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Photopic visual input is necessary for emmetropization in mice

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Abstract

It was recently demonstrated that refractive errors in mice stabilize around emmetropic values during early postnatal development, and that they develop experimental myopia in response to both visual form deprivation and imposed optical defocus similar to other vertebrate species. Animal studies also suggest that photopic vision plays critical role in emmetropization in diurnal species; however, it is unknown whether refractive eye development is guided by photopic vision in the mouse, which is a nocturnal species. We used an infrared mouse photorefractor and a high-resolution MRI to clarify the role of photopic visual input in refractive eye development in the mouse. Refractive eye development and form-deprivation myopia in P21-P89 C57BL/6J mice were analyzed under 12:12 h light-dark cycle, constant light and constant darkness regimens. Animals in all experimental groups were myopic at P21 (-13.2 ± 1.6 D, light-dark cycle; -12.5 ± 0.9 D, constant light; -12.5 ± 2.0 D, constant dark). The mean refractive error in the light-dark-cycle-reared animals was -0.5 ± 1.3 D at P32 and, and did not change significantly until P40 ($+0.3 \pm 0.6$ D, P40). Animals in this group became progressively hyperopic between P40 and P89 ($+2.2 \pm 0.6$, P67; $+3.7 \pm 2.0$, P89). The mean refractive error in the constant-light-reared mice was -1.0 ± 0.7 D at P32 and remained stable until P89 ($+0.1 \pm 0.6$, P40; $+0.3 \pm 0.6$, P67; 0.0 ± 0.4 , P89). Dark-reared animals exhibited highly hyperopic refractive errors at P32 ($+5.2 \pm 1.8$) and became progressively more hyperopic with age ($+8.7 \pm 1.9$, P40; $+11.2 \pm 1.4$, P67). MRI analysis revealed that emmetropization in the P40-P89 constant-light-reared animals was associated with larger eyes, a longer axial length and a larger vitreous chamber compared to the light-dark-cycle-reared mice. Constant-light-reared mice also developed 4 times higher degrees of form-deprivation myopia on average compared to light-dark-cycle-reared animals (-12.0 ± 1.4 , constant light; -2.7 ± 0.7 , light-dark cycle). Dark-rearing completely prevented the development of form-deprivation myopia (-0.3 ± 0.5). Thus, photopic vision plays important role in normal refractive eye development and ocular response to visual form deprivation in the mouse.

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Keywords

emmetropization; myopia; mouse model; form deprivation; photopic vision

1. Introduction

Postnatal refractive eye development is tightly controlled by visual input in a process of “emmetropization”, which adjusts ocular growth to ensure that the axial length of the eye matches its optical power (Curtin, 1985; Goss and Wickham, 1995; Hung and Ciuffreda, 2000; Kolata, 1985; Morgan, 2003; Norton and Siegwart, 1995; Troilo, 1990; Wallman, 1994; Wallman and Winawer, 2004). Emmetropization ensures that the refractive error present at birth is eliminated during early postnatal development (Curtin, 1985; Oyster, 1999; Wildsoet, 1997). Failure of emmetropization often leads to the development of myopia, which affects 33% of the adult population in the United States (Vitale et al., 2008) and up to 80% of adults in some parts of Asia (Lam et al., 2004; Lin et al., 2004).

Human population studies revealed that nearwork and reading associated with the lag of accommodation, which places the plane of best focus behind the retina (i.e., generates optical blur) when the subject performs nearwork tasks, play important role in the development of myopia (Goss, 2000; Gwiazda et al., 1993; Gwiazda et al., 2004; Hepsen et al., 2001; Parssinen and Lyyra, 1993; Saw et al., 2002; Seidemann and Schaeffel, 2003). Emmetropization and the role of optical blur (recapitulated in animal models by placing a diffuser or a lens in front of the eye) in refractive eye development have been described in species as diverse as the fish, chicken, tree shrew, monkeys, and guinea pig (Howlett and McFadden, 2006, 2009; Irving et al., 1991; Irving et al., 1992; Kroger and Wagner, 1996; Raviola and Wiesel, 1985; Shen and Sivak, 2007; Shen et al., 2005; Sherman et al., 1977; Smith et al., 1999; Smith and Hung, 1999; Wallman et al., 1978; Whatham and Judge, 2001; Wiesel and Raviola, 1977). The mouse also was recently introduced as a promising model for myopia research, which has a number of advantages compared to other animal models used for studies of myopia, such as availability of large number of well-characterized inbred strains and gene-targeted mutants, well-established techniques for genetic mapping and genome manipulation (Barathi et al., 2008; Schaeffel et al., 2004; Tejedor and de la Villa, 2003; Tkatchenko et al., 2010b).

Mice are classified as nocturnal animals; however, behavioral studies suggest that mice are also active during the day (Flores et al., 2007; Jacobs, 2013; Yang et al., 2009), and that vision is critical for accurate spatial navigation in mice (Buhot et al., 2001; Cook et al., 2001; de la Cera et al., 2006; Faulstich et al., 2004). The mouse basic spatial and temporal vision characteristics are fundamentally similar to those in humans (Histed et al., 2012; Umino et al., 2008). Mouse retina also has an area of increased density of ganglion cells and photoreceptors just temporal to the optic disc, which appears to represent an anatomical and functional analog of *area centralis* found in other mammals (Drager and Olsen, 1981; Jeon et al., 1998; Leamey et al., 2008). The fact that mice can follow a visual stimulus and use a specific fixation axis during Hartmann-Shack aberrometry (de la Cera et al., 2006) also supports the existence of a functional *area centralis*. In line with these observations, it was recently demonstrated that mice undergo emmetropization and develop experimental myopia in response to both visual form deprivation and imposed optical defocus (Barathi et al., 2008; Pardue et al., 2013; Schaeffel et al., 2004; Schmucker and Schaeffel, 2004; Tejedor and de la Villa, 2003; Tkatchenko et al., 2010b; Zhou et al., 2008).

Photopic vision plays a crucial role in emmetropization and ocular response to optical blur in diurnal animals because dark-rearing was found to interfere with emmetropization and

prevents the development of experimental myopia in chickens, monkeys, tree shrews and guinea pigs (Gottlieb et al., 1987; Guyton et al., 1989; Howlett and McFadden, 2006; Norton et al., 2006; Raviola and Wiesel, 1978); however, it is not clear what role (if any) photopic visual input plays in emmetropization and ocular response to the degradation of visual input in mice, which are classified as nocturnal animals. In the present study, we used high-resolution small animal MRI and automated eccentric infrared photorefractometry to analyze normal refractive eye development and form-deprivation myopia in C57BL/6J mice under different light regimens. The results show that photopic visual input is important for normal refractive eye development and plays a critical role in the ocular response to blur in mice.

2. Materials and methods

2.1. Animals and experimental design

C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and were maintained as an in-house breeding colony at the Wayne State University School of Medicine. C57BL/6J mice were recently shown not to have Rd8 mutation causing retinal degeneration in C57BL/6N mice (Mattapallil et al., 2012); however, C57BL/6J mice are known to have a relatively high incidence of microphthalmia, which affects from 4.4% to 10% of animals (Chase, 1942; Kalter, 1968). Therefore, animals were screened for the presence of microphthalmia and other ophthalmic abnormalities such as corneal opacities and anterior polar cataracts often associated with this condition (Koch and Gowen, 1939). Each animal was first examined visually, which is often sufficient to identify animals with microphthalmia or cataract. If no anomalies are identified after visual examination, eyes were examined under the dissecting microscope and using handmade slit lamp. This was followed by the examination of the pupil image with the photorefractor. Animals found to have microphthalmia, corneal opacities or cataract were removed from the study (~10% in our colony). All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Wayne State University Institutional Animal Care and Use Committee.

To analyze the effects of different light regimens on normal refractive eye development, three groups of mice (8 animals per group) were raised under light-dark cycle (12 h light/ 12 h dark) until P21. Daylight luminance was provided by fluorescent bulbs (F32T8/ TL841PLUS, Philips, Eindhoven, Netherlands; cage floor luminance 32 ± 2 cd/m², $n = 10$). Animals were refracted at P21 and then moved to rooms with different light regimens, i.e., 12 h light/12 h dark (light-dark cycle), 24 h light (constant light) and 24 h dark (constant dark), under which they were maintained until P89. Refractive errors in all experimental groups were measured at P21, P32, P40 and P67. In addition, animals maintained under light-dark-cycle and constant-light regimens were refracted at P89. After light-dark-cycle-reared and constant-light-reared animals were refracted at P89, random samples (5 animals each) were drawn from both experimental groups, and were used to analyze differences in ocular component dimensions using MRI.

To analyze ocular response to blur under different light regimens, three experimental groups (7 animals per group) were raised under the light-dark cycle until P24. At P24, diffusers were attached to the right eyes and animals were housed in transparent plastic cages under 12:12 h light-dark cycle, 24 hours light or 24 hours dark regimens for 21 days. At P45, diffusers were removed and animals were left in their cages to groom overnight. Refractive errors were measured next morning at P46.

2.2. Visual form deprivation

Frosted hemispherical plastic diffusers were hand-made using caps from 0.2 ml PCR tubes (Molecular BioProducts, San Diego, CA) and rings made of medical tape (inner diameter 6 mm; outer diameter 8 mm). A cap was frosted with fine sandpaper and attached to a ring with Loctite™ Super Glue (Henkel Consumer Adhesives, Avon, OH). On the first day of the experiment (P24), animals were anesthetized via intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg), and diffusers were attached to the skin surrounding the right eye with three stitches using size 5-0 ETHILON™ microsurgical sutures (Ethicon, Somerville, NJ) and reinforced with Vetbond™ glue (3M Animal Care Products, St. Paul, MN) (the left eye served as a control). Toenails were covered with adhesive tape to prevent mice from removing the diffusers. Animals recovered on a warming pad and were then housed in transparent plastic cages for the duration of the experiment.

2.4. Photorefracton

The refractive state of both left and right eyes was determined on alert animals using an automated eccentric infrared photorefractor (Schaeffel et al., 2004). The animal to be refracted was immobilized using a restraining platform, and each eye was refracted along the optical axis in dim room light (< 1 lux), 20-30 min. after instilling 1% tropicamide ophthalmic solution (Alcon Laboratories, Inc., Fort Worth, TX) to ensure mydriasis and cycloplegia as previously described (Tkatchenko et al., 2010a, b; Tkatchenko and Tkatchenko, 2010). Five independent measurement series (~ 300-600 measurements each) were taken for each eye. The measurements were automatically acquired by the photorefractor every 16 msec. Each successful measurement series (i.e., Purkinje image in the center of the pupil and stable refractive error for at least 5 sec.) was marked by a green LED flash, which was registered by the photorefractor software. Sixty individual measurements from each series, immediately preceding the green LED flash, were combined, and a total of 300 measurements (60 measurements × 5 series = 300 measurements) were used to calculate the refractive error mean and standard deviation (See Supplementary Information Section S1 for details regarding photorefractor resolution and accuracy).

2.3. High-resolution MRI

MRI was done as previously described (Luan et al., 2006; Tkatchenko et al., 2010a, b). Animals were refracted on the day preceding the MRI. On the day of the examination, animals were anesthetized via intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). A contrast agent gadopentetate dimeglumine (117 mg/ml, Magnevist®, Berlex Laboratories, Wayne, NJ) was used as eye drops to highlight the anterior chamber of the eye. Each mouse was then positioned on an MRI-compatible holder (Bruker, Karlsruhe, Germany). Animals were allowed to breathe spontaneously during the experiment. Rectal temperature was continuously monitored while the animal was inside the magnet. MRI data were acquired on a 7T ClinScan® MRI system (Bruker, Karlsruhe, Germany) using a two-turn transmit/receive surface coil (0.8-cm diameter, Bruker, Karlsruhe, Germany) placed over the eye. A preliminary low-resolution scan, in the horizontal plane of the eye going through the optic nerve, was used to position the high-resolution scan in the sagittal plane of the eye. The plane for the high-resolution scan was positioned to go through the center of the lens, the center of the cornea and the optic nerve, thus ensuring its close proximity to the optical axis of the eye. Seven serial low-resolution slices were collected using an adiabatic spin-echo imaging sequence (repetition time, 0.42 seconds; echo time, 12 ms; number of acquisitions, 1; matrix size, 128 × 128; slice thickness, 0.4 mm; field of view, 12 × 12 mm²; pixel size, 94 × 94 μm²; 56 seconds for 7 slices). High-resolution images were collected using an adiabatic spin-echo imaging sequence (repetition time, 1 second; echo time, 12 ms; number of acquisitions, 4; matrix size, 512 × 512; slice thickness, 0.60 mm; field of view,

12 × 12 mm²; pixel size, 23.4 × 23.4 μm²; 35 minutes/image). Virtual sagittal slices through the optical axis of the eye were obtained for each eye, and axial length (AL), equatorial diameter (ED), anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD) and circumference of the eye (Circ) were measured using ImageJ version 1.44p image processing software (Abramoff et al., 2004). Five independent measurements were taken for each parameter (with the exception of CRC – see below), and used to calculate the mean for each parameter. These animal-specific parameter means were then used to calculate the specific parameter mean and standard deviation for each experimental group. The AL was measured as a distance from the posterior (inner) surface of the cornea to the posterior (facing the RPE) surface of the retina. The ED was measured as the maximum sclera-to-sclera distance, perpendicular to the optical axis. The ACD was measured as the distance from the posterior surface of the cornea to the anterior pole of the lens. The LT was determined as the distance between the anterior and posterior poles of the lens. The VCD was measured as the distance from the posterior pole of the lens to the posterior surface of the retina along the optical axis. The CRC was determined by fitting the outline of the inner surface of the cornea (represented by a set of xy-coordinates of 30-40 equally spaced points residing on the inner surface of the cornea) to a circle using a nonlinear regression program written and run in MATLAB® (The MathWorks, Inc., Natick, MA). The program used a “trust-region” algorithm to minimize the sum-of-squares error (Ahn and Rauh, 1999; Ahn et al., 2001; Moura and Kitney, 1991). See Supplementary Information Section S2 for further details regarding MRI resolution and accuracy.

2.5. Data analysis

Data graphing was performed using SigmaPlot® version 10.0 (Systat Software, San Jose, CA). Power analysis was used to determine sample sizes for each experiment. Kolmogorov-Smirnov normality test was utilized to establish whether experimental data were normally distributed, and *F* test was used to check whether compared data sets had equal variances. All data sets presented here had normal distribution and equal variances, which was verified by Kolmogorov-Smirnov, Lilliefors, Shapiro-Wilk and *F* tests. We used ANOVA to analyze differences between dark-reared, light-dark-cycle-reared and constant-light-reared animals, to compare the effects of different light regimens on the development of form-deprivation myopia, as well as to analyze differences between multiple repeated measures in the same animals. We used paired *t* test to analyze differences between two repeated measures in the same animals, and *t* test for two independent samples to analyze differences between different groups of animals. All statistical analyses were performed using STATISTICA version 7.1 (StatSoft, Tulsa, OK). All data are presented as mean ± SD.

3. Results

3.1. Normal refractive eye development under different light regimens

Normal refractive eye development was analyzed in P21-P89 C57BL/6J mice under 12 h light/12 h dark (light-dark cycle), 24 h light (constant light) and 24 h dark (constant dark) regimens (Fig. 1, Table 1). Animals in all experimental groups were myopic at P21 (-13.2 ± 1.6 D, light-dark cycle; -12.5 ± 0.9 D, constant light; -12.5 ± 2.0 D, constant dark; $n = 8$). At P32, refractive errors were close to emmetropia in mice maintained under light-dark cycle and constant light regimen (-0.5 ± 1.3 D, light-dark cycle; -1.0 ± 0.7 D, constant light; $n = 8$), whereas we observed a significant hyperopic shift in the animals maintained in constant darkness compared to both light-dark-reared and constant-light-reared mice ($+5.2 \pm 1.8$ D; *t* test for independent samples, $p < 0.0001$; $n = 8$). At P40, animals maintained under light-dark cycle and constant light regimen remained emmetropic ($+0.3 \pm 0.6$ D, light-dark cycle; $+0.1 \pm 0.6$ D, constant light; $n = 8$), while dark-reared animals became even more hyperopic ($+8.7 \pm 1.9$; paired *t* test, $P40 \times P32$, $p < 0.0001$; $n = 8$). The refractive state of the eye in the

animals housed under constant light conditions was still emmetropic at P67 ($+0.3 \pm 0.6$ D; $n = 8$), whereas we observed a statistically significant hyperopic shift in refraction in the P67 animals maintained under light-dark cycle ($+2.2 \pm 0.6$ D; paired t test, $P67 \times P40$, $p < 0.0001$; $n = 8$) and a significantly higher hyperopia in the dark-reared group at P67 ($+11.2 \pm 1.4$ D; paired t test, $P67 \times P40$, $p = 0.004$; $n = 8$). A hyperopic shift in refraction in the animals maintained under light-dark cycle has increased from $+2.2 \pm 0.6$ D at P67 to $+3.7 \pm 2.0$ D at P89 (paired t test, $P89 \times P67$, $p = 0.02$, $n = 8$). Conversely, constant-light-reared mice remained emmetropic at P89 (0.0 ± 0.4 D, $n = 8$). The difference between these two groups of P89 mice was highly significant (t test for independent samples, constant light $\times$ light-dark cycle, $p < 0.0001$).

Light regimen had significant impact on refractive eye development (two-way ANOVA, $F(6, 129) = 53.48$, $p < 0.0001$). Refractive eye development in the animals maintained under light-dark cycle was significantly different from that in both mice housed under constant light regimen (two-way ANOVA, $F(4, 56) = 10.57$, $p < 0.0001$) and dark-reared animals (two-way ANOVA, $F(3, 42) = 33.53$, $p < 0.0001$). There were also significant differences in refractive eye development between constant-light-reared and dark-reared animals (two-way ANOVA, $F(3, 42) = 57.11$, $p < 0.0001$). Analysis of main effects and interaction effects of age and light regimen on refractive eye development in P32-P89 mice revealed that both age and light regimen had significant impact on refractive eye development; however, light regimen had a much stronger effect on refractive development ($F(2, 82) = 227.11$, $p < 0.0001$) compared to age ($F(3, 82) = 26.38$, $p < 0.0001$). Besides, age-related changes in refraction were strongly affected by the light conditions under which animals were raised ($F(5, 77) = 13.13$, $p < 0.0001$). A significant hyperopic shift in refraction was observed in P40-P89 mice raised under light-dark cycle (one-way ANOVA, $F(2, 39) = 28.63$, $p < 0.0001$) and in P40-P67 animals raised in constant darkness (one-way ANOVA, $F(1, 30) = 11.76$, $p = 0.0018$); however, no change in refractive error was observed in P40-P89 animals maintained in constant light (one-way ANOVA, $F(2, 45) = 0.66$, $p = 0.52$).

To get insight into the mechanism of this constant-light-promoted emmetropization, we compared ocular component dimensions in the light-dark-cycle-reared and constant-light-reared experimental groups at P89 using high-resolution MRI images of the right eye (Fig. 2, Table 2). The eyes in the constant-light-reared mice were significantly larger compared to the eyes of animals raised under light-dark cycle. The ocular enlargement was accompanied by significant increases in the Circ, AL, ACD and VCD. The Circ was 146 ± 27 μ m larger (t test for independent samples, $p = 0.0077$, $n = 5$) and the AL was 47 ± 2 μ m longer (t test for independent samples, $p = 0.0105$, $n = 5$) in the constant-light reared animals. The increase in the AL was primarily accounted for by the increases in the ACD (22 ± 8 μ m; t test for independent samples, $p = 0.0429$; $n = 5$) and VCD (27 ± 3 μ m; t test for independent samples, $p = 0.0004$; $n = 5$). No statistically significant changes were observed in the LT or CRC (see Tables 2 and 3 for CRC data).

3.2. Ocular response to diffuser-imposed blur under different light regimens

Susceptibility to form-deprivation myopia was analyzed in C57BL/6J mice under light-dark cycle, constant light and constant dark regimens (Fig. 3, Table 4). After 21 days of form deprivation, we detected -2.7 ± 0.7 D (paired t test, $p = 0.0002$, $n = 7$) of myopia in the deprived eyes compared to the contralateral control eyes in the animals maintained under light-dark-cycle regimen, and -12.0 ± 1.4 D of myopia in the constant-light-reared group of mice (paired t test, $p < 0.0001$; $n = 7$). The difference in the absolute value of refractive error in the form-deprived eye between these two groups of mice (-2.8 ± 0.7 D, light-dark cycle; -11.9 ± 0.9 D, constant light; $n = 7$) was highly significant (-2.8 ± 0.7 D under light-dark cycle versus -11.9 ± 0.9 D under constant light; t test for independent samples, $p < 0.0001$; $n = 7$). Refractive errors in the control eyes in the light-dark-cycle-reared and constant-light-

reared groups were -0.1 ± 0.5 D and $+0.1 \pm 0.6$ D respectively ($n = 7$). There was no statistically significant difference in the control eye refractive errors between these two groups of mice (t test for independent samples, $p = 0.51$). Contrary to the mice raised under light-dark cycle and constant light regimen, dark-reared animals developed high degrees of hyperopia in both control and visually deprived eyes ($+11.0 \pm 0.6$ D, control; $+10.7 \pm 0.8$ D, deprived; $n = 7$), similar to what we observed in the dark-reared non-deprived mice (Fig. 1, Table 1). The difference in refraction between form-deprived and control eyes in this group of mice was not significant (-0.3 ± 0.5 D; paired t test, $p = 0.10$; $n = 7$). One-way ANOVA revealed that the extent of myopia induced by form deprivation in C57BL/6J mice was strongly affected by light regimen ($F(2, 18) = 374.58$, $p < 0.0001$).

4. Discussion

4.1. Photopic vision and emmetropization in the mouse

Approximately 25% of human infants are born myopic, while the rest are either hyperopic (~68%) or emmetropic (~7%) (Cook and Glasscock, 1951; Oyster, 1999). Refractive errors in the one-day-old infants range from -12 D to +12 D (median refractive error is +1.5 D) (Cook and Glasscock, 1951); however, already at the primary school age both variability and magnitude of the refractive error are dramatically reduced. The median refractive error is reduced to +0.5 D and the range is reduced to 2 D (-0.7 D to $+1.3$ D) (Oyster, 1999). This trend continues into the adulthood, when the median refractive error stabilizes around zero diopters. This visually guided emmetropization process results in myopic children becoming less myopic and hyperopic children becoming less hyperopic. Our data indicate that mice undergo emmetropization too, i.e., both the variability and magnitude of the refractive error are reduced during the early postnatal period (P21-P40) in C57BL/6J and several other strains of mice (Tkatchenko et al., 2012; Tkatchenko et al., 2010a, b) independently of the magnitude and sign of the refractive error in the newborn animals. In C57BL/6J mice, refractive error stabilizes around zero diopters at P32-P40 in spite of highly myopic refractive errors observed in the newborn animals. C57BL/6J mice can also compensate for lens-imposed defocus (Tkatchenko et al., 2010b), which suggests active emmetropization.

Mice are classified as nocturnal animals and they are most active at night; however, mouse thresholds for rod-dominated (scotopic) and cone-dominated (photopic) vision, as well as the structure of the retinal scotopic and photopic pathways, are similar to those in humans (Leamey et al., 2008; Umino et al., 2008). Mice also have approximately 4 hours of activity during the daytime (Flores et al., 2007; Yang et al., 2009), and they are asleep for approximately 3 hours during the night (Flores et al., 2007; Yang et al., 2009). Considering that low intensity constant light (~300 lux) does not disrupt circadian rhythms in mice (Aschoff, 1981; Oliverio, 1980; Oliverio and Malorni, 1979) and that only approximately 0.062% of light penetrates the eyelids in C57BL/6J mice when the eyes are closed (see Supplementary Information, Section S4, Fig. S1), C57BL/6J mice placed in the constant light environment (~200 lux in this study) are estimated to be exposed to approximately 13 hours of photopic vision per 24-hour day because they are active on average for approximately 9 hours during the subjective 12-hour night (C57BL/6J mice are asleep for approximately 3 hours during the night) and approximately 4 hours during the subjective 12-hour day (C57BL/6J mice are asleep for approximately 8 hours during the day) (Flores et al., 2007; Yang et al., 2009).

Our results show that access to photopic vision is critical for emmetropization in mice because how well emmetropization functions appears to be directly related to the length of time per day during which mice are exposed to photopic visual stimulation. Animals raised under either light-dark cycle or constant light regimen exhibited gradual reduction in both magnitude and variability of refractive error during the early postnatal period (P21 – P40)

(Fig. 1, Table 1), which suggests active emmetropization. An estimated 4 hours of sporadic photopic vision (light-dark-cycle-reared mice) appears to be sufficient to provide adequate emmetropization signals at P21-P40, i.e., the age when the eye growth in mice was shown to be highly sensitive to visual input (Tkatchenko et al., 2010b). However, the same amount of photopic vision appears to be insufficient to maintain emmetropization in older animals (P40-P89), which were shown to be much less sensitive to visual input than younger mice (Tkatchenko et al., 2010b). The light-dark-cycle-reared (~4 hours of sporadic photopic vision) animals of this age (P40 – P89) exhibited increasing hyperopic shift in refraction and increasing variability in the refractive error, which suggests that emmetropization became less effective in the older animals raised with limited exposure to photopic vision. The magnitude and variability of the refractive error in the constant-light-reared (~13 hours of photopic vision) mice remained virtually unchanged between P32 and P89, which suggests that increased exposure to photopic vision provides sufficiently strong emmetropization signals in the older animals. Moreover, our data indicate that emmetropization in the older animals raised in constant light may be associated with active modulation of ocular growth because ocular growth was increased in the constant-light-reared mice. The $27 \pm 3 \mu\text{m}$ decrease in the VCD in the P89 light-dark-cycle-reared mice is predicted to translate into a hyperopic shift in refraction of approximately $+4.3 \pm 0.5 \text{ D}$, which is not significantly different from the hyperopic shift that we measured in the P89 animals raised under light-dark cycle compared to the P89 animals raised under constant light regimen ($+3.7 \pm 2.0 \text{ D}$) (Remtulla and Hallett, 1985; Schmucker and Schaeffel, 2004). In addition, the dark-reared mice, i.e., mice that had absolutely no access to photopic visual input during postnatal development, failed to undergo emmetropization and exhibited high degrees of hyperopia and high variability in refractive errors, which suggests lack of active emmetropization.

We also found that photopic visual input plays a critical role in the ocular response to blur, which underlies emmetropization. Constant-light-reared animals exhibited 4.4-fold stronger (0.57 D/day) response to visual form deprivation compared to the mice raised under the light-dark-cycle regimen (0.13 D/day), whereas no response was observed in the dark-reared animals. The extent of induced myopia observed in this study under light-dark-cycle regimen was similar to that reported by Schaeffel et al. (2004), who observed approximately 3.0 D myopic shift in form-deprived C57BL/6J mice under 12:12 h light-dark regimen. Other groups observed a slightly higher ($5\text{--}6 \text{ D}$) form-deprivation myopia in C57BL/6 mice under light-dark cycle (Pardue et al., 2008; Qian et al., 2009; Yu et al., 2011; Zhou et al., 2012); however, they performed form deprivation on younger animals and/or for longer periods of time (4–6 weeks).

Strong dependence of emmetropization on photopic vision was described in several diurnal species, such as chickens, monkeys and tree shrews (Gottlieb et al., 1987; Guyton et al., 1989; Howlett and McFadden, 2006; Norton et al., 2006; Raviola and Wiesel, 1978). Dark-rearing was also shown to block development of form-deprivation myopia in diurnal species (Gottlieb et al., 1987; Howlett and McFadden, 2006; Norton et al., 2006; Raviola and Wiesel, 1978). Similar to what we observed in mice, chickens and monkeys develop hyperopia if raised in the dark (Gottlieb et al., 1987; Guyton et al., 1989). These results seemingly contrast with the findings of Norton et al. (2006), who reported that 10-day exposure of tree shrews, which already completed emmetropization, to constant darkness results in a myopic refractive shift; however, these differences, perhaps, may be explained by differences in the ocular response to dark-rearing in the visually inexperienced animals, which had not yet completed emmetropization, and those, which already achieved emmetropia during postnatal emmetropization. Alternatively, the differences may be explained by the interspecies differences in the response to lighting conditions. Both of these possibilities warrant further investigation. Thus, our results show that photopic vision is necessary for normal refractive eye development in the mouse despite adaptation of mice to

nocturnal habitat, and that exposure to photopic visual environment is critical for induction of experimental myopia in mice.

4.2. Effect of constant light on refractive eye development in the mouse

In order to increase the extent of induced myopia during induced myopia experiments, we have been placing mice in the constant light environment to maximize exposure to photopic vision (Tkatchenko et al., 2010b). Exposure to constant light was shown to cause suppression of form-deprivation and lens-induced myopia in chickens, which is dependent on the intensity of light (Bartmann et al., 1994; Guo et al., 1996; Padmanabhan et al., 2007; Stone et al., 1995). Rearing of chicks under high intensity constant light (1000-3000 lux) resulted in the complete suppression of form-deprivation myopia (Bartmann et al., 1994; Stone et al., 1995), whereas low intensity constant light (70-140 lux) resulted in only minor suppression (Guo et al., 1996). The effect of constant light on the chicken eye is primarily manifested as hyperopia due to changes in the anterior chamber of the eye, which becomes shallower resulting in a significant increase in the corneal radius of curvature (Li et al., 1995; Liu et al., 2004; Oishi et al., 1987). However, these effects of constant light appear to be unique to chickens because constant light of comparable intensity (230 – 640 lux) did not have any significant effect on the refractive eye development in the infant rhesus monkeys (*Macaca mulatta*) (Smith et al., 2001; Smith et al., 2003). Our data also show that constant light did not cause flattening of the cornea, or development of hyperopia in mice. We also did not observe any suppression of form-deprivation myopia in the constant-light-reared animals.

These interspecies differences in response to constant light may result from important physiological differences between avian and mammalian circadian systems (Meijer et al., 1999; Natesan et al., 2002; Underwood et al., 2001; Yamazaki et al., 1999). The circadian system in all vertebrates is comprised of three main components, i.e., retina, suprachiasmatic nucleus (SCN) and pineal gland (PG) (Aronson et al., 1993; Brandstatter, 2003; Dibner et al., 2010). In mammals, SCN plays the role of master circadian pacemaker, which controls circadian rhythms and production of melatonin by PG; and the SCN circadian clock is entrained by the visual input from the retina (Challet, 2007; Dibner et al., 2010; Yamazaki et al., 2002). Organization of the avian circadian system differs from that in mammals in several ways (Brandstatter, 2003; Natesan et al., 2002; Underwood et al., 2001). The most important difference is that PG in birds can function autonomously because it contains light-sensitive photoreceptors and an endogenous circadian clock (Natesan et al., 2002). Therefore, production of melatonin by PG in birds can be controlled directly by the endogenous PG pacemaker that can be entrained and activated by the environmental light in the absence of retinal input (extraocular mechanism) (Deguchi, 1979; Kasal et al., 1979; Takahashi et al., 1989). The anterior chamber depth and corneal radius of curvature in the chicken are controlled by the PG-derived melatonin (Li and Howland, 1999, 2000a). This occurs via vision-independent mechanism because even complete elimination of all neural signals from the retina does not prevent characteristic ocular changes in the chicks reared in constant light (Lauber and Oishi, 1989, 1990; Li and Howland, 2000b). It is known that the PG-mediated extraocular mechanism is critical for the regulation of the anterior chamber growth in chickens because hoods, which shield PG from extraocular light, prevent the anterior segment changes in chicks reared in constant light (Li and Howland, 2000a, 2006). Conversely, extraocular light does not influence plasma melatonin levels or entrain circadian clock in mammals (Lee et al., 2003; Lockley et al., 1998; Lupi et al., 1999; Meijer et al., 1999; Yamazaki et al., 2002; Yamazaki et al., 1999). Furthermore, circadian rhythms and daily rhythms of melatonin synthesis remain normal in the laboratory mice (including C57BL/6J mice) under both light-dark-cycle and constant-light regimens in spite of reduced production of melatonin in the majority of inbred mouse strains (Aschoff, 1981; Conti and

Maestroni, 1996; Kasahara et al., 2010; Maestroni et al., 1986, 1987; Oliverio, 1980; Oliverio and Malorni, 1979; Vivien-Roels et al., 1998; Welp et al., 2010). Thus, we did not observe any reduction in the anterior chamber depth or flattening of the cornea in the mice raised under constant light regimen. Constant-light-reared mice also did not develop hyperopia. These results are in agreement with the data by Howland and Schaeffel that constant light rearing for 37 days did not have any effect on corneal curvature in mice (Schaeffel, 2008). Emmetropization observed in the constant-light-reared animals (compared to the light-dark-cycle-reared mice) is associated with increased growth of the eye, enlargement of the vitreous chamber, and no changes in the corneal radius of curvature.

4.3. Conclusions

Photopic vision plays important role in refractive eye development in the mouse in spite of adaptation of this species to nocturnal habitat. Similar to monkeys and chickens (Gottlieb et al., 1987; Guyton et al., 1989), the “default” trajectory of postnatal refractive eye development in the mouse in the absence of visual cues (i.e., under constant darkness conditions) is towards hyperopia. Photopic visual input plays important role in emmetropization in the mouse, as in diurnal species. It promotes emmetropization by regulating ocular growth and the size of the vitreous chamber of the eye. Furthermore, we found that photopic vision plays a critical role in the ocular response to blur, and that the degree of form-deprivation myopia is directly related to the duration of photopic visual exposure.

Our data strongly suggest that photopic vision plays important role in the refractive development of the mouse eye; however, a number of issues still need to be clarified. For example, analysis of the ocular parameters in the dark-reared mice would provide additional information about the effects of dark rearing on ocular growth in this species. In addition, exploring age-related changes in ocular component dimensions in the dark-reared mice and other species could shed light on the basic mechanisms of vision-independent eye growth and ocular component development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights for review

- Photopic vision is important for refractive eye development in the mouse
- Photopic vision promotes emmetropization in mice by regulating the size of the VCD
- The “default” of refractive eye development in the mouse is towards hyperopia
- Photopic vision plays important role in the ocular response to blur in the mouse

Fig. 1. Normal refractive eye development in C57BL/6J mice under different light regimens. Emmetropization completely failed in the dark-reared mice, and was much less effective in P40-P89 animals maintained under 12 h light/12 h dark regimen. The refractive errors in the animals raised in constant light remained stable between P32 and P89. Asterisks indicate statistically significant differences ($p < 0.01$, t test for two independent samples). Vertical error bars, SD; $n = 8$.

Fig. 2.

Analysis of changes in ocular component dimensions associated with emmetropization in C57BL/6J mice. (A) MRI images were used to measure the dimensions of various ocular components in P89 mice raised under either light-dark cycle or constant-light regimen (See Methods for details). (B) Emmetropization in the constant-light-reared animals was associated with increased ocular growth (larger eyes), increased AL, increased VCD, as well as slight increase in the ACD. Small decrease in the CRC was not statistically significant. ACD, anterior chamber depth; AL, axial length; Circ, circumference; CRC, corneal radius of curvature; LT, lens thickness; VCD, vitreous chamber depth. Asterisks indicate statistically significant differences (* $p < 0.05$, ** $p < 0.01$, t test for two independent samples). Vertical error bars, SD; $n = 5$.

Fig. 3.

Analysis of form-deprivation myopia in C57BL/6J mice under different light regimens. Susceptibility to experimentally induced form-deprivation myopia was significantly reduced in the light-dark-cycle-reared mice compared to the constant-light-reared animals. No myopia was detected in the dark-reared animals. Diffusers were applied to the right eyes at P24 (left eyes served as control) and form deprivation was carried out for 21 days. Experimental animals were maintained under one of the three light regimens for the duration of the experiment. Diffusers were removed at P45 and animals were refracted at P46. Asterisks indicate statistically significant differences ($p < 0.01$, paired t test). OD, right eye; OS, left eye; D, diopters; vertical error bars, SD; $n = 7$.

Table 1
Refractive eye development in C57BL/6J mice under various light regimens

Age (days)	12h light/12h dark	24h light	24h dark
21	-13.2 ± 1.6	-12.5 ± 0.9	-12.5 ± 2.0
32	-0.5 ± 1.3	-1.0 ± 0.7	+5.2 ± 1.8
40	+0.3 ± 0.6	+0.1 ± 0.6	+8.7 ± 1.9
67	+2.2 ± 0.6	+0.3 ± 0.6	+11.2 ± 1.4
89	+3.7 ± 2.0	0.0 ± 0.4	—

Data are shown as refractive error in diopters, mean ± SD. $n = 8$ animals (16 eyes; 2 eyes for each animal were averaged and then animal-specific means were used to calculate the mean and SD for the experimental group).

Table 2
Difference in ocular component dimensions between P89 C57BL/6J mice raised under 12h light/12h dark and 24h light regimens

Ocular component	12h light/12h dark (LD, μm)	24h light (LL, μm)	Difference (LL – LD, μm)	P-value
Circ	9802 $\pm$ 56	9947 $\pm$ 49	146 $\pm$ 27	0.0077
AL	3102 $\pm$ 18	3149 $\pm$ 19	47 $\pm$ 2	0.0105
ED	3202 $\pm$ 24	3219 $\pm$ 24	17 $\pm$ 6	0.3689
ACD	310 $\pm$ 14	332 $\pm$ 10	22 $\pm$ 8	0.0429
LT	2009 $\pm$ 9	2009 $\pm$ 4	0 $\pm$ 7	0.9845
VCD	786 $\pm$ 4	813 $\pm$ 6	27 $\pm$ 3	0.0004
CRC	1545 $\pm$ 14	1543 $\pm$ 22	-3 $\pm$ 9	0.8309

Data are shown as mean $\pm$ SD. ACD, anterior chamber depth; ACW, anterior chamber width; AL, axial length; Circ, eye circumference; CRC, corneal radius of curvature; ED, equatorial diameter; LD, light-dark cycle; LL, constant light; LT, lens thickness; VCD, vitreous chamber depth. $n = 5$.

Table 3
Corneal radius of curvature in P89 C57BL/6J mice raised under 12h light/12h dark and 24h light regimens

Regimen/Mouse ID	CRC	R ²
12h light/12h dark		
FL81	1552	0.9945
FL141	1536	0.9975
FL101	1526	0.9975
FL211	1554	0.9983
FL251	1560	0.9973
24h light		
CL41	1566	0.9971
CL121	1522	0.9990
CL171	1547	0.9637
CL231	1561	0.9979
CL271	1518	0.9812

Corneal radius of curvature (CRC, μm) was determined by fitting the outline of the inner surface of the cornea to a circle. R^2 , coefficient of determination (goodness of fit).

Table 4
Effect of visual form deprivation on refractive eye development in C57BL/6J mice under various light regimens

Light regimen	Control eyes (OS)	MOD eyes (OD)	Difference (OD - OS)	P-value
24h dark	+11.0 ± 0.6	+10.7 ± 0.8	-0.3 ± 0.5	0.0952
12h light/12h dark	-0.1 ± 0.5	-2.8 ± 0.7	-2.7 ± 0.7	0.0001
24h light	+0.1 ± 0.6	-11.9 ± 0.9	-12.0 ± 1.4	0.0004

Data are shown as refractive errors in diopters, mean ± SD.

MOD, monocular deprivation; OD, right eye; OS, left eye. $n = 7$.