetized with inhaled isoflurane
acclimated for 1 week
MATERIALS AND METHODS
Animals
We purchased 5-week-old female C57BL/6 mice from Jackson
Laboratories (Bar Harbor, ME, USA), acclimated for 1 week
prior to experimentation. All animals were treated in
accordance with National Institutes of Health (NIH) guidelines,
the ARVO Statement for the Use of Animals in Ophthalmic and
Vision Research, and the Baylor College of Medicine Institutional
Animal Care and Use Committee welfare guidelines.

Intraocular Pressure Elevation
To achieve a chronic elevation of IOP, mice were first
anesthetized with a weight-based intraperitoneal injection of
ketamine, xylazine, and acepromazine. One eye was dilated
with 1% tropicamide. Additional topical anesthesia was
provided with a drop of 0.5% proparacaine. Anterior chamber
injection of polystyrene beads into the dilated eye was
described previously.12,13 Following bead injection, a drop of 0.5% moxifloxacin was placed on the cornea.
The other eye was not injected and served as an intra-animal
control. Mice that were treated with IOP-lowering medications
received a single drop of either brimonidine tartrate 0.1%
(Alphagan P; Allergan, Inc., Dublin, Ireland) or brinzolamide 1%
(Azopt; Alcon, Fort Worth, TX, USA) once per day for the
duration of the experiment, including weekends. This drop was administered within a strict time window: between 10 AM
and 2 PM.

For the acute elevation of IOP, we performed an injection of
globules of hot water into the anterior chamber to occlude the trabecular meshwork and
cause a secondary elevation of IOP.12,16–18 Furthermore, we
applied IOP-lowering drops with distinct biological mechanisms in
conjunction with this model to separate out the
effects of RGC loss from RGC dysfunction. To generate high,
acute increases in IOP, we used a new model of unilateral sodium hyaluronate injection. With both models, we found that
scotopic contrast sensitivity, rather than photopic contrast
sensitivity, is preferentially impacted by IOP elevation. These
comparisons highlight potential changes to scotopic-specific
retinal circuitry caused by IOP elevation, and suggest a possible
role for scotopic contrast sensitivity in glaucoma diagnosis.

Measurement of OKRs
Animals were dark-adapted for at least 2 hours prior to testing.
Baseline photopic and scotopic contrast sensitivities were
tested prior to initial injection via either method with an
established OKR-based technique.20 Scotopic contrast sensitivities were determined at least 1 hour prior to photopic testing.
We repeated OKR experiments according to a precise schedule, depending on the mechanism of IOP elevation. For
chronic IOP elevation, contrast sensitivity was measured every 2 weeks (postinjection weeks 2, 4, and 6). For acute IOP
elevation, contrast sensitivity was measured immediately after
dark adaptation following sodium hyaluronate injection, and
then again on postinjection days 1 and 6 (which is the same as the
day prior to injection for the subsequent week of the cycle).

We tested OKRs using a custom-built apparatus.20 Mice were placed on an elevated platform inside a box of four
camera prevented the observer from seeing the stimulus
presented on the screens. The contrasts of the presented
gratings were controlled by a custom protocol written in
MATLAB. The protocol was a slope-constrained variant of the $\psi$
method, a Bayesian adaptive approach for estimating the
mouse’s psychometric function for contrast detection.27 The
contrast of the subsequent trial is chosen to maximize its
expected information by using a 1-step-ahead search. The
threshold was determined to be the contrast that evoked
correct responses of the animal half of the time. Contrast
sensitivity was then defined as the inverse of percent contrast
treshold. All experiments started with a prior that was based on
extended previous testing of C57BL/6 mice.20 The mean
photopic light intensity was 0.87 log10 cd/m2 (1.93 log10
photometric /rod). Light intensity was attenuated to the
scotopic range by placing neutral density filters in front of the
screens, thereby decreasing mean light intensity to ~2.3 log10
cd/m2 (~1.08 log10 photoshimations/rod).20 Relative intensities ranged approximately 2 log units from peak to peak at
highest contrast.

Experiments consisted of 100 stimuli, randomly drawn from
a pool of 50 leftward and 50 rightward moving gradients. Mice
respond only to stimuli moving in the temporal to nasal
direction, and following each stimulus, the trained observer—
who was masked to the direction of the stimulus—chose the
expected direction of the stimulus based on the mouse’s head
movement according to a two-alternative forced choice
paradigm.20,23,28 During the entire experiment, the observer
was also masked to which eye was exposed to IOP elevation to
minimize bias.

Immunohistochemistry and Cell Counting
Retinas were dissected, whole mounted, and fixed as previously described.20 Retinas were incubated in primary
antibody against the RGC-specific marker, class III beta-tubulin (TUJ1, 1:500; Covance, Princeton, NJ, USA).29 After washing,
secondary antibody (donkey-mouse 488, 1:300) and a fluores-
cent nuclear dye (TO-PRO3, 1:1000; Molecular Probes, Eugene,
Contrast Sensitivity and IOP

OR, USA) were added for counterstaining. Retinas were then washed and mounted in medium (Vectashield; Vector Laboratories, Burlingame, CA, USA). Retinas were imaged with a laser confocal microscope (LSM 510; Leica Microsystems, Wetzlar, Germany) and images were processed with commercial software (Zeiss LSM-PC; Carl Zeiss Microscopy, Jena, Germany). Retinal images for cell counting were obtained as previously described. Tubulin-positive marked cells were manually counted by a single masked observer, assisted by ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). These numbers were used to convert the cell counts into cells/mm². A second masked observer recounted several regions to verify the results.

Statistical Analysis

All analysis was performed using statistical software (SPSS Statistics Version 21; IBM Corp., Armonk, NY, USA). Values in text and figures are presented as averages ± SEM and a P-value < 0.05 was considered as statistically significant. We used ANOVAs to compare the difference between different groups and repeated measured ANOVAs to compare between groups at different times. When there was a significant effect on the group level, additional Bonferroni post hoc analyses were performed to detect pairwise differences between groups. Cumulative IOP difference was calculated as the sum of the differences in IOP between uninjected and injected eyes of the same animal. Similarly, log contrast sensitivity loss was calculated as the difference between uninjected and injected eyes of the same animal.

Results

The measurement of optokinetic responses (OKRs) can be used in mice to accurately detect changes in visual function that occur following various experimental interventions, including IOP elevation. Since contrast sensitivity may be disrupted in patients with early stage glaucoma, we chose to initially study contrast sensitivity in mice in which IOP was chronically elevated to mild levels (uninjected eyes: 10.1 ± 0.3 mm Hg; injected eyes: 12.1 ± 1.2 mm Hg). In wild-type mice, OKRs occur in response to a wide range of spatiotemporal information and exhibit high sensitivity to moderate values, but low sensitivity to extreme highs or lows, with responses degrading gradually as these extremes are approached.

To determine whether contrast sensitivity was lost uniformly across the spatiotemporal frequency spectrum, four mice with elevated IOP were tested over a wide range of spatiotemporal frequencies. Following 6 weeks of IOP elevation, we found that the expected band-pass pattern of contrast sensitivity was present (lowest at minimal and maximal spatial and temporal frequencies, highest in between), and that contrast sensitivity was consistently reduced at all tested spatial and temporal frequencies under both photopic and scotopic conditions (Figs. 1A–D).

However, the patterns of contrast sensitivity reduction differed with lighting condition. Under photopic conditions, neither the spatial (P = 0.51) nor temporal (P = 0.07) frequency impacted the reduction in contrast sensitivity (Figs. 1E–F, blue lines), whereas under scotopic conditions, the reduction in contrast sensitivity was highest at peak spatial and temporal frequencies (P < 0.001 for both; Figs. 1E–F, red lines). Thus, we found that contrast sensitivity was not only highest at peak spatiotemporal frequencies, but might also be most affected at these frequencies. We therefore tested animals at peak spatiotemporal frequencies (spatial frequency = 0.08 c/y/deg; temporal frequency = 2 cyc/sec) for all additional experiments.

While it is well established that anterior chamber bead injections result in elevation of IOP in mice, few studies confirm that IOP-lowering medications work to lower IOP and preserve anatomy after bead injection. Furthermore, no studies have assessed visual function after lowering the IOP of bead-injected eyes. Since daily administration of both brimonidine and brinzolamide has been confirmed to reduce IOP in a similar bead injection model, we first confirmed their abilities to lower IOP in our model. Both agents blunted the cumulative IOP increase seen from bead injection, but neither eliminated it. Over the course of the 6-week study, the mean daily IOP percentage increases (<SEM) in eyes that received only bead injection, bead injection + daily brimonidine, and bead injection + daily brinzolamide were 26.9% ± 2.3%, 21.9% ± 5.0%, and 20.0% ± 2.6%, respectively. Viewed as a cumulative IOP difference over time, this effect was statistically significant only for brinzolamide (Fig. 2A).

To determine if the reduction in IOP caused by brimonidine and brinzolamide also preserved visual function, we compared the OKRs at peak spatiotemporal frequencies of bead-injected eyes to those of bead-injected eyes treated daily with either IOP-lowering agent. At each time point (baseline and 2, 4, and 6 weeks post injection), we calculated the intra-animal difference in contrast sensitivity between the treated and untreated eye to determine the contrast sensitivity difference (Figs. 2B, 2C). Bead-injected eyes showed marked contrast sensitivity loss under both photopic and scotopic conditions by 2 weeks after injection, which remained constant through the rest of the experiment (P < 0.001). Interestingly, both brinzolamide and brimonidine mitigated this contrast sensitivity loss, yet did so in statistically distinct manners. Under photopic conditions, brimonidine, but not brinzolamide, caused an attenuation of contrast sensitivity loss that was statistically significant (ANOVA with repeated measures, Bonferroni post hoc: brinzolamide, P = 0.159; brimonidine, P < 0.001). Under scotopic conditions, however, both brinzolamide and brimonidine protected against contrast sensitivity loss (ANOVA with repeated measures, Bonferroni post hoc: brinzolamide, P = 0.002; brimonidine, P < 0.001). Under both photopic and scotopic conditions, there was minimal effect of brinzolamide at the earliest time point (2 weeks), but moderate and similar effectiveness at later time points (4 and 6 weeks). Taken together, these data suggest that both brinzolamide and brimonidine can preserve vision in vivo, and that the contrast sensitivity loss seen following IOP elevation via bead injection, but the pattern of this preservation is different and may occur via distinct mechanisms.

Next, we sought to compare the anatomic impact of IOP reduction by brinzolamide and brimonidine after bead injection. Thus, at the end of the 6-week study period, after all OKR measurements, we assessed the number of RGCs present in retinal flat mounts with a well-established antibody to class III beta-tubulin, which is thought to label all RGCs (Fig. 3). Interestingly, we found that bead-injected eyes, and bead-injected eyes treated with brinzolamide, showed a reduction in RGC count from uninjected control eyes (control, 4767 ± 187 RGCs/mm² [mean ± SEM]; beads, 4138 ± 232 RGCs/mm²; beads + brinzolamide, 4105 ± 131 RGCs/mm²; P < 0.05 for beads and beads + brinzolamide when compared with control). Conversely, no reduction in RGC count was seen in bead-injected eyes treated with brimonidine, suggesting that daily brimonidine treatment prevented IOP-mediated RGC loss, whereas brinzolamide did not (beads + brimonidine, 4547 ± 160 RGCs/mm²; P > 0.05 when compared with control). Interestingly, as brimonidine was less effective at lowering IOP in our model, we found that contrast sensitivity was not only highest at peak spatial and temporal frequencies, but might also be most affected at these frequencies. We therefore tested animals at peak spatiotemporal frequencies (spatial frequency = 0.08 c/y/deg; temporal frequency = 2 cyc/sec) for all additional experiments.
than brinzolamide, this suggests that RGCs may be preserved in these eyes by an IOP-independent mechanism.

We then tried to further explain the relationship between IOP, RGC count, and contrast sensitivity difference. To do so, we calculated the mean photopic and scotopic contrast sensitivity for all control (uninjected) eyes and plotted these values against either the average IOP (average of the 12 time points post injection) or the RGC count. We then determined whether these relationships were changed by the use of drops by populating the correlation with all control eyes and the respective data points from each group. We found very different results for scotopic and photopic contrast sensitivity. Under scotopic conditions, there was a consistent and strong correlation with IOP, as well as a consistent absence of a relationship with RGC count, regardless of treatment group.

These findings suggest that scotopic contrast sensitivity is impacted most prominently by IOP level and not by RGC count. Under photopic conditions, however, correlations were present for both IOP and RGC count, but only for bead-injected eyes and bead-injected eyes treated with brinzolamide. These data suggest a more complex relationship among IOP, RGC count, and contrast sensitivity under photopic conditions that is altered by the use of brimonidine but not brinzolamide (Fig. 4; Table).

Finally, to further explore our observation that IOP level had a stronger effect on scotopic than photopic contrast sensitivity, we developed an alternative model of IOP elevation based on anterior chamber injection of highly cohesive sodium hyaluronate to acutely increase IOP. With this model, we were able to directly test the hypothesis that acute increases in IOP preferentially impact scotopic contrast sensitivity, because...
Figure 2. Cumulative IOP difference and peak contrast sensitivity loss after bead injection. (A) Cumulative IOP difference of bead-injected eyes was increased across the entire range of the study (beads, green) and was attenuated by both brinzolamide (orange) and brimonidine (purple). A post hoc analysis revealed that only brinzolamide-treated animals had a statistically significant decrease in cumulative IOP difference compared with animals that did not receive IOP-lowering drops (1-way ANOVA, Bonferroni post hoc; \( P = 0.041 \); bracket with \#). While brimonidine treatment trended toward lowering IOP, this effect was not significant compared with animals that did not receive IOP-lowering drops (Bonferroni post hoc; \( P = 0.184 \); \( n = 17 \) for beads only, 9 for brinzolamide, and 12 for brimonidine). (B, C) Contrast sensitivity loss was observed under both photopic (B) and scotopic (C) conditions in animals that received a unilateral bead injection but not IOP-lowering eye drops (green). While brinzolamide treatment (orange) attenuated this contrast sensitivity loss in both lighting conditions, it only resulted in a significant difference compared with animals that did not receive treatment in scotopic conditions (ANOVA, Bonferroni post hoc: photopic, \( P = 0.159 \); scotopic, \( P = 0.002 \); bracket with \#). Brimonidine (purple) attenuated the contrast sensitivity loss even further and resulted in contrast sensitivity loss that was statistically smaller than the loss observed in bead-treated animals in both light intensities (ANOVA, Bonferroni post hoc: \( P < 0.001 \) for both photopic and scotopic; brackets with \^; none, \( n = 7 \); brinzolamide, \( n = 9 \); brimonidine, \( n = 7 \)). Mean ± SEM is shown for all panels.
FIGURE 3. Retinal ganglion cell numbers after bead injection. (A) Box-and-whisker plots showing RGC number in flatmounted retinas. Eyes injected with beads had a statistically significant smaller number of RGCs compared with control (uninjected) eyes. * Bonferroni post hoc: $P = 0.045$ (green; $n = 7$ eyes). A similar loss of RGCs was observed in eyes injected with beads that received treatment with brinzolamide. * Bonferroni post hoc: $P = 0.031$ (orange; $n = 6$ eyes), but was not observed in injected eyes were treated with brimonidine (Bonferroni post hoc: $P = 1$; purple; $n = 7$ eyes). All post hoc analyses were performed where injected eyes were compared with RGC number in uninjected control eyes (black, $n = 13$ eyes). (B–E) Representative retinal flatmount images of class III beta-tubulin staining (green).
IOP became elevated shortly after injection and then rapidly recovered to and remained at baseline pressures (Fig. 5A). We measured contrast sensitivity prior to, immediately after, and 1 and 6 days after IOP elevation to determine acute and prolonged effects, and repeated this cycle of IOP elevation and contrast sensitivity measurement four times to determine any additive effects. We found that both photopic and scotopic contrast sensitivity were diminished immediately after IOP elevation (testing 2 hours after injection, 3-way ANOVA [day of experiment × week of repetition × lighting condition]: effect of day on contrast sensitivity: \( P < 0.001 \), Bonferroni post hoc, injection day is different from preinjection day and postinjection day: \( P < 0.001 \) and \( P = 0.001 \), respectively), but that the reduction in contrast sensitivity was more profound and more temporally linked with acute IOP elevation under scotopic than photopic conditions (Figs. 5B, 5C). Furthermore, scotopic contrast sensitivity was found to be reduced significantly more over a longer period of follow-up time than photopic contrast sensitivity, even when treated with brimonidine (\( R^2 \) values, slopes, and \( P \) values are reported in the Table).

**TABLE.** Correlation Coefficients, Slopes, and \( P \) Values

<table>
<thead>
<tr>
<th>Correlated Factors</th>
<th>Beads</th>
<th>Beads + Brinzolamide</th>
<th>Beads + Brimonidine</th>
</tr>
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<tbody>
<tr>
<td>IOP: photopic difference</td>
<td>0.23</td>
<td>−0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IOP: scotopic difference</td>
<td>0.25</td>
<td>−0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RGCs: photopic difference</td>
<td>0.33</td>
<td>0.00054</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>RGCs: scotopic difference</td>
<td>0.04</td>
<td>0.00016</td>
<td>0.282</td>
</tr>
</tbody>
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Bold indicates statistically significant data.
FIGURE 5. Peak contrast sensitivity after sodium hyaluronate injection. Intraocular pressure in injected eyes was measured on the day before IOP elevation (pre), immediately after injection (injection), and on the day following elevation (1 day post) for four weekly cycles (weeks 1–4). Log contrast sensitivity of injected eyes at peak spatiotemporal frequencies was measured on the day before IOP elevation (pre), 2 hours after injection (injection), and on the day following elevation (1 day post) for four weekly cycles (weeks 1–4). The label "6 days post" for each week is the same measurement as "pre" for the following week. (A) Mean IOP; IOP was only elevated immediately after sodium hyaluronate injection (asterisk). (B) Mean log scotopic contrast sensitivity. Scotopic contrast sensitivity loss occurred uniformly at each week of treatment, with a single reduction on the day of injection. *P = 0.001, all weeks. (C) Mean log photopic contrast sensitivity. Photopic contrast sensitivity loss was less during the first week of treatment (week 1, black) than in the following weeks (all other colors; repeated measures ANOVA, P = 0.02) and did not follow a specific pattern within the weeklong injection cycle (n = 8 for all panels). Mean ± SEM is shown for all panels.
contrast sensitivity also recovered rapidly to baseline levels once IOP normalized, whereas photopic contrast sensitivity did not (interaction between day of experiment × lighting condition: P = 0.004). These repeated, acute increases in IOP did not affect postmortem RGC counts (class III beta-tubulin positive cells: 4604 ± 72 cells/mm² for injected eyes versus 4644 ± 59 cells/mm² for control eyes), suggesting that scotopic contrast sensitivity loss occurs independently of RGC number. These data support our findings following chronic IOP elevation, namely that scotopic visual impairment may be directly affected by IOP level and is independent of RGC number, and that photopic visual impairment may be mediated by a more complex relationship among several factors.

**DISCUSSION**

In this study, we used a modified version of an established OKR-based technique to estimate both photopic and scotopic contrast sensitivity in mice after IOP elevation. Optokinetic reflex–based testing has been used previously to distinguish photopic and scotopic phenotypes. However, the use of OKR-based testing in animal models of glaucoma has been limited to chronic models and has focused exclusively on RGC-based testing in animal models of glaucoma has been limited to chronic models and has focused exclusively on RGC-based testing in animal models of glaucoma. These data support our findings following chronic IOP elevation, namely that scotopic visual impairment may be directly affected by IOP level and is independent of RGC number, and that photopic visual impairment may be mediated by a more complex relationship among several factors.

**FIGURE 6.** Summary of experimental data.

<table>
<thead>
<tr>
<th></th>
<th>RGC</th>
<th>Scotopic CS</th>
<th>Photopic CS</th>
</tr>
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<tbody>
<tr>
<td>Chronic IOP</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>+ Brimonidine</td>
<td>─</td>
<td>↓</td>
<td>─</td>
</tr>
<tr>
<td>+ Brinzolamide</td>
<td>↓</td>
<td>(rapid)</td>
<td>(delayed)</td>
</tr>
<tr>
<td>Acute IOP</td>
<td>─</td>
<td>↓</td>
<td>↓</td>
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</tbody>
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We also found that brinzolamide and brimonidine are both able to lower IOP, but only brimonidine prevented IOP-induced RGC loss. This discrepancy suggests that brimonidine has an additional, IOP-independent, and possibly neuroprotective mechanism, which has been postulated in both animal and clinical studies. This preservation of RGCs in mice treated with brimonidine allowed us to determine that scotopic contrast sensitivity changed in response to IOP level itself, whereas photopic contrast sensitivity changed as a consequence of IOP and additional factors, such as RGC count. These findings were supported by measurements made after acute IOP elevation, in which we detected a severe reduction of scotopic contrast sensitivity that recovered as IOP normalized. Since scotopic contrast sensitivity relies on a complex relationship of RGCs and various retinal interneurons, in particular All amacrine cells (AIIACs), these findings suggest that IOP may have preferential effects on specific connections in the retina, such as those mediated by amacrine cells that are critical for rod pathway–mediated visual function. Previous work with single cell electrophysiology and transgenic animals in models of experimental glaucoma have already implicated AIIACs as a critically affected cell type, and our data strongly support this relationship, which appears to exist at both chemical synapses and connexin-mediated gap junctions. This is also consistent with a recent ERG study in rats that found that the scotopic ERG was more sensitive to IOP elevation than the photopic ERG, as well as additional ERG studies in rodents that detected abnormal scotopic responses in the setting of elevated IOP. Another possible explanation is that direction-selective retinal ganglion cells (DSGCs), which are critical for the contrast-dependent OKR and can be impacted by IOP elevation, display variable susceptibility to IOP elevations depending on their adaptive state. That is, DSGCs function relatively normally under conditions of high IOP when light-adapted, but poorly when dark-adapted. Finally, there is evidence for RGC subtype-specific IOP related phenotypes, as well as indirect evidence that RGC light sensitivity can change according to adaptive state. These subtype differences include differential impacts of elevated IOP on RGC dendrite structure, this could be mediated by a loss of specific intraretinal synaptic contacts. Future studies will be required to distinguish from among these possibilities.

**Acknowledgments**

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