Published in final edited form as:

Invest Ophthalmol Vis Sci. 2007 February ; 48(2): 745–751.

The pattern electroretinogram as a tool to monitor progressive retinal ganglion cell dysfunction in the DBA/2J mouse model of glaucoma

Vittorio Porciatti, Maher Saleh, and Mahesh Nagaraju

Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL 33136

Abstract

Purpose—To determine the baseline characteristics, reliability and dynamic range of the pattern electroretinogram (PERG) as a tool to monitor progressive RGC dysfunction in the DBA/2J mouse model of glaucoma with spontaneously elevated intraocular pressure (IOP).

Methods—PERGs were recorded from 56 undilated eyes of 28 anesthetized (Ketamine-Xylazine-Acepromazine) DBA/2J mice of different ages (2–4 months, $n = 44$ eyes ; 12–14 months, $n = 12$ eyes) in response to contrast-reversal of gratings that maximize PERG amplitude (95% contrast, 1 Hz reversal, 0.05 cycles/degree spatial frequency, 50×56 deg field size). Robust averaging (1800 sweeps) was used to isolate PERG from background noise. Cone-driven ERGs in response to diffuse light flashes superimposed on a rodadapting background (FERG) were also recorded.

Results—PERGs had consistent waveforms and were reproducible across batches of mice and operators. In 2–4 month-old mice (pre-hypertensive stage), the PERG amplitude (mean $8.15 \mu\text{V}$, SEM 0.4) was considerably larger than the noise (mean $1.18 \mu\text{V}$, SEM 0.1). The test-retest variability (two different sessions one week apart) and interocular asymmetry of PERG amplitude was about 30%, and that of PERG latency was about 17%. In 12–14 month old mice (advanced hypertensive stage) the PERG amplitude (mean $1.29 \mu\text{V}$, SEM 0.12) was close to that of noise. In 12–14 month old mice the FERG was reduced to a lesser extent compared to the PERG.

Conclusions—The PERG has adequate signal to noise ratio, reproducibility, and dynamic range to monitor progression of functional changes of inner retina in DBA/2J mice.

Introduction

The use of mouse models of glaucoma is currently limited by the paucity of non-invasive methods able to monitor retinal ganglion cell (RGC) dysfunction and its progression. Electrical activity in the retina associated with RGC function can be evaluated by means of the pattern electroretinogram (PERG). The PERG is obtained in response to contrast-reversal of patterned visual stimuli (gratings, checkerboards), rather than uniform flashes of light.¹ RGCs are necessary for the PERG generation, since their selective loss after optic nerve transection in mammals,^{2, 3} including mice,⁴ abolishes the response. In experimental primate models of optic nerve transection³ and glaucoma,⁵ the amount of PERG amplitude reduction is consistent with the degree of damage apparent by counting either RGCs or optic nerve fibers. In the same experimental animals the a- and b-waves of the conventional bright flash-ERG are little or not affected. An important distinguishing characteristic of the PERG is that it requires the physiological integrity of viable RGCs to be generated. The PERG amplitude can be reversibly

Corresponding author: Vittorio Porciatti, DSc e-mail vporciatti@med.miami.edu Tel. 305-326-6050, Fax 305-482-4567.

Financial support: NIH RO3 EY016322, NIH center grant P30-EY14801, unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc.

reduced by intravitreal injections of tetrodotoxin which block the spiking activity in the inner retina.^{6, 7} Short-term elevation of the intraocular pressure (IOP) has the effect of reversibly reducing the PERG amplitude but not the flash-ERG.^{8, 9} Altogether, these findings indicate that reduction in PERG amplitude may reflect both the reduced activity of dysfunctional, yet viable, RGCs as well as the lack of activity of lost RGCs. The PERG, therefore, may represent an important tool to detect and monitor the onset and the progression of RGC dysfunction in mouse models of glaucoma. In addition, the PERG allows establishing retinal resolution and contrast threshold^{4, 10} that have a counterpart in cortical visual acuity and contrast sensitivity.¹¹ Finally, the PERG stimulus can be precisely located on the retina, thereby allowing quantitative structure-function correlations for specific retinal regions,¹² as well as topological assessment of RGC losses in glaucomatous DBA/2J mice.¹³ These PERG properties may provide important information to characterize glaucomatous disease and the effect of neuroprotective agents on retinal function. For the above reasons we focused on the PERG rather than other potentially useful techniques in rodents such as the scotopic threshold response (STR) of the full-field flash ERG.¹⁴⁻¹⁶

Despite its great potential for monitoring RGC dysfunction in glaucoma models, the PERG has not been widely adopted probably because considered technically difficult to record.¹⁴ The purpose of this study was to determine the baseline characteristics, reliability and dynamic range¹¹ of an optimized version of the PERG as a tool to systematically monitor progressive RGC dysfunction in the DBA/2J mouse model of glaucoma with spontaneously elevated intraocular pressure.^{17, 18} DBA/2J mice develop an iris disease characterized by iris atrophy and pigment dispersion due to mutations of two genes, *Gpnmb* and *Tyrp1*.¹⁹ The iris disease is apparent at 6 months and progresses with age, resulting in elevated intraocular pressure (IOP).¹⁸ Young (2-4 month-old) DBA/2J mice have normal IOP and normal histological appearance of RGCs and optic nerve.^{17, 18} Glaucoma damage in the optic nerve is apparent in about 75% of eyes by 13 months,¹⁸ and in about 90% of eyes by 18 months,¹⁸ and appears to be IOP dependent.²⁰ By 16 months cataract may develop.¹⁷ Preliminary results of this study have been previously shown in abstract form.²¹

Methods

A total of 28 DBA/2J mice (n=56 eyes) of both sexes and different ages obtained by Jackson Labs, Bar Harbor, Maine, were recorded. All animals were treated according to protocols established by the Association for Research in Vision and Ophthalmology. The experimental protocol was approved by the Animal Care and Use Committee of the University of Miami. Mice were anesthetized with I.P injections (0.5-0.7 ml/Kg) of a mixture of Ketamine, 42.8 mg/ml; Xylazine, 8.6 mg/ml; Acepromazine, 1.4 mg/ml. The head of anesthetized mice was gently restrained in a modified stereotaxic apparatus¹¹ in which the tips of ear bars are covered with flexible plastic material. A mouth bite and a small nose holder, which allows unobstructed vision, were used to restrain the anterior part of the head. 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 proptosed, with undilated pupils pointing laterally and upwardly.

PERG recording

The recording electrode was made of a thin silver wire (0.25 mm diameter) configured to a semi-circular loop of 2 mm radius (figure 1). Electrodes were simply obtained by bending the silver wire around a screwdriver with 2 mm diameter. Electrodes for the right eye and left eye had mirror geometry, the vertical portion of the electrode connected with the electrode holder being on the temporal side of the eye in order not to interfere with vision. The electrodes were gently leaned on the corneal surface by means of a micromanipulator under microscopic control

and positioned in such a way to encircle the undilated pupil without limiting the field of view (figure 1). Electrode positioning entailed minimal manipulation of the eye. The thin diameter of the silver wire allowed the electrode to follow small forward-backward eye movements associated with breathing that might otherwise increase the noise and even cause corneal abrasion. Corneal stimulation is known to easily induce cataract in the mouse, which would preclude further PERG testing.²² This electrode placement allowed easily checking for possible development of cataract, as well as controlling that the pupil projection was centered on the pattern stimulus. Instillations of BSS every 30' were sufficient to maintain the cornea and lens in excellent conditions for the duration of recording (about 1 hour). After a long experience with mouse PERG⁴ this simple electrode geometry and placement was found to give the most consistent results. Reference and ground electrodes were small stainless steel needles inserted in the skin of the back of head and the back of the body, respectively.

Previous studies in C57BL6/J mice have shown that the PERG amplitude is maximal at 0.05 cycles per degree and low reversal frequency (1 Hz).²³ The PERG amplitude progressively decreases with increasing temporal frequency (temporal resolution about 13 Hz),²⁵ increasing spatial frequency (spatial resolution about 0.5 cycles/deg),^{4, 10, 23} decreasing contrast (contrast threshold about 10%),^{4, 23} decreasing luminance,²³ and decreasing stimulus area.²³ The spatio-temporal properties of the 2-4 month-old DBA/2J mouse PERG closely corresponded to those reported for C57BL6/J (not shown in figures). To maximize PERG amplitude, patterned visual stimuli were therefore high contrast (98%) horizontal gratings of 0.05 cycles/deg spatial frequency with square-wave profile, abruptly reversing in spatial phase at 1 Hz (2 contrast reversals/s) and 50 cd/m² mean luminance. Stimuli were generated by a Cambridge Research System VSG3 video card (Cambridge Research Systems Ltd, Kent, UK) and displayed on a 21" TV monitor (Sony Multiscan G500). To further maximize PERG amplitude, a large stimulus area (50.2 × 58.0 deg) was obtained by presenting pattern stimulus from short distance (20 cm). The stimulus display contained 3 full stimulus cycles. Each bar subtended a visual angle of 8.4 deg, which approximately corresponds to the average size of the receptive field centers of mouse retinal ganglion cells.^{24, 25} The center of visual stimulus was aligned with the projection of the pupil. 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. Uniform stimuli for FERG recording consisted of strobe flashes stimuli of (20 cd/m²/s) superimposed on a steady background light (12 cd/m²) and presented within a Ganzfeld bowl. Under these conditions rod activity is largely suppressed while cone activity is minimally suppressed.²⁶

Recording protocol

Anesthetized mice were mounted on the holder, a corneal electrode was placed on the right eye, a small drop of BSS was added to prevent corneal dryness, and body temperature was allowed to stabilize at 37.0 °C. Typical protocol consisted of a series of 3 consecutive responses to 600 contrast reversals each (PERG recording). The responses were superimposed to check for consistency and then averaged. Overall, 1800 sweeps were averaged, thereby reducing the uncorrelated electrical activity by a factor of $\sqrt{1800} = 42$. To have an estimate of the residual noise, the PERG recording was preceded by an additional recording of 600 sweeps, in which the contrast was set to zero. Immediately after PERG recording, the stimulus was switched to the strobe flash. Three consecutive responses to 30 flashes each (FERG) were recorded. After visual inspection for consistency, responses were averaged (the uncorrelated electrical activity was reduced by a factor of $\sqrt{90} = 9.5$). After PERG and FERG recording from the right eye, additional anesthesia was given as needed, a new electrode was placed on the left eye, and the procedure was repeated. The entire protocol required about one hour. Averaged PERGs and FERGs were automatically analyzed to evaluate the major positive and negative waves

(figure1). For both PERG and FERG, response amplitude was evaluated peak-to-trough, and the latency was the time-to-peak of the major positive deflection.

Results

PERG and FERG waveforms obtained in 44 eyes of 22 DBA/2J mice aged 2-4 months (pre-glaucoma stage) are displayed in figure 2. Thin lines correspond to individual waveforms, and thick lines correspond to the averaged waveform. PERG and FERG waveforms are reasonably reproducible in different eyes. Note that consistent waveforms are obtained from all tested eyes. Also note that two different batches of mice, recorded by two different operators, have very similar PERG and FERG average waveforms.

Amplitudes and latencies of individual waveforms are displayed in figure 3 as amplitude-latency scattergrams. Since PERGs and FERGs have markedly different amplitudes and latencies, $\log_{10}$ scales were used to compare them on the same plot. Note in fig. 3 that, on average, PERGs have smaller amplitudes and longer latencies compared to cone-driven FERGs. However, the spread of data is similar for both kinds of responses.

Interindividual variability

Mean PERG and FERG amplitudes are reported in Table I. Note that the Coefficient of Variations (SD/Mean) are comparable between the responses two eyes, and that the CV of PERG amplitude and latency are of the same order of magnitude as those of the FERG. For both PERG and FERG, the CV of latency is smaller than that of amplitude. As shown in fig. 3, PERG and FERG amplitudes can vary over a wide range among individuals. If an eye with a large PERG also had a large FERG, then normalizing the PERG amplitude to the FERG amplitude (computing a PERG/FERG amplitude ratio) would in principle reduce interindividual variability. However the Pearson correlation between PERG amplitude and FERG amplitude was weak ($R=0.23$, $p=0.15$). The CV of the PERG/FERG ratio (Table I) was of the same order of magnitude or even larger than that of raw PERG and FERG amplitudes. Therefore there is no apparent advantage in computing a PERG/FERG amplitude ratio.

Reproducibility of PERG

A subset of 8 mice aged 2-4 months has been tested twice in two different sessions with one week interval. The results of the first session have been included in the core sample (Table I). We compared the variation occurring between the responses of the same eyes recorded in the two different sessions (test retest variability) with that occurring between the right and the left eye recorded in sequence (about 1/2 hour apart) during the same session (interocular asymmetry). Variation has been calculated according to the formula [Variation = $|test1 - test2|/2$ (test1 + test 2)]. The results are reported in Table II. For both PERG and FERG amplitude, the test retest variability and interocular asymmetry are of the order of 0.3 (30%). The variability of PERG and FERG latency is typically smaller than that of PERG and FERG amplitude. As for interindividual variability, the within-individual variability of the PERG/FERG amplitude ratio is larger than that of PERG and FERG amplitude.

Dynamic range of PERG

The dynamic range of PERG amplitude can be defined as the interval between the level of signal when a normal number of functioning RGCs are present (i.e., young DBA/2J mice) and the level of noise (the PERG amplitude in response to a pattern stimulus with zero contrast).

PERG and FERG waveforms obtained in 12 eyes of 6 DBA/2J mice aged 12-14 months (advanced glaucoma stage) are displayed in figure 4. Thin lines correspond to individual waveforms, and thick lines correspond to the averaged waveform. Note that PERGs of old

mice have dramatically reduced amplitude compared to those of young mice depicted in fig. 2 A and B. By contrast, the FERG is still recordable although with reduced amplitude. The average latency and latency scatter are approximately similar to those of young DBA/2J mice.

Average PERG and FERG amplitudes of young and old mice evaluated from individual waveforms are compared in figure 5. The PERG amplitude of 2-4 month old mice (mean 8.15 μ V, SEM 0.4, n =44 eyes) is higher than the noise amplitude (mean 1.18 μ V, SEM 0.1, n =12) by a factor of 6.9. The PERG amplitude of older mice (1.29 μ V, SEM, n =12) is close to that of the noise. Overall, the PERG amplitude is reduced by 6.8 μ V (84%) between 3 and 13 months of age in DBA/2J mice. If the percentage of amplitude loss is rescaled on the response dynamic range (difference between average amplitude of young mice and the average noise amplitude), then the PERG amplitude of 13 month-old mice appears to have lost approximately 98% of its dynamic range. Older mice presented typical iris stromal atrophy, slightly enlarged pupil and moderate corneal precipitates.¹⁸ Corneal precipitates involved only minimally the pupil area, however, and no apparent cataract was present. It is unlikely that reduced PERG amplitude in older mice is due optical factors that degrade the stimulus contrast. At 13 months of age, the FERG is also significantly ($P < 0.01$) reduced in amplitude by 38%. The amount of FERG amplitude reduction is less than that of the PERG, indicating that outer retina function may be relatively spared in advanced stages of glaucoma. A previous study also reported changes of comparable magnitude in the diffuse-flash ERG in old DBA/2NNia mice.²⁷

Discussion

Functional outcome measures are sorely needed for following the progression of glaucoma in mice, and for assessing therapeutic effects of potential neuroprotective treatments. In this study we determined the baseline characteristics, reproducibility, and dynamic range of an optimized version of the PERG as a tool to monitor progressive RGC dysfunction in the DBA/2J mouse model of glaucoma. The PERG technique may have the potential to specifically indicate RGC dysfunction or loss, since RGC death induced by optic nerve transection in the C57BL/6J mouse abolishes the PERG response, whereas it leaves the luminance-flicker ERG unaltered.⁴ The present study indicates that by about 13 months of age the PERG is virtually abolished in the DBA/2J mouse glaucoma model. PERG alterations are unlikely to depend on optical factors such as corneal/lens opacities, or defocus. A detailed histological study of the DBA/2J mouse retina with advanced glaucoma revealed no cell loss in any retinal neurons other than ganglion cells.¹³ In that study, no group of RGCs was found to be especially vulnerable or resistant to degeneration. Axonal atrophy, dendritic remodeling, and somal shrinkage seemed to precede ganglion cell death. It is conceivable that PERG losses found in our study are associated to RGC loss and/or abnormalities of RGC architecture. We cannot exclude, however, a possible association between PERG losses and ischemic effects as well as glial activation reported for some old DBA/2J mice obtained from Charles River (Sulzfeld, Germany).²⁸

Optimization of the PERG technique

In order to provide a robust index of RGC function for systematic use in neuroprotection studies, in this study we used a PERG protocol that maximizes the response amplitude and minimizes the residual noise. Under the present recording conditions, the PERG is recordable in pre-glaucomatous DBA/2J mice with high signal to noise ratio (about 7:1 on average). This level of signal to noise ratio is considerably higher than that reported in a previous pilot PERG study of DBA/NNia mice using different experimental conditions.²⁹ The absolute level of PERG amplitude in pre-glaucomatous DBA/2J mice is of the same order of magnitude as that reported for the rat STR.¹⁴

Response reproducibility

Comparable PERG waveforms were obtained in all tested eyes of 2-4 month old mice, independently of whether they belonged to different batches, or were recorded from different operators. In addition, PERG waveforms were comparable between eyes of the same animals during the same session and between the same eyes recorded in different sessions.

Interindividual variability

There was a wide range of PERG amplitudes among eyes of 2-4 month old mice, resulting in a CV (SD/mean) of about 30%. This value is at least twice as large as the interindividual variability of RGC number reported in young DBA/2J³⁰ and young DBA/2NNia.³¹ This suggests that there may be other sources of PERG variability, such as anesthesia, electrode placement, and residual noise. A CV of 30%, however, is of the same order of magnitude as that reported for human PERG³²⁻³⁴ as well as for human ordinary flash-evoked ERG.^{35, 36} In our own study, the interindividual variability of cone-driven flash ERG amplitude is comparable to that of the PERG amplitude. Similar variability for FERG and PERG would exclude optical factors (incorrect stimulus alignment, reduction in stimulus contrast due to image blurring) as a major source of PERG variability, since the flash-ERG is virtually independent from these optical factors.

Test-retest variability

The intersession variability of PERG amplitude is about 30%. These values are somewhat larger than those reported for the monkey PERG³⁷ and some studies of human PERG.^{38, 39} However, other human PERG studies report similar ranges of variability.⁴⁰⁻⁴²

Interocular asymmetry

The progression of glaucoma is often asymmetrical in both human patients⁴³ and DBA/2J mice.¹⁸ Differences in PERG amplitude and latency between the two eyes may therefore represent an important criterion in the evaluation of changes in RGC function. In future neuroprotection studies, the level of interocular asymmetry may set the sensitivity limit for comparing the effect of treatment between the treated- and the sham-treated eye. Our results show that the variability due to interocular asymmetry (within session) is comparable to the test retest variability (between sessions). It has to be taken into account that in the present protocol the two eyes are recorded in sequence during the same session. Therefore, it is possible that animal conditions (primarily the level of anesthesia) may vary during the same session to an extent comparable to that occurring when testing the same eyes in two different sessions. This may limit the precise measurement of interocular asymmetry. We are currently testing a new system in which the PERG is simultaneously recorded from both eyes. This would allow recording both eyes under identical conditions and would shorten the recording time. Preliminary results obtained by simultaneous PERG recording of both eyes indicated that PERG interocular asymmetry in young DBA/2J is substantially smaller compared to that obtained using the present protocol.

Dynamic range

The PERG amplitude of 12-14 months old mice is much reduced compared to that 2-4 month-old DBA/2J mice, having lost about 98% of its dynamic range. This suggests that after one year of age the residual activity of RGCs and/or the inner retina circuitry impinging on them is small or absent. At 13 months of age histological damage of the optic nerve is apparent in about 75% of eyes.¹⁸ Preliminary results of our group^{21, 44} indicate that in DBA/2J mice the PERG is already much reduced between 10 and 11 months of age, when histological damage is evident in only a minority (40%) of eyes. This would suggest that RGC dysfunction may precede histologically visible damage of RGCs and the optic nerve.

Conclusions

The PERG can be reliably recorded in DBA/2J mice. With our optimized protocol the PERG has adequate signal to noise ratio, reproducibility, and dynamic range. In addition, the PERG is dramatically reduced in glaucomatous mice. These represent adequate conditions to monitor progression of functional changes of inner retina in DBA/2J mice. We are currently performing a longitudinal PERG study indicating that monitoring progression of RGC dysfunction is indeed possible in individual DBA/2J mice. This would open the possibility of using the DBA/2J mouse model as a template for systematic studies of neuroprotection including functional endpoints. It remains to be established whether the PERG represents an adequate technique for monitoring RGC dysfunction in other mouse glaucoma models.⁴⁵

Acknowledgements

We wish to thank Allergan, Inc. for supporting Maher Saleh.

References

1. Riggs LA, Johnson EP, Schick AML. Electrical responses of the human eye to moving stimulus pattern. *Science* 1964;144:567–8. [PubMed: 17836392]
2. Maffei L, Fiorentini A. Electoretinographic responses to alternating gratings before and after section of the optic nerve. *Science* 1981;211(4485):953–5. [PubMed: 7466369]
3. Maffei L, Fiorentini A, Bisti S, Hollander H. Pattern ERG in the monkey after section of the optic nerve. *Exp Brain Res* 1985;59(2):423–5. [PubMed: 4029317]
4. 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 U S A* 1996;93(25):14955–9. [PubMed: 8962163]
5. Johnson MA, Drum BA, Quigley HA, et al. Pattern-evoked potentials and optic nerve fiber loss in monocular laser-induced glaucoma. *Invest Ophthalmol Vis Sci* 1989;30(5):897–907. [PubMed: 2722446]
6. Trimarchi C, Biral G, Domenici L, et al. The Flash- and pattern electroretinogram generators in the cat: a pharmacological approach. *Clin. Vision Sci* 1990;6:19–24.
7. 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(9):2797–810. [PubMed: 10937600]
8. 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(4):558–65. [PubMed: 3356513]
9. Feghali JG, Jin JC, Odom JV. Effect of short-term intraocular pressure elevation on the rabbit electroretinogram. *Invest Ophthalmol Vis Sci* 1991;32(8):2184–9. [PubMed: 2071332]
10. Rossi FM, Pizzorusso T, Porciatti V, et al. Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc Natl Acad Sci U S A* 2001;98(11):6453–8. [PubMed: 11344259]
11. Porciatti V, Pizzorusso T, Maffei L. The visual physiology of the wild type mouse determined with pattern VEPs. *Vision Res* 1999;39(18):3071–81. [PubMed: 10664805]
12. Drasdo N, Thompson DA, Arden GB. A comparison of pattern ERG amplitudes and nuclear layer thickness in different zones of the retina. *Clin Vision Sciences* 1990;5(4):415–20.
13. Jakobs TC, Libby RT, Ben Y, et al. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. *J Cell Biol* 2005;171(2):313–25. [PubMed: 16247030]
14. Fortune B, Bui BV, Morrison JC, et al. Selective ganglion cell functional loss in rats with experimental glaucoma. *Invest Ophthalmol Vis Sci* 2004;45(6):1854–62. [PubMed: 15161850]
15. Bui BV, Fortune B. Ganglion cell contributions to the rat full-field electroretinogram. *J Physiol* 2004;555(Pt 1):153–73. [PubMed: 14578484]
16. Saszik SM, Robson JG, Frishman LJ. The scotopic threshold response of the dark-adapted electroretinogram of the mouse. *J Physiol* 2002;543(Pt 3):899–916. [PubMed: 12231647]

17. 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(6):951–62. [PubMed: 9579474]
18. 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(5):637–48. [PubMed: 16332275]
19. 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(1):81–5. [PubMed: 11743578]
20. 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(3):986–96. [PubMed: 16505033]
21. Porciatti V, Tapia M, Lee RK. Age-related Changes Of The Pattern lectroretinogram In The DBA/2J Mouse Model Of Glaucoma. 2006ARVO # 4011
22. Fraunfelder FT, Burns RP. Acute reversible lens opacity: caused by drugs, cold, anoxia, asphyxia, stress, death and dehydration. *Exp Eye Res* 1970;10(1):19–30. [PubMed: 5456775]
23. Porciatti V, Falsini B. Physiological Properties of the Mouse Pattern Electroretinogram. 2003ARVO #2705
24. Balkema GW Jr, Pinto LH. Electrophysiology of retinal ganglion cells in the mouse: a study of a normally pigmented mouse and a congenic hypopigmentation mutant, pearl. *J Neurophysiol* 1982;48(4):968–80. [PubMed: 7143036]
25. Stone C, Pinto LH. Response properties of ganglion cells in the isolated mouse retina. *Vis Neurosci* 1993;10(1):31–9. [PubMed: 8424927]
26. 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(28):3235–51. [PubMed: 15535992]
27. Bayer AU, Neuhardt T, May AC, et al. Retinal morphology and ERG response in the DBA/2NNia mouse model of angle-closure glaucoma. *Invest Ophthalmol Vis Sci* 2001;42(6):1258–65. [PubMed: 11328737]
28. Schuetttauf F, Rejdak R, Walski M, et al. Retinal neurodegeneration in the DBA/2J mouse-a model for ocular hypertension. *Acta Neuropathol (Berl)* 2004;107(4):352–8. [PubMed: 14745571]
29. Bayer AU, Brodie SE, Mittag T. Pilot study of pattern-electroretinographic changes in the DBA/2NNia mouse. Animal model of congenital angle-closure glaucoma. *Ophthalmologe* 2001;98(3):248–52. [PubMed: 11320811]
30. Williams RW, Strom RC, Rice DS, Goldowitz D. Genetic and environmental control of variation in retinal ganglion cell number in mice. *J Neurosci* 1996;16(22):7193–205. [PubMed: 8929428]
31. Danias J, Lee KC, Zamora MF, et al. Quantitative analysis of retinal ganglion cell (RGC) loss in aging DBA/2NNia glaucomatous mice: comparison with RGC loss in aging C57/BL6 mice. *Invest Ophthalmol Vis Sci* 2003;44(12):5151–62. [PubMed: 14638711]
32. Graham SL, Drance SM, Chauhan BC, et al. Comparison of psychophysical and electrophysiological testing in early glaucoma. *Invest Ophthalmol Vis Sci* 1996;37(13):2651–62. [PubMed: 8977479]
33. Garway-Heath DF, Holder GE, Fitzke FW, Hitchings RA. Relationship between Electrophysiological, Psychophysical, and Anatomical Measurements in Glaucoma. *Invest Ophthalmol Vis Sci* 2002;43(7):2213–20. [PubMed: 12091419]
34. Ventura LM, Porciatti V, Ishida K, et al. Pattern electroretinogram abnormality and glaucoma. *Ophthalmology* 2005;112(1):10–9. [PubMed: 15629814]
35. Birch DG, Anderson JL. Standardized full-field electroretinography. Normal values and their variation with age. *Arch Ophthalmol* 1992;110(11):1571–6. [PubMed: 1444914]
36. Bradshaw K, Hansen R, Fulton A. Comparison of ERGs recorded with skin and corneal-contact electrodes in normal children and adults. *Doc Ophthalmol* 2004;109(1):43–55. [PubMed: 15675199]
37. Bui BV, Fortune B, Cull G, et al. Baseline characteristics of the transient pattern electroretinogram in non-human primates: inter-ocular and inter-session variability. *Exp Eye Res* 2003;77(5):555–66. [PubMed: 14550397]
38. Bach M. Electrophysiological approaches for early detection of glaucoma. *Eur J Ophthalmol* 2001;11(Suppl 2):S41–9. [PubMed: 11592530]

39. Porciatti V, Ventura LM. Normative data for a user-friendly paradigm for pattern electroretinogram recording. *Ophthalmology* 2004;111(1):161–8. [PubMed: 14711729]
40. Holopigian K, Snow J, Seiple W, Siegel I. Variability of the pattern electroretinogram. *Doc Ophthalmol* 1988;70(1):103–15. [PubMed: 3229288]
41. Bartel P, Becker P, Robinson E. The intrasession repeatability of pattern electroretinograms and the effects of digital filtering. *Doc Ophthalmol* 1991;76(4):351–8. [PubMed: 1935543]
42. Jacobi PC, Walter P, Brunner R, Kriegelstein GK. Reproducibility and intraindividual variability of the pattern electroretinogram. *Ger J Ophthalmol* 1994;3(45):216–9. [PubMed: 7804106]
43. Zeyen TG, Caprioli J. Progression of disc and field damage in early glaucoma. *Arch Ophthalmol* 1993;111(4):62–5. [PubMed: 8424726]
44. Libby RT, Porciatti V, Tapia M, et al. Perg Analysis Detects Physiological Dysfunction Prior To Ganglion Cell Loss In DBA/2J Glaucoma. 2006ARVO # 4005
45. Lindsey JD, Weinreb RN. Elevated intraocular pressure and transgenic applications in the mouse. *J Glaucoma* 2005;14(4):318–20. [PubMed: 15990617]

Figure 1.
Geometry and placement of the PERG electrode.

Figure 2.

PERG and FERG waveforms recorded in 22 young (3-4 month old) DBA/2J mice (n=44 tested eyes). Waveforms shown on the left panels (A, C) and on the right panels (B, D) have been obtained from different operators in independent batches of mice. Thin lines correspond to individual waveforms. Thick lines correspond to the average waveform.

Figure 3. Amplitude-latency scattergrams of PERGs (filled symbols) and FERGs (open symbols) recorded from 44 eyes of 22 DBA/2J mice of 2-4 months of age.

Figure 4. PERG (A) and FERG (B) waveforms recorded in 6 old (12-14 month old) DBA/2J mice (n=12 tested eyes). Thin lines correspond to individual waveforms. Thick lines correspond to the average waveform.

Figure 5. Mean PERG (A) and FERG (B) amplitudes recorded in young (2-4 month old, n=44 eyes) and aged (12-14 month old, n=12 eyes) DBA/2J mice. Noise represents the PERG amplitude recorded in young mice (n=12 eyes) in response to a stimulus of zero contrast. Error bars represent the standard error of the mean.

Table 1
Average PERG and FERG amplitudes, latencies and PERG/FERG amplitude ratios evaluated in 22 DBA/2J mice aged 2-4 months.

	N=22 right eyes			N=22 left eyes		
	Mean	SD	CV	Mean	SD	CV
PERG amplitude	8.25	2.51	0.30	PERG amplitude	8.05	0.38
PERG latency	154.18	32.68	0.21	PERG latency	159.00	0.19
FERG amplitude	67.37	27.85	0.41	FERG amplitude	49.56	0.34
FERG latency	74.25	17.00	0.23	FERG latency	86.76	0.15
PERG/FERG amplitude ratio	0.14	0.05	0.36	PERG/FERG amplitude ratio	0.18	0.44

Table II

Variation $[(\text{test1} - \text{test2})/\frac{1}{2}(\text{test1} + \text{test2})]$ between the two eyes within the same session (interocular asymmetry) and between the same eyes in two different sessions (test retest variability).

	Interocular asymmetry	SD	Test retest variability	SD
PERG amplitude	0.30	0.23	0.29	0.21
PERG latency	0.16	0.15	0.17	0.20
FERG amplitude	0.25	0.15	0.34	0.23
FERG latency	0.20	0.12	0.21	0.16
PERG/FERG amplitude ratio	0.4	0.31	0.49	0.27
number of observations	16		16	