Quantification of Deficits in Spatial Visual Function of Mouse Models for Glaucoma

Stephanie L. Burroughs, Simon Kaja, and Peter Koulen

PURPOSE. DBA/2J mice are a standard preclinical glaucoma model, which spontaneously developed mutations resulting in chronic age-related pigmentary glaucoma. The goals of this study were to identify the degree of visual impairment in DBA/2J mice before and after disease onset by quantifying the optokinetic reflex responses and to compare them to the less-researched strain of DBA/2NHsd mice.

METHODS. Visual performance was measured in healthy, non-glaucomatous, and glaucomatous male DBA/2NHsd or DBA/2J mice using a visuospatial testing box. The optokinetic reflex resulting in optomotor head tracking was manually detected. Measured threshold levels equate to the maximum contrast or spatial frequency the mouse responds to. Intraocular pressure (IOP) was measured by applanation tonometry.

RESULTS. IOP increased with age in both DBA/2J and DBA/2NHsd mice and was not different between the two substrains. Both visual acuity and ability to detect contrast decreased significantly, and similarly with age in both substrains. However, DBA/2NHsd had poorer visual acuity even at a younger age compared to age-matched DBA/2J mice.

CONCLUSIONS. Both DBA/2J and DBA/2NHsd mice show a progressive glaucomatous phenotype of age-related increases in IOP and loss of visual acuity and contrast sensitivity when compared to other inbred or outbred strains. Given the similar increases in IOP and contrast sensitivity threshold and loss of visual acuity between these two DBA/2 substrains, we also conclude that DBA/2NHsd mice are a suitable alternative model for pigmentary glaucoma. (*Invest Ophthalmol Vis Sci*. 2011;52:3654–3659) DOI:10.1167/iovs.10-7106

Glaucoma is a major leading cause of blindness worldwide and is defined as a heterogeneous group of optic neuropathies manifesting as progressive damage to the optic nerve head (ONH), irreversible loss of retinal ganglion cells (RGCs), and ultimately visual field loss.^{1–5} Glaucoma has a distinctive pattern of visual field loss for which increased intraocular pressure (IOP) is an important risk factor.^{4,6} The molecular mechanisms of glaucoma are still poorly understood, and elevated IOP is the only variable risk factor.⁷

Thus, current medical treatments for glaucoma solely target increased IOP. Rodents represent a smaller, inexpensive alternative to animal models such as the Argon laser-induced monkey and rabbit models and have similar optic nerve morphology to humans.

DBA/2J mice⁸ are the standard, most widely published, and well-characterized preclinical glaucoma model, which spontaneously developed mutations resulting in chronic age-related pigmentary glaucoma that shares many similarities with the human condition.⁹

Disease onset in DBA/2J mice can vary, largely depending on environmental factors.^{8,10} IOP levels are reported to increase linearly between 2 and 10 months of age;¹¹ however, similar to human pigmentary glaucoma,¹² not all DBA/2J mice display elevated IOP with age.¹¹ Interestingly, however, animals with elevated IOP levels display a similar loss of RGC axons and increased corneal thickness regardless of age.¹¹ In addition to elevated IOP levels, DBA/2J mice suffer iris disease that typically manifests at 6–8 months of age⁹ as well as progressive structural changes that include the thinning of the inner and outer retina as well as a reduction of the ONH width by 11 months of age.⁵ Furthermore, DBA/2J mice show deficits in axonal transport that occur early during the disease (at 3 to 5 months of age)¹³ as well as optic nerve degeneration resulting from elevated IOP and progressively diminishing metabolic capacity.¹⁴

Given that glaucomatous retinopathy directly affects visual performance and that DBA/2J mice are the best characterized of glaucoma mouse models, it is surprising that visual function has not been investigated in this strain in more detail.

Therefore, one aim of this study was to provide a detailed report of visual function and the visuospatial responses of DBA/2J mice. Visual function can be measured in rodents by exploiting two natural reflexes: eye movement (optokinetic nystagmus) and head movement (optomotor tracking), in response to rotating stimuli of black gratings against a white background.¹⁵ In our experiments, we used a specific optokinetic testing apparatus for mice^{15–17} to investigate visual acuity and contrast sensitivity in DBA/2J mice.

Many isogenic substrains of DBA/2 mice exist; however, these have not been studied in the context of glaucoma. For instance, DBA/2NHsd mice have not been investigated for much other than chronic infection.^{18,19} Therefore, the second aim of this study was to characterize and compare the IOP and visual function of DBA/2NHsd mice with DBA/2J mice, to determine whether the DBA/2NHsd mice may be suitable as a complementary glaucoma mouse model.

The present study reports the behavioral outcomes of glaucomatous retinopathy in two substrains of DBA/2 mice using a comprehensive, noninvasive assessment of the full visual system. This knowledge will enable the broader use of these genetic glaucoma models in future studies investigating long-term sustainability of benefits from and efficacy of novel antiglaucoma therapies in longitudinal studies.

From the Vision Research Center and Departments of Ophthalmology and Basic Medical Science, University of Missouri–Kansas City, School of Medicine, Kansas City, Missouri.

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Corresponding author: Peter Koulen, University of Missouri–Kansas City, School of Medicine, Vision Research Center, 2411 Holmes St., Kansas City, MO 64108; koulenp@umkc.edu.

MATERIAL AND METHODS

Animals

Six-week- and 8-month-old male DBA/2NHsd or DBA/2J mice were obtained from the Harlan Laboratory (Indianapolis, IN) or Jackson Laboratory (Bar Harbor, ME), respectively. Mice were housed with one to four mice per cage, where all animals had unlimited access to food and water and were maintained on a 12-hour light/dark cycle. All animal husbandry and experimental procedures were performed in accordance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Six-week- and 8-month-old mice were chosen as representative age groups for healthy, nonglaucomatous animals and animals suffering moderate to severe glaucomatous retinopathy, respectively. Based on the literature available and our own preliminary studies, 8-month-old DBA/2J mice display significantly elevated IOP, iris disease, and deficits in axonal transport and metabolism.^{5,11,13,14,20}

IOP Measurements

Intraocular pressures were measured by applanation tonometry (TONO-PEN XL, Reichert Ophthalmic Instruments, Depew, NY), as validated previously.²¹ Three readings were taken per eye, and the average of these readings was used in the analysis.

Optokinetic Reflex Measurements

Visual function and contrast sensitivity were assessed using an optokinetic testing system (OptoMotry, Cerebral Mechanics, Lethbridge, Alberta, Canada). This noninvasive method utilizes the optokinetic tracking response to assess functional vision and has been described and validated previously.¹⁵⁻¹⁷

The testing apparatus essentially consists of an acrylic box ($39 \times 39 \times 32.5$ cm) with 20-inch computer monitors attached to each of the four walls facing inwards and a central, elevated platform (5.3 cm diameter, 15 cm elevation). A video camera is positioned in the ceiling of the apparatus transmitting the image to the connected computer. Whisper fans control the ambient air temperature. The system is operated through a desktop computer (G5 Power Macintosh, Apple Computer Corp., Cupertino, CA) running the optokinetic testing system mentioned above. At the beginning of each test session and between each trial, a gray homogenous stimulus was projected onto all screens of the chamber. For testing, an animal was placed onto the platform and allowed to briefly acclimate. Visual stimuli are projected onto the monitors such that a virtual cylinder with rotating gratings is produced. Based on the operator input, whether a tracking response was detected or not, the software will automatically change the gratings until a reliable threshold has been reached.

The threshold of maximum spatial frequency that will result in an optokinetic tracking response is a correlate of visual acuity.¹⁵⁻¹⁷ Testing was initiated by projecting a grating of low spatial frequency (0.042 cycles/degree [c/d]), rotating at $12^\circ/\text{s}$ at maximum 100% contrast; that is, the bars of the gratings are maximally black, and the background is maximally white.¹⁵⁻¹⁷

Altering the contrast of the grating allows the determination of the contrast sensitivity threshold, where a higher threshold is indicative of lesser ability to distinguish contrast.¹⁵⁻¹⁷ The contrast sensitivity threshold was determined with the spatial frequency set at 0.042 c/d.¹⁵⁻¹⁷

The asymmetry of the optokinetic motor reflex allows testing visual function for each eye separately.¹⁵ Specifically, Douglas et al.¹⁵ demonstrated that closure by suture of the left eye during visuospatial testing (using the same testing system) abolished tracking when grating stimulus was in a clockwise (CW) direction, and similarly, that tracking was abolished in the counterclockwise (CCW) direction when the right eye was sutured. Spatial frequency thresholds were similar when tested with one or both eyes, suggesting complete asymmetry of tracking, likely due to the absence of cortical activity during tracking motion.^{15,22}

Compared with the more traditional mechanical drum, the use of a computer-generated visual stimulus pattern in the optokinetic testing system has several advantages benefiting both the experimental design as well as contributing to animal welfare¹⁵⁻¹⁷: (1) a crosshair cursor on the live video stream from the camera allows adjustment of the stimulus displayed during testing such to ensure that visual perception remains constant to the freely moving mouse on the platform; and (2) the software reacts to the operator's decision whether or not tracking has occurred, automatically adjusting the display until a reliable threshold is reached, thus ensuring fast and accurate measures for visual acuity or contrast sensitivity thresholds.

Statistics

Data were statistically analyzed using a one-way ANOVA with a Bonferroni post hoc test to compare all pairs of columns. Data are shown as mean $\pm$ SE. For correlation analyses, a Pearson product-moment correlation coefficient (r) calculation to evaluate the strength of the association was used.^{23,24}

To avoid investigator bias, the investigators performing the various assays of the study, including the IOP measurements and behavioral assessment of visual function, were blinded for substrain, age, and gender information. All animals were assigned a random number and the coding was only revealed for statistical comparisons of the data. Furthermore, we have validated the results of our behavioral tests on a consecutive set of experiments with the operator blinded for the rotation of the stimulus (see also Ref. 15).

RESULTS

IOP Measurements

Eight-month-old DBA/2J (26.0 ± 2.7 mm Hg; $n = 5$) and eight-month-old DBA/2NHsd (21.1 ± 3.6 mm Hg, $n = 5$) mice showed a significant increase in IOP values compared to 6-week-old DBA/2J (10.8 ± 0.6 mm Hg; $P < 0.01$, $n = 5$) and to 6-week-old DBA/2NHsd mice (9.3 ± 0.5 mm Hg; $P < 0.05$, $n = 5$), respectively (Fig. 1A). There was no significant difference in IOP between age-matched (6-week- or 8-month-old) DBA/2J or DBA/2NHsd mice (Fig. 1A).

There was no difference between IOP measurements in the left (L) and right (R) eye of 6-week-old DBA/2J (L, 10.2 ± 0.7 mm Hg; R, 11.4 ± 0.7 mm Hg) or 6-week-old DBA/2NHsd (L, 8.8 ± 0.5 mm Hg; R, 9.8 ± 0.7 mm Hg) or 8-month-old DBA/2J (L, 25.4 ± 2.4 mm Hg; R, 26.6 ± 3.6 mm Hg) or 8-month-old DBA/2NHsd (L, 19.6 ± 3.4 mm Hg; R, 22.6 ± 3.8 mm Hg) (Fig. 1B).

Visual Acuity

Eight-month-old DBA/2J (0.371 ± 0.021 c/d; $n = 5$) or DBA/2NHsd (0.287 ± 0.009 c/d, $n = 5$) mice showed a significant decrease in visual acuity (c/d) compared to 6-week-old DBA/2J (0.557 ± 0.021 c/d; $P < 0.001$, $n = 5$) or DBA/2NHsd mice (0.458 ± 0.017 c/d; $P < 0.05$, $n = 5$), respectively (Fig. 2A). DBA/2NHsd at both 6 weeks old and 8 months old showed a decrease in visual performance compared to age-matched DBA/2J mice (6 weeks old, $P < 0.01$; 8 months old, $P < 0.05$) (Fig. 2A).

There was no difference between visual acuity in the left and right eye of 6-week-old DBA/2J (L, 0.560 ± 0.020 c/d; R, 0.555 ± 0.023 c/d) or DBA/2NHsd (L, 0.459 ± 0.012 c/d; R, 0.458 ± 0.029 c/d) or 8-month-old DBA/2J (L, 0.392 ± 0.034 c/d; R, 0.349 ± 0.048 c/d) or DBA/2NHsd (L, 0.267 ± 0.022 c/d; R, 0.306 ± 0.018 c/d) (Fig. 2B).

Contrast Sensitivity

Eight-month-old DBA/2J ($30.8 \pm 1.3\%$; $n = 5$) or DBA/2NHsd ($29.9 \pm 0.6\%$, $n = 5$) mice showed a significant reduction in contrast sensitivity thresholds compared to 6-week-old DBA/2J

FIGURE 1. DBA/2J and DBA/2NHsd sub-strains had a similar age-related increase in IOP. Column graph showing IOP measurements in (A) combined (average of left and right) eye or (B) between the left and right eye of 6-week-old and 8-month-old male DBA/2J or DBA/2NHsd mice. Mean values $\pm$ SE, $n = 5$ for each group, * $P < 0.05$, ** $P < 0.01$. Data were statistically analyzed using a one-way ANOVA with a Bonferroni post-test.

($11.8 \pm 1.0\%$; $P < 0.001$; $n = 5$) or DBA/2NHsd mice ($14.5 \pm 3.3\%$; $P < 0.001$, $n = 5$), respectively (Fig. 3A). There was no significant difference in contrast sensitivity between age-matched DBA/2J or DBA/2NHsd mice of either age (6-week- or 8-month-old age groups, respectively) (Fig. 3A).

There was no difference between contrast sensitivity thresholds in the left and right eye of 6-week-old DBA/2J (L, $11.5 \pm 1.1\%$; R, $12.1 \pm 1.1\%$) or DBA/2NHsd (L, $15.0 \pm 4.1\%$; R, $14.0 \pm 3.7\%$) or 8-month-old DBA/2J (L, $32.6 \pm 2.3\%$; R, $29.1 \pm 2.9\%$) or DBA/2NHsd (L, $33.0 \pm 1.1\%$; R, $26.7 \pm 2.2\%$) (Fig. 3B).

Correlation Analysis

A Pearson product-moment correlation coefficient (r) calculation was used to establish the correlation between variables, where preset parameters to evaluate the strength of the association were determined as follows: no correlation for $0.0 \leq r \leq 0.2$, weak correlation for $0.2 \leq r \leq 0.4$, moderate correlation for $0.4 \leq r \leq 0.6$, and strong correlation for $0.6 \leq r \leq 1.0$.^{23,24}

An increase in IOP produced a weak negative correlation ($r^2 = -0.378$; slope, -0.008 ± 0.002 ; $P < 0.05$) with a decrease in visual acuity for 6-week- or 8-month-old DBA/2J and DBA/2NHsd mice (totaling 20 mice all together) (Fig. 4A).

An increase in IOP produced a moderate positive correlation ($r^2 = 0.487$; slope, 0.7874 ± 0.1906 ; $P < 0.001$) with an increase in contrast sensitivity thresholds for 6-week- or

8-month-old DBA/2J and DBA/2NHsd mice (totaling 20 mice all together) (Fig. 4B).

A decrease in visual acuity displayed a strong negative correlation ($r^2 = -0.659$; slope, -71.720 ± 12.150 ; $P < 0.0001$) with an increase in contrast sensitivity thresholds for 6-week- or 8-month-old DBA/2J and DBA/2NHsd mice (totaling 20 mice all together) (Fig. 4C).

DISCUSSION

The aims of this study were, first, to report the visuospatial readings of the standard DBA/2J glaucoma mouse model using a specific optokinetic testing system (OptoMotry), and, second, to compare the IOP and visuospatial readings of the DBA/2J mouse with the less characterized DBA/2NHsd sub-strain, to determine whether the DBA/2NHsd may represent a viable alternative to the DBA/2J mouse model.

IOP Levels in DBA/2J and DBA/2NHsd Mice

DBA/2J mice display elevated IOP levels as disease progresses, similar to the human condition.^{11,12} The initial experimental aim was to measure the IOP of 6-week-old (control) and 8-month-old (glaucomatous) DBA/2J and DBA/2NHsd mice, to establish whether these two DBA/2 sub-strains show IOP elevations at the same time point. Previously published data report that DBA/2J mice at 6 to 12 weeks of age have an IOP of ~ 9 – 17 mm Hg compared to older mice at approximately 8 to 12 months of age, which measure an IOP of ~ 20 – 30 mm

FIGURE 2. Visual acuity was poorer in DBA/2NHsd compared to age-matched DBA/2J mice. Column graph showing visual acuity in (A) combined (average of left and right) eye or (B) between the left and right eye of 6-week-old and 8-month-old male DBA/2J or DBA/2NHsd mice. Mean values $\pm$ SE, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data were statistically analyzed using a one-way ANOVA with a Bonferroni post-test.

FIGURE 3. Contrast sensitivity thresholds were similar in DBA/2NHsd compared to age-matched DBA/2J mice. Column graph showing contrast sensitivity thresholds in (A) combined (average of left and right) eye or (B) between the left and right eye of 6-week-old and 8-month-old male DBA/2J or DBA/2NHsd mice. Mean values $\pm$ SE, *** P < 0.001. Data were statistically analyzed using a one-way ANOVA with a Bonferroni post-test.

Hg.^{8,11,25–30} The IOPs from this study corroborate these published data, as the average IOP readings for 6-week-old DBA/2J mice are 10.8 mm Hg and older, 8-month-old, mice are 26.0 mm Hg. The average IOP of 6-week-old DBA/2NHsd is 9.3 mm Hg and for 8-month-old mice is 21.1 mm Hg, results that are slightly lower than DBA/2Js within this study; however, they fall within the published parameters of IOPs in DBA/2J mice, showing that the DBA/2NHsd substrain appears to be glaucomatous at the same time point as the standard DBA/2J glaucoma mouse model.

Functional Vision in DBA/2J and DBA/2NHsd Mice

Although the DBA/2J mouse substrain is the most characterized of the glaucoma mouse models in terms of IOP and aspects of degeneration, including RGC cell loss, optic nerve deterioration, and metabolic and transport defects, aspects of functional vision have not yet been studied in DBA/2J mice. Thus, following confirmation of age-related IOP increases in both DBA/2 substrains, we here provide a quantitative report of visuospatial measurements of DBA/2J and DBA/2NHsd mice. Visual acuity in both mouse substrains significantly decreased with age, where DBA/2NHsd mice performed poorer in both young and old age groups compared to age-matched DBA/2J mice. Both young DBA/2 substrains showed visual acuity readings between 0.458 and 0.557 c/d, which is slightly higher than those published for C57 mice, which have a reported visual acuity of 0.4 c/d from P26 onward.^{16,17} However, older mice in the present study have acuity thresholds between 0.287 and

0.371 c/d, showing the age-related deficit in visual acuity correlates with previously published data. In contrast, a study by Zhou et al.³¹ used the same visuospatial testing system and showed DBA/2J mice maintained visual acuity levels at approximately 0.4 c/d from 1 to 12 months of age. The same study demonstrates that retinal ganglion cell density is substantially decreased in 6-month- and 15-month-old mice compared to 1-month-old DBA/2J mice in flat-mounted retina.³¹ In the visual water task, visual acuity of 6-month-old DBA/2J mice was 0.54 c/d, yet an earlier time point was not examined in that particular study.³² In another study by the same group,^{32,33} it was determined that 16- to 21-week-old DBA/2J had a visual acuity threshold of 0.375 c/d using the visual water task. Variation in the threshold levels between the present study and other published data may be due to a variety of factors, including user, test, parameters, strain, and age. To minimize variation in our own study, we have performed all measurements with the experimenter blinded for substrain, age, and gender information to reduce bias. Furthermore, relevant controls have been established within the present study, and the method of testing

FIGURE 4. Strong correlation between age-related increases in IOP and attenuated visuospatial performance in both DBA/2 substrains. Visual acuity in the two DBA/2 substrains had a strong negative correlation with contrast sensitivity thresholds. Scatter graphs showing the statistical correlation between (A) visual acuity and IOP, (B) contrast sensitivity thresholds and IOP, or (C) contrast sensitivity threshold and visual acuity in 6-week-old or 8-month-old male DBA/2J or DBA/2NHsd mice. Data were statistically analyzed using a Pearson product-moment correlation coefficient (r) in order to evaluate the strength of the association between groups.

was described in detail to allow easy reproduction of our test settings.

In correlation with visual acuity levels, contrast sensitivity thresholds increased with age, indicative of visual performance worsening with age. However, there was no difference in contrast sensitivity thresholds between age-matched substrains.

Correlation of IOP and Visual Function with Age and Disease Progression

The results from the present study suggest that there is a correlation of visuospatial performance (visual acuity and contrast sensitivity) with increasing IOP. The visual acuity in the two substrains had a strong negative correlation with contrast sensitivity ($r^2 = 0.659$), in agreement with published findings in glaucoma and ocular patients^{34,35}; these studies reported a moderate to strong correlation of “contrast sensitivity versus total visual field” and “contrast sensitivity versus central visual field” (with r^2 coefficients of 0.42–0.63 and 0.53–0.76, respectively).^{34,35} Another study showed that in 100 individuals with a reading index of 0.59–0.67, contrast sensitivity had a moderate to strong correlation with distance visual acuity ($r^2 = 0.5–0.76$) and near visual acuity ($r^2 = 0.59–0.72$).³⁶ Similarly, Elliot and Situ³⁷ found a strong correlation between contrast sensitivity and visual acuity in 37 eyes of elderly individuals ($r^2 = 0.7$). The correlation between visuospatial parameters identified and quantified in this study and published data demonstrates a significant relationship between visual acuity and contrast sensitivity that is present in both rodent disease models and humans. As such, the tested DBA/2 substrains represent a powerful model for this aspect of human pigmentary glaucoma.

However, although the present study shows loss of visual performance in two DBA/2 substrains in 8-month-old mice compared to 6-week-old mice, further longitudinal studies are required to determine when visual deficits are initiated. Both functional and anatomic changes in DBA/2 mice may serve as indicators of disease onset and progression. One study correlating anatomic changes with electroretinogram (ERG) outcomes, reported that DBA/2 retina thinning started at approximately 4 months of age, while RGC loss and thinning of the outer plexiform were observed later at approximately 7 months of age, correlating with decreases in electroretinogram (ERG) α - and β -wave amplitudes.³⁸ Previous studies also show that visuospatial readings of other disease models and strains appear to correlate with such parameters as ERG readings.^{33,38,39} For instance, 2.5-year-old B6D2F1/J mice had decreased scotopic visual acuity (0.43–0.23 c/d) and contrast sensitivity thresholds (10.3%–5.9%), when compared with 4-month-old mice,⁴⁰ which correlated with the reduction of ERG readouts as well as measurements of rod numbers and outer segment length of the retina.⁴⁰ Yet published data for the time point of RGC cell loss in DBA/2J mice is varied. Ultrastructural analysis revealed that by 3 months old, DBA/2J mice experience RGC apoptosis, peaking at 6 months of age, followed by necrosis.⁴¹ Overall, IOP and Müller glia activation increased with age, and older animals display myelin-like bodies, possibly representing phospholipid aggregates from injured cells, suggesting that retinal degeneration in the DBA/2J mouse model may partially resemble human pigment dispersion syndrome and pigmentary glaucoma.⁴¹ A recent elegant study demonstrated that RGC axonal dysfunction and degeneration occur in 13-month-old DBA/2J mice, preceding RGC somatic cell loss, which occurred at 18 months of age.²⁰ Our present study shows that such drastic late-stage changes are preceded by earlier elevation of the IOP levels, in accordance with previous studies,^{8,11,25–30} and a concomitant decline of visuospatial performance in DBA/2J and DBA/2Hsd mice.

CONCLUSION

Both DBA/2J and DBA/2Hsd mice show a progressive glaucomatous phenotype of age-related increases in IOP and loss of visual acuity and contrast sensitivity. Our study allows us for the first time to characterize the effects of glaucomatous retinopathy with a comprehensive behavioral assay that allows noninvasive assessment of the full visual system beyond just the measurement of retina function and that is repeatable in individual animals allowing advancing preclinical studies that follow disease development and therapy outcomes over time. The findings will enable the broader use of the DBA/2J and DBA/2Hsd models in future studies investigating long-term sustainability of health benefits from and efficacy of novel antiglaucoma therapies. Given the similar increases in IOP and contrast sensitivity threshold and loss of visual acuity between these two DBA/2 substrains, we also conclude that DBA/2Hsd mice are a suitable alternative model for pigmentary glaucoma.

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