IOP-dependent retinal ganglion cell dysfunction in glaucomatous DBA/2J mice

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Abstract

Purpose: To characterize the effect of postural IOP elevation and pharmacological IOP lowering on retinal ganglion cell (RGC) function in the DBA/2J mouse model of glaucoma.

Methods: Four groups of DBA/2J mice (3 month old, n=7; 5 month old, n=7, 10 month old, n=7, 11 month old, n=8) were anesthetized with intraperitoneal injections (0.6 ml/Kg) of a mixture of Ketamine (42.8 mg/ml), Xylazine (8.5 mg/ml) and Acepromazine (1.4 mg/ml). IOP and Pattern Electroretinogram (PERG) were sequentially measured with mice in horizontal position (0°), 60° head-down, and again at 0°. IOP and PERG were also measured before and after intraperitoneal mannitol 25% (2.5 g/Kg) administration with mice in horizontal position.

Results: Head-down position induced reversible IOP elevations of 32-38% in all age groups (P<0.01), and age-dependent reductions of PERG amplitude (3 months: +3%; 5 months: − 47%, P<0.01; 10 months: −65%, P<0.01). Mannitol administration in 11 month-old mice resulted in IOP lowering of about 38% (P<0.01) and PERG amplitude improvement of about 83% (P<0.001). IOP and PERG amplitude changes were inversely correlated (10 months head-down $r^2 = 0.58$, P<0.001; 10 month-old mannitol $r^2 = 0.41$, P<0.001). For all conditions, the light-adapted flash ERG was unaltered.

Conclusions: In the DBA/2J mouse, RGC susceptibility to artificial IOP elevation increases with age. Abnormal RGC function in older mice may be improved with IOP lowering. Evaluation of PERG changes in response to artificial IOP modulation may represent a powerful tool to non-invasively assess RGC susceptibility to IOP insult in genetically distinct mouse models of glaucoma.

Keywords

Retinal ganglion cells; glaucoma; pattern electroretinogram; mouse; DBA/2J; cell death

Introduction

Mouse models with genetic alterations relevant to glaucoma are receiving increasing attention to better understand the complex nature of the disease and design specific treatments to prevent death of retinal ganglion cells (RGCs) and their axons.[1-4]

The DBA/2J mouse is a well established model of spontaneous glaucoma. Recessive mutations in two genes, Gpnmb and Tyrp1, cause iris atrophy and pigment dispersion.[5] The iris disease is apparent at 6 months and progresses with age, resulting in elevated intraocular pressure (IOP), loss of RGCs and optic nerve axons, and optic disk excavation.[6] Young (2-3 month-old) DBA/2J mice have normal IOP and normal histological appearance of RGCs and optic nerves. By 8-9 months of age RGCs show signs of apoptotic death.[7, 8] Axonal damage in the

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optic nerves is apparent in about 50% of eyes by 10 to 11 months of age, and in about 90% of eyes by 18 months.[6]

Surviving RGCs may not be functional. Functional events associated to IOP elevation and RGC progressive degeneration in DBA/2J mice need to be elucidated. RGC function in mice can be evaluated by means of the Pattern Electroretinogram (PERG).[9-12] The non invasive nature of the PERG allows serial recordings as a function of changing conditions (e.g., age, IOP levels). Using PERG, we have been able to characterize the natural history of RGC dysfunction and its association with IOP in a 12-month longitudinal study of BDA/2J mice. [13] On average, the IOP increased from 14 mm Hg to 18 mm Hg between 2 and 6 months and then more steeply to level off by 11 months at a value of about 28 mm Hg.[13] After 3 months, the PERG amplitude decreased progressively with age to approach the noise level at about 10-11 months.[13] The time-course of IOP elevation and PERG amplitude reduction were closely associated. Histological analysis of eyes with abolished PERG showed that the retinal nerve fiber layer (RNFL) had lost only about 40% of the normal thickness, and the cone-flash ERG did not significantly change.[13] Taken together, these results indicated that DBA/2J mice develop a progressive functional damage in the inner retina (abnormal PERG) but not in the outer retina (normal flash-ERG) that seems to precede anatomical damage of the optic nerve (relatively spared RNFL).

Recently, Aihara and coll.[14] reported in NIH white Swiss mice that head-down position induces substantial elevation of IOP associated with elevation of the episcleral venous pressure (EVP). Head-down position induces very similar IOP and EVP changes in human.(e.g.,15) In the present study we tested the hypothesis that IOP elevation in the head-down position causes reduction of PERG amplitude in susceptible eyes of older DBA/2J mice. We also tested the hypothesis that impaired RGC function in older DBA/2J mice may be improved by pharmacologically reducing IOP with mannitol.[16] Preliminary results of this study have been presented at ICER, 2006 [17] and ARVO, 2007.[18]

Methods

Animals and husbandry

A total of 29 DBA/2J mice of different ages — 3 month old, n=7 (7 eyes); 5 month old, n=7 (7 eyes), 10 month old, n=7 (7 eyes), 11 months, n=8, (16 eyes) — obtained by Jackson Labs, Bar Harbor, Maine were tested. Mice were maintained in a cyclic light environment (12-h 50 lux, 12-h dark) and fed *ad libitum*. For both IOP and PERG measurements, mice were weighed and anesthetized with intraperitoneal 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. All procedures were performed in compliance with the ARVO statement for 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.

IOP measurement

IOP was measured with an induction/impact tonometer (Tonolab Colonial Medical Supply, Franconia, NH).[19] The tonometer was fixed in a vertical position to a support stand by means of clamps. Anesthetized mice were placed on an adjustable stand and the tail restrained with adhesive tape. The probe tip was aligned with the optical axis of the eye at 1-2 mm distance using a magnifier lamp. Five consecutive IOP readings were averaged. Importantly, the impact of the Tonolab probe on the cornea is minimal, such as local corneal anesthesia is not necessary. [20] In a previous longitudinal study over one year [13] we did not find obvious corneal damage at biomicroscopic examination. IOP readings obtained with Tonolab have been proven to be accurate and reproducible in different mouse strains including DBA/2J.[13, 20]

PERG recording

Detailed description of the PERG technique is reported elsewhere.[11, 12] In brief, anesthetized mice were gently restrained using a mouth bite bar and a nose holder that allowed unobstructed vision,[11, 21] and kept at a constant body temperature of 37.0 ° C using a feedback-controlled heating pad. The body of the mouse could be oriented horizontally as well as 60 deg head-down on an adjustable platform by holding the tip of the tail with adhesive tape. The eyes of anesthetized mice were typically wide open and in a stable position, with undilated pupils pointing laterally and upwardly. The ERG electrode (0.25 mm diameter silver wire configured to a semi-circular loop of 2 mm radius) was placed 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 the scalp and tail, respectively. 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: 50×58 deg; mean luminance 50 cd/m^2; spatial frequency: 0.05 cycles/deg, contrast: 98%; temporal frequency: 1Hz) was aligned with the projection of the undilated pupil at 20 cm distance. Eyes were not refracted for the viewing distance since the mouse eye has a large depth of focus due to the pinhole pupil.[22, 23] Retinal signals were amplified (10,000 fold) and band-pass filtered (1-30 Hz). Three consecutive responses to 600 contrast reversals each were recorded. The responses were superimposed to check for consistency and then averaged. 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/m^2/s) superimposed on a steady background light (12 cd/m^2) and presented within a Ganzfeld bowl. Under these conditions rod activity is largely suppressed while cone activity is minimally suppressed.[24] Averaged PERGs and FERGs were automatically analyzed to evaluate the major positive and negative waves (Figure 1). For both PERG and FERG, peak-to-trough response amplitude was automatically evaluated on the major positive-negative complex, and the latency was the time-to-peak of the major positive deflection. Separate analysis of positive and negative components of responses was not systematically performed since unambiguous recognition of these components was not possible in older mice with reduced PERG signal.

Pupil size

Anesthesia may cause pupil dilation, [25] and older DBA/2J may have slight pupil dilation or deformation due to the pathologic iris condition.[5] The size of the pupil was continuously checked during sequential IOP measurements using a magnifier lamp. Pupil size was also checked with a microscope (25x) at the beginning and at the end of the PERG recording together with assessment of correct positioning of ERG electrodes. A four-point grading system was used to approximately describe the pupil size: small, slightly dilated, moderately dilated, dilated. All mice had small or slightly dilated baseline pupils; the pupil size did not appreciably change after either head-down posture or mannitol administration.

Experiment #1 — IOP elevation

After two baseline IOP measurements performed with the animal resting in a horizontal position, the adjustable platform was tilted in such a way as to put mice in a head-down (60 deg) position for 30 minutes and then repositioned horizontally for at least 20 minutes (recovery). Sequential IOP measurements (1 to 5 minutes apart) were obtained over the whole tilted-recovery period. The adjustable platform was then inserted in the stereotaxic holder for PERG recording. Since recording time for PERG/FERG recording was approximately 15 minutes, only tree sequential responses could be obtained during the observation period: baseline, 15 to 30 minutes after head-down position, and 15 to 30 minutes after horizontal repositioning.

Experiment #2 — IOP lowering

With the animal resting in a horizontal position, two baseline IOPs were measured. One intraperitoneal injection of mannitol 25% (2.5 g/Kg; Hospira, Inc. Lake Forest, USA) was then administered, and IOP was measured approximately every 5 minutes over one hour. Mice were then allowed to recover from anesthesia and returned to their cages. One week later, IOP was again measured with the animal resting in a horizontal position as a control that IOP recovered initial baseline values. Mice were then inserted in the stereotaxic holder for PERG recording in horizontal position. After a baseline PERG/FERG were recorded, one intraperitoneal injection of mannitol 25% was administered and PERG/FERG were recorded 20-60 minutes later. No further IOP measures were taken.

Time-course of IOP changes

In most eyes of each age group, the number of data points was sufficient to mathematically fit the time-course of IOP rise and decay. Data were well fitted with exponential rise and decay functions [exponential rise: $IOP_{(t)} = {}_pIOP + {}_dIOP \cdot (1 - e^{-x/tau})$; exponential decay: $IOP_{(t)} = {}_pIOP + {}_dIOP \cdot (e^{-x/tau})$], where $_pIOP$ represents the plateau IOP, $_dIOP$ represents the delta IOP, and tau represents the time constant of IOP change. The time constant is the time required to change the baseline value by a 1/e (63%) factor.

Results

Experiment #1 — Effect of IOP increase

Figure 2 displays the time-course of IOP changes measured in the left eyes of 21 mice of different ages under baseline condition (horizontal), tilted (60 deg head-down), and recovery (horizontal). Upon head-down positioning, the IOP progressively increased in all eyes and reached a relatively stable value. Upon horizontal repositioning, the IOP progressively returned to baseline values. The pupil size did not appreciably change during the entire observation period. The time-courses of IOP rise and decay were symmetric, with a time constant on the order of 4 minutes on average (IOP rise tau = 3.6 ± 2.2; IOP decay tau = 4.6 ± 3.3).

Average PERG amplitudes recorded in horizontal position (baseline), 10-30 minutes after head-down position (tilted), and 10-30 minutes after repositioning mice in horizontal position (recovery) are displayed together with corresponding IOP and FERG amplitude (Figure 3). Data are plotted on log scales to normalize differences with respect to baseline conditions as well as relative changes of PERG amplitude with respect to IOP changes. The mean baseline IOP increased with increasing age (3 months: 13.2 ± 1.1 mm Hg; 5 months: 14.6 ± 3.2 mm Hg; 10 months: 20.0 ± 3.4 mm Hg, ANOVA, P<0.01). The increase in baseline IOP with age was significant between 10 month-old mice and 3 month-old mice (P<0.001) but not between 5 month-old mice and 3 month-old mice (P=0.33). The delta IOP (tilted minus horizontal baseline) was on the order of 5 mm Hg and the relative change (tilted /horizontal baseline*100) did not change significantly with age (3 months: 33.8 ± 13.9%; 5 months: 37.8 ± 17.9 %; 10 months: 32.4 ± 10.7 %, ANOVA, P=0.77). The mean baseline PERG amplitude decreased with increasing age (3 months: 10.8 ± 3.5 μV; 5 months: 8.7 ± 2.4 μV; 10 months: 4.5 ± 1.5 μV, ANOVA, P<0.01). The decrease in baseline PERG amplitude with age was significant between 10 month-old mice and 3 month-old mice (P<0.001) but not between 5 month-old mice and 3 month-old mice (P=0.28). Differently from IOP, the % PERG amplitude change (tilted/horizontal baseline*100) was strongly dependent on age (3 months: 3.4 ± 16.5 %; 5 months: − 46.8 ± 15.4 %; 10 months: − 65 .4 ± 14.0 %, ANOVA, P<0.001). In 3 month-old mice the PERG amplitude change was not significantly different from zero, whereas in both 5 month-old mice and 10 month-old mice PERG amplitude reductions in head-down position were highly significant (P<0.001). Relative PERG amplitude reductions in 10 month-old mice were significantly (P<0.05) larger than those of 5 month-old mice. Note, by comparing panel A with

panel B, the age-dependent disproportion between logarithmic differences of IOP and PERG amplitude. In all mice, the PERG amplitude fully recovered baseline values when mice were tested again in horizontal position. The head-down position did not cause significant changes in FERG amplitude. The peak latency of both PERG and FERG did not change significantly across conditions (not shown in figures).

Mean PERG amplitudes recorded in baseline and tilted positions have been plotted against corresponding mean IOP values, and data fitted with linear regressions (Figure 4). The correlation between PERG amplitude and IOP increases with increasing age of mice (3 month-old: $r^2 = 0.04$, P=0.53; 5 month-old: $r^2 = 0.17$, P=0.14; 10 month-old: $r^2 = 0.58$, P<0.001).

Experiment #2 —Effect of IOP decrease

Figure 5A displays the time-course of IOP changes measured in 8 mice (16 eyes) aged 11 months in horizontal position after intraperitoneal injection of 25% mannitol. The time-course of IOP decay had a time constant on the order of 5-6 minutes (average tau = 5.5 ± 3.5). The pupil size did not appreciably change during the entire observation period. As shown in Figure 5 B the untreated IOP was 26.3 ± 4 mm Hg on average. Mannitol treatment consistently reduced the IOP to a relatively stable average value of 16.3 ± 2.8 mm Hg (−38%) (P<0.001) over the next 20-60 minutes. When the same mice were tested one week later, IOP had recovered baseline values (mean 26.0 ± 4.3). PERGs and FERGs were then recorded before and 20-60 minutes after mannitol treatment. The baseline PERG amplitude of these 11 month-old DBA/2J mice (Figure 5 C) was typically very low[13] and close to the noise level, as measured by recording a response to a pattern stimulus of zero contrast.[11] After mannitol treatment the PERG amplitude improved by about 83% (P<0.01) (Figure 5 C). Mannitol treatment had no significant effect on FERG amplitude (Figure 5 E), PERG latency, and FERG latency (not shown in figures).

Mean PERG amplitudes recorded before and after mannitol have been plotted against corresponding mean IOP values (Figure 5E), and data fitted with a linear regression ($r^2 = 0.41$, P<0.001).

Discussion

In agreement with previous results,[14] this study shows that it is possible to induce transient and consistent elevations of IOP in Ketamine-Xylazine anesthetized DBA/2J mice of different age by positioning the animals 60 deg head-down. The amount of IOP increase (mean about 5 mm Hg) is larger than that (mean 2.6 mm Hg) reported in Ketamine-Xylazine anesthetized NIH Swiss white mice positioned 60 deg head-down.[14] The reason for this difference might be due to the different technique of IOP measurement. The present data were obtained with a non-invasive rebound tonometer (Tonolab) whereas previous results [14] were obtained by cannulating the anterior chamber (AC) with a water-filled microneedle connected to a pressure transducer.[26] However, baseline IOP values measured in this study (13.2 ± 1.1 mm Hg in 3 month-old mice) are similar to those measured with AC cannulation in 3 month-old NIH Swiss white mice (14.8 ±2.3 mmHg,[27] 16.5 ± 0.6 mm Hg [14]). In addition, IOP readings obtained with rebound tonometer compare very well with IOP readings obtained with AC cannulation. [20, 28] Overall, it appears more likely that the differences in posture-induced IOP elevation are due to genetic differences between DBA/2J and NIH Swiss white mice rather than different technique of IOP measurement.

Posture-induced IOP elevation is closely related to increase of the episcleral venous pressure (EVP) as experimentally demonstrated in human [29, 30] as well as mouse. [14] EVP increase in human subjects and mice is likely caused by increase in the volume of the choroidal vascular bed.[14, 30, 31] The time course of choroidal engorgement upon head-down position resulting

in elevated EVP is expected to be relatively brief (seconds). Our data show that the time course of IOP elevation is much longer (minutes). The gradual rise of IOP probably reflects the dynamic interaction between the rate of aqueous humor formation and the establishment of a new equilibrium IOP to compensate for the increase in afterload to aqueous outflow.(discussed in [32, 33]) A similar reasoning applies for the slow decay of IOP upon horizontal repositioning. The mouse anterior chamber [34] and the turnover of aqueous humor [35] are comparable to those of human. In human subjects, postural changes also induce changes in systemic blood pressure (e.g., 36) and ophthalmic artery pressure that may have a role in IOP regulation. These measures are not available for the mouse. However, Savinova and collaborators, 2001[37] did not find a positive correlation between blood pressure and IOP in different mouse strains whose blood pressure differed up to 36 mm Hg.

This study shows that postural IOP elevation induces reversible, age-dependent reductions of PERG amplitude in DBA/2J mice. The baseline IOP, the amount of IOP elevation upon tilting, and the baseline PERG amplitude were similar in 3 month-old and 5 month-old mice. However, clear PERG amplitude reduction occurred in 5 month-old mice but not in 3 month-old mice. This indicates increased susceptibility of retinal neurons to IOP insult in 5 month-old mice compared to 3 month olds. In 10 month-old mice the baseline IOP was increased, and the PERG amplitude decreased, compared to 3 month olds. IOP elevation upon tilting exacerbated the pre-existing PERG amplitude deficit to larger extent compared to 5 month-old mice. In mice of all ages, posture-induced IOP elevation did not cause FERG changes, thereby excluding a generalized retinal effect and indicating that PERG changes reflect inner retina dysfunction.

This study also shows that intraperitoneally administered mannitol consistently reduced IOP by 38% on average. Both the amount of IOP reduction and the time course of IOP changes are in good agreement with previous results.[16] In 11 month-old DBA/2J mice, in which the baseline PERG was close to the noise level, IOP reduction resulted in a marked improvement of PERG amplitude by about 83% on average. Altogether, results indicate that reduced PERG signal in 11 month-old DBA/2J mice results, at least in part, from reduced responsiveness of viable retinal neurons rather than lack of signal of dead neurons. Reduced responsiveness is alleviated by IOP reduction. IOP reduction does not cause FERG changes, thus indicating that PERG changes reflect selective improvement of inner retina dysfunction.

Thus, in older DBA/2J mice RGC dysfunction appears to be strongly IOP-dependent since it can be either exacerbated or alleviated, respectively, by experimental elevation or lowering of the IOP. This does not imply that IOP is the only causal factor of RGC dysfunction. In addition to elevated IOP, different insults may conjure up to induce RGC death in glaucoma.[38, 39] In DBA/2J mice these include excitotoxicity, axonal injury, glial activation, ischemia, and autoimmunity. [40-42 43] Age-dependent, IOP-independent, increase of RGC susceptibility in mice has also been reported in response to optic nerve crush.[44] Finally, RGC susceptibility to optic nerve crush in mice depends on the genetic background.[45] Altogether, these results indicate that IOP initiates or amplifies pathogenetic processes of RGCs resulting in progressive reduction of electrical responsiveness. Reduction of RGC electrical responsiveness is at least in part reversible by lowering IOP, thus indicating that a stage of IOP-dependent, reversible RGC dysfunction may precede cell death. The present findings support previous reports of PERG improvement after IOP reduction in patients with glaucoma and ocular hypertension. (reviewed in [46])

Evaluation of PERG changes in response to artificial IOP modulation may represent a powerful tool to non-invasively evaluate RGC susceptibility to IOP insult in genetically distinct mouse models of glaucoma.

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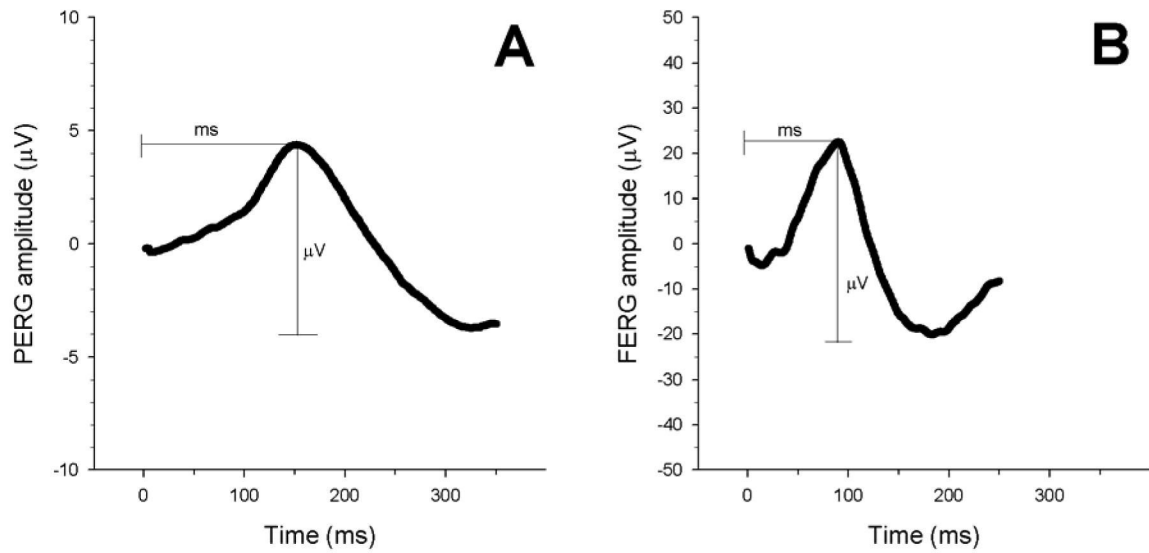
Figure 1.
Examples of PERG (A) and FERG (B) recorded in DBA/2J mice aged 3 months. For both PERG and FERG, the amplitude was measured from the positive peak to the negative trough. The latency was evaluated from the stimulus onset (contrast reversal for the PERG, strobe flash for the FERG) to the peak of the positive wave. Note that the PERG has smaller amplitude and longer latency compared to the FERG.
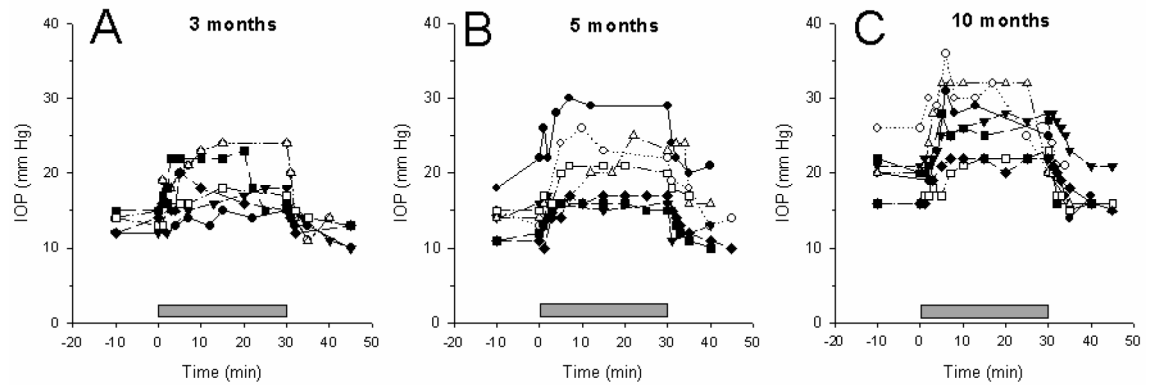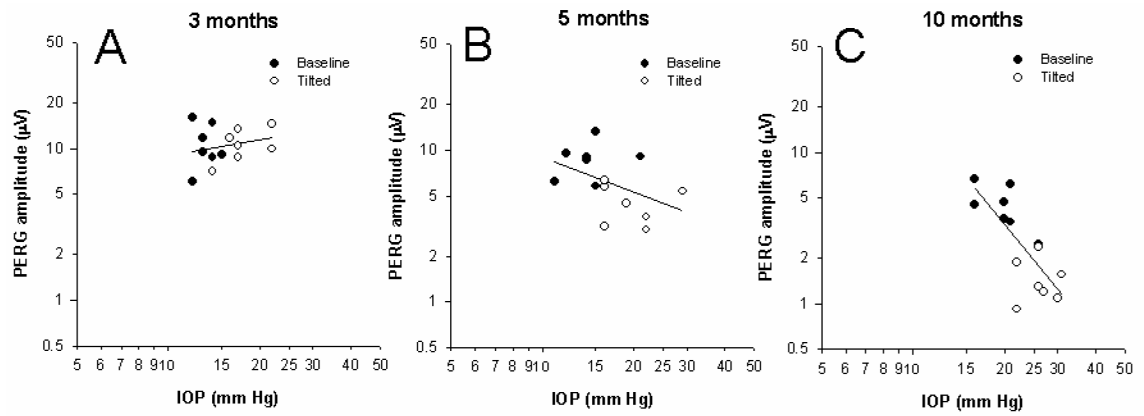
Figure 2.
Time course of IOP changes during head down (60 deg) body tilting (grey bar over the x-axis) in DBA/2J mice of different ages (A: 3 months; B: 5 months; C: 10 months). Different symbols correspond to different mice.
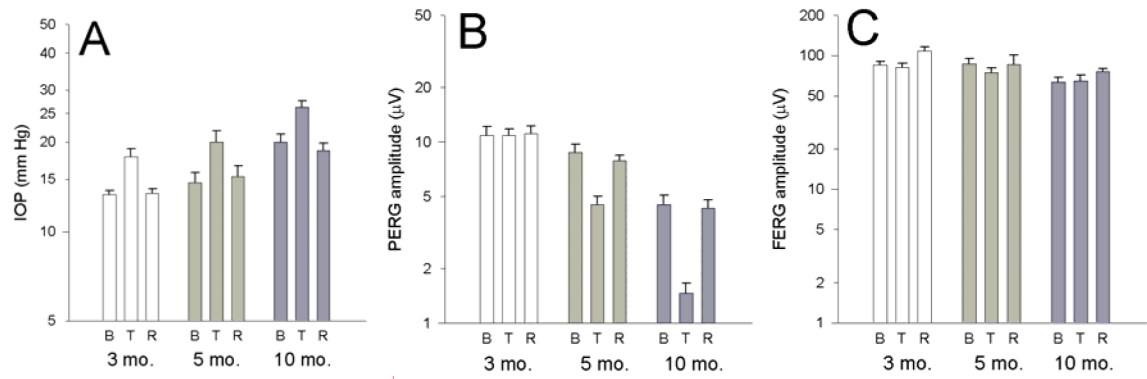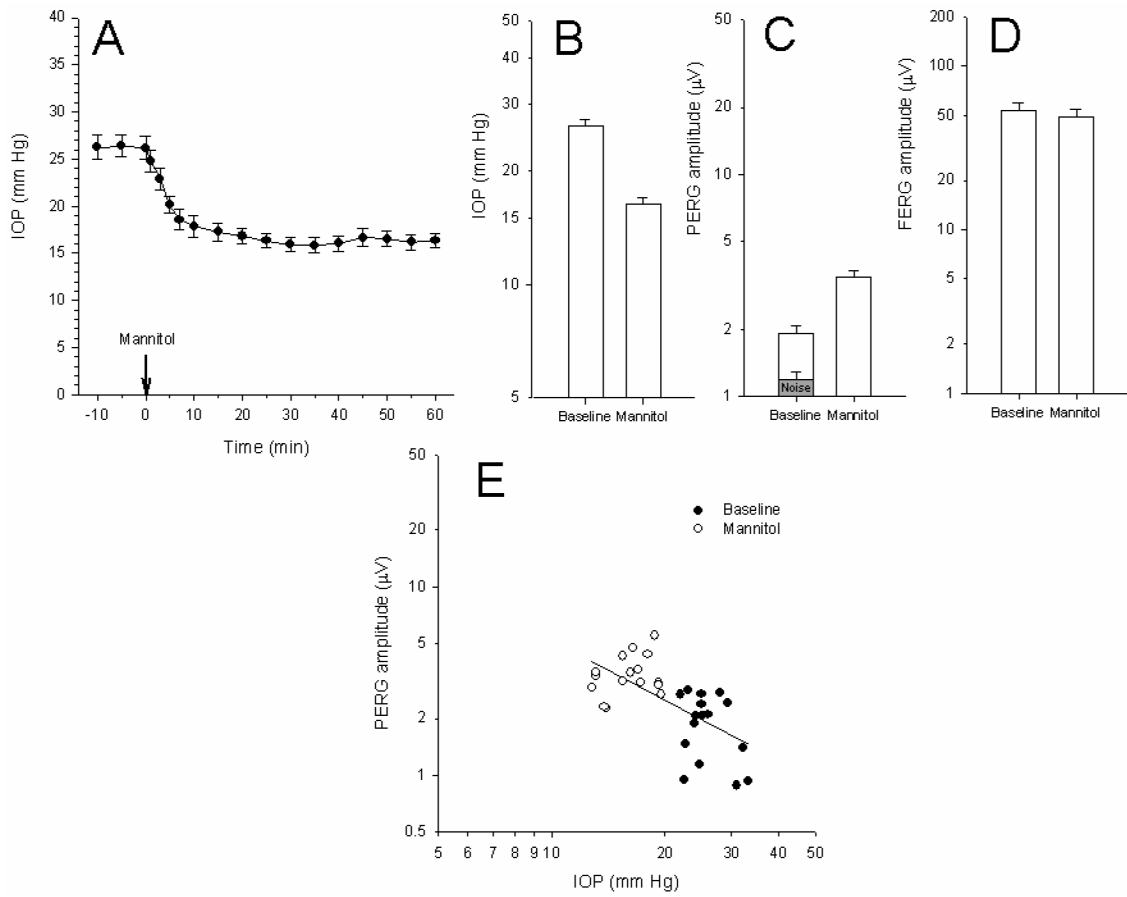
Figure 3.
A: Average (±SEM) values for IOP (A), PERG amplitude (B) and FERG amplitude (C) in DBA/2J mice of different ages measured in horizontal baseline condition (B), during head-down body tilting (T), and during recovery (R) in horizontal position. Data are plotted on log scales to normalize relative changes.

Figure 4.
Changes in PERG amplitude and IOP after 60 deg head-down body tilting in DBA/2J mice of different ages (A: 3 months; B: 5 months; C: 10 months). Data have been fitted with linear regression lines. Closed symbols: baseline condition; open symbols: tilted condition.

Figure 5.
Effect of mannitol treatment on IOP and PERG in 11 month-old DBA/2J mice. **A**: Time-course of IOP after mannitol treatment. **B, C, D**: Mean (± SEM) IOP (B), PERG amplitude (C), and FERG amplitude (D). The grey bar in (C) represents the average (± SEM) noise level. Data are plotted on log scales to normalize relative changes. **E:** Changes in PERG amplitude and IOP after mannitol treatment. Data have been fitted with linear regression lines. Closed symbols: baseline condition; open symbols: mannitol treatment.