Selecting Ganglion Cell Functional Loss in Rats with Experimental Glaucoma

Brad Fortune,1 Bang V. Bui,1 John C. Morrison,2 Elaine C. Johnson,2 Jin Dong,1 William O. Cepurna,2 Lijun Jia,2 Stacey Barber,2 and George A. Cioffi1

Purpose. To characterize retinal functional consequences of elevated intraocular pressure (IOP) in a rat model of experimental glaucoma.

Methods. Unilateral elevation of IOP was produced by hypertonic saline injection into an episcleral vein in 20 adult male Brown-Norway rats. IOP was measured in both eyes of awake animals four to five times per week. After 5 weeks, animals were dark adapted overnight (>12 hours) and full-field electroretinograms (ERGs) were obtained simultaneously from both eyes. Scotopic ERG stimuli were brief white flashes (−6.64–2.72 log cd·s/m²). Photopic responses were also obtained (0.97–2.72 log cd·s/m²) after 15 minutes of light adaptation (150 cd/m²). Eyes were processed the following day for histologic evaluation by light microscopy, including masked determination of optic nerve injury grade (ONIG; 1, normal; 5, severe, diffuse damage).

Results. Among experimental eyes, the group average IOP (±SD) was 34.5 ± 4.1 mm Hg, whereas the average for control eyes was 28.1 ± 0.5 mm Hg (t = 7.1, P < 0.0001). The average ONIG for experimental and control eye groups, respectively, was 3.4 ± 1.7 and 1.0 ± 0.02 (t = 6.3, P < 0.0001). The ONIG increased with mean IOP in experimental eyes (r² = 0.78, P < 0.0001) and was unrelated to mean IOP in control eyes (r² = 0.09, P = 0.18). In experimental eyes with relatively mild IOP elevation (mean IOP < 31 mm Hg) and no structural (histologic) damage to the optic nerve evident by light microscopy (the ONIG = 1.1 ± 0.2, n = 5), there was a selective reduction of the positive scotopic threshold response (pSTR; P < 0.001), whereas other ERG components remained unaltered. In four of the five eyes, pSTR amplitude was reduced by more than 50%, whereas all five had normal scotopic a-wave, b-wave, and OP amplitudes. Eyes with mean IOP of more than 35 mm Hg had reduced a-wave, b-wave, and oscillatory potential (OP) amplitudes.

Conclusions. As demonstrated by prior studies, selective loss of the pSTR is indicative of selective retinal ganglion cell (RGC) injury. In this rat model of experimental glaucoma, selective RGC functional injury occurred before the onset of structural damage, as assessed by light microscopy of optic nerve tissue. The highest IOP levels resulted in nonselective functional loss. Thus, in rodent models of experimental glaucoma, lower levels of chronically elevated IOP may be more relevant to human primary chronic glaucoma. (Invest Ophthalmol Vis Sci. 2004; 45:1854–1862) DOI:10.1167/iovs.03-1411

The use of rodents in experimental models of glaucoma and other diseases that affect the optic nerve and retinal ganglion cells (RGCs) has grown steadily over the past two decades for a recent review, see Goldblum and Mittag1). Generally, such studies rely on histologic assessment of RGC survival or optic nerve injury as the primary outcome measure, although, several laboratories have also used the electroretinogram (ERG) to monitor retinal function in vivo (WoldeMussie E, et al. IOVS 2003;44:ARVO E-Abstract 41).2–15 Functional outcome measures such as the ERG are attractive because they are not terminal and can thus be used longitudinally. Furthermore, they may provide important complementary information when meaningful discrepancies exist between anatomic and functional status.14,15

The ERG technique most typically used to study retinal function in rodent models is the dark-adapted (scotopic) full-field ERG. However, it has long been recognized that the full-field ERG is not particularly useful for assessment of G function.2 Rather, the pattern-ERG (PERG) has received extensive scrutiny and prevailed as one of the most useful techniques for this purpose.2,16,17 Although it has been used successfully in rodents,2,18 the PERG is technically much more difficult to record than the full-field ERG. Hence, it is of interest that careful investigations of the dark-adapted, full-field ERG have shown that the response to a very dim stimulus, near the scotopic threshold, is a reflection of inner retinal activity in cats,19–21 mice,22 rats,23,24 monkeys,25,26 and humans.27,28 Accordingly, this ERG response has been called the scotopic threshold response (STR).19 Recently, the STR of the rat has been shown to be directly dependent on intact RGC function,24 suggesting that it may be suitable for assessment of function in experimental models of glaucoma in the rat.

Previously, studies that have used the full-field ERG to monitor retinal function in rat experimental models of glaucoma have examined only the response to relatively bright stimulus flashes under scotopic or photopic (light-adapted) conditions.7,13,15 However, alterations in the rat ERG response to these brighter full-field flashes, particularly under scotopic conditions, are most likely to represent functional damage to retinal cells other than RGCs.24 Therefore, the purpose of the present study was to examine the full-field ERG response over a wide range of stimulus intensity, to include the STR, after 5 weeks of elevated intraocular pressure (IOP) in a rat model of experimental glaucoma. In particular, our goal was to compare functional changes across various levels of chronically elevated IOP and to determine what range might result in selective RGC functional loss.
Methods

Animals and Glaucoma Model

All experimental and animal care procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee (IACUC) at both the Legacy Health System and the Oregon Health Sciences University. Unilateral elevation of IOP was produced in 20 adult male Brown Norway rats (Rattus norvegicus, weights 300–400 g) as previously described.29 In brief, while under general anesthesia (1 mL/kg rat cocktail: 5:2.5:1 ketamine [100 mg/mL], xylazine [20 mg/mL], and acepromazine [10 mg/mL]), one eye of each animal was chosen randomly and injected with 50 μL of a 1.75 M hypertonic saline solution through the episcleral venous route. Four animals received a second injection 14 days after the first because the initial injection failed to elevate IOP. During the 5-week postinjection period, animals were provided with food and water ad libitum while housed in rooms that were maintained at 21°C with a low constant light level (40–90 lux) to stabilize circadian IOP oscillations.30,31 IOP was determined in both eyes of awake animals four to five times per week using a calibrated tonometer (TonoPen XL; Mentor, Norwell, MA) after 1 drop of proparacaine hydrochloride (0.5%; Alcon Laboratories, Inc., Fort Worth, TX) was applied to each eye.31,32 The IOP history of each eye was described by two measures, the mean IOP (area under the curve describing IOP versus days after injection divided by the number of days after injection) and the peak IOP (highest IOP measured during the postinjection period). This was collected for histologic analysis at 5 weeks after injection. One to 3 days before tissue collection, electrotetrognography was performed according to the following protocol.

Electroretinography

Animals were dark adapted overnight (>12 hours) and prepared for recording under dim red light (λ > 600 nm). Anesthesia was initially induced with an intramuscular injection of ketamine (55 mg/kg; Ketaset; Fort Dodge Animal Health, Fort Dodge, IA), xylazine (5 mg/kg; X-ject; Phoenix Scientific Inc., St. Joseph, MO) and acepromazine maleate (1 mg/kg; Aceproject; Phoenix Scientific, Inc.). Supplementation anesthesia was provided approximately 50 minutes after initial induction using a mixture of ketamine, xylazine, and acepromazine (30:2:1 mg/kg, intramuscularly). Pupils were dilated with 1 drop each of 0.5% tropicamide (Alcon Laboratories, Inc.) and phenylephrine (2.5%, Bausch and Lomb Pharmaceuticals, Inc., Tampa, FL). Corneal anesthesia was achieved with 1 drop of 0.5% proparacaine hydrochloride (Alcon Laboratories, Inc.). Animals were lightly secured to a stage with Velcro strips across the upper and lower back to ensure a stable, reproducible position for ERG recording. Body temperature was maintained between 37°C and 38°C with a pumped-water heating pad (TPS500 T/Pump; Gammex Industries, Orchard Park, NY) that was fixed to the top of the stage. The duration of the ERG recording session was 75 minutes for each animal, after which animals fully recovered from anesthesia while resting on a heated pad.

Full-field ERGs were recorded (UTAS-E3000; LKC Technologies, Gaithersburg, MD) in both eyes simultaneously with custom silver-chloride electrodes. The tip of the active electrode was placed at the corneal apex and was referenced to a ring-shaped electrode positioned against the scleral conjunctiva around the equator of the eye. Eyes were lubricated after electrode placement and periodically throughout the session with 1.0% carboxymethylcellulose sodium (Allergan, Irvine, CA). A platinum electrode (Grass-Telefactor, West Warwick, RI) placed in the tail served as the ground. Simultaneous recording from both eyes allowed the recording time and allowed ERGs to be obtained from the control and treated eyes under identical states of anesthesia and adaptation.

Stimuli were brief white flashes (xenon arc discharge, x = 0.32, y = 0.33) delivered through a Ganzfeld integrating sphere (UTAS-E3000; LKC Technologies). Stimulus intensities were measured with a calibrated photometer (Spectra Pritchard PR-1980B; Photo Research, Chatsworth, CA) with a (human) scotopic luminosity filter in place.

Selective Loss of STR in a Rat Glaucoma Model

STR responses were obtained for flash intensities ranging from −6.64 to −3.30 log cd·s/m² in 0.2-log-unit increments, by averaging 20 to 60 responses per intensity (60 for the dimmest and 20 for the higher intensities), with an interstimulus interval of 2 seconds. Scopotic ERGs obtained for all intensities above −3.50 log cd·s/m² were recorded as single flash responses. For stimulus intensities between −3.04 to 2.72 log cd·s/m² the interval between flashes was progressively lengthened from 10 to 120 seconds to allow complete recovery of b-wave amplitude. After completion of the scotopic ERG intensity series, animals were light-adapted for 15 minutes to a steady white background (150 cd/m², x = 0.44, y = 0.41). Photopic flash responses were recorded for intensities between 0.97 and 2.72 log cd·s/m² in 0.25-log-unit increments. Each record was an average of 20 responses obtained with a 2-second interstimulus interval. STR records were acquired at 1 kHz with high- and low-pass filters set to 0.3 and 50 Hz, respectively. All other ERG records were acquired at 2 kHz with high- and low-pass filters set to 0.3 and 500 Hz, respectively.

ERG Data Analysis

ERG component amplitudes were measured relative to prestimulus baseline at fixed times after the stimulus. Criterion times were chosen to correspond with the peak (120 ms) and trough (220 ms) of control responses to dim flashes. For responses to brighter flashes, an 8-ms criterion time was used to measure a-wave amplitude. The amplitude of the photopic b-wave was measured using a 50 ms criterion time. To measure the amplitudes of scotopic and photopic oscillatory potentials (OPs), raw data were first band-pass filtered (−5 dB at 50 and 280 Hz), then the root-mean-square (RMS) amplitude of the entire OP complex, beginning at the trough preceding the first OP and ending at the trough after the last OP, was summed.

Statistics

Analysis of variance (ANOVA; Prism, ver. 4.0; GraphPad Software, Inc., San Diego, CA) was applied to test the null hypotheses, which in general, could be stated as no difference between control and experimental eyes (i.e., no treatment effect). Two-way ANOVA (ERG amplitude vs. treatment and intensity) was used to evaluate the effect of experimental treatment for each ERG parameter. In all cases, the α level was adjusted to 0.01 to correct for multiple comparisons (i.e., to limit type 2 errors, given that seven ERG parameters were evaluated). One-way ANOVA (ERG amplitude versus treatment) was applied to the analysis presented in Figure 5. Bonferroni post hoc tests were used to evaluate differences between experimental groups by intensity. Nonparametric ANOVAs with Dunn post hoc tests were used to evaluate differences between experimental groups for optic nerve injury grade.

Histopathology

Optic Nerve Histology and Injury Grading. At 5 weeks after injection, animals were anesthetized with halothane and the tissues fixed by transcardial perfusion with 4% paraformaldehyde.35 Optic nerve segments taken 2 mm from the globe were postfixed with 5% glutaraldehyde, and cross sections were prepared for masked grading of optic nerve injury as previously described and validated.36 In brief, five masked observers graded each nerve on a scale of 1 (normal) to 5 (degenerating axons or glialosis filling the entire nerve cross-section). Grade 2 indicates a small, focal lesion with approximately 15% of axons degenerating, grade 3 a larger focal lesion occupying approximately 30% of the neural area with increased density of degenerating axons and grade 4 indicates that approximately half of the optic nerve axons are degenerating. The mean of the five individual observers was taken as the optic nerve injury grade (ONIG).

Optic Nerve Head and Retinal Histology. All eyes were postfixed in a 4% formaldehyde-parafomaldehyde solution after enucleation. Eyes were processed by paraffin embedding, and longitudinal sections (6 μm) were cut through the globe along the anterior-posterior axis. Thus, retinal sections were vertically oriented, containing both inferior and superior retina. Sections were deparaffinized and rehydrated, stained with 0.1% Mayer hematoxylin (Sigma-Aldrich, St.
RESULTS

A wide range of experimental injury was achieved in this group of 20 rats (Fig. 1). Figure 1A shows the IOP history for one animal. For the initial analyses of retinal function, eyes were grouped by degree of IOP elevation: group 1 consisted of all control eyes ($n = 20$); group 2, experimental eyes with mean IOP less than 31 mm Hg ($n = 5$); group 3, IOP 31 to 35 mm Hg ($n = 6$); group 4, IOP more than 35 mm Hg ($n = 9$). Table 1 provides summary data for these four groups. Figure 2 shows representative histologic findings for each of these groups. The left column (Fig. 2A) shows findings for a control eye. Data for the mean IOP in each of the remaining three groups are shown in Figures 2B through 2D. In the top row, portions of the optic nerve cross sections used for masked grading are shown along with the ONIGs for each of the four eyes, respectively. The middle row shows longitudinal (vertical) sections through the anterior optic nerve. The bottom row shows a portion of the same retinal cross section from a field centered 0.5 mm inferior to the optic nerve. Note that all images within a column are taken from the same eye.

By definition, optic nerve cross-sections from experimental eyes with an injury grade equal to 1 (normal) were indistinguishable from those of control eyes. The similarity between the optic nerve cross sections shown in Figures 2A and 2B. The summary data in Table 1 show that the average ONIG for the group of experimental eyes whose mean IOP was less than 31 mm Hg was not statistically different from that of the control group ($P > 0.05$, Dunn post hoc comparison test). Similarly, experimental eyes with an ONIG of 1 were indistinguishable from control eyes on the basis of either anterior optic nerve or retinal morphology (compare Figure 2A with 2B).

Figure 1. Intraocular pressure (IOP) history for one animal (A). Saline injection in the experimental eye occurred on day 0. Mean IOP (a weighted mean derived from the area under the curve) was 34.3 mm Hg in the experimental eye and 27.9 mm Hg in the control eye. Peak IOP versus mean IOP for the entire group (B). Optic nerve injury grade versus mean IOP for the entire group (C). Filled symbols: experimental eyes; open symbols: control eyes.
In contrast, the two groups with higher mean IOP developed significant morphologic changes in the optic nerve ($P < 0.0001$, Kruskal-Wallis ANOVA, see Table 1). Group 3 eyes had an average ONIG of 3.1 ($P < 0.01$ vs. control eyes), and group 4 eyes had an average ONIG of 4.8 ($P < 0.001$ vs. control eyes). As shown in Figures 2C and 2D (middle row), eyes with higher ONIGs also manifested morphologic changes in the anterior optic nerve. In group 3 eyes with mild-to-moderate focal optic nerve damage (ONIG $= 1.5–4.5$), neural degeneration, and gliosis were evident within the anterior optic nerve, as shown by disorganization of the normal columnar structure and increased glial cell density (Figure 2C, middle). Retinal changes in this group, however, were not striking (Figure 2C, bottom).

Experimental eyes in group 4 with the highest IOP and the most severe optic nerve injury also had the greatest damage evident within the anterior optic nerve and retina (Figure 2D). In this group, longitudinal sections through the anterior optic nerve revealed enlargement of the scleral canal and extensive gliosis. Retinal sections from most eyes in this group were thinner overall, with marked reduction of ganglion cell layer density. In some group 4 eyes, the inner nuclear layer appeared to be thinner and to contain fewer nuclei than control eyes.

Figure 3 shows ERG results for individual animals whose experimental eyes are representative for group 2 (column 3A), group 3 (column 3B), and group 4 (column 3C). Dark-adapted (scotopic) responses to increasing stimulus intensity are shown, beginning with the STR at the bottom of each column ($-6.04$ log cd-s/m$^2$, approximately 0.6 log units above the ERG threshold), through the middle of each column where responses to bright flashes are shown (up to $2.22$ log cd-s/m$^2$), and on to the top of each column where light-adapted (photopic) responses are shown. In all cases, the bold records represent the responses of experimental eyes, and the thin traces show control responses from the fellow control eye. The isolated OPs are also shown to the right of the corresponding raw waveforms.

Comparison of control responses (thin traces) across columns demonstrates good reliability between individuals for responses to all stimulus intensities. The ERGs for all control eyes were well within the range of previously published normative data collected under identical conditions.$^{24}$ In control eyes, the STR contained a positive component followed by a negative component, designated as the pSTR and nSTR, respectively. The amplitude of both STR components grew with increasing stimulus intensity. The amplitude of the nSTR reached saturation at approximately $-5$ log cd-s/m$^2$. Above this intensity, another less sensitive positive component began to influence the shape and timing of the waveform. Initially, this component merged with the pSTR, but at higher intensities the sum of positive potentials became indistinguishable from the classic scotopic b-wave. The a-wave threshold occurred at approximately $-2$ log cd-s/m$^2$, and its maximum amplitude (baseline to trough) was reached at $2.22$ log cd-s/m$^2$. Above this intensity, the slope of the leading edge became steeper. Scotopic OPs first appeared just below a-wave threshold and grew with intensity until saturation behavior became evident at approximately $0.0$ log cd-s/m$^2$. Scotopic OP implicit times decreased smoothly as flash intensity increased. The photopic ERG was dominated by the b-wave, which also displayed amplitude saturation. Photopic OPs were evident for all intensities investigated, but small a-waves were observed only in response to the brightest flashes on this relatively bright background. The average implicit time of the photopic OPs did not change with intensity.

The bold traces in Figure 3 show ERG data for one representative experimental eye from each of the three groups. The

**Table 1.** Summary Data for Mean IOP, Peak IOP and ONIG, by Experimental Group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean IOP (mm Hg)</th>
<th>Peak IOP (mm Hg)</th>
<th>ONIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control eyes)</td>
<td>20</td>
<td>28.1 ± 0.5</td>
<td>31.2 ± 2.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>2 (IOP &lt; 31 mm Hg)</td>
<td>5</td>
<td>29.3 ± 0.8</td>
<td>38.1 ± 2.5</td>
<td>1.1 ± 0.2*</td>
</tr>
<tr>
<td>3 (IOP 31 to 35 mm Hg)</td>
<td>6</td>
<td>53.0 ± 1.5</td>
<td>44.0 ± 2.3</td>
<td>3.1 ± 1.3†</td>
</tr>
<tr>
<td>4 (IOP &gt; 35 mm Hg)</td>
<td>9</td>
<td>38.4 ± 1.3</td>
<td>48.9 ± 1.5</td>
<td>4.8 ± 0.5‡</td>
</tr>
</tbody>
</table>

Probabilities are by the Kruskal-Wallis ANOVA with Dunn’s post hoc comparison test.

* $P > 0.05$, NS group 2 versus control.
† $P < 0.01$, group 3 versus control.
‡ $P < 0.001$, group 4 versus control.
ERGs for a group 2 eye with relatively mild IOP elevation (mean IOP = 30.2) are shown in the left column (Fig. 3A). Despite a lack of morphologic evidence for optic nerve or retinal damage (ONIG = 1.05), the pSTR was markedly reduced, a finding that was especially evident at the dimmest stimulus intensities. The nSTR was also substantially smaller than that of the control eye, but a small remnant negative potential persisted even at the dimmest stimulus intensity. All aspects of the scotopic responses to brighter flashes; however, were very similar between this eye and its fellow control: the a- and b-wave implicit times were identical with control levels, whereas amplitudes were approximately 5% to 10% lower. Although the summed amplitudes of the scotopic OPs were also similar to those of the control, the shape and timing of individual wavelets were slightly altered for moderate flash intensities. The photopic b-waves were also slightly smaller, compared with the control eye, but the photopic OPs were similar in the two eyes.

Figure 3B demonstrates the ERG findings for a group 3 animal with slightly greater IOP elevation (mean IOP = 31.1 mm Hg) and clear signs of optic nerve damage (ONIG = 3.1, mild-to-moderate focal optic nerve damage). In this eye, the pSTR appeared to be nearly completely eliminated, as the waveform never rose above baseline, whereas again, a small remnant nSTR persisted for flashes above −6.04 log cd·s/m². The scotopic b-wave was substantially smaller and delayed in the damaged eye, as were a-waves for moderately bright flashes. However, the a-wave for the brightest stimulus flashes was only slightly smaller and slower than the control eye. Both scotopic and photopic OPs were well below control eye values, and the photopic b-wave was reduced by approximately 40%.

The ERGs for a group 4 animal with more substantial IOP elevation (mean IOP = 37.8 mm Hg) and severe diffuse optic nerve damage (ONIG = 5.0) are shown in Figure 3C. At this stage, the pSTR was completely abolished, leaving only a small negative waveform for responses up to −3.72 log cd·s/m². The amplitudes of scotopic a- and b-waves, as well as photopic b-waves, were all reduced to less than 30% of the control eye. Similarly, both scotopic and photopic OPs were markedly attenuated.

The amplitude versus intensity functions for each ERG parameter, measured across the full spectrum of stimulus intensity, are shown in Figure 4 (note, amplitudes are measured at criterion times as described in the Methods section). Data for these intensity–response functions are grouped by IOP level as described in Table 1 and plotted as the group mean (±SEM).

The group data shown in Figure 4 confirm the results for the individual examples given in Figure 3. The STR was abnormal even in group 2 experimental eyes (filled circles) despite only a modest IOP elevation (P = 0.01 vs. control eyes) and no morphologic evidence of optic nerve damage. The amplitude...
Selective Loss of STR in a Rat Glaucoma Model

Figure 4. ERG amplitude versus stimulus intensity. (A) pSTR, (B) nSTR, (C) scotopic b-wave, (D) scotopic a-wave, (E) photopic b-wave, (F) scotopic OPs, (G) photopic OPs. Shown is the group mean (±SEM) in control eyes (□, n = 20) and experimental eyes from groups 2 (●, n = 5), 3 (○, n = 6), and 4 (▲, n = 9).

of the pSTR (Fig. 4A) was essentially 0 up to −5.36 log cd-s/m². Its threshold appeared to be elevated (shifted rightward) nearly 10-fold (~1 log unit) in comparison to the control (P < 0.0001). Post hoc evaluation by intensity revealed that the pSTR amplitude differences between group 2 eyes and control eyes were most significant for intensities between −5.36 and −4.97 log cd-s/m² (P < 0.001).

The nSTR amplitude (Fig. 4B) in group 2 eyes was also smaller than control amplitudes for all intensities up to −4.36 log cd-s/m², where its saturated amplitude ultimately became equal to the control group maximum amplitude. The nSTR intensity–response function was also shifted to the right by nearly 1 log unit. The larger negative values in the group 2 eyes, compared with the control, for stimuli near −4.38 log cd-s/m², represent loss of opposing positive potentials at the 220-ms criterion time.

As flash intensity increased, the amplitude of the scotopic b-wave (Fig. 4C) in group 2 eyes became more like that of the control group, eventually saturating at approximately 80% of the latter. Similarly, scotopic a-wave amplitude (Fig. 4D) was reduced by only approximately 10% in the group with mild damage. The amplitudes of both scotopic and photopic OPs were unaffected by this mild degree of injury (Figs. 4F, 4G, respectively), whereas reduction of the photopic b-wave (Fig. 4E) was evident only at the highest flash intensities. Post hoc analysis by intensity revealed that none of the ERG parameters other than pSTR amplitude exhibited significant differences between group 2 eyes and control eyes. Thus, the pSTR amplitude differences represent selective functional loss in the lowest IOP group.

ERG abnormalities in group 3 eyes (Fig. 4, open diamonds) were more widespread. The pSTR amplitude was 0 (or negative) until flash intensity surpassed −4 log cd-s/m²; representing more than a 2-log-unit increase in threshold. The negative pSTRs observed between −5 and −4 log cd-s/m² represent the effect of an unopposed remnant negative potential which is apparent in Figure 3B. In fact, Figure 4B shows that there was a small remnant nSTR with a maximum amplitude that was approximately half that of the control eye group. Moderate elevation of mean IOP was also associated with a slightly larger reduction of the scotopic b-wave saturated amplitude (Fig. 4C), as well as substantial losses in the scotopic a-wave (Fig. 4D) and OPs (Fig. 4F). Scotopic b-wave (P < 0.001) and OP amplitude changes (P < 0.01) were most significant for lower intensities, whereas a-wave changes (P < 0.001) were most significant at higher intensities. The photopic b-wave (Fig. 4E) and OPs (Fig. 4G) were also attenuated in this group. Amplitude changes in these two photopic ERG parameters were most significant for flash intensities between 1.71 and 2.72 log cd/m² (P < 0.001).

Lastly, the group of eyes with the highest IOP (>35 mm Hg; filled triangles) manifested the most severe functional abnormalities. Figures 4A and 4B show that the amplitudes of the pSTR and nSTR, respectively, were further reduced in group 4 eyes and that their intensity response functions were shifted farther to the right. Similarly, the scotopic b-wave and OP amplitudes were more significantly affected in group 4 eyes, as were photopic b-wave and OP amplitudes. For these parameters, relative amplitude differences and the intensity range over which significant amplitude differences were observed were both larger in group 4 eyes compared with the other three groups. In contrast, there was little additional decline in the scotopic a-wave amplitude between groups 3 and 4 (Fig. 4D).

The sensitivity to detect functional loss was compared across the various ERG parameters. Figure 5 shows the distribution of amplitudes observed among the group of control eyes (n = 20) for each of the seven ERG components measured (scotopic ERG: pSTR, nSTR, a-wave, b-wave, OPs; photopic ERG: b-wave and OPs). In each case, the box plot represents the mean (horizontal hash mark) and interquartile range, while the whiskers outline the 5th and 95th percentiles of the control eye distribution. The symbols to the right of each control group distribution represent the individual data for the three groups of experimental eyes described earlier. Those data that fall below the lower limit of the “normal” range (lower whisker) can be considered abnormal relative to a fixed specificity of 95%. For clarity, the amplitude of each ERG component is shown for only a single representative intensity (pSTR and nSTR: −4.97; all others: 2.22 log cd-s/m²); amplitudes were measured as described in the Methods section. The overall effect of experimental treatment was significant (P < 0.0001) for each of the seven ERG parameters.

Figure 5 shows that the two groups of experimental eyes with the highest mean IOP had the poorest function, on average, across all ERG parameters, as expected from Figure 4. Post hoc analyses revealed no significant differences between the
mean amplitudes of group 3 (diamonds) and group 4 (triangles) for any of these seven ERG parameters. When the percentages of eyes with below-normal function were compared, groups 3 and 4 were also quite similar. Both of these groups had 100% of eyes below the normal limit for the pSTR and photopic OPs, and most eyes in both groups also had abnormally small photopic b-waves. Approximately half of the eyes in both groups had scotopic a-wave, b-wave, and OP amplitudes below normal limits.

More important, the results for group 2 (circles) revealed a more selective pattern of functional abnormalities. All the eyes in this group (n = 5) were within normal limits for the scotopic a-wave, b-wave, and OPs and most had normal amplitudes for the photopic b-wave and OPs as well. But the pSTR amplitude was normal in only one of these group 2 eyes and only two had normal nSTR amplitudes. Only the amplitude of the pSTR was found to be significantly different after post hoc comparisons between the mild damage group and control group (t = 3.8, P < 0.01).

The amplitudes of each ERG parameter (for the same intensities as Fig. 5) are plotted against mean IOP in Figure 6. For each of the 20 experimental eyes in the study, amplitude is expressed relative to the amplitude of the fellow eye (%) so that comparisons can be made between ERG parameters. In addition, the 95% limits of agreement for interocular amplitude—that is, the range of interocular reliability—was calculated for each parameter based on data collected separately in 20 naive animals. One direction of the range is plotted in each panel (Fig. 6, shaded zone) to show the criterion used to determine significance (P < 0.05) of a reduction in relative amplitude (note, the range is not symmetric around 1.0 on the linear scale used here).

Figure 6 shows that relative pSTR amplitude was significantly reduced in all but one animal and that even mildly increased IOP resulted in substantial functional loss as measured by pSTR amplitude (Fig. 6A). The nSTR data showed a similar pattern, although the magnitude of loss was not as large (Fig. 6B). Relative amplitudes of scotopic a- (Fig. 6C) and b-waves (Fig. 6D) were within normal limits in three or four animals with minimally increased IOP, but as mean IOP increased, both parameter amplitudes declined in a linear fashion, and functional losses became significant. The photopic b-wave (Fig. 6E) showed a similar dependence on mean IOP compared with scotopic a- and b-wave amplitudes. Both scotopic and photopic OP amplitudes (Fig. 6F) were also correlated negatively with mean IOP; however, they were more variable between pairs of normal eyes and thus less likely to be useful for detection of functional change.

**Discussion**

The results of this study demonstrate that the most sensitive ERG parameter for detection of functional abnormalities in this rodent model of glaucoma is the STR. In particular, loss of the pSTR was more prevalent and its magnitude even larger than that of the nSTR. The pSTR was significantly reduced at the earliest stage of experimental glaucoma studied here (Figs. 5, 6). In fact, because pSTR amplitude was reduced by approximately 70% at even the lowest level of elevated IOP, the function relating its amplitude to mean IOP (Fig. 6) was nearly flat (its slope was not significantly different from zero after applying the Bonferroni correction for multiple comparisons). Significant pSTR abnormalities were present even in the group of experimental eyes that had no histologic evidence of structural damage. As demonstrated by our prior studies, selective loss of the pSTR is indicative of selective RGC injury. As the cumulatively sustained injury (mean IOP) increased, additional functional abnormalities became apparent, along with clear histologic signs of structural damage. After 5 weeks at the highest mean IOP levels, both functional and structural changes were indicative of damage to outer retinal layers, including photoreceptors.

These results suggest that selective RGC damage is produced by a relatively mild elevation of IOP. The rat STR, particularly the pSTR, is dependent on intact RGC function, but scotopic ERG responses to brighter flashes are only negligibly affected by substantial RGC loss. Taken together, these studies suggest that the highest levels of elevated IOP result in nonselective retinal damage, perhaps directly, but perhaps also by indirect mechanisms such as ischemia. Retinal ischemia is well known to affect the scotopic ERG b-wave in rodents and if severe enough, also the a-wave—components that reflect inner nuclear layer and photoreceptor function, respectively.

Previous studies that have used the full-field ERG to monitor retinal function in rat experimental models of glaucoma have
only examined the response to relatively bright stimulus flashes (WoldeMussie E, et al. IOVS 2003;44:ARVO E-Abstract 41). Alterations in the response to brighter flashes would be indicative of effects on retinal cells other than RGCs.24 This suggests that lower levels of elevated IOP should be sought in rodent models of experimental glaucoma for such models to be most relevant to human chronic open-angle glaucoma.

For mild elevations of IOP in the present model, RGC functional losses assessed using the STR were observed before structural changes evaluated using standard histologic techniques. The exquisite sensitivity of the scotopic psychophysical threshold, and subsequently also of the STR, are thought to depend on extensive pooling and progressive convergence of retinal signals from rods to rod bipolar cells and eventually to RGCs through all amacrine cells and cone bipolar cells. Retraction of RGC dendritic arbors, as has been shown to occur in experimental glaucoma before RGC death, may well reduce the efficiency of signal processing within the inner plexiform layer and thus raise the threshold of the STR. In the present study, the STR intensity-response function was shifted rightward, suggesting a threshold elevation of approximately one log unit in the mild (selective) damage group. This is substantially larger than the effect that would be produced by a small reduction of maximally dilated pupil diameter (from 4.5 to 3.0 mm) observed in some of these eyes (the latter could account for ~0.35 log units elevation). Elevated scotopic thresholds have been measured psychophysically in early human glaucoma and selective reduction of the STR has been observed in nonhuman primates with experimental glaucoma. However, selective STR abnormalities have not yet been reported in human glaucoma. Indeed, the human STR may not be as sensitive to RGC loss compared with the STR of rats or monkeys.

In summary, selective RGC functional loss was observed in this experimental model of glaucoma before the onset of optic nerve structural changes, as assessed by standard histologic techniques and light microscopy of optic nerve tissue. Selective loss of the STR occurred at low levels of chronically elevated IOP. The highest IOP levels resulted in nonspecific functional loss. Thus, in rodent models of experimental glaucoma, careful monitoring of IOP in awake animals is crucial, because lower levels of chronically elevated IOP may be more relevant to human primary chronic glaucoma.

References


