Refractive Changes Induced by Form Deprivation in the Mouse Eye

Jaime Tejedor1 and Pedro de la Villa2

PURPOSE. Form-deprivation myopia has been induced in many different animal species. Underlying mechanisms are not well understood to date. In the current study the possibility of inducing refractive errors by form deprivation in the mouse (C57 BL/6) was investigated.

METHODS. Thirty animals underwent a 13- to 20-day monocular form-deprivation period by suture of the left eyelid before or after natural eye opening. A subsequent survival period was allowed in a group of animals. Retinoscopic refraction was performed at lid reopening and after the subsequent survival period, when applicable. Animals were killed and the eyes enucleated. The axial length of the eyes was measured in histochemically processed horizontal eye sections. A group of 30 age-matched normal control mice was also studied.

RESULTS. Depressed eyes showed significant development of myopia compared with the contralateral fellow eye after transient hypermetropia, regardless of whether they were deprived before or after natural eye opening. The refractive difference between form-deprived and corresponding fellow eyes was significantly correlated with the difference in axial length, which indicates that myopia is mainly axial. The differences exceeded those between eyes of age-matched normal control mice.

CONCLUSIONS. Form-deprivation myopia can be induced in the mouse. This model may be useful to investigate underlying mechanisms of myopia in mammals, because of easier handling and availability of genetically manipulated strains. (Invest Ophthalmol Vis Sci. 2003;44:32–36) DOI:10.1167/iovs.01-1171

Axial myopia can be induced in different species by depriving the eye of form vision during a period of susceptibility.1–11 Hypermetropia occurs as an initial transient shift before myopia develops in some animal species, and it may be caused by rearing the animals in the dark.9,16,18,19 These procedures are frequently used as models of human myopia and rarely as models of hypermetropia. In chicks and primates, changes in retinal level of several neurotransmitters and scleral changes are frequently used as models of hypermetropia. Regardless of whether they were deprived monocularly or binocularly, myopia develops in some animal species, and it may be caused by a ciliary muscle at least in general terms. We believe it would be of interest to study the response of the mouse eye to form deprivation, because this animal model enables easier pharmacologic or genetic manipulation than other species. This feature is considered an advantage to elucidate the mechanisms of refractive errors in vertebrate mammals. The purpose of the present study is to investigate the consequences and timing of the response to form deprivation in the mouse eye.

METHODS

Subjects

C57 BL/6 mice reared in a 12-hour light–dark cycle were used in the study. Management of the animals was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Refractive errors were induced monocularly in 30 mice by suturing the eyelid. The contralateral untreated eyes of these animals served as an interocular control. Untreated eyes of 30 animals (age, 30 days) provided normal control data for refractive errors, and eyes from 9 of these animals were used for axial length measurements.

Retinoscopy and Axial Length Measurements

With the animals restrained, the refractive state was determined by streak retinoscopy at 50 cm of working distance, using lens bars to neutralize the two principal meridians. Measurements were made in darkness without cycloplegia, because a ciliary muscle has not been demonstrated in the mouse29 and to avoid the possible influence of anticholinergics on scleral growth.30 Reliability of these measurements was good, according to the intraclass correlation coefficient (0.72, 95% CI: 0.6–0.9) computed after five replicate measures of the same eye in five different animals, obtained in a masked fashion by a single observer (average SD: 0.69 D, 95% CI: 0.5–0.9). All refraction data presented are spherical equivalents.

To test the hypothesis that potential refractive errors induced were axial, as indicated by previous animal models of form-deprivation myopia, we measured axial length of the eye. Eyes were enucleated immediately after death by 5% isoflurane inhalation, immersed in phosphate-buffered 4% paraformaldehyde (pH 7.4) for 24 hours at 4°C and subsequently in phosphate-buffered 30% sucrose (pH 7.4) overnight, and preserved at −20°C. Horizontal sections (20 μm) were cut in a cryostat at −20°C, mounted, and stained with hematoxylin-eosin. The fixation process was chosen for compatibility with immunocytochemical procedures. Images of horizontal sections obtained through the optic nerve head were transferred to a computer by a charge-coupled device (CCD) camera (Fig. 1). The eye perimeter was measured along the anterior corneal surface and retinochoroid interface with image analysis software (MIP-4 Advanced; MicroComputers, Barcelona, Spain). The axial length of the eye (anterior cornea–retinochoroid interface) was deemed to be the diameter of the circle with the same perimeter as the contour measured from the photographs. Five sections per eye were measured. The mean of the five measurements was used as the actual axial length.

Induction of Refractive Changes

Monocular form deprivation (MD) was achieved by suturing the left eye lid. Lid-suturing surgery was performed while the animals inhaled 2% isoflurane. Natural eye-opening occurs usually at approximately 12

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From the 1Department of Ophthalmology, Hospital Ramón y Cajal, Madrid, Spain; and the 2Department of Physiology, University of Alcalá, Madrid, Spain.

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Corresponding author: Jaime Tejedor, Department of Ophthalmology, Hospital Ramón y Cajal, C. Colmenar km 9100, Madrid 28034, Spain; tejedor@hrc.insalud.es.
days of age in mice. In 20 mice, the lids of the left eye were divided, trimmed, and sutured with 5-0 nylon, at 10 to 11 days of age (no previous visual experience). In 11 of these animals (group A), the lids were reopened along the fusion line at 30 days of age (20 days of MD) and retinoscopic refraction and axial length measurements were performed. Reopening of sutured lids was performed with mice under brief inhalation anesthesia. In the remaining nine animals (group B), parting of the left eye lid was performed 7 days earlier (13 days of MD), when we performed retinoscopy, and bilateral form vision was permitted until 30 days of life, when we performed retinoscopic refraction and axial length measurement.

In 13 additional mice, the left eye lids were sutured 4 days after eye opening (at approximately 16 days of postnatal life), thus allowing previous binocular visual experience. The free margins were trimmed away and sutured with 5-0 nylon under inhalation anesthesia. Ten animals survived until the lids were reopened at 30 days of age (14 days of MD). In five animals, we performed retinoscopy and enucleation for measurement of axial length at lid reopening (group C). In the remaining five mice, we also performed retinoscopy at 30 days of age but allowed them to survive for 7 more days, at the end of which retinoscopy and axial length measurement were performed (group D).

**Statistical Analysis**
Comparisons between control and deprived (lid-sutured) eyes were made with the paired *t*-test. Comparisons between unpaired groups of subjects were made with the unpaired *t*-test, when the observations in each group were normally distributed, or the Mann-Whitney test, when the underlying distribution could not be assumed to be normal. Regression lines were fitted to the data by simple linear regression. Statistical computation was performed using SPSS (SPSS Inc., Chicago, IL).

**RESULTS**

**Control Animals**
The retinoscopic findings and axial length dimensions found in eyes of control animals are shown as a scatterplot in Figure 2 and summarized in Table 1. Mean retinoscopic refraction in control animals (spherical equivalent) was approximately +13.5 D (+13.68 D in right eyes and +13.32 D in left eyes; 30 animals). Apparent hypermetropia is a retinoscopic artifact due to small eye size, but it is known that these values correspond to a nearly emmetropic state. Mean axial length was approximately 3.264 mm (3.256 mm in right eyes and 3.273 mm in left eyes; nine animals). Correlation between retinoscopic refraction and postfixation axial length was significant (*r* = −0.62; *P* < 0.01).

**Experimental Animals**
The axial dimensions and refractive findings of all eyes in experimental groups (A through D) are plotted in Figure 2. Intercocular differences in refraction and axial length of mice in the control and experimental groups are displayed as a scatterplot in Figure 3. A summary of these data is shown in Table 1.

**Effect of MD Not Followed by Form Vision**
In animals of group A (lid suture before visual experience), deprived eyes were significantly more hyperopic than contralateral control eyes (mean ± SD; 16.61 ± 2.36 D compared with 12.88 ± 1.44 D; *P* < 0.01, paired *t*-test). The measured axial lengths in deprived eyes were shorter than in the contralateral eyes (mean ± SD; 13.17 ± 0.195 mm compared with 13.32 ± 0.21 mm; *P* < 0.01, paired *t*-test). Correlation between interocular refractive difference and axial length difference was significant (*r* = −0.92, *P* < 0.01), and 84% of the variability in refractive difference could be explained by difference in axial length (coefficient of determination, *R*²). The differences in interocular refractive error and axial length were greater than the disparities between the two eyes in age-matched control animals (*P* < 0.01, Mann-Whitney test, and *P* = 0.01, unpaired *t*-test, respectively).

In group C (lid suture after visual experience), deprived eyes were also significantly hyperopic compared with contralateral control eyes (mean ± SD; 20.15 ± 4.45 D compared with 13.5 ± 1.23 D; *P* = 0.04, paired *t*-test). Measured axial length of the experimental eye was significantly smaller than that of the fellow control eye (mean ± SD; 2.961 ± 0.142 mm vs. 3.174 ± 0.195 mm, *P* = 0.01, paired *t*-test). The measured axial length of the experimental eye was significantly smaller than that of the fellow control eye (mean ± SD; 2.961 ± 0.142 mm vs. 3.174 ± 0.195 mm, *P* = 0.01, paired *t*-test).
TABLE 1. Axial Dimensions and Refractive Errors

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Eye</th>
<th>Left Eye</th>
<th>Difference§</th>
<th>P</th>
<th>Right Eye</th>
<th>Left Eye</th>
<th>Difference§</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.256 ± 0.138</td>
<td>3.273 ± 0.147</td>
<td>0.017 ± 0.114</td>
<td>0.75</td>
<td>13.68 ± 2.04</td>
<td>13.32 ± 1.98</td>
<td>-0.36 ± 0.9</td>
<td>0.06</td>
</tr>
<tr>
<td>A</td>
<td>3.524 ± 0.210</td>
<td>3.574 ± 0.195</td>
<td>-0.051 ± 0.100</td>
<td>&lt;0.01</td>
<td>12.88 ± 1.44</td>
<td>16.61 ± 2.36</td>
<td>3.73 ± 2.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B</td>
<td>3.226 ± 0.209</td>
<td>3.478 ± 0.122</td>
<td>0.252 ± 0.155</td>
<td>&lt;0.01</td>
<td>15.52 ± 1.41</td>
<td>7.19 ± 2.86</td>
<td>-6.33 ± 3.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C</td>
<td>3.228 ± 0.186</td>
<td>2.961 ± 0.142</td>
<td>-0.266 ± 0.215</td>
<td>0.04</td>
<td>15.5 ± 1.25</td>
<td>20.15 ± 4.45</td>
<td>6.65 ± 5.28</td>
<td>0.04</td>
</tr>
<tr>
<td>D</td>
<td>3.197 ± 0.169</td>
<td>3.305 ± 0.188</td>
<td>0.108 ± 0.103</td>
<td>0.04</td>
<td>13.96 ± 1.74</td>
<td>11.87 ± 3.97</td>
<td>-2.09 ± 1.65</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are mean ± SD, diopters; AL, axial length; R, refraction; MD, monocular deprivation.

* Measurements at 30 days of age (no MD interval). n = 9 (AL); n = 30 (R).
‡ Measurements at 37 days of age (MD interval, days: 16–30). n = 5.
§ Left eye minus right eye (deprived eye in groups A through D is left eye).
|| Paired t-test.

compared with 3.228 ± 0.186 mm; P = 0.04, paired t-test). The interocular difference in axial length correlated with the difference in refractive error (r = -0.85, P < 0.01) and explained 72% of the variability observed in interocular refractive difference. The differences in interocular refractive error and axial length in group C could not be explained by the naturally occurring difference in control mice of the same age (P = 0.047, Mann-Whitney test, and P = 0.03, unpaired t-test, respectively).

The relative induced hyperopia was greater in group C (6.65 D) than in group A (5.73 D), but the difference was not significant (P = 0.26, Mann-Whitney test). Therefore, whether the animals had or did not have visual experience before MD did not significantly influence the relative hyperopia observed at the end of MD.

Effect of MD Followed by Form Vision

In group B (lid suture before visual experience), deprived eyes had significant relative myopia compared with contralateral control eyes (mean ± SD: 7.19 ± 2.86 D compared with 13.52 ± 1.41 D; P < 0.01, paired t-test). The axial length of experimental eyes was significantly larger than the contralateral control eyes (3.478 ± 0.122 compared with 3.226 ± 0.209; P < 0.01, paired t-test). The difference in axial length between experimental and control eyes correlated significantly with the difference in refractive error (r = -0.88, P < 0.01), and it accounted for 77% of the variability in the difference in refractive error. Interocular difference in axial length and refractive state were greater than differences found in age-matched control subjects (P = 0.01, unpaired t-test, and P = 0.02, Mann-Whitney test, respectively). It is of interest that retinoscopic refraction performed on the day lid sutures were removed revealed relative hyperopia in deprived eyes than in fellow control eyes (16.08 ± 2.30 D compared with 13.20 ± 1.39 D; P < 0.01, paired t-test). This difference may be attributed to transient corneal flattening or to smaller axial length of the deprived eyes (or a contribution of the two factors). However, it is clear that the slightly hyperopic deprived eyes at removal of the lid suture became relatively myopic when compared with the contralateral control eyes at 30 days of age, after a post-MD period of bilateral form vision (Fig. 4).

**FIGURE 3.** Relationship between refractive error differences and axial length differences in control and monocularly lid-sutured mice: deprived (left) eye minus control (right) eye. A strong correlation was found in the control (r = -0.8, P < 0.01) and experimental groups (r = -0.9, P < 0.01). Groups are as described in Figure 2.

**FIGURE 4.** Spherical equivalent refractive error plotted for individual eyes at the first measurement against the second measurement made in groups B (at 23 and 30 days of age, respectively) and D (at 30 and 37 days of age, respectively). Dashed line: equality between the two measures; open symbols: right eye; filled symbols: left (deprived) eye. Symbols to the left of the dashed line indicate hyperopic shifts, and those to the right indicate myopic changes.
In group D (lid suture after visual experience), deprived eyes were also significantly hyperopic at lid reopening compared with control fellow eyes (mean ± SD; 18.51 ± 2.88 D compared with 13.52 ± 1.24 D; \( P = 0.04 \), paired \( t \)-test) but changed over time (7 days) to relative myopia (11.87 ± 3.97 D compared with 13.96 ± 1.74 D; \( P = 0.05 \), paired \( t \)-test) as shown in Figure 4. Axial length determined after myopia had developed was significantly more in deprived eyes than in contralateral eyes (mean ± SD; 3.305 ± 0.188 mm compared with 3.197 ± 0.169 mm; \( P = 0.04 \), paired \( t \)-test). Interocular differences in axial length were correlated with differences in refraction \( (r = -0.79, P < 0.01) \) and accounted for 62% of the variability in refractive error.

The myopia observed at the end of the binocular vision period was greater in group B (−0.53 D) than in group D (−2.15 D) but this difference did not reach statistical significance \( (P = 0.08, \text{Mann-Whitney test}) \), which indicates that the variable of visual experience before lid suturing did not have a detectable effect on the resultant myopia.

**DISCUSSION**

The main finding of this study is that refractive error may be experimentally manipulated in mice by form deprivation, thereby producing myopia after transitory hypermetropia. Induction of refractive changes has been described in many different species,\(^1\)\(^-\)\(^19\),\(^32\) but it has not been reported in the mouse. This fact is probably related to small eye size, resultant retinoscopic artifact, and difficulty for axial length measurement. However, Schaeffel and Burkhardt (Schaeffel F, ARVO Abstract, 182, 2002) have recently shown, in a poster presentation, amounts of myopia similar to those obtained in the current study, determined by eccentric infrared photorefraction, after fixing monocularly glued plastic occluders in black wild-type mice, but refractions in both eyes were tightly matched—that is, the fellow eye also became myopic. In the rat, phototoxic degeneration of the retina by continuous light exposure results in a mean myopic shift as a consequence of several changes in refractive surfaces and eye size, beyond pure retinal thinning.\(^32\)

The refractive and axial length measurements we obtained in control mice are in keeping with those previously reported, taking into account the small eye effect.\(^29\),\(^31\) This effect caused an apparent hypermetropia of approximately 13.5 D by retinoscopy, but previous data indicate that it corresponds to a nearly emmetropic state in mice.\(^29\) A more recent study in the rat suggests that correction factors for retinoscopy in small eyes may be smaller than previously assumed, based on the fact that retinoscopic reflex might be located at the outer retina rather than at the inner limiting membrane.\(^35\) In experimental cases the effect of the eye’s small size may be disregarded, because we compared the form-deprived eye with its corresponding nondeprived fellow eye. Small differences with published figures in axial length (3.26 mm in this study versus 3.37 mm in Remtulla and Hallett\(^25\)) may be attributed to the age of the animals, postfixation changes, and differences in the measurement methods. There is a significant correlation between postfixation axial length and the immediately preceding retinoscopic refraction \( (r = -0.6) \). Ultrasound biometry is theoretically ideal, because it is performed in vivo, but it was not reliable enough in our attempts (e.g., positioning of the transducer in such a small eye was arbitrary).

The effect of form deprivation on the eye varies among animal models. The behavior of the mouse eye when deprived of form vision resembles that of some species,\(^16\) in that it takes a few days after deprivation is discontinued for myopia to develop. Our findings indicate that there was a transient period of hypermetropia detected at the end of the monocular-deprivation period. Neither this hypermetropia nor the subsequent development of myopia was affected by whether the animals had had visual experience before lid suture. The transient hyperopic shift, previously reported in other species,\(^9\),\(^16\) may be due to transient corneal flattening, lesser axial length of the deprived eye, or some other optical factors. In transitory hyperopes, interocular difference in axial length correlates with interocular refractive difference, which suggests that axial length is involved in the relative transitory hypermetropia (see group C). Thus, the most plausible hypothesis to explain the described findings is that form deprivation induces myopia, but corneal flattening, with or without prevention of normal elongation of the eye by the lid-suture, causes transient hyperopia followed by axial myopia when the eye recovers its natural shape and growth. Whether this is a pure mechanical effect of prevention by lid suturing of normal elongation, after which the eye overcompensates without influence of vision, cannot be totally discounted by our data. However, the significant amount of axial length changes in deprived eyes after removal of the suture favors the possibility of a net myopic and vision-related effect. The difference in axial length of the deprived left eyes between group A (measurement at the end of MD) and group B (measurement after a subsequent period of vision) is significant \( (0.304 \text{ mm}, P < 0.01, \text{unpaired} \ t\text{-test}) \) and greater than the equivalent difference for the nondeprived right eyes of the same groups \( (0.098 \text{ mm}, P = 0.32, \text{unpaired} \ t\text{-test}) \). A similar conclusion may be obtained after comparing the left and right eyes of groups C and D (difference for left eyes: 0.344 mm, \( P < 0.01, \text{unpaired} \ t\text{-test}; \text{difference in right eyes: 0.031 mm, P = 0.79, unpaired} \ t\text{-test}) \). These data indicate that the deprived eyes grow longer after form deprivation ends.

Still another explanation of our findings is that deprivation induced hyperopia, compensated by the eye when the lids are opened, but the compensation continued into myopia, because the depth of focus of the eye is such that the hyperopia is detectable but slight myopia is not, or because defocus always induces enlargement of the eye. The depth of focus of the mouse eye is greater than 10 D (Artal P, personal communication, March 2002), in part because of poor quality of image in this optical system, implying that slight to moderate defocus is not likely to be detected by mice. The induced myopia appears to be axial, because the interocular difference in axial length correlated significantly and strongly with interocular refractive difference and explains most of its variability (results from groups B and D). When compared with other animal species, the main advantages of the present mouse model of form deprivation myopia include a better knowledge of the genetic mapping and architecture, the availability of genetically manipulated mice strains, and easier handling. These features make it useful in elucidating the molecular mechanisms involved in the genesis of refractive errors. The relative importance of genetic and environmental factors that contribute to differences in normal growth of the eye, lens, and retina have been studied in 50 mouse strains, showing a continuous growth of the adult eye and a high correlation between eye weight and retinal area and between lens weight and size of the posterior segment.\(^34\) Heritability is sufficiently high to justify the mapping of genes that modulate growth of different parts of the eye. Eye1 and Eye2, mapped to mouse chromosomes 5 and 17, respectively, are the first known loci that control normal variation in eye size in mammals.\(^35\) Further studies are needed to better the understanding of the response of the mouse eye to form deprivation, including the possibility of recovery from myopia, and to test the effect of lens defocus on refractive state of the eye. Possible applications to human idiopathic myopia (or hypermetropia),
both in its pathogenesis and therapy, remain a challenge for future studies.

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References