Published in final edited form as:

Invest Ophthalmol Vis Sci. 2007 October ; 48(10): 4564–4572. doi:10.1167/iov.07-0483.

Longitudinal Evaluation of Retinal Ganglion Cell Function and IOP in the DBA/2J Mouse Model of Glaucoma

Maher Saleh^{1,2}, Mahesh Nagaraju^{1,2}, and Vittorio Porciatti¹

¹ Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida

Abstract

Purpose—To characterize progressive changes of retinal ganglion cell (RGC) function and intraocular pressure (IOP) in the DBA/2J mouse model of spontaneous glaucoma.

Methods—Serial pattern electroretinograms (PERGs) and IOPs measures were obtained from both eyes of 32 anesthetized DBA/2J mice over an age range of 2 to 12 months at 1-month intervals. Cone-driven flash-ERGs (FERGs) were also recorded. The endpoint was defined as the age at which the PERG amplitude reached the noise level in at least one eye. At that point, both eyes were histologically processed to evaluate the thickness of the retinal fiber layer (RNFL).

Results—IOP increased moderately between 2 and 6 months (~14–17 mm Hg) and then more steeply, until it leveled off at approximately 28 mm Hg by 9 to 11 months. The mean PERG amplitude decreased progressively after 3 months of age to reach the noise level (85% reduction of normal amplitude) at approximately 9 to 12 months in different animals. When the PERG was at noise level, the RNFL showed a relatively smaller reduction (40%) in normal thickness. The FERG displayed minor changes throughout the observation period. IOP and PERG changes were highly correlated ($r^2 = 0.51$, $P < 0.001$).

Conclusions—Results indicate that inner retina function in DBA/2J mice progressively decreases after 3 months of age, and it is nearly abolished by 10 to 11 months, whereas outer retina function shows little change and the RNFL thickness is relatively spared. This result suggests that surviving RGCs may not be functional. Progression of inner retinal dysfunction is strongly associated with increased IOP.

Glaucoma is a progressive neurodegenerative disease of retinal ganglion cells (RGCs) that represents the second most prevalent cause of blindness worldwide.^{1–3} Although elevated intraocular pressure (IOP) is considered to be one of the major risk factors for development and progression of glaucoma,⁴ many other factors are known to contribute to the complex nature of the disease.^{5–10} Knowledge gained in the biological basis of glaucomatous neurodegeneration by using mouse models^{7,9,11–14} may yield to specific neuroprotective treatments that are also relevant for other degenerative diseases of the central nervous system.^{15–17}

The inbred DBA/2J mouse strain is a well-established model of spontaneously elevated IOP, progressive glaucomatous loss of RGCs, and optic disc excavation, which represent the hallmarks of glaucoma.^{7,18–20} Photoreceptor, horizontal, and bipolar cells remain histologically intact.^{21,22} DBA/2J mice develop iris atrophy and pigment dispersion due to

Corresponding author: Vittorio Porciatti, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL 33136; vporciatti@med.miami.edu.

²Contributed equally to the work and therefore should be considered equivalent authors.

Disclosure: M. Saleh, Allergan, Inc. (F); M. Nagaraju, None; V. Porciatti, None

mutations of two genes, *Gpnmb* and *Tyrp1*.²³ The iris disease is visible at 6 months, progresses with age, and is associated with increased IOP.^{20,24,25} Young (2–3-month-old) DBA/2J mice have normal IOP and normal histologic appearance of RGCs and optic nerve.^{19,20} RGC and optic nerve degeneration is first apparent between 8 and 9 months of age.^{20,26} By 18 months of age, approximately 90% of the optic nerves show advanced degeneration.²⁰

Little is known about the sequence of functional events associated with elevation of IOP and progressive degeneration of RGCs in DBA/2J mice. Surviving RGCs may not be functional. The lack of information on the natural history of RGC dysfunction represents a major limitation to the use of the DBA/2J mouse model. We noninvasively characterized the changes in RGC function and IOP in a longitudinal study of 32 DBA/2J mice (64 eyes) from 2 to 12 months of age at 1-month intervals. RGC function was quantified by means of the pattern electroretinogram (PERG).^{27–29} IOP was measured with an induction–impact tonometer (Tonolab Colonial Medical Supply, Franconia, NH).^{30,31} The thickness of the retinal nerve fiber layer (RNFL) was measured from histologic sections of the optic nerve head in eyes with abolished PERGs. The results showed that, in DBA/2J mice, progressive RGC dysfunction precedes anatomic loss of RGC axons by at least 2 months. The time course of RGC dysfunction is strongly associated with that of progressive increase in IOP.

Methods

Animals and Husbandry

A total of 32 DBA/2J female mice ($n=64$ eyes) of 2 months of age obtained by Jackson Laboratories (Bar Harbor, ME) were longitudinally tested. Mice were aged in a cyclic light environment (12 hours 50 lux; 12 hours dark) and fed ad libitum. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Animal Care and Use Committee of the University of Miami.

The Pattern Electroretinogram

Electrical activity in the retina associated with RGC function can be evaluated by means of the pattern electroretinogram (PERG). The PERG is typically obtained in response to contrast reversal of patterned visual stimuli (gratings, checkerboards), rather than uniform flashes of light.^{32,33} RGCs are necessary for PERG generation, since their selective loss after optic nerve transection in mammals abolishes the response.²⁷ The conventional flash ERG, which originates in the outer retina, is little or not affected. This crucial experiment has been successfully performed in several mammals including cat,^{27,34} monkey,^{35,36} rat,^{37,38} and mouse.^{28,39,40} The inner retina origin of the mammalian PERG has also been confirmed by current-source analysis of intraretinal recordings.^{41,42} Further support for an RGC origin of the PERG is that the response amplitude is maximized when pattern stimuli have a spatial frequency that matches the size of the average RGC receptive field center in humans^{43,44} and mice.²⁹ For a given retinal eccentricity and stimulus area, the PERG amplitude to the peak spatial frequency is linearly proportional to the expected volume of RGCs.⁴⁵

An important distinguishing characteristic of the PERG is that, to be generated, it requires the physiological integrity of viable RGCs. The PERG amplitude can be reversibly reduced by intravitreal injections of tetrodotoxin, which blocks spiking activity in the inner retina.^{46,47} Short-term elevation of the IOP has the effect of reversibly reducing the PERG amplitude but not the flash-ERG.^{48,49} Altogether, these findings indicate that reduction in PERG amplitude may reflect both the reduced activity of dysfunctional yet viable RGCs and the lack of activity of lost RGCs. The PERG, therefore, may represent an important tool for detecting and monitoring the onset and the progression of RGC dysfunction in mouse models of glaucoma.

The PERG, in addition, allows establishing retinal resolution and contrast threshold^{28,50} that have a counterpart in cortical visual acuity and contrast sensitivity.⁵¹ Finally, the PERG stimulus can be precisely located on the retina, thereby allowing quantitative structure–function correlations for specific retinal regions,⁴⁵ as well as topological assessment of RGC losses in glaucomatous DBA/2J mice.²¹ These PERG properties may therefore provide important information that can be used in characterizing glaucomatous disease and the effect of neuroprotective agents on retinal function.

PERG Recording

A detailed description of the PERG technique is reported elsewhere.²⁹ In brief, the recording electrode (0.25-mm diameter silver wire configured to a semicircular loop of 2-mm radius) was laid on the corneal surface by means of a micromanipulator and positioned in such a way as to encircle the undilated pupil without limiting the field of view. Reference and ground electrodes were stainless steel needles inserted under the skin of the scalp and tail, respectively. Positioning of the corneal electrode entailed minimal manipulation of the eye, which would otherwise induce cataract and preclude further PERG testing.^{52,53} A small drop of balanced saline maintained the cornea and lens in excellent conditions for the duration of recording. A visual stimulus of contrast-reversing bars (field area: $50^\circ \times 58^\circ$; mean luminance 50 cd/m²; spatial frequency: 0.05 cyc/deg, contrast: 98%; temporal frequency: 1 Hz) was aligned with the projection of the undilated pupil at a 20-cm distance. Eyes were not refracted for the viewing distance, because the mouse eye has a large depth of focus due to the pinhole pupil.^{54,55} Retinal signals were amplified (10,000-fold) and band-pass filtered (1–30 Hz). After body temperature was stabilized at 37.0°C, three consecutive responses to 600 contrast reversals each were recorded. The responses were superimposed to check for consistency and then averaged. To have an estimate of the residual noise, an additional recording was made in which the pattern contrast was set to 0. The PERG is a light-adapted response. To have a corresponding index of outer retina function, a light-adapted ERG (FERG) was also recorded with undilated pupils in response to strobe flashes of 20 cd/m²/s superimposed on a steady background light of 12 cd/m² and presented within a Ganzfeld bowl. Under these conditions, rod activity is largely suppressed while cone activity is minimally suppressed.⁵⁶ Averaged PERGs and FERGs were automatically analyzed to evaluate the peak-to-trough amplitude and peak latency of the positive wave (Fig. 1). Separate analysis of positive and negative components of the PERG waveform was not performed because unambiguous recognition of these components was not possible in older mice with reduced signal. In mice in which the PERG had a relatively good signal-to-noise ratio, both the positive and negative waves were affected. The peak-to-trough FERG amplitude showed little change throughout the study, on average. In individual eyes, we were unable to identify systematic changes in the FERG negative component, compared with the positive component. However, the peak-to-trough amplitude measurements we made included both the positive and the negative wave, so that any glaucoma-induced change in either component was reflected in the FERG. To evaluate relative PERG changes compared with FERG, we calculated individual PERG/FERG amplitude ratios.

IOP Measurement

IOP was measured with an induction–impact tonometer (Tonolab Colonial Medical Supply)³⁰ immediately after induction of general anesthesia (~5 minutes). The tonometer was fixed in a vertical position to a support stand by means of clamps. The mouse was gently restrained by hand on an adjustable stand, and the eye was oriented in such a way as to align the probe tip with the optical axis of the eye at a 1- to 2-mm distance with a magnifier lamp. Six consecutive IOP readings were averaged. IOP readings obtained with Tonolab have been shown to be accurate and reproducible in different mouse strains, including DBA/2J.³¹ The impact of the Tonolab probe on the cornea is minimal and does not cause either corneal damage or progressive changes in IOP, even after many repeated readings.

Protocol

The mice were weighed and anesthetized with IP injections (0.5–0.7 mL/kg) of a mixture of ketamine, 42.8 mg/mL; xylazine, 8.6 mg/mL; and acepromazine, 1.4 mg/mL. After IOP measurements in each eye, the mice were restrained in a modified stereotaxic apparatus that allowed unobstructed vision.^{29,51} The body of the mouse rested on a feedback-controlled heating pad able to maintain the body temperature at 37.0°C. Under these conditions the eyes of mice were naturally wide open and in a stable position, with undilated pupils pointing laterally and upwardly. A PERG and an FERG were recorded from the right eye. Additional anesthesia was given as needed, and a PERG and an FERG were recorded from the left eye. All IOP, PERG, and FERG recordings were made between 10 AM and 4 PM. After recording, the mice were allowed to recover on a heating pad and put back in their cages. The entire protocol required about 1 hour. IOP, PERG, and FERG recordings were repeated at every successive month until the PERG amplitude was reduced to the noise level in at least one eye (9–12 months of age). Both eyes of mice reaching the endpoint PERG amplitude were carefully inspected for lens and corneal opacities,^{25,53} given additional dose of anesthesia, euthanatized by cervical dislocation, and the eyes processed for histologic measurements of the RNFL. Forty-one eyes of the initial 64 were analyzed at the PERG endpoint. For the remaining eyes, follow-up was truncated because of the premature death of the animal or development of cataracts that precluded further testing.

RNFL Thickness Measurement

The superior pole of the eyes was marked with an electrical cautery without perforating the cornea. Eyes were carefully enucleated avoiding traction on the optic nerves. Right eyes and left eyes were separately fixed overnight at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The fixative was then replaced with 0.4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 and stored at 4°C.⁵⁷ Fixed eyes were conventionally dehydrated in graded ethyl alcohols. Alcohol was then substituted with xylene and the eyes embedded in paraffin. Serial sections (8 μ m thick) through the optic nerve head were obtained maintaining the plane of cut parallel to the optical axis of the eye. Sections were stained with hematoxylin-eosin, bright-field imaged with an upright fluorescence microscope (BX51; Olympus, Melville, NY), and photographed (Qicam 12-bit Fast model 1394 camera; QImaging, Surrey, BC, Canada). Digital images were analyzed on computer (ImageJ 1.36b freeware available by ftp at zippy.nimh.nih.gov/ or at <http://rsb.info.nih.gov/nih-image>; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD), to measure the thickness of the RNFL at the boundary between retina and optic nerve (Fig. 1). The two sections at which the optic nerve had maximum size were selected for measurements. Overall, four RNFL thicknesses per optic nerve head section (section 1: superior, inferior; section 2: superior, inferior) were measured and averaged to obtain a single entry. RNFL thicknesses of eyes reaching the PERG endpoint were compared with average RNFL thicknesses of independent groups of 2-month-old DBA/2J mice ($n = 5$) and 16-month-old mice ($n = 5$). To account for interocular differences, RNFL data have been analyzed separately for eyes with worse PERG amplitude (first eye) and better PERG amplitude (second eye) at the endpoint. Since the number of axons entering the optic nerve is equal to the number of RGCs, RNFL thickness measurements in a given optic nerve head sector represents a specific index of both RGC and axon number originating from the corresponding retinal sector. In principle, for a given RNFL thickness the number of axons/RGCs could be calculated if axon density/diameter at the entrance of the optic nerve head were known. An approximate estimate of RNFL axon density might be obtained from axon densities reported for the retrobulbar portion of the optic nerve.^{25,58,59} However, evaluation of the absolute number of axons from RNFL measurements may yield to ambiguous results since the RNFL becomes thicker as it approaches the optic nerve due to stratification of nerve fibers originating from different retinal eccentricities. RNFL thickness may be reduced due to either glaucomatous axon loss or increased distance from the optic nerve head. To avoid this

uncertainty, we analyzed relative changes of RNFL thickness at a fixed optic nerve head location between mice of different ages. It was shown that relative changes in RNFL thickness in DBA/2J mice of different ages are in good agreement with corresponding changes of optic nerve axons.^{20,60} We focused on RNFL thickness measurements, rather than axon/RGC counts, for the potential advantages offered by noninvasive RNFL measurements obtained with imaging techniques such as optical coherence tomography (OCT) that are now available for the mouse (Ruggeri M, *IOVS* 2007;48:ARVO E-Abstract 1199).⁶¹ Longitudinal evaluation of RGC function and RNFL thickness using PERG and OCT, respectively, represent powerful tools for neuroprotection studies of mouse glaucoma models. It should take into account, however, that RNFL thickness includes nonaxonal elements (i.e., glia, vessels) whose relative contribution may increase with increasing severity of glaucoma due to progressive axonal loss.⁶² It is also possible that gliosis may replace axons,^{63–66} and show a thicker than expected residual RNFL thickness in advanced stages of glaucoma, where few RGC axons are expected to be present.

Statistical Treatment of Data

Data have been collected by performing repeated measures on the same mice over time. However, due to substantial attrition the loss of data, together with a reduction in sample size, increases with observation time. This loss may be associated with inhomogeneity of variance and departures from normality. To minimize these problems, we used a nonparametric statistical approach throughout the study (Spearman rank-order correlation; Kruskal-Wallis ANOVA; Mann-Whitney rank-sum test) for age-related effects. Right eyes and left eyes were analyzed separately.

Results

Age-Related PERG Changes

Longitudinal changes in PERG and FERG amplitude and latency are summarized in Figure 2. The major feature of Figure 2 is that, on average, PERG amplitude progressively decreases with age until it reaches the upper limit of the noise range at approximately 11 months of age. Age-related PERG amplitude changes are highly significant (Spearman [R_s] coefficient of correlation between amplitude and age, OD: $R_s = -0.8$, $P < 0.0001$; OS: $R_s = -0.754$, $P < 0.0001$). By 4 months of age, the PERG amplitude was significantly smaller than that of 2-month-old mice in either eye (Mann-Whitney Rank sum test $P < 0.05$). The Spearman correlation between PERG latency and age is not significant in either eye. Age-related FERG amplitude changes are borderline significant in the right eyes (Spearman coefficient of correlation between amplitude and age, OD: $R_s = -0.165$, $P = 0.03$) and not significant in the left eyes (Spearman coefficient of correlation between amplitude and age, OS: $R_s = -0.089$, $P = 0.25$). Compared with that in 2-month-old mice, the FERG amplitude is significantly smaller in the right eyes of mice aged 10 and 11 months (Mann-Whitney rank sum test $P < 0.05$), but it is not significantly different in the left eyes at any age. The Spearman correlation between FERG latency and age is not significant in either eye. Age-related PERG amplitude losses may be accounted for, at least in part, by FERG amplitude losses. To evaluate specific PERG losses, a PERG/FERG amplitude ratio was calculated. For both right eyes and left eyes, the PERG/FERG amplitude ratio significantly decreased with age (Kruskal-Wallis ANOVA, $P < 0.001$) from an average of 13% at 2 months to an average of 3% at 11 months. The Spearman coefficient of correlation between PERG/FERG amplitude ratio and age was, OD: $R_s = -0.62$, $P < 0.0001$; OS: $R_s = -0.494$, $P < 0.0001$. Results indicate that the cone-flash ERG, including both the positive and negative components, is not much affected in this glaucoma model. Age-related PERG changes may be largely explained by changes occurring in the inner retina.

PERG-RNFL Relationship

At the PERG endpoint, the RNFL was relatively spared from thinning. In Figure 3 the mean RNFL thickness of eyes with relatively smaller and higher PERG amplitude at the endpoint is compared with that of preglaucomatous 2-month-old mice as well as with that of 16-month-old mice with advanced glaucoma. At the PERG endpoint, the mean RNFL thickness was reduced in either eye, when compared with the thickness in 2-month-old mice (first eyes: -41.5% , $P < 0.001$; second eyes: -36.8% , $P < 0.001$) but still much thicker than that of 16-month-old mice (first eyes: $+127.4\%$, $P < 0.001$; second eyes: $+145.8\%$, $P < 0.001$).

Figure 4 compares average PERG amplitude changes with average RNFL thicknesses (both eyes) on a normalized scale. Previous cross-sectional data from Libby et al.²⁰ and Anderson et al.⁶⁰ (Simon John's laboratory) in mice of different ages have been combined and included in Figure 4 to make a comparison between PERG, RNFL thickness, and axon counts of optic nerves. An approximate axon count evaluation was possible, because Libby et al.²⁰ graded severity of optic nerve damage in a large population of DBA/2J mice, and Anderson et al.⁶⁰ reported the mean axon count for each severity grade. The major features of Figure 4 are that (1) relative changes of RNFL thickness with age were in very good agreement with corresponding changes in axon counts in the optic nerve, and that (2) loss of RNFL thickness/axon number lagged behind loss of PERG amplitude by approximately 3 months, on average. By 8 months of age, when the PERG amplitude had lost approximately 50% of its initial value, the number of optic nerve axons was still unchanged.^{20,60} By 11 months of age, when the PERG had already reached the noise level in many eyes, RNFL thickness/axon number was decreased by approximately 50% only. There was a small overlap between SD bars of PERG amplitude in the endpoint range (9–12 months) and RNFL thickness at the PERG endpoint.

PERG-IOP Relationship

Longitudinal changes in IOP are displayed in Figure 5. Average PERG amplitude data shown in Figure 2A are also displayed for comparison. For IOP data, the y-axis corresponds to millimeters of mercury; for PERG data, the y-axis corresponds to microvolts. Note that IOP, on average, tended to increase over the entire age range tested. The increase in IOP was moderate between 2 (14 ± 1.9 mm Hg) and 6 (17 ± 2.9 mm Hg) months. After 6 months, there was an acceleration of age-related IOP increase. IOP tended to level off between 25 and 30 mm Hg at 9 and 11 months of age. Age-related IOP changes are highly significant (Spearman coefficient of correlation between IOP and age, OD: $R_s = 0.77$, $P < 0.0001$; OS: $R_s = 0.745$, $P < 0.0001$). By 3 months in the right eyes and by 5 months in the left eyes, IOP was significantly higher than that in 2-month-old mice (Mann-Whitney rank sum test, $P < 0.05$). Also note the good association between the time course of IOP increase and PERG amplitude decrease, except for older ages, at which the PERG is close to the noise level.

In Figure 6 PERG amplitudes in both eyes have been plotted against corresponding IOP values. As predicted from Figure 5, PERG amplitude changes as a function of IOP were not linear (Fig. 6A). To linearize PERG changes, PERG amplitudes were plotted on a log scale and the data fitted with a linear regression (Fig. 6B). It can be noted that the correlation between PERG amplitude and IOP is reasonably good: $\log(\text{amplitude}) = 1.48 - 0.036 \times \text{IOP}$; $r^2 = 0.51$, $P < 0.001$. As shown in Figures 6A and 6B, PERG amplitude decreased as a function of IOP increase even in the IOP range of 10 to 20 mm Hg, which is considered the range of normal-tension glaucoma in human.⁶⁷ For IOPs of 30 mm Hg and higher, the PERG amplitude was in the noise range. The PERG amplitude did not show significant correlation with IOP (data not shown).

Discussion

The main results of this study are that progressive RGC dysfunction precedes loss of RNFL thickness and progressive RGC dysfunction is closely associated with progressive increase in IOP.

RGC Dysfunction Preceding Loss of RNFL Thickness

Loss of RNFL thickness is comparable to loss of optic nerve axons, which lags behind loss of PERG amplitude by approximately 3 months on average. This represents approximately one tenth of the mouse's lifespan.⁶⁸ If results obtained in the mouse model were extrapolated to human glaucoma, then one tenth of a lifespan would represent a time window wide enough to offer the opportunity of detecting RGC dysfunction and treating the condition before irreversible RGC death. Although the survival time of dysfunctional RGCs in early human glaucoma is not known, recent results have shown that PERG amplitude losses are relatively larger than RNFL losses measured by OCT,⁶⁹ and that PERG amplitude losses in early glaucoma can be at least in part restored by lowering IOP with eye drops.⁷⁰ Altogether, these results suggest that anatomic loss of RNFL thickness is preceded by a sizable period of RGC dysfunction that is potentially reversible.

By 8 months of age, when the PERG amplitude has lost approximately 50% of its initial value, the number of optic nerve axons is still unchanged.²⁰ In fact, clear signs of RGCs undergoing apoptotic death are first detected at 8 to 9 months of age with both TUNEL and annexin-V staining.^{71,72} Preapoptotic anatomic alterations such as truncated or misdirected dendrites, shrunken somata, abnormal accumulation of neurofilaments, and thin axons have been demonstrated in surviving RGCs of DBA/2J mice.²¹ These observations suggest that generalized neuropathy may precede RGC death. In addition, the number of RGCs labeled by retrograde transport of marked compounds injected into the superior colliculus may decrease, even before 8 to 9 months of age in DBA/2J mice.^{21,73,74} This suggests that failure of axonal transport may precede RGC death. In primate experimental glaucoma, Weber and Harmon⁷⁵ demonstrated, using intracellular recording and staining techniques in isolated retinas, that RGCs are less responsive, both spatially and temporally, to visual stimuli. The authors concluded that the reduction in visual responsiveness most likely results from significant changes in dendritic architecture, which affects their level of innervation by more distal retinal neurons. PERG alterations in mice aged 4 to 8 months, therefore, may represent reduction of RGC responsiveness associated with any or all the above preapoptotic abnormalities of RGCs and their axons. An additional possibility for PERG amplitude reduction might be associated with the failure of the nonneural support system that results from glial activation^{73,76–78} or retinal ischemia.^{74,78} In fact, Müller cells are believed to contribute to PERG generation,^{42,79,80} and retinal ischemia impairs the PERG.^{48,81}

Finally, PERG amplitude reduction may result from nonspecific causes such as defocus and corneal/lens opacities that reduce stimulus contrast⁵³ or nonglaucomatous age-related retinal degeneration. These causes seem unlikely, however. Corneal and lens opacities were checked with suitable magnification ($\times 25$) during each recording and at the endpoint. At the level of light anesthesia used for our noninvasive protocol, lens opacities represented an occasional occurrence; eyes with cataracts or corneal opacities invading the pupil area were excluded from the analysis. The pupils remained undilated, which assured an adequate depth of focus.^{54,55} Previous work has shown that PERGs²⁸ and pattern VEPs⁵¹ are not modified by trial lenses of ± 10 spherical diopters placed before the eyes. Older animals had slightly enlarged pupils compared with the young ones. This difference may reduce the depth of focus as well as the optical quality of the eye,^{82,83} eventually causing reduction of image contrast at the retinal level and may result in an exaggerated reduction of PERG amplitude with age. However, mouse eyes have a very large depth of focus, even when pupils are artificially dilated.⁸² Imposed

amounts of spherical defocus do not alter optical image significantly.⁸³ In contrast, an enlarged pupil results in greater retinal illuminance and associated increase of PERG amplitude (Porciatti et al. *IOVS* 2005;46:ARVO E-Abstract 2705) that tends to counteract the effects of reduction of image contrast. Altogether, optical factors are unlikely to be responsible for the age-related effects on PERG in DBA/2J mice. Nonspecific, age-related PERG changes could also be excluded since previous work has shown that congenic controls (wild-type for the *Gpnmb* mutation) of 10.5 months of age and without glaucoma have normal PERG (Libby et al. *IOVS* 2006;47:ARVO E-Abstract 4005).

RGC Dysfunction Associated with Increased IOP

Progressive loss of RGC function is closely associated with a progressive increase in IOP. This is also true in the 14 to 20 mm Hg IOP range, which represents the range of normal tension glaucoma in humans.⁸⁴ Moderate, progressive IOP increase in DBA/2J mice aged 2 to 6 months has been also shown by other laboratories in a longitudinal evaluation with a handheld corneal applanation tonometer (Tonopen; Medtronic Xomed, Jacksonville, FL)^{25,77} as well as in a cross-sectional evaluation using a corneal induction–impact tonometer identical with that used in the present study.²⁴ Noninvasive corneal induction–impact tonometry yields reliable IOP measurements that correlate very well with induced IOP changes obtained by cannulation of the anterior chamber in mouse eyes, in vivo and ex vivo.^{31,85} It is possible that the absolute IOPs we measured under ketamine-xylazine anesthesia are somewhat lower than those occurring in nonsedated mice.⁸⁶ The IOP-lowering effect of anesthesia, however, is not expected in our protocol, in which IOP has been measured in a time window of up to 5 minutes after anesthetic administration.^{87–89} Progressive IOP increase in DBA/2J mice aged 2 to 6 months might be related to the corneal thickening²⁵ and corneal calcifications^{19,25} reported in older DBA/2J mice that may result in changes in elastic properties of the cornea and spurious IOP readings. Corneal alterations, however, are unlikely to occur in mice aged 2 to 6 months.^{19,20} IOP readings from in vivo direct cannulation of the anterior chamber, which bypasses the cornea, are often higher in 6-month-old females compared to 2-month-old females.^{19,20} In the present study, only females were used.

That PERG amplitude changes are closely associated with IOP changes in both time-course and relative amount of change does not necessarily imply that IOP is the causal factor of RGC dysfunction. Different insults, including ischemia, excitotoxicity, axonal injury, glial activation, and autoimmunity,⁹ may combine to impair RGC function in glaucoma. DBA/2J eyes have deficiencies in some aspects of immune response before dispersed pigment is evident,^{24,90} but there is no evidence of macrophage infiltration into the retina.^{7,78} Microarray analysis shows evidence of glial activation and immune-related response associated with IOP elevation.⁷⁷ Signs of ischemia have been reported in DBA/2J mice.⁷⁸ Treatments that target all these factors are generally effective in alleviating glaucomatous progression. These include treatments that do not alter IOP such as glial-derived neurotrophic factor (GDNF),⁷³ the N-methyl-D-aspartate (NMDA) receptor blocker memantine,^{74,91} the ischemia protectant erythropoietin,⁷⁴ immune system reconstitution through bone marrow transfer with cells that express wild-type, *Gpnmb*,⁹⁰ and IOP-lowering eye drop treatments.⁹¹

Altogether, these results suggest that IOP initiates or amplifies pathogenetic processes in RGCs that result in progressive reduction of electrical responsiveness and eventually apoptotic death. That IOP exacerbates existing RGC dysfunction is clearly shown by recent results obtained by our group (Nagaraju M et al. *IOVS* 2007;48:ARVO E-Abstract 212). IOP can be temporarily and reversibly increased by changes in the body posture in mice due to increase of the episcleral venous pressure.⁹² In DBA/2J mice aged 3 to 10 months, the head-down position (60°) results in an IOP increase of approximately 35%. Artificial IOP elevation does not modify PERG amplitudes in 3-month-old mice, but induces marked PERG reductions in older mice. Finally,

artificially decreasing IOP to normal values in 11-month-old mice partially restores PERG amplitude (Nagaraju M et al. *IOVS* 2007;48:ARVO E-Abstract 212). It remains to be established whether chronic IOP-lowering treatment alters the time course of PERG reduction in parallel with that of RGC death. Such a determination would represent strong proof that PERG alteration represents an early predictor of impending cell death.

Conclusions

In the DBA/2J mouse model of glaucoma, RGCs undergo a prolonged stage of progressive dysfunction that seems to precede loss of RGC axons. IOP elevation appears to initiate or amplify RGC distress resulting from a variety of other insults. These results have important implications for human glaucoma. Systematic neuroprotection studies using the DBA/2J mouse model as a template would benefit from the inclusion of PERG endpoints, since rescued RGCs may not be functional.

Acknowledgments

Supported by National Eye Institute Grants R03 EY016322 and R01EY014957 and Center Grant P30EY14801; and an unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc. MS was supported by Allergan, Inc.

References

1. Quigley HA. Open-angle glaucoma. *N Engl J Med* 1993;328:1097–1106. [PubMed: 8455668]
2. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;363:1711–1720. [PubMed: 15158634]
3. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90:262–267. [PubMed: 16488940]
4. Leske MC. The epidemiology of open-angle glaucoma: a review. *Am J Epidemiol* 1983;118:166–191. [PubMed: 6349332]
5. Quigley HA. Neuronal death in glaucoma. *Prog Retin Eye Res* 1999;18:39–57. [PubMed: 9920498]
6. Quigley HA. Glaucoma: macrocosm to microcosm: the Friedenwald lecture. *Invest Ophthalmol Vis Sci* 2005;46:2662–2670. [PubMed: 16043835]
7. John SW. Mechanistic insights into glaucoma provided by experimental genetics: the Cogan lecture. *Invest Ophthalmol Vis Sci* 2005;46:2649–2661. [PubMed: 16043833]
8. Tezel G, Wax MB. The immune system and glaucoma. *Curr Opin Ophthalmol* 2004;15:80–84. [PubMed: 15021215]
9. Libby RT, Gould DB, Anderson MG, John SW. Complex genetics of glaucoma susceptibility. *Annu Rev Genomics Hum Genet* 2005;6:15–44. [PubMed: 16124852]
10. 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. [PubMed: 15953750]
11. John SW, Anderson MG, Smith RS. Mouse genetics: a tool to help unlock the mechanisms of glaucoma. *J Glaucoma* 1999;8:400–412. [PubMed: 10604301]
12. Levin LA, Gordon LK. Retinal ganglion cell disorders: types and treatments. *Prog Retin Eye Res* 2002;21:465–484. [PubMed: 12207946]
13. Levkovitch-Verbin H. Animal models of optic nerve diseases. *Eye* 2004;18:1066–1074. [PubMed: 15534591]
14. Lindsey JD, Weinreb RN. Elevated intraocular pressure and transgenic applications in the mouse. *J Glaucoma* 2005;14:318–320. [PubMed: 15990617]
15. Morrison BM, Hof PR, Morrison JH. Determinants of neuronal vulnerability in neurodegenerative diseases. *Ann Neurol* 1998;44:S32–44. [PubMed: 9749571]
16. McKinnon SJ. Glaucoma: ocular Alzheimer's disease? *Front Biosci* 2003;8:s1140–1156. [PubMed: 12957857]

17. Tatton W, Chen D, Chalmers-Redman R, Wheeler L, Nixon R, Tatton N. Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. *Surv Ophthalmol* 2003;48(suppl 1):S25–S37. [PubMed: 12852432]
18. Sheldon WG, Warbritton AR, Bucci TJ, Turturro A. Glaucoma in food-restricted and ad libitum-fed DBA/2Nnia mice. *Lab Anim Sci* 1995;45:508–518. [PubMed: 8569148]
19. John SW, Smith RS, Savinova OV, et al. Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. *Invest Ophthalmol Vis Sci* 1998;39:951–962. [PubMed: 9579474]
20. Libby RT, Anderson MG, Pang IH, et al. Inherited glaucoma in DBA/2J mice: pertinent disease features for studying the neurodegeneration. *Vis Neurosci* 2005;22:637–648. [PubMed: 16332275]
21. Jakobs TC, Libby RT, Ben Y, John SW, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. *J Cell Biol* 2005;171:313–325. [PubMed: 16247030]
22. Moon JI, Kim IB, Gwon JS, et al. Changes in retinal neuronal populations in the DBA/2J mouse. *Cell Tissue Res* 2005;320:51–59. [PubMed: 15714280]
23. Anderson MG, Smith RS, Hawes NL, et al. Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet* 2002;30:81–85. [PubMed: 11743578]
24. Zhou X, Li F, Kong L, Tomita H, Li C, Cao W. Involvement of inflammation, degradation, and apoptosis in a mouse model of glaucoma. *J Biol Chem* 2005;280:31240–31248. [PubMed: 15985430]
25. Inman DM, Sappington RM, Horner PJ, Calkins DJ. Quantitative correlation of optic nerve pathology with ocular pressure and corneal thickness in the DBA/2 mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 2006;47:986–996. [PubMed: 16505033]
26. Schlamp CL, Li Y, Dietz JA, Janssen KT, Nickells RW. Progressive ganglion cell loss and optic nerve degeneration in DBA/2J mice is variable and asymmetric. *BMC Neurosci* 2006;7:66. [PubMed: 17018142]
27. Maffei L, Fiorentini A. Electrophoretographic responses to alternating gratings before and after section of the optic nerve. *Science* 1981;211:953–955. [PubMed: 7466369]
28. Porciatti V, Pizzorusso T, Cenni MC, Maffei L. The visual response of retinal ganglion cells is not altered by optic nerve transection in transgenic mice overexpressing Bcl-2. *Proc Natl Acad Sci USA* 1996;93:14955–14959. [PubMed: 8962163]
29. Porciatti V, Saleh M, Nagaraju M. The pattern electroretinogram as a tool to monitor progressive retinal ganglion cell dysfunction in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci* 2007;48:745–751. [PubMed: 17251473]
30. Danias J, Kontiola AI, Filippopoulos T, Mittag T. Method for the noninvasive measurement of intraocular pressure in mice. *Invest Ophthalmol Vis Sci* 2003;44:1138–1141. [PubMed: 12601041]
31. Wang WH, Millar JC, Pang IH, Wax MB, Clark AF. Noninvasive measurement of rodent intraocular pressure with a rebound tonometer. *Invest Ophthalmol Vis Sci* 2005;46:4617–4621. [PubMed: 16303957]
32. Riggs LA, Johnson EP, Schick AML. Electrical responses of the human eye to moving stimulus pattern. *Science* 1964;144:567–568. [PubMed: 17836392]
33. Zrenner, E. The physiological basis of the pattern electroretinogram. In: Osborne, N.; Chader, G., editors. *Progress in Retinal Research*. Oxford, UK: Pergamon Press; 1990. p. 427–464.
34. Hollander H, Bisti S, Maffei L, Hebel R. Electrophoretographic responses and retrograde changes of retinal morphology after intracranial optic nerve section: a quantitative analysis in the cat. *Exp Brain Res* 1984;55:483–493. [PubMed: 6468554]
35. Maffei L, Fiorentini A, Bisti S, Hollander H. Pattern ERG in the monkey after section of the optic nerve. *Exp Brain Res* 1985;59:423–425. [PubMed: 4029317]
36. Morrone C, Fiorentini A, Bisti S, Porciatti V, Burr DC. Pattern-reversal electroretinogram in response to chromatic stimuli: II. Monkey. *Vis Neurosci* 1994;11:873–884. [PubMed: 7947401]
37. Berardi N, Domenici L, Gravina A, Maffei L. Pattern ERG in rats following section of the optic nerve. *Exp Brain Res* 1990;79:539–546. [PubMed: 2340873]
38. Domenici L, Gravina A, Berardi N, Maffei L. Different effects of intracranial and intraorbital section of the optic nerve on the functional responses of rat retinal ganglion cells. *Exp Brain Res* 1991;86:579–584. [PubMed: 1761091]

39. Chierzi S, Cenni MC, Maffei L, et al. Protection of retinal ganglion cells and preservation of function after optic nerve lesion in bcl-2 transgenic mice. *Vision Res* 1998;38:1537–1543. [PubMed: 9667018]
40. Ratto GM, Bonfanti L, Cenni MC, et al. Retinal ganglion cell anatomy and physiology after section of the optic nerve in mice overexpressing bcl-2. *Adv Neurol* 1997;72:87–94. [PubMed: 8993687]
41. Sieving PA, Steinberg RH. Proximal retinal contribution to the intraretinal 8-Hz pattern ERG of cat. *J Neurophysiol* 1987;57:104–120. [PubMed: 3559667]
42. Baker CL Jr, Hess RR, Olsen BT, Zrenner E. Current source density analysis of linear and non-linear components of the primate electroretinogram. *J Physiol* 1988;407:155–176. [PubMed: 3256615]
43. Hess RF, Baker CL Jr. Human pattern-evoked electroretinogram. *J Neurophysiol* 1984;51:939–951. [PubMed: 6726319]
44. Drasdo N, Thompson DA, Thompson CM, Edwards L. Complementary components and local variations of the pattern electroretinogram. *Invest Ophthalmol Vis Sci* 1987;28:158–162. [PubMed: 3804646]
45. Drasdo N, Thompson DA, Arden GB. A comparison of pattern ERG amplitudes and nuclear layer thickness in different zones of the retina. *Clin Vision Sci* 1990;5:415–420.
46. Trimarchi C, Biral G, Domenici L, Porciatti V, Bisti S. The flash- and pattern electroretinogram generators in the cat: a pharmacological approach. *Clin Vision Sci* 1990;6:19–24.
47. Viswanathan S, Frishman LJ, Robson JG. The uniform field and pattern ERG in macaques with experimental glaucoma: removal of spiking activity. *Invest Ophthalmol Vis Sci* 2000;41:2797–2810. [PubMed: 10937600]
48. Siliprandi R, Bucci MG, Canella R, Carmignoto G. Flash and pattern electroretinograms during and after acute intraocular pressure elevation in cats. *Invest Ophthalmol Vis Sci* 1988;29:558–565. [PubMed: 3356513]
49. Feghali JG, Jin JC, Odom JV. Effect of short-term intraocular pressure elevation on the rabbit electroretinogram. *Invest Ophthalmol Vis Sci* 1991;32:2184–2189. [PubMed: 2071332]
50. Rossi FM, Pizzorusso T, Porciatti V, Marubio LM, Maffei L, Changeux JP. Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc Natl Acad Sci USA* 2001;98:6453–6458. [PubMed: 11344259]
51. Porciatti V, Pizzorusso T, Maffei L. The visual physiology of the wild type mouse determined with pattern VEPs. *Vision Res* 1999;39:3071–3081. [PubMed: 10664805]
52. Fraunfelder FT, Burns RP. Acute reversible lens opacity: caused by drugs, cold, anoxia, asphyxia, stress, death and dehydration. *Exp Eye Res* 1970;10:19–30. [PubMed: 5456775]
53. Ridder W 3rd, Nusinowitz S, Heckenlively JR. Causes of cataract development in anesthetized mice. *Exp Eye Res* 2002;75:365–370. [PubMed: 12384099]
54. Remtulla S, Hallett PE. A schematic eye for the mouse, and comparisons with the rat. *Vision Res* 1985;25:21–31. [PubMed: 3984214]
55. Schmucker C, Schaeffel F. A paraxial schematic eye model for the growing C57BL/6 mouse. *Vision Res* 2004;44:1857–1867. [PubMed: 15145680]
56. Lyubarsky AL, Daniele LL, Pugh EN Jr. From candelas to photoisomerizations in the mouse eye by rhodopsin bleaching in situ and the light-rearing dependence of the major components of the mouse ERG. *Vision Res* 2004;44:3235–3251. [PubMed: 15535992]
57. Smith, RS.; John, SWM.; Nishina, PM.; Sundberg, JP. *Systematic Evaluation of the Mouse Eye: Anatomy, Pathology, and Biomethods*. Boca Raton, FL: CRC Press; 2002.
58. Mabuchi F, Aihara M, Mackey MR, Lindsey JD, Weinreb RN. Optic nerve damage in experimental mouse ocular hypertension. *Invest Ophthalmol Vis Sci* 2003;44:4321–4330. [PubMed: 14507876]
59. Mabuchi F, Lindsey JD, Aihara M, Mackey MR, Weinreb RN. Optic nerve damage in mice with a targeted type I collagen mutation. *Invest Ophthalmol Vis Sci* 2004;45:1841–1845. [PubMed: 15161848]
60. Anderson MG, Libby RT, Gould DB, Smith RS, John SW. High-dose radiation with bone marrow transfer prevents neurodegeneration in an inherited glaucoma. *Proc Natl Acad Sci USA* 2005;102:4566–4571. [PubMed: 15758074]

61. Kocaoglu OP, Ulhorn SR, Hernandez E, et al. Simultaneous fundus imaging and optical coherence tomography of the mouse retina. *Invest Ophthalmol Vis Sci* 2007;48:1283–1289. [PubMed: 17325174]
62. Ogden TE. Nerve fiber layer of the primate retina: thickness and glial content. *Vision Res* 1983;23:581–587. [PubMed: 6612997]
63. Neufeld AH. Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma. *Arch Ophthalmol* 1999;117:1050–1056. [PubMed: 10448748]
64. Wang L, Cioffi GA, Cull G, Dong J, Fortune B. Immunohistologic evidence for retinal glial cell changes in human glaucoma. *Invest Ophthalmol Vis Sci* 2002;43:1088–1094. [PubMed: 11923250]
65. Wang X, Tay SS, Ng YK. An immunohistochemical study of neuronal and glial cell reactions in retinae of rats with experimental glaucoma. *Exp Brain Res* 2000;132:476–484. [PubMed: 10912828]
66. Naskar R, Wissing M, Thanos S. Detection of early neuron degeneration and accompanying microglial responses in the retina of a rat model of glaucoma. *Invest Ophthalmol Vis Sci* 2002;43:2962–2968. [PubMed: 12202516]
67. CTNGSG. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol* 1998;126:487–497. [PubMed: 9780093]
68. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone *klotho*. *Science* 2005;309:1829–1833. [PubMed: 16123266]
69. Ventura LM, Sorokac N, De Los Santos R, Feuer WJ, Porciatti V. The relationship between retinal ganglion cell function and retinal nerve fiber thickness in early glaucoma. *Invest Ophthalmol Vis Sci* 2006;47:3904–3911. [PubMed: 16936103]
70. Ventura LM, Porciatti V. Restoration of retinal ganglion cell function in early glaucoma after intraocular pressure reduction: a pilot study. *Ophthalmology* 2005;112:20–27. [PubMed: 15629815]
71. Libby RT, Li Y, Savinova OV, et al. Susceptibility to neurodegeneration in a glaucoma is modified by *Bax* gene dosage. *PLoS Genet* 2005;1:17–26. [PubMed: 16103918]
72. Reichstein D, Ren L, Filippopoulos T, Mittag T, Danias J. Apoptotic retinal ganglion cell death in the DBA/2 mouse model of glaucoma. *Exp Eye Res* 2007;84:13–21. [PubMed: 17074320]
73. Ward MS, Khoobehi A, Lavik EB, Langer R, Young MJ. Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres. *J Pharm Sci* 2007;96:558–568. [PubMed: 17177208]
74. Zhong L, Bradley J, Schubert W, et al. Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice. *Invest Ophthalmol Vis Sci* 2007;48:1212–1218. [PubMed: 17325165]
75. Weber AJ, Harman CD. Structure-function relations of parasol cells in the normal and glaucomatous primate retina. *Invest Ophthalmol Vis Sci* 2005;46:3197–3207. [PubMed: 16123419]
76. Neufeld AH, Liu B. Glaucomatous optic neuropathy: when glia misbehave. *Neuroscientist* 2003;9:485–495. [PubMed: 14678581]
77. Steele MR, Inman DM, Calkins DJ, Horner PJ, Vetter ML. Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. *Invest Ophthalmol Vis Sci* 2006;47:977–985. [PubMed: 16505032]
78. Schuettauf F, Rejdak R, Walski M, et al. Retinal neurodegeneration in the DBA/2J mouse—a model for ocular hypertension. *Acta Neuropathol (Berl)* 2004;107:352–358. [PubMed: 14745571]
79. Sieving PA, Steinberg RH. Contribution from proximal retina to intraretinal pattern ERG: the M-wave. *Invest Ophthalmol Vis Sci* 1985;26:1642–1647. [PubMed: 4055298]
80. Frishman LJ, Yamamoto F, Bogucka J, Steinberg RH. Light-evoked changes in $[K^+]_o$ in proximal portion of light-adapted cat retina. *J Neurophysiol* 1992;67:1201–1212. [PubMed: 1317916]
81. Kremmer S, Tolksdorf-Kremmer A, Stodtmeister R. Simultaneous registration of VECG and pattern ERG during artificially raised intraocular pressure (in German). *Ophthalmologica* 1995;209:233–241. [PubMed: 8570144]
82. Artal P, Herreros de Tejada P, Munoz Tedo C, Green DG. Retinal image quality in the rodent eye. *Vis Neurosci* 1998;15:597–605. [PubMed: 9682864]
83. de la Cera EG, Rodriguez G, Llorente L, Schaeffel F, Marcos S. Optical aberrations in the mouse eye. *Vision Res* 2006;46:2546–2553. [PubMed: 16516259]

84. CNTGSG. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol* 1998;126:498–505. [PubMed: 9780094]
85. Nissirios N, Goldblum D, Rohrer K, Mittag T, Danias J. Noninvasive determination of intraocular pressure (IOP) in nonsedated mice of 5 different inbred strains. *J Glaucoma* 2007;16:57–61. [PubMed: 17224751]
86. Avila MY, Carre DA, Stone RA, Civan MM. Reliable measurement of mouse intraocular pressure by a servo-null micropipette system. *Invest Ophthalmol Vis Sci* 2001;42:1841–1846. [PubMed: 11431452]
87. John SW, Hagaman JR, MacTaggart TE, Peng L, Smithes O. Intraocular pressure in inbred mouse strains. *Invest Ophthalmol Vis Sci* 1997;38:249–253. [PubMed: 9008647]
88. Savinova OV, Sugiyama F, Martin JE, et al. Intraocular pressure in genetically distinct mice: an update and strain survey. *BMC Genet* 2001;2:12. [PubMed: 11532192]
89. Aihara M, Lindsey JD, Weinreb RN. Reduction of intraocular pressure in mouse eyes treated with latanoprost. *Invest Ophthalmol Vis Sci* 2002;43:146–150. [PubMed: 11773025]
90. Mo JS, Anderson MG, Gregory M, et al. By altering ocular immune privilege, bone marrow-derived cells pathogenically contribute to DBA/2J pigmentary glaucoma. *J Exp Med* 2003;197:1335–1344. [PubMed: 12756269]
91. Schuettauf F, Quinto K, Naskar R, Zurakowski D. Effects of anti-glaucoma medications on ganglion cell survival: the DBA/2J mouse model. *Vision Res* 2002;42:2333–2337. [PubMed: 12350421]
92. Aihara M, Lindsey JD, Weinreb RN. Episcleral venous pressure of mouse eye and effect of body position. *Curr Eye Res* 2003;27:355–362. [PubMed: 14704919]

Figure 1.

Representative examples of PERG (A) and FERG (B) recorded in DBA/2J mice aged 3 months. For both PERG and FERG, the amplitude was measured from the positive peak to the negative trough. The latency was evaluated from the stimulus onset (contrast reversal for the PERG, strobe flash for the FERG) to the peak of the positive wave. Note that the PERG had a lower amplitude and longer latency compared to the FERG. (C) Axial section through the optic nerve head of a DBA/2J mouse aged 10 months and stained with hematoxylin–eosin. The thickness of the RNFL was measured at point of curvature of axons entering the optic nerve head.

Figure 2.

Progressive changes of PERG amplitude (A), PERG latency (B), FERG amplitude (C), and FERG latency (D) with age. *Thick symbols connected by thick lines*: mean $\pm$ SEM. *Thin lines*: amplitudes longitudinally measured in individual eyes. *Dashed lines*: upper limit of the noise range. Note that the PERG amplitude (A), but not FERG amplitude (C) progressively decreased with age and reached the noise level by approximately 11 months. *Interrupted thin lines* at intermediate ages represent eyes for which follow-up could not be completed due to death or development of cataract.

Figure 3.

Residual RNFL thickness at the PERG endpoint. By the time the PERG was at noise level in at least one eye (age range, 9–12 months) the RNFL had lost ~40% of normal thickness (2-month-old mice) but was still much thicker (~135%) than that in 16-month-old mice with advanced glaucoma. RNFL data have been analyzed separately for eyes with worse PERG amplitude (first eye) and better PERG amplitude (second eye) at the endpoint.

Figure 4.

Average PERG amplitude, RNFL thickness for both eyes as a function of age. Data are plotted on a normalized scale. Error bars, SD. Axon counts at different ages obtained from previous studies are also plotted for comparison. Note that progressive loss of RGC axons lags behind progressive loss of PERG amplitude by approximately 3 months. Also note that relative changes in RNFL thickness are in agreement with corresponding changes in RGC axons.

Figure 5.

Progressive changes of IOP as a function of age. *Thick symbols connected by thick lines*: mean $\pm$ SEM. *Thin lines*: longitudinally measured IOP in individual eyes. *Interrupted thin lines* at intermediate ages represent eyes for which follow-up could not be completed due to death or cataract development. Average PERG amplitude ($\pm$ SEM) data shown in Figure 2A (*bottom trace*) are also displayed for comparison. For IOP data, the y-axis corresponds to mm Hg; for PERG data, the y-axis corresponds to μ V. Note that the close association between IOP increase and PERG amplitude decrease.

Figure 6. PERG amplitude as a function of IOP in individual eyes. (A) Linear PERG amplitude scale; (B) log PERG amplitude scale; *solid lines*: linear regression of data and $\pm 95\%$ CI. *Dashed lines*: the upper limit of the noise range. Note the good correlation between PERG amplitude and IOP.