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ON pathway mutations increase susceptibility to form-deprivation myopia

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ABSTRACT

The ON pathway mutation in *nob* mice is associated with altered refractive development, and an increased susceptibility to form-deprivation (FD) myopia. In this study, we used *mGluR6*-/- mice, another ON pathway mutant, to determine whether the *nob* phenotype was due to the *Nyx* mutation or abnormal ON pathway transmission. Refractive development under a normal visual environment for *mGluR6*-/- and age-matched wild-type (WT) mice was measured every 2 weeks from 4 to 16 weeks of age. The response to monocular FD from 4 weeks of age was measured weekly in a separate cohort of mice. Refraction and ocular biometry were obtained using a photorefractor and optical coherence tomography. Retinas were harvested at 16 weeks, and analyzed for dopamine (DA) and DOPAC using high-performance liquid chromatography. Under normal conditions, *mGluR6*-/- mice were significantly more myopic than their WT controls (refraction at 12 weeks; WT: 9.40 ± 0.16 D, *mGluR6*-/-: 6.91 ± 0.38 D). Similar to *nob* mice, two weeks of FD resulted in a significant myopic shift of −5.57 ± 0.72 D in *mGluR6*-/- mice compared to −1.66 ± 0.19 D in WT animals. No significant axial length changes were observed with either normal or FD visual conditions. At 16 weeks, *mGluR6*-/- retinas showed significantly lower DOPAC levels (111.2 ± 33.0 pg/mg) compared to their WT counterparts (197.5 ± 11.2 pg/mg). Retinal DA levels were similar between the different genotypes. Our results indicate that reduced retinal DA metabolism/turnover may be associated with increased susceptibility to myopia in mice with ON pathway defect mutations.

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Emmetropization is an active, visually-guided process whereby the axial length and the optical power of the eye precisely match each other to eliminate neonatal refractive errors, and bring the eye into perfect focus (Smith, 1998; Wallman and Winawer, 2004). Any disruption to this mechanism of ocular growth results in the development of refractive errors; eyes being either too short (hyperopia) or too long (myopia) (Wallman and Winawer, 2004).

A large body of animal research using lens-induced (Hung et al., 1995; Irving et al., 1992; Schaeffel et al., 1988; Wildsoet and Wallman, 1995) and form-deprivation (FD) (Nickla et al., 1998; Smith and Hung, 2000; Wallman et al., 1978, 1995) paradigms have shown the importance of the visual environment in the regulation of ocular growth. The ocular response to FD (Troilo et al., 1987) or lens-induced defocus (Wildsoet and Wallman, 1995) after optic nerve section in chickens indicates that the visual mechanisms involved in regulating refractive development localize primarily [if not exclusively, (Troilo et al., 1987; Wildsoet, 2003)] to the retina. Furthermore, partial diffusers only cause changes in the defocused area of the visual field in chickens (Diether and Schaeffel, 1997; Hodos and Kuenzel, 1984; Wallman et al., 1987) and primates (Smith et al., 2009), resulting in focal changes in refraction. Therefore, any defect in visual transmission through the retina could potentially influence ocular growth and lead to development of refractive errors.

Several studies have suggested a role for various retinal cell types and pathways in normal eye development. In chickens, physiological and morphological changes in photoreceptors are associated with experimentally induced myopia (Crewther, 2000). Pharmacological elimination of the OFF pathway in chicken retina

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using the D isomer of a gliotoxin α amino adipic acid (DαAAA) resulted in an enhanced rate of axial elongation under normal visual conditions, as well as with negative lenses (Crewther and Crewther, 1990). Conversely, inhibition of the ON pathway with the L isomer (LαAAA) caused a reduction in axial eye growth for both visually normal and lens-reared conditions (Crewther and Crewther, 1990). Furthermore, alternations in ON and OFF responses using 2-amino-4 phosphonobutyric acid (APB) or cis 2,3 piperidine-dicarboxylic acid (PDA) in chickens influence the ocular growth patterns and refractive errors (Crewther et al., 1996). More recent studies using various mutant mouse models have provided stronger evidence of retinal involvement in ocular refractive development (Chakraborty et al., 2014; Pardue et al., 2008; Park et al., 2013, 2014). Mouse models ensure complete and selective blockage of a single pathway, and allow examination of the interaction between genetic background and visual environment in refractive development (Pardue et al., 2013).

ON and OFF pathways are important for efficiently transferring information about changes in light stimuli to the higher visual centers, and processing contrast sensitivity information (Schiller, 1992; Schiller et al., 1986). These pathways have been implicated in refractive development of mutant mice with selective ON or OFF pathway defects (Chakraborty et al., 2014; Pardue et al., 2008). A non-functional ON pathway (Gregg et al., 2003; Pardue et al., 1998) in *nob* mice due to a mutation in *Nyx* (Gregg et al., 2003) causes low retinal dopamine (DA) levels and has previously been associated with a small myopic shift under normal visual conditions, and increased susceptibility to myopia in response to visual FD (Pardue et al., 2008). Conversely, non-functional OFF pathways in *Vsx1*-/- mice (Chow et al., 2001, 2004) had no significant effect on either normal or visually deprived refractive development (Chakraborty et al., 2014). These findings suggest that abnormal visual transmission through the ON pathway causes a greater refractive effect than disruption of the OFF pathway, perhaps due to changes in retinal dopaminergic activity, and may be more critical for normal ocular development in mammals.

In this study, we examined the refractive development and dopamine levels of *mGluR6*-/- mice (Masu et al., 1995; Sugihara et al., 1997; Tagawa et al., 1999; Takao et al., 2000), with a null mutation in the metabotropic glutamate receptor (*mGluR6*), which is located on the postsynaptic membrane of ON bipolar cells in both rod and cone systems (Nakajima et al., 1993; Vardi and Morigiwa, 1997). As a result of defective synaptic transmission through the ON-bipolar cells, *mGluR6*-/- mice have normal electroretinogram (ERG) a-waves, but non-recordable b-waves without significant change in responses from the OFF pathway (Masu et al., 1995). Additionally, *mGluR6* mutants show unmeasurable ON-responses from the superior colliculus (Masu et al., 1995). Furthermore, the loss of *mGluR6* produces these functional abnormalities without morphological changes in the retina (Tagawa et al., 1999; see reviews of mouse b-wave mutants McCall and Gregg, 2008; Pardue and Peachey, 2014). In humans, *mGluR6* mutations are associated with complete autosomal recessive congenital stationary night blindness (CSNB) (Dryja et al., 2005; Zeitz et al., 2005), abnormal cone ERG ON responses (Dryja et al., 2005) and high myopia (Xu et al., 2009) suggesting a potential link between the genetic mutation and refractive error development. In this study, we measured refractive changes in *mGluR6*-/- mice to determine whether altered refractive development in this mutant was similar to *nob* mice, which might implicate abnormal ON pathway transmission (and related changes in retinal DA levels) in refractive development versus some other aspect of the mutations.

An in-house breeding colony with both male and female homozygous *mGluR6* mutants (Jackson Laboratory, Bar Harbor, ME) on C57BL/6J background was maintained at the Atlanta Department of Veterans Affairs Medical Center. Mice were kept in 12:12 h light cycles of ~17 lux with mouse chow and water *ad libitum*.

Age-matched male and female *mGluR6*-/- and C57BL/6J wild-type (WT) mice were subjected to one of two different experimental conditions: a normal visual environment or form-deprivation (FD). For mice raised in a normal visual environment (WT n = 10; *mGluR6*-/- n = 10), refractive measurements were obtained every 2 weeks from 4 to 16 weeks of age. For FD experiments, baseline refractive error measurements for WT (goggled n = 6; naïve controls n = 6) and *mGluR6*-/- (goggled n = 5; naïve controls n = 5) mice were obtained at 4 weeks of age and then the mice were subjected to monocular visual deprivation in the right eye using head-mounted diffuser goggles, as described previously (Faulkner et al., 2007). Weekly measurements were performed on the FD cohort for a period of 2 weeks (i.e. to 6 weeks of age). All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local Institutional Animal Care and Use Committee.

For both a normal visual environment and FD conditions, refractive error and axial length measurements (measured from the anterior cornea to the retinal pigment epithelium) were acquired using an automated infrared photorefractor (Schaeffel et al., 2004) and a 1310 nm spectral-domain optical coherence tomography system (SD-OCT; Bioptigen Inc., Durham, NC), as described previously (Chakraborty et al., 2014; Pardue et al., 2008; Park et al., 2012, 2013). Statistical analyses were performed using commercial software (SigmaStat 3.5, Aspire Software International, Ashburn, VA). Changes in refractive error and axial length between the *mGluR6*-/- and C57BL/6J WT animals across age under normal and FD visual conditions were analyzed by repeated-measures two-way analysis of variance (ANOVA), and Holm-Sidak post-hoc tests for statistical significance.

Under a normal visual environment, both *mGluR6*-/- and WT mice showed significantly increased hyperopic refractions (values averaged between the two eyes for each mouse) from 4 to 16 weeks of age (Fig. 1A; two-way repeated-measures ANOVA main effect of age, $F(6,135) = 58.69$, $p < 0.001$). However, *mGluR6*-/- mice were significantly more myopic (mean refraction at 12 weeks ± standard error of the mean (SEM); WT: 9.40 ± 0.16 D, *mGluR6*-/-: 6.91 ± 0.38 D) than their age-matched WT controls throughout the developmental period (two-way repeated-measures ANOVA main effect of genotype, $F(1,135) = 75.04$, $p < 0.001$), suggesting that the *mGluR6* mutation had a significant effect on normal refractive development of the eye.

From 4 to 16 weeks of age, both genotypes exhibited a significant increase in axial length (mean change in axial length between 4 and 16 weeks of age; WT: 0.37 ± 0.01, *mGluR6*-/-: 0.37 ± 0.007 mm; two-way repeated-measures ANOVA main effect of genotype, $F(6,135) = 1214.2$, $p < 0.001$). However, no significant differences were observed between the two genotypes at any measured time point (two-way repeated-measures ANOVA main effect of genotype, $F(1,135) = 0.25$, $p = 0.623$). In order to elucidate the refractive changes in *mGluR6* mutants, we further examined the changes in corneal curvature using automated keratometry. However, no significant differences in corneal curvatures were observed between the *mGluR6*-/- and WT mice under either normal visual or FD conditions (data not shown). Given the inadequacy of the axial length and corneal curvature changes in explaining the myopic refractive error in *mGluR6*-/- mice, we hypothesize that it could be due to differences in other ocular optical parameters, such as changes in thickness, curvature or refractive index of the crystalline lens.

To examine the interaction between visual environment and genetic background, mice were form-deprived from 4 to 6 weeks of age, and the effect of goggling on refraction were compared