



Gene expression signatures in tree shrew choroid in response to three myopiagenic conditions



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ARTICLE INFO

Article history:

Received 15 May 2014

Received in revised form 23 June 2014

Available online 27 July 2014

Keywords:

Myopia

Choroid

Emmetropization pathway

Gene expression

Animal models

Refractive error

ABSTRACT

We examined gene expression in tree shrew choroid in response to three different myopiagenic conditions: minus lens (ML) wear, form deprivation (FD), and continuous darkness (DK). Four groups of tree shrews ($n = 7$ per group) were used. Starting 24 days after normal eye opening (days of visual experience [DVE]), the ML group wore a monocular -5 D lens for 2 days. The FD group wore a monocular translucent diffuser for 2 days. The DK group experienced continuous darkness binocularly for 11 days, starting at 17 DVE. An age-matched normal group was examined at 26 DVE. Quantitative PCR was used to measure the relative (treated eye vs. control eye) differences in mRNA levels in the choroid for 77 candidate genes. Small myopic changes were observed in the treated eyes (relative to the control eyes) of the ML group (-1.0 ± 0.2 D; mean $\pm$ SEM) and FD group (-1.9 ± 0.2 D). A larger myopia developed in the DK group (-4.4 ± 1.0 D) relative to Normal eyes (both groups, mean of right and left eyes). In the ML group, 28 genes showed significant differential mRNA expression; eighteen were down-regulated. A very similar pattern occurred in the FD group; twenty-seven of the same genes were similarly regulated, along with five additional genes. Fewer expression differences in the DK group were significant compared to normal or the control eyes of the ML and FD groups, but the pattern was similar to that of the ML and FD differential expression patterns. These data suggest that, at the level of the choroid, the gene expression signatures produced by “GO” emmetropization signals are highly similar despite the different visual conditions.

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1. Introduction

It is well established that a visually-guided emmetropization mechanism operates during post-natal visual development in a wide range of vertebrate species, including humans (Mutti et al., 2005; Norton, 1999; Schaeffel & Howland, 1988; Smith, Hung, & Harwerth, 1999; Wallman & Winawer, 2004). This mechanism uses refractive error, detected by the retina, to adjust the axial elongation rate of the growing eye to achieve a match between the location of the retina and that of the focal plane, reducing the refractive error. Studies that either cut the optic nerve (Raviola & Wiesel, 1985; Troilo, Gottlieb, & Wallman, 1987) or suppressed retinal output (Norton, Essinger, & McBrien, 1994) have found that there is a direct pathway within the eye: emmetropization signals originate in the retina, pass into the retinal pigment epithelium (RPE) and then into the choroid, finally reaching the sclera. In tree shrews (mammals closely related to primates), stim-

ulation of this pathway produces remodeling of the scleral extracellular matrix that alters its biomechanical properties, increasing the viscoelasticity and the axial elongation rate. We will refer to this as the “direct emmetropization pathway” because it can operate, albeit less well, in the absence of an “indirect” pathway comprised of connections from the retina, through central visual structures, that controls accommodation and other potential outputs to the eye and can affect refractive development (Dillingham, Guggenheim, & Erichsen, 2013; McFadden & Wildsoet, 2009; Schaeffel et al., 1990; Wildsoet, 2003).

Minus lens (ML) wear and form deprivation (FD) are two treatments often used to stimulate the emmetropization mechanism (Wallman & Winawer, 2004). Wearing a minus lens, held in place in front of the eye in a goggle frame, shifts the focal plane away from the cornea, creating an artificially hyperopic refractive state. This produces what has been described as a “GO” condition (Rohrer et al., 1993; Schaeffel & Howland, 1988). In response, the lens-wearing eye increases its axial elongation rate, moving the retina to the shifted focal plane at which point the hyperopia is eliminated and the GO condition has dissipated. When the minus lens is removed, the treated eye is myopic. Form deprivation with a translucent diffuser provides ample retinal illuminance but

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removes the possibility that sharply-focused images can occur on the retina. This also is a GO condition that causes an increase in the axial elongation rate and myopia in the treated eye. However, because elongation cannot restore clear retinal images, the GO condition continues and the elongation rate remains elevated throughout the treatment period.

A third procedure, treatment with a period of continuous darkness (DK), also produces increased axial elongation in tree shrews and in chicks. Tree shrews that were first raised in standard colony lighting with light-on and light-off periods, and then transferred to a completely dark environment, develop an increased axial elongation rate and become myopic compared with age-matched normally-raised animals (Norton, Amedo, & Siegwart, 2006). Chicks placed in DK also exhibit increased axial elongation (Troilo & Wallman, 1991). However, prolonged DK treatment also produces flattening of the cornea so that the birds eventually become refractively hyperopic despite having elongated eyes (Lauber, 1991). The retinal mechanism by which darkness produces a GO condition is still unclear.

Based on behavioral and electrophysiological studies, ML and FD treatments produce different, distinct patterns of excitation and inhibition in the retina that are communicated through the geniculostriate visual pathway to produce differing visual responses. Several studies have suggested that the retinal emmetropization-related signaling produced by these two GO conditions can be distinguished (Bartmann et al., 1994; Bitzer, Feldkaemper, & Schaeffel, 2000; Fujikado et al., 1997; Kee, Marzani, & Wallman, 2001; Schaeffel et al., 1994; Wildsoet, 2003; Yew & Wildsoet, 2003). However, in the sclera, it has been found that ML and FD produce nearly identical gene expression signatures; DK treatment also produces a similar gene expression signature (Guo et al., 2013). It appears that the different retinal activity produced by these three myopiagenic conditions may be converted into a common set of emmetropization signals as it passes through the direct RPE-choroid-sclera emmetropization pathway. Has this consolidation into a common signal occurred at the level of the choroid, or does the choroidal “compartment” of the emmetropization pathway still distinguish amongst the visual conditions that produce a retinally-generated GO condition?

Although changes in levels of proteins or other molecules presumably are key to actually transmitting signals from choroid to sclera, it has been found that changes in mRNA levels can identify the responses of the cells in tissues and are useful in identifying pathways of interest (Gao et al., 2011, 2013; Schippert et al., 2006; Shelton et al., 2008; Siegwart & Norton, 2005; Stone et al., 2011; Zhang, Liu, & Wildsoet, 2012). In a previous paper in tree shrew choroid (He et al., 2014), we examined the gene expression signatures produced by ML wear (GO) and by recovery from induced myopia (a STOP condition). Short-term ML treatment produced a GO gene expression signature that was distinct from the STOP gene expression signature. These results, involving altered gene expression in many genes, have shown that emmetropization-related signaling can be detected in the choroidal compartment of the direct emmetropization pathway. The goal of the present study was to examine alterations in gene expression in the choroid after 2 days of ML wear, 2 days of FD, and after 11 days of DK treatment. The question asked was whether the three GO conditions would produce the same, or very similar, gene expression signatures?

2. Materials and methods

2.1. Experimental groups

The methods employed in this study were generally identical to those in our previous paper (He et al., 2014). The juvenile tree shrews (*Tupaia glis belangeri*) used in this study were produced in

our breeding colony and raised by their mothers on a 14 h light/10 h dark cycle. Tree shrew pups open their eyes about three weeks after birth. The first day both eyes are open is day one of visual experience (DVE). All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Experimental groups were balanced to include both males and females, and avoided pups from the same parents wherever possible.

There were four groups of animals ($n = 7$ per group) (Fig. 1). Starting at 24 ± 1 DVE, the ML group wore a monocular -5 D (spherical power) lens for 2 days; the FD group wore a monocular translucent diffuser for 2 days; the DK group was housed in continuous darkness for 11 days, starting at 17 ± 1 DVE. In the ML and FD groups, the untreated fellow eye served as a control. A normal group (26N) was also examined at 26 DVE. Data from the ML and 26N groups were reported in the previous study (He et al., 2014) and are shown here for direct comparison with the FD and DK group results.

2.2. Visual treatments

Animals in all groups were anesthetized (17.5 mg ketamine, 1.2 mg xylazine; supplemented with 0.5–2.0% isoflurane as needed) and received a dental acrylic pedestal. For the ML and FD groups, this occurred at 21 ± 1 DVE; in the DK group, the pedestal was installed at 16 ± 1 DVE. After pedestal installation, all animals were placed in individual cages with standard colony fluorescent lighting (GE F34CW WM ECO cool white or F32T8/25W/SPX41/ECO), 100–300 lux on the floor of the cage. In the ML and FD groups, 3 days after pedestal installation, a goggle frame holding a -5 D lens (12 mm diameter PMMA contact lens; Conforma Contact Lenses, Norfolk, VA) or a translucent diffuser was clipped to the pedestal, firmly holding the lens or diffuser in front of the randomly selected treated eye. The untreated fellow control eye had unrestricted vision through the open goggle frame. Lenses were cleaned twice daily (approximately 9:30 AM and 4:30 PM) while diffusers were cleaned only in the morning. During cleaning, goggles were briefly (<3 min) removed under dim illumination and animals were kept in a darkened nest box to minimize exposure to visual stimuli. Animals in the DK group were transferred to continuous darkness 1 day after pedestal installation (at 17 ± 1 DVE) and checked daily with night-vision goggles and infrared illumination; DK treatment ended after 11 days. The 26N group received a pedestal at 21 ± 1 DVE but did not wear a goggle.

2.3. Refractive and axial measures

Non-cycloplegic refractive measures were made, in awake animals, at the start and end of the treatment period with a Nidek

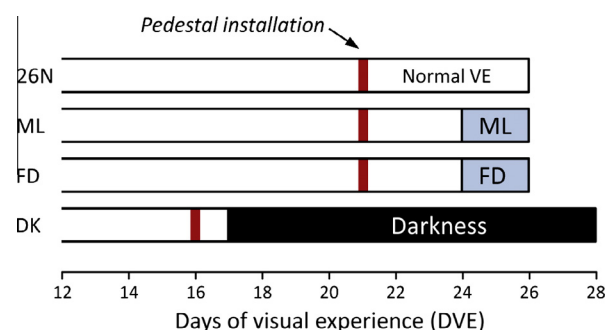


Fig. 1. Experimental groups and duration of treatments. The vertical bar indicates the point when a dental acrylic pedestal was installed under anesthesia. Filled regions indicate the type and duration of visual treatment. The right end of each bar indicates the time point when mRNA levels were measured.

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