Age- and Sex-Related Differences in Contrast Sensitivity in C57Bl/6 Mice

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PURPOSE. To measure contrast sensitivity in C57Bl/6, the most commonly used mouse in behavioral neuroscience, and to study the effect of sex, age, and miotic drugs on the contrast sensitivity function. In addition, the authors tested a mutant in which plasticity in the cerebellum is impaired by expressing a protein kinase C inhibitor. This inhibitor is also expressed in the retina, possibly affecting vision.

METHODS. The gain of the optokinetic reflex (OKR) decreases as stimuli become more difficult to see. Recording OKR gains evoked by moving sine gratings shows whether the stimulus was distinguished from a homogeneous background and how well the stimulus was distinguished.

RESULTS. Female mice have lower OKR gains than male mice (both groups: n = 10, P = 0.001). A similar difference was observed between 4-month-old (n = 10) and 9-month-old (n = 5) C57Bl/6 mice (P = 0.001). These differences could not be detected with earlier dichotomic tests. C57Bl/6 mice are able to see contrasts as low as 1%, well below the previously reported 5% threshold. Pilocarpine had no significant effect on contrast sensitivity (both groups: n = 10, P = 0.89). Vision in L7-PKCi mutants was unaffected (both groups: n = 10, P = 0.82).

CONCLUSIONS. OKR gains decrease as stimuli become more difficult to see, making the OKR a powerful tool to quantify contrast sensitivity. In C57Bl/6 these response magnitudes vary greatly between sexes and between mice that differ only a few months in age. Therefore, it is important to match groups according to age and sex in experiments that require unimpaired vision. Otherwise, impaired vision can be misinterpreted as a learning or motor problem. (Invest Ophthal Vis Sci. 2009;50:2451-2458) DOI:10.1167/iovs.08-2594

The contrast sensitivity function (CSF) shows which contrasts can be seen at a range of spatial frequencies (the number of sine gratings in 1° of visual angle). Maze tests and optomotor tests are most commonly used to test the CSF of mice. Both however, have their drawbacks.

In maze tests, a mouse is trained to distinguish sine gratings from a uniform gray field to obtain a food reward or to find a submerged platform in a two-armed water maze.2-4 The distance from animal to stimulus varies in this test, making it difficult or even impossible to define exactly the perceived spatial frequency. In optomotor tests a mouse is surrounded by a panoramic sine grating. As the stimulus rotates about the animal, it evokes an optomotor response when the mouse distinguishes the stimulus pattern from a homogeneous background. This optomotor response can consist of optokinetic nystagmus or full-body rotations.6-9 The behavior in optomotor tests is often not quantified (though one group measured full-body rotations); the experimenter observes the animal and tries to distinguish which stimuli evoke responses.

The optomotor response scales with how well the stimulus is perceived and becomes less vigorous as stimuli are more difficult to perceive.8 This, in turn, makes it more difficult for observers to see which stimuli evoke responses close to threshold.

Furthermore, maze tests and optomotor tests give different results. Generally, maximum spatial acuity is reported to be higher in maze tests whereas optomotor tests show higher contrast sensitivity.6 In addition, measurements of contrast sensitivity are basically dichotomous. When an animal responds by choosing the correct arm in a maze or by rotating along with the stimulus, it is inferred that the animal sees the stimulus.

We used a novel method to measure contrast sensitivity in mice. By recording how the gain of the optokinetic reflex (OKR, a gaze stabilization reflex) decreases as moving sine grating stimuli become more difficult to see, we were able to look not just at whether an animal reacted to a stimulus but also at the magnitude of that response.

Our first objective was to quantify vision in the most commonly used mouse in behavioral neuroscience, the C57Bl/6 mouse. In addition, we investigated the effects of sex and age on contrast sensitivity function.

To show applicability in pharmacologic and genetic studies, we also investigated how the contrast sensitivity of mice was affected by pilocarpine, a drug that decreases pupil size and is often used in mice video-oculography.10 Additionally, we studied the L7-PKCI mouse, a mutant in which plasticity in the cerebellum is impaired,11 by bringing a protein kinase C inhibitor to expression. This mutant was reported to perform worse in the Morris water maze, a navigation task requiring accurate vision. This decrease in performance did not occur in a star maze, a maze in which navigation is less dependent on vision.12 It was suggested that the mutation might have an effect on spatial learning. However, the promoter used to express the PKC inhibitor is also found in the retina.13 Here we tested whether vision was affected in the L7-PKCI mutant.

MATERIALS AND METHODS

Animals

We used 25 adult mice (ten 4-month-old males, five 9-month-old males, ten 4-month-old females) of the C57Bl/6 background (Jackson Laboratory, Bar Harbor, ME) strain and 10 male L7-PKCI mutants and 10 male wild-type littermates. The L7-PKCI transgenic mouse lacks long-term depression at the parallel fiber Purkinje cell synapse.11 All mice were housed on a 12-hour light/12-hour dark cycle and had unrestricted access to food and water. Experiments were conducted during their light phase with approval of the local ethics committee and in accordance with the European Communities Council Directive (86/
The stimulus was programmed in such a way that it appeared as a virtual cylinder to the mouse. (B) Front view. To keep the field of view of the mouse unobstructed, we recorded eye movements with an infrared camera placed under the setup. The eye was tracked using a hot mirror (a mirror that is transparent to visible light but reflects infrared light).

609/EEC) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgery
Animals were prepared for fixation by head restraint by the attachment of two metal nuts to the skull with a construct made of a microglass composite (Charisma; Heraeus Kulzer GmbH, Hanau, Germany). All surgical procedures were performed while the animal was anesthetized by a mixture of isoflurane (Isofloran 1%–1.5%; Rhodia Organique Fine Ltd., Bristol, UK) and oxygen. After anesthesia induction, a sagittal incision was made across the scalp, exposing lambda and bregma. The peristemeum was removed and the skull was briefly etched using a gel containing phosphorous acid. Then the skull was dried thoroughly, and a drop of primer (Optibond Prime; Kerr USA, Orange, CA) was applied to the etched portion of the skull, followed by a layer of adhesive (Kerr USA) that was light cured until it formed a hard, shiny layer to facilitate a strong bond between skull and composite. Two layers of composite were used to make a pedestal on which a prefabricated construct of two metal nuts (M4) was attached.

Stimulus Setup
Optokinetic stimuli were created using a modified CRT projector (Electrohome Marquee 9000; Christie Digital Systems, Cypress, CA). Red and blue CRT tubes were replaced by green tubes. The three tubes were mounted on the ceiling at 120° angles from each other and projected their images by mirrors onto the three screens. The stimulus was programmed in such a way that it appeared as a virtual cylinder to the mouse.

A stimulus was first projected and was kept stationary for 1 minute, allowing the eye to adjust to changes in stimulus brightness. Then the stimulus started to move at a constant velocity of 6°/s. It moved to one direction for 2 seconds and then changed direction and moved in the opposite direction for 2 seconds. This was repeated five times, yielding 10 changes in direction. As the stimulus moved, the position of the left eye was recorded. The screens were bright enough to allow pupil recordings over the small range of eye movement amplitude.

Data Analysis
Recorded eye positions were transformed offline using commercial programming language (Matlab; The MathWorks, Natick, MA) in a velocity signal and by taking the first derivative, smoothed with a Gaussian filter with a low-pass cutoff of 1.25 Hz. Fast phases were removed with a velocity threshold of twice the stimulus velocity (12°/s). The first 200 ms after stimulus onset and before and after each change in direction were removed as well. Because the stimulus velocity was constant and eye data in the first 200 ms after the stimulus direction changes were ignored, average absolute eye velocity could be divided directly by the stimulus velocity to calculate a gain value for each combination of spatial frequency and contrast. The gain is the

Recoding Setup
Each animal was placed in a plastic tube attached to a set of linear stages that allowed translations in three dimensions and rotation about the naso-occipital axis. These stages were used to position the mouse eye in the center of the visual stimulus and in front of the eye position recording apparatus, before the experiment. Linear stages and recording setup were attached to a table.

Image Acquisition
The system resembled that described by Stahl et al.\textsuperscript{16} with some modifications. Eye movements were recorded with an infrared video system (ETL-200; Iscan, Burlington, MA). Images of the eye were captured at 120 Hz with an infrared-sensitive CCD camera. From this image, X and Y positions of the center of the pupil and the corneal reflection (first Purkinje image) were recorded in pixel positions, giving their location on the 512 × 256 pixel grid, with a resolution of one-third pixel horizontally and one-tenth pixel vertically. These positions were low-pass filtered and had a cutoff frequency of 300 Hz (Cyberamp 380; Axon Instruments, Union City, CA), sampled at 1 kHz and stored for offline analysis. To keep the field of view as free from obstacles as possible, the camera and lens were mounted under the table surface, and recordings were made with a hot mirror that was transparent to visible light and reflective to infrared light (Fig. 1B). The eye was illuminated with two infrared LEDs at the base of the hot mirror. Camera, mirror, and LEDs were mounted on an arm that could rotate about the vertical axis over a range of 26.12° (peak to peak). Eye movement recordings and calibration procedures were similar to those described by Stahl et al.,\textsuperscript{16} yielding eye position in degrees of visual angle.

Testing Procedure
The animal was placed in a plastic tube with its head fixed to a small metal bar. By fixing its head, the exact distance from the eye to the screen is known, which allows optimal definition of the stimulus. Additionally, head fixing the mouse is required to record its eye movements with an infrared camera. The left eye of the mouse was positioned at the center of the virtual cylinder.

Each stimulus showed a sine grating composed of a combination of 1 of 7 spatial frequencies (0.03, 0.05, 0.08, 0.17, 0.25, 0.33, 0.42 cyc/deg) and 1 of 6 contrast values (100%, 75%, 50%, 25%, 10%, 1%). The 42 stimulus combinations were presented in random order.

A stimulus was first projected and was kept stationary for 1 minute, allowing the eye to adjust to changes in stimulus brightness. Then the stimulus started to move at a constant velocity of 6°/s. It moved to one direction for 2 seconds and then changed direction and moved in the opposite direction for 2 seconds. This was repeated five times, yielding 10 changes in direction. As the stimulus moved, the position of the left eye was recorded. The screens were bright enough to allow pupil recordings over the small range of eye movement amplitude.
ratio between average eye velocity and stimulus velocity, and a gain of 1 means that the eye has the same velocity as the stimulus.\textsuperscript{14}

Experimenters were masked to the experimental conditions. Trials were randomized, mice were assigned numbers, and the data analysis scripts were automated. All data were analyzed after the experiment.

**Statistical Analysis**

Effects of age, sex, pilocarpine, and L7-PKCi mutation were analyzed (SPSS software; SPSS Inc., Chicago, IL) with repeated-measures ANOVA with three factors: one between-subjects factor with two levels (e.g., sex with two levels, male and female) and two within-subjects factors (e.g., contrast with six levels and spatial frequency with seven levels). Post hoc groups were compared at each contrast by averaging OKR gains over spatial frequencies. Differences between groups were analyzed for significance with a Student’s t-test. Values of the F-statistic with according degrees of freedom are supplied together with the P-values.

**Contrast Sensitivity in C57BL/6j Mice**

To investigate whether sex had an effect on contrast sensitivity, we compared OKR gains between ten male and ten female 4-month-old C57BL/6j mice. To gain more insight in the effect of age on contrast sensitivity, we compared OKR gains between the ten 4-month-old male mice and five 9-month-old male C57BL/6j mice.

**Effect of Miotic Drugs on Contrast Sensitivity**

When the gaze stabilization reflexes of mice were tested with video-oculography, pupil size was often reduced for the eye movement recordings to work properly. This was achieved by applying miotic drugs such as pilocarpine to the cornea.

Pilocarpine acts on a subtype of the muscarinic receptor (M\textsubscript{3}) found on the iris sphincter muscle, causing the muscle to contract and the pupil to constrict. A drawback to pilocarpine use while investigating the gaze stabilization reflex was that the treatment could affect visual function. We investigated the effect of artificial pupil constriction on contrast sensitivity by applying a 1% pilocarpine hydrochloride solution (Minims, Chauvin Benelux NV, Belgium) to the eyes. We compared OKR gains before and after treatment of ten 4-month-old male C57BL/6j mice with the use of multivariate repeated-measures ANOVA

**Contrast Sensitivity of L7-PKCi Mutants**

In L7-PKCi mice, a protein kinase C inhibitor is expressed in Purkinje cells under the control of the pcP-2(L7) gene promoter.\textsuperscript{11} However, the protein pcP-2 (L7) is expressed not only in Purkinje cells but also in bipolar cells in the retina.\textsuperscript{13} This means that the protein kinase C inhibitor may be expressed in the retina of the L7-PKCi transgenic mouse and could result in deteriorated vision. We investigated whether contrast sensitivity function was affected in the L7-PKCi mutant by comparing OKR gains between ten 4-month-old male L7-PKCi mouse mutants and ten 4-month-old wild-type littermates.

**RESULTS**

**Effect of Sex on Contrast Sensitivity**

We determined OKR gain in 10 male and 10 female mice for the 42 combinations of contrast and spatial frequency and found a main effect of sex (between-subject; \( F = 17.6, P = 0.001 \)). No significant interaction effects were observed on spatial frequency \( \times \) sex \( (F(6,13) = 2.56, P = 0.073) \) or contrast \( \times \) sex \( (F(5,14) = 2.82, P = 0.058) \). Within subjects we found a main effect of spatial frequency \( (F(6,13) = 141, P < 0.001) \) and a main effect of contrast \( (F(5,14) = 177, P < 0.001) \).

Post hoc analysis showed that for each of the six contrasts, the OKR gains averaged over spatial frequencies were signiﬁcantly lower in female than in male mice (t-test: \( P < 0.05 \) at 100\% contrast; \( P < 0.01 \) at 1\%, 25\%, 50\%, and 75\% contrast; \( P < 0.001 \) at 10\% contrast; see Figs. 3B–3H). Optimum response occurred at 0.17 cyc/deg in both sexes for all contrasts. With maximum contrast and optimal spatial frequency, the OKR gain was not different between male and female mice \((0.97 \pm 0.13 \text{ vs.} 0.85 \pm 0.15; \ t-test, P = 0.1) \).

When contrast was reduced, the gain of the OKR decreased (Fig. 2). For all contrasts, the OKR response was highest in only a small range of spatial frequencies at approximately 0.17 cyc/deg (Figs. 3C–3H). Even stimuli with 1\% contrast were able to evoke an OKR (Fig. 3H). A stimulus with 0.17 cyc/deg and 1\% contrast evoked an OKR with a gain of 0.2, indicating that the eye was moving at 20\% of the stimulus speed, or 1.2\%/s for 2 seconds.

Velocity gains were extrapolated by elongating the line through the last two points (0.33 and 0.42 cyc/deg) to zero gain to estimate the maximum contrast sensitivity for both groups. At 100\% contrast, the estimated maximal contrast sensitivity of male mice was 0.52 cyc/deg. For females, extrapolation showed an estimated maximum contrast sensitivity of 0.50 cyc/deg at 100\% contrast.

**Effect of Age on Contrast Sensitivity**

Figure 4 shows the OKR gains of 10 young adult males (4 months old) and 5 adult males (9 months old). Between subjects, there was a main effect of age \((F = 17.7, P = 0.001) \). Within subjects there were main effects of spatial frequency \((F(6,8)=300, P < 0.001) \) and contrast \((F(5,9) = 168, P < 0.001) \). One significant interaction effect occurred: spatial frequency \( \times \) age \((F(6,8)=2.56, P = 0.01) \). The interaction of contrast \( \times \) age was not significant \((F(5,9)=2.61, P = 0.109) \).

Post hoc analysis showed that in 4 of 6 contrasts, the OKR gains of 4-month-old mice were significantly lower than those of 9-month-old mice \((t-test, P < 0.01 \text{ at} 10\%, 25\%, 50\%, \text{and} 75\% \text{contrast}; \text{Fig. 3}) \). No significant differences between the two groups were observed only at the highest and lowest contrasts (Figs. 4C, 4H). At maximum contrast and optimal spatial frequency (0.17 cyc/deg), OKR gains were not different between younger and older mice \((0.97 \pm 0.12 \text{ vs.} 0.94 \pm 0.03; \ t-test, P = 0.76) \).

Extrapolation by elongation of the line through the last two points (0.33 and 0.42 cyc/deg) to zero shows estimated maximum contrast sensitivities of 0.52 for 5-month-old mice and 0.54 for 9-month-old mice at 100\% contrast.

**Effect of Miotic Drugs on Contrast Sensitivity**

In the group of ten 4-month-old male C57BL/6j mice, we tested the effect of pilocarpine. Application of pilocarpine reduced pupil diameter from 0.366 ± 0.096 (SD) mm to 0.216 ± 0.053 mm \((P < 0.001) \). The main effect of pilocarpine \((F = 3.64, P = 0.89) \) and the interactions between pilocarpine and contrast \((F(5,14) = 2.20, P = 0.20) \) and pilocarpine and spatial frequency \((F(6,13) = 1.95, P = 0.27) \) were not significant. Significant main effects of the factors contrast \((F(5,14) = 214, P < 0.0001) \) and spatial frequency \((F(6,13) = 123, P < 0.0001) \) were observed.

Velocity gains were extrapolated by elongating the line through the last two points (0.33 and 0.42 cyc/deg) to zero gain to estimate the maximum contrast sensitivity for both groups. At 100\% contrast, the estimated maximal contrast sensitivity was 0.51 cyc/deg for both groups.

**Effect of L7-PKCi Mutation on Contrast Sensitivity**

To test the effect of the L7-PKCI mutation on visual performance, we compared the OKR gains of 10 mutants with those of 10 of their wild-type littermates. The main effects of the
established, making the OKR a sensitive tool for probing easily quantified with eye tracking. Such methods are well suitable to responses, however, the magnitude of the OKR can be more difficult to perceive. Unlike other optomo-motor responses, turning rodents. The response did not diminish by a merely perceptual threshold, however; it diminished over a much larger range of stimuli (Fig. 2). Motor circuits underlying the OKR appeared unaffected in all mice because their OKR has a gain close to 1 at 0.17 cyc/deg and 100% contrast, identical with the gain in younger mice (Figs. 3C–6C). The OKR response to repeated stimulus presentations was highly uniform for all mice (Fig. 2).

To use the OKR as a tool for investigating contrast sensitivity requires an understanding of the properties of this tool. The OKR is a gaze stabilization reflex evoked by image motion in the visual field. It causes the eye to move along with the movement in the visual field, thus minimizing retinal slip. Rabbit data show that one-quarter of the retinal ganglion cells are sensitive to a pattern moving in a particular direction. These direction-selective ganglion cells are most sensitive to low stimulus velocities just like in other avo-cate species like rats and goldfish. The OKR of mice is also velocity tuned and decreases with increasing stimulus velocity. Therefore, the OKR is most useful as a probe to study contrast sensitivity when it is used with low stimulus velocities, where gain is high.

Umino et al. also report velocity tuning for the optomotor response under photopic conditions but find a bell-shaped response curve, with an optimum response at a stimulus velocity of 12°/s. An explanation for these differences in velocity tuning might be found in their experimental paradigm. Mice were subjected to stimuli that moved clockwise or counterclockwise for 5 seconds, at velocities between 1.5°/s and 24°/s, and an observer decided whether each mouse rotated its body along with the stimulus. However, like the OKR, the optomotor response decreases as stimulus become more difficult to perceive. A mouse that responds optimally to a stim-

FIGURE 2. Eye velocity and stimulus velocity traces. (A) Eye and stimulus velocities were plotted for a single C57Bl/6J male (4 months old) mouse at seven spatial frequencies. Contrast was 100%. Here the response shows an optimum at 0.17 cyc/deg and decreases as the gratings become larger or smaller than 0.17 cyc/deg. (B-D) Eye and stimulus velocities of the same mouse at 6 contrast values. Spatial frequencies were 0.03 cyc/deg (B), 0.17 cyc/deg (C), and 0.42 cyc/deg (D). As contrast decreased, the stimulus became more difficult to see and eye velocity decreased. At very low contrasts, the eye velocity decreased to zero.

mutation were not significant \((F = 0.055, P = 0.82)\) and neither were the interactions of spatial frequency with the mutation \((F(6,15)=0.90, P = 0.51)\) or of contrast with the mutation \((F(5,14)=0.4, P = 0.84)\). Within subjects there were main effects of spatial frequency \((F(6,15)=115, P < 0.001)\) and contrast \((F(5,14) = 184, P < 0.001)\). We were able to illustrate (see Fig. 6) that there were no difference in OKR gains between the two groups. Both groups had similar gains close to 1 at 100% contrast and 0.17 cyc/deg (0.93 ± 0.05 vs. 0.93 ± 0.19, \(P = 0.998)\).

Extrapolation by elongation of the line through the last two points (0.33 and 0.42 cyc/deg) to zero shows an estimated maximum contrast sensitivity of 0.49 for wild-type and 0.51 for mutant mice at 100% contrast.

DISCUSSION

The contrast sensitivity function can be inferred by measuring how the gain of the OKR varies with different contrasts and spatial frequencies. With this approach, we observed differences in contrast sensitivity between male and female C57BL/6J mice and between younger and older adult C57BL/6J mice. As expected, the effects of spatial frequency and contrast on OKR were highly significant in all experiments. Like other optomotor responses, the OKR becomes less vigorous as stimuli become more difficult to perceive. Unlike other optomo-tor responses, however, the magnitude of the OKR can be easily quantified with eye tracking. Such methods are well established, making the OKR a sensitive tool for probing visual capabilities without the need for an observer to decide whether an animal responds.

The OKR response evoked by gratings of different contrasts and spatial frequencies decreased, similar to the optomotor response of turning rodents. The response did not diminish by a merely perceptual threshold, however; it diminished over a much larger range of stimuli (Fig. 2). Motor circuits underlying the OKR appeared unaffected in all mice because their OKR has a gain close to 1 at 0.17 cyc/deg and 100% contrast, identical with the gain in younger mice (Figs. 3C–6C). The OKR response to repeated stimulus presentations was highly uniform for all mice (Fig. 2).
FIGURE 3. OKR gains of 4-month-old male and female C57BL/6J mice. (A, B) The color reflects the OKR gains at 42 combinations of contrast and spatial frequency (*white crosses*). Spaces between the measured points are linearly interpolated. (C-H) Six cross-sections of A and B are plotted, one at each contrast. Error bars indicate SD. Stars: significant differences between male and female mice (t-test, *P < 0.05; **P < 0.01; ***P < 0.001). Dotted lines: extrapolation to zero gain.

FIGURE 4. OKR gains of ten 4-month-old and five 9-month-old male C57BL/6J mice. (A, B) White crosses show OKR gains at 42 combinations of contrast and spatial frequency. The color shows the OKR gain at each point. Spaces between the measured points are linearly interpolated. (C-H) Six cross-sections of (A) and (B) are plotted, one at each contrast. Error bars indicate SD. Stars: points significantly different between groups (t-test, *P < 0.05; **P < 0.01; ***P < 0.001). (D–G) OKR response curves are significantly different (t-test, P < 0.01). Dotted lines: extrapolation to zero gain.
**Figure 5.** The effect of pilocarpine on the OKR gains in 10 male C57BL/6J mice. (A, B) White crosses show OKR gains at 42 combinations of contrast and spatial frequency. The color shows the OKR gain at each point. Spaces between the measured points are linearly interpolated. (C–H) Six cross-sections of (A) and (B) are plotted, one at each contrast. Error bars show SD. Stars: points significantly different between groups (*P < 0.05, t-test). Dotted lines: extrapolation to zero gain.

**Figure 6.** OKR gains of 10 L7-PKCi mutants and 10 wild-type littermates. (A, B) White crosses show OKR gains at 42 combinations of contrast and spatial frequency. The color shows the OKR gain at each point. Spaces between the measured points are linearly interpolated. (C–H) Six cross-sections of (A) and (B) are plotted, one at each contrast. Error bars show SD. There are no significant differences in contrast sensitivity between L7-PKCi mutants and their wild-type littermates (t-test). Dotted lines: extrapolation to zero gain.
ulus that moves for 5 seconds with 1.5°/s will rotate 7.5°. This is analogous to the hand of a clock that moves in 5 seconds from 12:00 to halfway between 12:01 and 12:02. If a mouse responds with a gain of 0.5, it moves at 0.75°/s, and the full body rotation of 3.75° in 5 seconds becomes difficult to detect by eye.

In this study, we did not search for the highest possible acuity. The highest spatial frequency, 0.417 cyc/deg, evoked an OKR with gains above 0.2 in all animals at 100% and 75% contrast. However, extrapolation to zero gain showed an absolute threshold of 0.49 to 0.52 cyc/deg for all groups, similar to the 0.5 to 0.6 cyc/deg reported in other experiments that used optomotor responses to probe the visual system.

We found a significant optokinetic response at the lowest contrast (1%) in all animals that was lower than the threshold of 5% contrast reported in maze tests and optomotor tests. This lower threshold almost closes the contrast detection gap between mice and humans. Humans are able to see up to approximately 0.5% contrast and up to 60 cyc/deg. They have much better visual acuity than mice (0.5 cyc/deg). Hence, the acuity of humans is more than 100 times better than that of mice, whereas the lowest detectable contrast varies by only a factor of 2. The mouse eye has notoriously poor optics. Two studies measured modulation transfer functions of the mouse eye. Both show that the ability of the lens to transfer contrast decreases rapidly as spatial frequency increases. However, the stimuli used in our experiments were no higher than 0.42 cyc/deg, at which the reported modulation transfer functions were close to optimal.

We did not correct for refractive errors of the mouse eye; therefore, the data reported in this article may be an underestimation of the contrast sensitivity of C57BL/6J mice. However, these data are useful for designing behavioral experiments that require vision and when it is not possible to correct for the refractive errors of the mouse eye. In addition, not much is known about whether mice can accommodate; therefore, the close distance to the screen could have resulted in suboptimal contrast performance.

Effect of Sex on Contrast Sensitivity
Female C57BL/6J mice showed consistently lower gains than male mice in almost all stimulus conditions except for a few spatial frequencies at maximum or minimum contrast. Because the OKR was similar for males and females under optimal conditions (0.17–0.25 cyc/deg, 100% contrast; Fig. 3C), gain differences could not be explained by differences in the ocular motor system. This result suggests that female mice have lower contrast sensitivity than male mice. The sex-related difference in CSF cannot be easily explained. It may be a particular trait of the C57BL/6J strain or it may occur for mice in general. One way to gain more insight into this sex-related difference is to test whether it also occurs in other strains.

However, this sex-related difference strongly argues for a proper matching of animals on sex in any test involving vision, such as water maze tests. Differences in contrast sensitivity are likely to have an impact of the outcome of these tests. If not controlled for differences in visual function, these outcomes can be misinterpreted as related to, for instance, learning or motor processes.

Effect of Age on Contrast Sensitivity
In humans, vision deteriorates with aging. Acuity decreases, as does contrast sensitivity. Contrast sensitivity decreases with age even in those with normal or corrected to normal acuity. The visual acuity threshold of C57BL/6J mice also decreases as they age. In 6-month-old C57BL/6J mice, acuity has been reported to be 0.48 cyc/deg and to decrease to 0.38 cyc/deg in 12-month-old C57BL/6J mice.

Contrast sensitivity function was lower for 9-month-old C57BL/6J males than for 4-month-old C57BL/6J males in most stimulus conditions, whereas their maximum response was unaffected (Fig. 4C). Even with a 5-month age difference, large changes occur in the contrast sensitivity function. A decrease in visual threshold from 0.48 to 0.38 cyc/deg was reported earlier for C57BL/6J mice aged 6 and 12 months. Here we show that the effect of age on vision is not limited to a decrease in acuity and that contrast sensitivity decreases dramatically in 5 months. Rod and cone numbers and densities do not decrease as mice age.

Pilocarpine, a receptor agonist in the parasympathetic nervous system, is often used in mice video-oculography. When applied to the eye, it caused the iris sphincter muscle to contract and the pupil to constrict. Because of this, the contrast sensitivity function decreased slightly but not significantly (Fig. 5). Both groups had gains close to 1 at 100% contrast and 0.17 cyc/deg; therefore, pilocarpine application had no effect on motor performance. These results suggest that pilocarpine can be used to reduce pupil size without significant impact on contrast sensitivity.

I1-PKCi Mutants on Contrast Sensitivity
The contrast sensitivity function for I1-PKCi mutants is similar to that of their wild-type littermates. Mutants are reported to be slower than wild-types at finding a hidden platform in the Morris water maze task but not in the star maze task. Our data show that mutant and wild-type mice have identical contrast sensitivities (Fig. 6). Differences in navigating the Morris water maze, therefore, cannot be explained by differences in contrast sensitivity.

In summary, the methodology outlined in this article is more sensitive than the methods currently available. Behavior in response to visual stimuli is no longer recorded as an all-or-nothing response but as a graded response. By quantifying eye movement behavior evoked by moving sine gratings, information about response magnitudes can be obtained from optomotor responses. A drawback is that this method is not suitable for use in very young (younger than 3 weeks) animals or for valuable mutants. In those cases optomotor tests with freely moving animals are better suited.

We show that in C57BL/6J mice, these response magnitudes varied greatly between sexes and between mice that differed only a few months in age. Therefore, it is important to match groups according to age and sex in experiments that require unimpaired vision, such as water maze experiments and ocularomotor studies. Otherwise, impaired vision can be misinterpreted as a learning or a motor problem.

This new and sensitive method is useful for characterizing mouse models in which vision is affected as a result of mutations, aging, retinal degeneration, or neurologic impairment of the visual system.
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