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ScienceDirect
Vision Research 48 (2008) 904–916
Vision Research
www.elsevier.com/locate/visres

Two models of experimental myopia in the mouse

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Received 15 May 2007; received in revised form 25 December 2007

Abstract

Purpose: The purpose of this study was to test the response of the mouse eye to two methods for the induction of experimental myopia.

Methods: Growth patterns of eyes were determined by axial length measurements from birth to adult in eyes of both sexes of normal mice examined on post-natal day 1 to 6 months and at 1 year. For the induction of experimental myopia, Balb/cJ mice were prepared with either unilateral lid suture or by a −10 D spectacle lens placed over one eye at post-natal day 10. Other mice received a plano lens as a control for lens wear. Refraction was carried out at post-natal days of 28, 42 and 56 in lid suture and spectacle lens wear group by streak retinoscopy. Axial length was measured by a combination of video image photography, digital caliper, or Optical Low Coherence Interferometry (OLCI). Corroborative optical modeling of the mouse eye was carried out using ZEMAX ray tracing software.

Results: Axial length (AL) increased linearly between post-natal day 1 to day 56, plateauing at about 140 days. After 18 days of unilateral lid suture initiated 10 days after birth, the AL of experimental eyes was 3.032 ± 0.003 mm, while AL in contra-lateral control eyes was 2.981 ± 0.005 mm (mean ± sem, $p < 0.05$, $n = 40$), after 32 days, the AL of experimental eyes was 3.290 ± 0.004 mm, and the AL of control eyes was 3.104 ± 0.002 mm ($p < 0.001$, $n = 60$). After 46 days of lid closure AL of experimental eyes was 3.592 ± 0.003 mm, while AL of control eyes was 3.363 ± 0.003 mm ($p < 0.001$, $n = 80$). Spectacle lens wear of 46 days duration increased AL in experimental eyes to 3.721 ± 0.002 mm, while AL in control eyes was 3.354 ± 0.003 mm ($p < 0.001$, $n = 100$). Refraction and ray tracing analysis substantiated the dimensional changes to be consistent with increased AL.

Conclusions: Two procedures to induce experimental myopia, initiated at eye opening, produced significant myopic shifts corresponding to increases in axial lengths after 32 and 46 days of lid suture and after 46 days wearing a −10 D spectacle lens.

Keywords: Development; Eye; Optics; Mouse; Myopia; Imaging

1. Introduction

Myopia is a leading cause of visual disability throughout the world (Midelfart, Kinge, Midelfart, & Lydersen, 2002; Saw, Carkeet, Chia, Stone, & Tan, 2002; Saw et al., 2002; Takashima et al., 2001; Tan, 2002). The prevalence of myopia has increased in many populations particularly in South East Asia (Saw et al., 2005; Woo et al., 2004). High myopia, which is the condition with more than a 6 D change from anisometropia can be sight threatening, and is associated with conditions such as choroidal atrophy, retinal detachment, glaucoma and strabismus (Fredrick, 2002; Ichibe et al., 2003; Ramakrishnan et al., 2003; Sarra et al., 2003; Hergesberg, 2003).

In the development of animal models of myopia, Wiesel and Raviola (1977) found that suturing the eyes of monkeys would induce myopia. This important result stimulated studies of experimental myopia in a number of species to understand the optics and biological basis of myopia. Subsequently, it has been found that experimental myopia can be induced in a wide variety of animal species including

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doi:10.1016/j.visres.2008.01.004

chickens (Irving, Callender, & Sivak, 1991; Lauber, 1991; Osol, Schwartz, & Foss, 1986; Yinon, Rose, & Shapiro, 1980), several species of primates (Tigges, Tigges, Fernandez, Eggers, & Gammon, 1990; Troilo & Judge, 1993), tree shrews (Sherman, Norton, & Casagrande, 1977; Marsh-Tootle & Norton, 1989; McBrien & Norton, 1992; Norton, 1990), rabbit (Tokoro, 1970), cat (Gollender, Thorn, & Erickson, 1979) and more recently in the guinea pig (Howlett & McFadden, 2006) and mouse (Beuerman, Barathi, Weon, & Tan, 2003; Faulkner, Kim, Iuvone, & Pardue, 2007; Schaeffel, Burkhardt, Howland, & Williams, 2004; Tejedor & Pedro, 2003).

The induction period varies from species to species. One to 2 weeks or even 1–2 h (Zhu, Park, Winawer, & Wallman, 2005) is sufficient to develop form deprivation or defocus myopia in chicks with a high degree of reproducibility. The tree shrew requires 4 weeks to develop myopia when lid suture is carried out on the day of eye opening (Siegwart & Norton, 1998) while monkeys require several months (Raviola & Wiesel, 1985). Mammalian models of myopia have an advantage over avian models, as the structure and biochemistry of their eyes are similar to that of humans; however, some species such as the mouse are particularly advantageous as a great deal is known about the biology and genetics as well. Zhou and Williams (1999) reported that the eye and lens of the mouse continues to grow after sexual maturity (40–60 days of age). The mouse as a potential model of experimental myopia is interesting as the sclera structure is similar to humans and mouse fibroblasts have all five types of muscarinic receptors as in the human (Barathi, Weon, Kam, Wess, & Beuerman, 2007; Qu et al., 2006).

In the present study, we used form deprivation and spectacle lens induced defocus to induce myopia in the Balb/cJ mouse, a strain little studied as a model for experimental myopia. As optical modeling using the data would be useful to confirm the biological measurements, we also developed a method to measure the major refractive surfaces in the mouse eye. This has allowed us to model the optics of the eye in the normal and myopic states. Early data has been presented indicating that the mouse may be suitable for studies of experimental myopia (Beuerman et al., 2003; Faulkner et al., 2007; Schaeffel et al., 2004; Schmucker & Schaeffel, 2004a; Schmucker & Schaeffel, 2004b; Tejedor & Pedro, 2003). In this study, we show that axial elongation and a myopic refractive shift can be induced in Balb/cJ mice after 4–6 weeks of lid suture or 6 weeks of −10 D spectacle lens wear when treatments were started at eye opening. Optical modeling was carried out to study image formation and the effect of a spectacle lens on the mouse eye.

2. Materials and methods

2.1. Animals

Pregnant Balb/cJ mice (*Mus musculus*) were obtained from the animal holding unit of the National University of Singapore. The maternal animals gave birth in our animal holding unit. The eyes of each animal were examined clinically to confirm that the cornea was clear and that there were no infections or injuries of the eye or lids. Naive control animals were housed in groups of 6 while experimental animals were housed individually after the age of 28 days in standard mouse cages at 25 °C on a schedule of 12:12 h of light on and off, with mouse pellets and water available ad libitum. Approval was obtained from the SingHealth IACUC and all procedures performed in this study complied with the Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmology and Vision Research.

2.2. Treatment groups and experimental design

Mice were used in groups of 20. Treatment commenced for both lid suture and spectacle lens wear on post-natal day 10. The groups and times of lid suture were as follows: 18 days ($n = 40$); 32 days ($n = 60$) and 46 days ($n = 80$). In spectacle lens wear, 5 groups of 20 animals were attached with −10 D lens and 20 mice was with a plano lens for 46 days. The groups and age of untreated or naive control were as follows: 28 days ($n = 20$); 42 days ($n = 20$) and 56 days ($n = 40$). The treatment groups and number of animals tested are shown in Table 1.

2.3. In vivo measurement of axial length

Axial length measurements were performed between 9–11 a.m. and 2–3 p.m. Prior to the measurements, mice were anaesthetized with 0.05–0.1 ml (IP) of a mixture of 0.2 ml 10% ketamine hydrochloride and 0.1 ml 2% xylazine hydrochloride, dissolved in 1.0 ml sterile saline. Subsequently, the animals were positioned on an adjustable platform that was placed close to the chinrest of the Optical Low Coherence Interferometer (OLCI), adapted for short measurement distances by Meditec, AC-Master (Carl Zeiss, Jena, Germany) and axial length measurements were made as previously described by Schmucker and Schaeffel (2004b).

2.4. Developmental morphometry of untreated mice

Eye and body weights were determined in 84 (6 mice/post-natal age) naive mice of both sexes, post-natal days 1, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, 140, 168 and 1 year. Axial length (AL) was measured using the AC-Master and by video image morphometry.

2.5. Methods for inducing experimental myopia

Lid suture: Right eyes were sutured with 7° nylon nonabsorbable sutures, black monofilament (A.C.S®, Alcon® Surgical, TX, USA) at day 10 after birth just prior to eye opening. Right eyes (OD) were used as the experimental eye. Left eyes (OS) were not sutured and served as experimental controls. Mice were used in groups of 20. The duration of

Table 1
Experimental design and number of animals tested

Groups	No. of animals used	Starting age (days)	Ending age (days)	Treatment (days)
Lid suture	40	10	28	18
	60	10	42	32
	80	10	56	46
−10 D spectacle lens induced	100	10	56	46
Plano spectacle lens induced	20	10	56	46
Untreated	20		28	
	20		42	
	40		56	
Ray tracing analysis	3		56	46