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Short communication

Ketamine–xylazine anesthesia causes hyperopic refractive shift in mice

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ABSTRACT

Mice have increasingly been used as a model for studies of myopia. The key to successful use of mice for myopia research is the ability to obtain accurate measurements of refractive status of their eyes. In order to obtain accurate measurements of refractive errors in mice, the refraction needs to be performed along the optical axis of the eye. This represents a particular challenge, because mice are very difficult to immobilize. Recently, ketamine–xylazine anesthesia has been used to immobilize mice before measuring refractive errors, in combination with tropicamide ophthalmic solution to induce mydriasis. Although these drugs have increasingly been used while refracting mice, their effects on the refractive state of the mouse eye have not yet been investigated. Therefore, we have analyzed the effects of tropicamide eye drops and ketamine–xylazine anesthesia on refraction in P40 C57BL/6J mice. We have also explored two alternative methods to immobilize mice, i.e. the use of a restraining platform and pentobarbital anesthesia. We found that tropicamide caused a very small, but statistically significant, hyperopic shift in refraction. Pentobarbital did not have any substantial effect on refractive status, whereas ketamine–xylazine caused a large and highly significant hyperopic shift in refraction. We also found that the use of a restraining platform represents good alternative for immobilization of mice prior to refraction. Thus, our data suggest that ketamine–xylazine anesthesia should be avoided in studies of refractive development in mice and underscore the importance of providing appropriate experimental conditions when measuring refractive errors in mice.

1. Introduction

Several vertebrate species, including non-human primates (Wiesel and Raviola, 1977), tree shrews (Sherman et al., 1977) and chickens (Wallman et al., 1978), have been historically used for myopia research. Myopia can be experimentally induced in these species by eyelid fusion, diffusers or negative spectacle lenses. Several recent reports have also suggested that visual form deprivation and imposed hyperopic defocus can lead to development of myopia in mice (Barathi et al., 2008; Schaeffel et al., 2004; Tejedor and de la Villa, 2003; Tkatchenko et al., 2010b). Therefore mice increasingly become a popular model for myopia research. However, small body size, small size of mouse eyes and lack of well-established procedures for restraining the animals cause difficulty in obtaining accurate measurements of the refractive state of the eye, which complicates the use of mice for myopia research.

Several different approaches have been used to measure refractive errors in mice, i.e. streak retinoscopy of animals anesthetized with ketamine–xylazine (Barathi et al., 2008), photorefraction of animals anesthetized with ketamine–xylazine (Pardue et al., 2008), and photorefraction of freely moving (Schaeffel et al., 2004; Schmucker and Schaeffel, 2004; Zhou et al., 2008) or physically restrained (Tkatchenko et al., 2010a,b) alert mice using a high-resolution automated eccentric infrared photorefractor. In some of these studies, animals have been immobilized by ketamine–xylazine anesthesia before refraction (Barathi et al., 2008; Pardue et al., 2008), and/or tropicamide was used to induce mydriasis (Pardue et al., 2008; Schaeffel et al., 2004).

Ketamine hydrochloride is a N-methyl-D-aspartate (NMDA) receptor antagonist, which is used for induction of analgesia and anesthesia. Xylazine hydrochloride is an α2-adrenergic receptor agonist, which causes sedation, anesthesia, muscle relaxation and analgesia. Tropicamide, a muscarinic acetylcholine receptor antagonist, is widely used in clinical practice to induce mydriasis and cycloplegia.

Although ketamine–xylazine anesthesia is increasingly used to immobilize mice and rats before refraction, xylazine alone and in combination with ketamine was shown to cause transient opacification of the crystalline lens of the eye in rodents (Calderone et al., 1986; Kufoy et al., 1989) that may interfere with the accurate measurement of refractive errors. The effects of tropicamide on refractive state of the mouse eye have not been investigated.

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In the present study, we have analyzed the effects of tropicamide eye drops, ketamine–xylazine and pentobarbital anesthesia on refractive state of the eye in C57BL/6J mice. We found that pentobarbital did not have any substantial effect on refractive status, whereas ketamine–xylazine caused a large hyperopic shift in refraction. Tropicamide caused statistically significant, but very small hyperopic shift in refraction.

2. Materials and methods

2.1. Animals

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. The experimental group was composed of 40-day-old (P40) animals from the same litter to minimize the impact of individual variations within the mouse population. 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). 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.

2.2. Drug treatment and dosages

Mydriasis was induced by 1% tropicamide ophthalmic solution (Alcon Laboratories, Fort Worth, TX), which was used as eye drops. One drop of the solution was instilled in each eye; excess of the solution was carefully removed with Kimwipes (Kimberly-Clark, Roswell, GA). Mydriasis was complete in 2–3 min and remained stable for at least 2 h (the longest we have carried out the observation). Application of the eye drops caused transient eye irritation, resulting in partial eyelid closure that lasted for 10–15 min; therefore, mice were refracted 20 min after application of tropicamide. Following the measurement, animals were anesthetized via intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg), and refracted again within 10 min after the injection. The same animals were anesthetized via intraperitoneal injection of pentobarbital (40 mg/kg) next day and refracted again within 10 min after injection. The pentobarbital injection was preceded by the application of tropicamide eye drops as described above.

2.3. Photorefraction

The refractive state of both left and right eyes was determined using a high-resolution automated eccentric infrared photorefractor as previously described (Schaeffel et al., 2004; Tkatchenko et al., 2010a). The animal to be refracted was immobilized using either restraining platform (alert mice) or drug-induced anesthesia (ketamine–xylazine- or pentobarbital-anesthetized mice), and each eye was refracted in dim room light (<1 lx). To ensure refraction along the optical axis, the first Purkinje image was aligned with the center of the pupil. In case of off-axis refraction, the first Purkinje image was positioned at the periphery of the pupil (~23° off the optical axis) by rotating the animal in the horizontal plane. Five independent measurements (5–10 s-long each) were taken for each eye. Each successful measurement was marked by a green LED flash, which was registered by the photorefractor software. Sixty points (automatically acquired by the system every 16 ms) from each measurement immediately preceding the green LED flash were combined, and a total of 300 points were used to calculate the mean and standard deviation.

2.4. Data analysis

Data modeling was performed with SigmaPlot version 10.0 (Systat Software, San Jose, CA). *P*-values were calculated using paired *t* test for two independent samples as implemented in Statistica version 7.1 (StatSoft, Tulsa, OK). All data are presented as mean ± SD.

3. Results

We found that animals in our population of 40-day-old C57BL/6J mice were emmetropic (0.0 ± 0.4 D, $n = 9$) when refracted along the optical axis of the eye, and highly hyperopic (+11.6 ± 2.4 D, $P < 0.0001$, $n = 9$) when refracted off the optical axis (Fig. 1, Table 1). Topical application of a 1% tropicamide ophthalmic solution resulted in only a slight, but statistically significant, hyperopic shift in refraction. The average on-axis refractive errors after the tropicamide treatment were +0.8 ± 0.8 D ($P = 0.02$, $n = 9$) (Table 1). Tropicamide did not have any adverse effects on the reflective properties of the cornea or the crystalline lens. However, ketamine–xylazine anesthesia delivered via intraperitoneal injection resulted in the development of a transient cataract as previously reported (Calderone et al., 1986; Kufoy et al., 1989). The cataract developed within 10–20 min after the ketamine–xylazine injection and persisted for several hours. In spite of the cataract development, animals could still be refracted between the onset of anesthesia (3–5 min after injection) and the appearance of cataract (10–20 min after injection). We found that ketamine–xylazine

Table 1
Refractive errors in P40 C57BL/6J mice before and after application of drugs.

Eye no.	Control[a]	Off-axis[b]	Tropicamide	Ketamine/xylazine[c]	Pentobarbital[c]
1	−0.4 ± 1.5	+8.7 ± 1.2	+1.2 ± 0.5	+7.1 ± 0.8	+0.3 ± 1.3
2	−0.2 ± 1.0	+15.8 ± 2.0	−0.4 ± 1.3	+5.4 ± 1.1	+0.5 ± 0.5
3	+0.1 ± 0.8	+9.8 ± 1.1	+1.0 ± 1.2	+6.2 ± 1.1	+0.4 ± 0.9
4	0.0 ± 1.3	+13.6 ± 3.9	+1.1 ± 0.8	+6.9 ± 0.8	−0.7 ± 0.7
5	+0.2 ± 1.3	+13.6 ± 2.9	+1.2 ± 1.0	+5.7 ± 1.6	+1.2 ± 0.6
6	−0.6 ± 1.0	+9.1 ± 4.1	−0.4 ± 1.0	+4.1 ± 0.6	−0.1 ± 0.5
7	+0.1 ± 1.0	+10.1 ± 2.3	+0.9 ± 1.3	+9.0 ± 2.0	+0.1 ± 0.5
8	−0.4 ± 1.1	+12.8 ± 2.7	+2.1 ± 0.6	+5.2 ± 0.9	−0.5 ± 0.8
9	+0.8 ± 1.1	+11.2 ± 1.4	+0.7 ± 1.0	+12.1 ± 0.7	−0.4 ± 0.8
Mean	0.0 ± 0.4	+11.6 ± 2.4	+0.8 ± 0.8	+6.9 ± 2.4	+0.1 ± 0.6

Refractive errors are shown in diopters as mean ± SD.
[a] Control refractions were measured along the optical axis of the eye.
[b] Maximum off-axis values (~23° off the optical axis) registered by the photorefractor are reported.
[c] Anesthetic injection followed tropicamide application (see Section 2).