Transfer from blue light or green light to white light partially reverses changes in ocular refraction and anatomy of developing guinea pigs

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Relative to the broadband white light (BL), postnatal guinea pigs develop myopia in a monochromic middle-wavelength light (ML, 530 nm) environment and develop hyperopia in a monochromic short-wavelength light (SL, 430 nm) environment. We investigated whether transfer from SL or ML to BL leads to recuperation of ocular refraction and anatomy of developing guinea pigs. Two-week-old guinea pigs were given (a) SL for 20 weeks, (b) SL recuperation (SLR, SL for 10 weeks then BL for 10 weeks), (c) ML for 20 weeks, (d) ML recuperation (MLR, ML for 10 weeks then BL for 10 weeks), or (e) BL for 20 weeks. Two weeks after transfer from ML to BL (MLR group), ocular refraction increased from $1.95 \pm 0.35$ D to $2.58 \pm 0.24$ D, and vitreous length decreased from $3.48 \pm 0.06$ mm to $3.41 \pm 0.06$ mm. Two weeks after transfer from SL to BL (SLR group), ocular refraction decreased from $5.65 \pm 0.61$ D to $4.33 \pm 0.49$ D, and vitreous length increased from $3.18 \pm 0.07$ mm to $3.26 \pm 0.11$ mm. The MLR and SLR groups had final ocular refractions that were significantly different from those of the ML and SL groups at 20 weeks (ML vs. MLR: $p < 0.0001$; SL vs. SLR: $p < 0.0001$) but were still significantly different from the BL group (BL vs. MLR: $p = 0.0120$; BL vs. SLR: $p = 0.0010$). These results suggest that recuperation was not complete after return to BL for 10 weeks.
Introduction

Previous studies of primates (Graham & Judge, 1999; Hung, Crawford, & Smith, 1995; Smith & Hung, 1999; Wiesel & Raviola, 1977), tree shrews (Norton & McBrien, 1992; Shaikh, Siegwart, & Norton, 1999; Siegwart & Norton, 1999), guinea pigs (Howlett & McFadden, 2006; Jacobs & Deegan, 1994; Liu et al., 2011), chicks (Crewther, Crewther, & Xie, 1996; Irving, Callender, & Sivak, 1991; Irving, Sivak, & Callender, 1992; Schaeffel, Glasser, & Howland, 1988; Schaeffel & Howland, 1991; Wildsoet, 1997; Wildsoet & Wallman, 1995), and fish (Kröger & Fernald, 1994; Kröger & Wagner, 1996) indicate that early visual experience plays an important role in refractive development of the eye. In particular, previous researchers have examined the effect of exposure to monochromatic light of different wavelengths upon longitudinal chromatic aberration (LCA), which refers to a wavelength-dependent refractive error (Liu et al., 2011). The results indicated that LCA affects emmetropization during refractive development of the eyes of chickens and fish (Kröger & Wagner, 1996; Rucker & Wallman, 2009).

As a result of LCA, the cornea and lens refract short-wavelength light (SL) more than long-wavelength light, so shorter wavelengths (blue) are focused closer to the lens than longer wavelengths (red) (Rucker & Wallman, 2009). The results of previous studies showed that the ocular refraction and ocular dimensions of animals adjust to the wavelength of light (Kröger & Fernald, 1994; Kröger & Wagner, 1996; Rohrer, Schaeffel, & Zrenner, 1992; Rucker & Wallman, 2008; Rucker & Wallman, 2009; Schaeffel & Howland, 1991; Seidemann & Schaeffel, 2002; Wildsoet, Howland, Falconer, & Dick, 1993). In particular, Rucker and Wallman (2008) showed that the long-wavelength pathway is more effective in modulating choroidal thickness, and the short-wavelength pathway is more effective in modulating ocular elongation. Other investigations suggested that choroid and axial compensatory responses underlie this effect (Rucker & Kruger, 2006; Rucker & Wallman, 2009; Seidemann & Schaeffel, 2002).

In a recent study (Liu et al., 2011), we raised guinea pigs under 430 nm and 530 nm monochromatic light and examined longitudinal changes in refraction and eye growth. The results were in accordance with the predicted direction of LCA–guided refractive development as reported in previous studies of fish and chicken. However, the refractive difference was several times greater than the LCA. This overcompensation manifests as an exaggerated increase in refraction.

Processes that function in active fine-tuning of the photoreceptors and postreceptoral processing of chromatic information during ontogenetic development may be similar in all color vision systems (Wagner & Kröger, 2005). Previous research has reported changes in refraction following changes in the spectral composition of the environment. For example, a rapid change in the refractive state of chickens occurs when the environment changes to red light (680 nm) following two days in blue light (430 nm) (Seidemann & Schaeffel, 2002). Additional research also reported considerable developmental plasticity in the color vision system of a fish, the trichromatic blue acara (Aequidens pulcher) (Kröger & Wagner, 1996).

Materials and methods

Animals and experimental design

Fifty pigmented guinea pigs (Cavia porcellus, ~2 weeks old; body weight = 100.38 ± 6.55 g) were purchased from the animal facility of Fudan University. All rearing and experimental procedures were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision research. The research project was approved by the Animal Care and Ethics Committee at the Eye & ENT Hospital of Fudan University (Shanghai, China).

The animals were randomly assigned to one of five groups with 10 animals per group: (a) SL, (b) SL recuperation (SLR), (c) ML, (d) ML recuperation (MLR), and (e) BL. The SLR and MLR groups were reared for 10 weeks under monochromatic light at 430 nm (SL) or 530 nm (ML) and then shifted into BL for 10 weeks. Animals were removed from their light treatment containers to a white light environment for measurements of ocular refraction, corneal curvature, and axial length, which typically lasted for 30 min. Measurements were performed every two weeks for 20 weeks.

Illumination conditions and housing

Illumination was from three different types of LED light tubes (Shanghai Rui Gao Xiang Light and
Electronic Corporation, Shanghai, China): blue ($\lambda_{\text{max}} = 430 \text{ nm, half-bandwidth} = 20 \text{ nm}$), green ($\lambda_{\text{max}} = 530 \text{ nm, half-bandwidth} = 30 \text{ nm}$), and white (color temperature = 5000 K). The blue and green LEDs were chosen based on the spectral sensitivity of the S-cone system (429 nm) and the M-cone system (529 nm) of the guinea pig (Jacobs & Deegan, 1994). Data from the manufacturer indicated that the white light had no bias toward the blue or green and perfectly covered the output of the two monochromatic sources. The illumination conditions and housing were designed as previously described (Liu et al., 2011). Four to six LED tubes were placed on each interior surface (including the ceiling and floor) of specially designed wooden crates with white paint on the inner walls to ensure homogenous illumination. Guinea pigs were reared in cages (dimensions: 80.0 × 50.0 × 40.0 cm, mesh size: 1.5 × 5.0 cm$^2$) that were placed inside these crates. Light intensity was controlled by LED voltage, and the light irradiance and photon flux density were measured with an IL1700 Research Radiometer (International Light Inc., Chula Vista, CA). Our previous study indicated that narrow-band lights had the same effect on refractive development when conditions were matched for equal luminance or equal absolute quantum number (Liu et al., 2011). Thus, the photon flux density of each treatment was selected to produce equal quantal numbers for each group and was set at $3 \times 10^{-4} \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ (about 1770 mW$\cdot$m$^{-2}$ for blue light, 700 mW$\cdot$m$^{-2}$ for green light, and 740 mW$\cdot$m$^{-2}$ for white light). All animals were maintained under a 12 h : 12 h light/dark cycle (8 a.m. to 8 p.m.) at a temperature of 22°C–26°C and at a relative humidity of 55%–65%.

Optical and biometric measurements

Optical and biometric measurements were conducted by two researchers who were blinded to animal allocation. General anesthesia was not used for either the baseline or measuring procedures because the opening was noninvasive and the animals were very cooperative during measurements. The cornea was anesthetized by topical application of 0.4% oxybuprocaine hydrochloride (Santen, Japan) before ultrasonography. Ocular refraction was measured in a dark environment. One hour before measurement, one drop of 1% cyclopentolate hydrochloride (ALCON, Belgium) was administered four times every 5 min to achieve cycloplegia and a completely dilated pupil. The refractive state was established by streak retinoscopy with trial lenses and recorded as the mean of the horizontal and vertical refractions. Previous study determined that refractive accuracy was 0.25 D (Zhou et al., 2006).

The corneal radius of curvature was measured by a keratometer (Topcon OM-4, Japan) with a +8.0 D spherical lens on the anterior surface so that measurements were performed on the steep cornea. Data was the mean of the horizontal and vertical measurements. The keratometer was calibrated with a series of stainless-steel balls (diameters of 5.5 to 11.0 mm). The corneal radius of curvature was determined from the mean of three readings on the balls (Norton & McBrien, 1992; Zhou et al., 2006).

A-scan ultrasonography was performed after keratometer measurements. The axial length of the eye was measured by A-scan ultrasonography (11 MHz; Optikon Hiscan A/B). During the axial measurement, the ultrasound probe rested directly in contact with the corneal surface to provide measurements of the anterior segment length (depth of anterior chamber and corneal thickness), thickness of the crystalline lens, and vitreous chamber length. For the anterior segment and vitreous chamber, the conducting velocity was 1540 m/s, and for the crystalline lens the conducting velocity was 1645 m/s (Howlett & McFadden, 2006; Zhou et al., 2006). A valid measurement was confirmed when echoes of the various intraocular surfaces were clear (Zhou et al., 2006). The axial length was the mean value of 10 measurements.

Statistics

All data were from the left eyes. Results are expressed as means ± standard deviations for descriptive purposes. A one-way ANOVA was used for comparisons of the three groups at different weeks. A repeated measure one-way ANOVA was used for comparisons of different weeks within a group. When a significant difference between groups was apparent, multiple comparisons were performed using the Bonferroni procedure with type-I error adjustment. Data were analyzed with SPSS version 15.0 (Chicago, IL), and a $p$ value less than 0.05 was considered significant.

Results

Changes in ocular refraction

Figure 1A and B shows the changes in ocular refraction of all five groups over the 20-week experimental period. The mean ocular refraction at the onset was about 4.4 D for all groups ($p = 0.9985$), but there were significant differences among the groups at the end of the experiment ($p < 0.0001$). Soon after initiation of the experiment, ocular refraction decreased in the ML, MLR, and BL groups (Figure 1A) but
increased in the SL and SLR groups (Figure 1B). At 20 weeks, the ocular refraction decreased to 2.78 ± 0.68 D in the BL group and to 0.90 ± 0.61 D in the ML group (BL vs. ML: p < 0.0001, Figure 1A) but increased to 6.95 ± 1.25 D in the SL group (BL vs. SL: p < 0.0001, Figure 1B).

The transfer of animals from ML to BL (MLR group) and from SL to BL (SLR group) at 10 weeks (ML vs. MLR: p = 0.7730; SL vs. SLR: p = 0.0720) led to a rapid and significant recuperation of ocular refraction at 12 weeks (ML vs. MLR: p < 0.0001; SL vs. SLR: p < 0.0001, Figure 1A and B). At 2 weeks after transfer of the MLR group to BL, the ocular refraction increased from 1.95 ± 0.35 D (10 weeks) to 2.58 ± 0.24 D (12 weeks). At 2 weeks after transfer of the SLR group to BL, the ocular refraction decreased from 5.65 ± 0.61 D (10 weeks) to 4.33 ± 0.49 D (12 weeks). At 20 weeks, the MLR group had an ocular refraction of 2.10 ± 0.32 D, and the SLR group had an ocular refraction of 4.18 ± 0.47 D (Figure 1A and B), which were significantly different from the values of the ML and SL groups (ML vs. MLR: p < 0.0001; SL vs. SLR: p < 0.0001). These observations indicated that transfer of animals from ML to BL or from SL to BL partially normalized the ocular refraction (Figure 1A and B). However, at 20 weeks, the SLR and MLR groups had ocular refractions that were still significantly different from the BL group (BL vs. MLR: p = 0.0120; BL vs. SLR: p = 0.0010), suggesting that the emmetropization process was not complete after transfer to BL for 10 weeks.

**Changes in corneal radius of curvature, anterior segment length, and lens thickness**

The corneal radius of curvature increased in a similar manner in all five groups from about 3.15 mm to about 3.77 mm during the 20-week experimental period (Figure 2A and B). In addition, all five groups had
similar increases in the length of the anterior segment from about 1.65 mm to about 1.79 mm (Figure 3A and B) and in lens thickness from about 2.65 mm to about 3.57 mm (Figure 4A and B). However, there were no significant differences between the groups at any time in any of these parameters, and transfer of animals to BL had no apparent effect on any of these parameters ($p > 0.05$ for all comparisons).

Changes in vitreous chamber length

Figure 5A and B shows the changes in the length of the vitreous chamber during the 20-week study period. The vitreous length was about 3.15 mm in all groups at the onset of the experiment and increased throughout the experimental period in the BL, ML, and MLR groups (Figure 5A). From week zero to week four, the vitreous length increased by $0.03 \pm 0.13$ mm in the BL group, by $0.08 \pm 0.12$ mm in the ML group, and by $0.11 \pm 0.12$ mm in the MLR group. However, the vitreous length decreased by $0.06 \pm 0.11$ and $0.07 \pm 0.09$ mm during the first 4 weeks in the SL and SLR groups (Figure 5B).

The mean vitreous lengths of the ML and MLR groups were significantly longer than that of the BL group from week six to week 10. At week 10, the vitreous length of the MLR group ($3.48 \pm 0.06$ mm) was significantly longer than that of the BL group ($3.33 \pm 0.06$ mm, $p < 0.0001$, Figure 5A). At 2 weeks after transfer of the MLR group to BL (12 weeks), the vitreous length decreased from $3.48 \pm 0.06$ mm to $3.41 \pm 0.06$ mm ($-0.07$ mm), which was significantly different from the ML group (ML vs. MLR: $p = 0.0005$). The mean vitreous lengths then increased at a rate similar to that of the BL group for the remaining 8 weeks. The MLR group had a longer vitreous length than the BL group during this period, but this was not statistically significant ($p > 0.05$ for all comparisons). However, the vitreous length of the MLR group was significantly shorter than that of ML group from week 12 to the end of the experiment, suggesting that transfer of animals from ML to BL normalized the length of the vitreous chamber (Figure 5A).
At 10 weeks, the vitreous length of the SLR group (3.18 ± 0.07 mm) was significantly less than that of the BL group (3.33 ± 0.06 mm, p = 0.0001, Figure 5B). At 2 weeks after transfer of the SLR group to BL, the vitreous length increased from 3.18 ± 0.07 mm (10 weeks) to 3.26 ± 0.11 mm (12 weeks) (+0.08 mm), but this was not significantly different from the vitreous length of either the SL group or the BL group. The vitreous length of the SLR group was significantly less than that of the BL group at 20 weeks (p = 0.0050) but not significantly different from that of the SL group, suggesting that transfer of animals from SL to BL had no significant effect on the length of the vitreous chamber.

**Discussion**

In this study, 2-week-old guinea pigs were reared under five different lighting conditions for 20 weeks. As we reported previously (Liu et al., 2011), the eyes of animals reared under BL underwent a gradual developmental decrease in ocular refraction (emmetropization) that was accompanied by an increase of the vitreous length, but the eyes of animals reared under SL or ML had markedly different developmental changes in ocular refraction and axial length. Relative to animals in the BL group, animals in the ML group became more myopic, and those in the SL group became more hyperopic. These changes occur when the two groups were reared under conditions of equal illumination or equal photon flux density (Liu et al., 2011).

The novelty of the present study is that we examined the effect of transfer from ML to BL and from SL to BL at a time when guinea pig eyes were still developmentally plastic, in that changes in ocular refraction and biometry can still occur. Our results indicate that transfer of guinea pigs from SL to BL or ML to BL led to rapid changes in ocular refraction (within 2 weeks) without changes in the radius of corneal curvature, lens thickness, or length of the anterior segment. These parameters change during normal ocular development (Liu et al., 2011) but were not affected by the different illumination conditions that we employed. The SLR and MLR groups had ocular refractions that approached that of the BL group. We propose that this reversal in refraction status following transfer to BL can be explained by recuperation of the emmetropization process, which normally occurs during postnatal development. Our results agree with those of previous reports that chromatic aberration induced by monochromatic light in the developing animal did not interfere with the recovery from myopia (Wildsoet et al., 1993).

In the discussion of our previous article, we suggested that our finding of sudden changes in eye-growth direction after changing of the monochromatic light can be the result of the formation of a new signal or loss of the original signal (Qian et al., 2013). If there was no new signal after the shift and loss of the original signal was responsible for the observed refractive drift, then a shift to any other spectrum should yield similar results. The results in Qian et al. showed that the eyes shifted from 430 nm light to 530 nm light did not grow to the same refractive states as the eyes that were maintained at 530 nm. Actually, the final refractive states of the two experimental groups were not significantly different and were closer to the refractive state of the BL group. In this manuscript, we report the similar observation that the MLR and SLR groups had final ocular refractions that were closer to the refractive state of the BL group. This suggests that loss of the original signal resulted in the similar observed refractive drift. However, in this study, both groups were...
shifted to BL. If generation of a new signal after the shift was solely responsible for the observed changes, then both groups should also have similar results because both groups received the same new BL signal. Thus, we cannot rule out that the refraction shifts were really due to the new lighting stimulations from BL (new signal), not just recovery from removal of the original SL or ML stimulation (loss of the original signal). Whether the mechanism of emmetropization plasticity is different when the eye is switched to different lighting (white light vs. monochromatic lights) needs to be clarified by further study.

We currently have no data to explain why the initial change in refraction at the start of the experiment (4 weeks for ML, 6 weeks for SL) was slower than the change in refraction after the crossover (within 2 weeks for each group). We observed the similar phenomenon for vitreous chamber length change. Based on the previous finding that emmetropization is mainly related to changes in the vitreous chamber length in guinea pigs (Zhou et al., 2006), the animals we used to initiate our experiment (age ~2 weeks) might be still immature and require more than 10 weeks (age ~12 weeks) to change the developmental direction of the vitreous chamber length. In our study, the ML group had a significant difference from the BL group in vitreous chamber length at 6 weeks after initiation of the experiment (Figure 5). However, the vitreous length significantly decreased at 2 weeks after transfer of the MLR group to BL. Increasing plasticity with increasing age seems unlikely, so the focus/defocus mechanism in the accommodation system may play a role. In other words, visual emmetropization may be guided by a balance between multiple chromatic focal lines rather than a single dominant chromatic focus (Rucker, 2013). Previous study found that accommodation to monochromatic targets is not as accurate as accommodation to a white-light target (Aggarwala, Nowbotsing, & Kruger, 1995). Initially, the guinea pigs shifted from BL to either SL or ML light, which caused the retina—a much narrower location—to be focused and took the emmetropization mechanism a while to determine which direction should grow. Changing from SL or ML to BL at 10 weeks, however, might present the emmetropization mechanism with a clearer definition of which direction should be developed.

The compensatory changes that we observed did not allow full recovery of the refractive power or vitreous length relative to the BL group at the end of the study period (at 20 weeks). In particular, the SLR group had ocular refraction that was about 1.3 D greater than the BL group, and the MLR group had ocular refraction that was about 0.6 D less than the BL group. The incomplete reversal of ocular refraction and vitreous length following transfer from ML or SL to BL may be explained by overcompensation during the 10 weeks of growth under SL and ML (Liu et al., 2011) or by insufficient developmental plasticity of animals at the time of transfer to BL. Previous research applied −4.00 D lenses to guinea pigs from the age of 3 weeks to 7 weeks to induce defocus-induced myopia. Biometric measurements at 14 days after lens removal (at the age of 9 weeks) indicated complete recovery (Lu et al., 2009). In contrast, the guinea pigs in the present study were exposed to SL or ML for 10 weeks before transfer to BL, so they should still have sufficient developmental plasticity.

When guinea pigs were transferred from ML to BL, the rapid and significant change in ocular refraction (within 2 weeks) was accompanied by a significant change in vitreous length. Previous research (Rucker & Kruger, 2006; Rucker & Wallman, 2008; Seidemann & Schaeffel, 2002) indicated that the rapid change of vitreous length following experimental manipulation could result from modification of choroid thickness. Thus, the changes that we observed in vitreous length are presumably related to inverse changes in choroid thickness. Changes in vitreous length and choroidal thickness are closely associated with myopia and recovery (Lu et al., 2009). During the process of recovery from induced myopia in adolescent guinea pigs, refractive changes corresponded to a slower vitreous lengthening and a rapid thickening of the choroid (Lu et al., 2009). Changes in the axial elongation of the vitreous chamber allowed the juvenile guinea pigs to recover from monocular myopia induced by a face mask (Zhou et al., 2007).

However, the findings for the SLR group were different. Transfer from SL to BL did not significantly alter vitreous length and did not correlate with the rapid and significant change in ocular refraction at 12 weeks. The vitreous length of the SLR group at 20 weeks was significantly less than that of the BL group but was not significantly different from that of SL group and also did not correlate with the change in ocular refraction at 20 weeks. This finding indicated that factor(s) other than vitreous length (e.g., choroidal thickness) might be responsible for the short-term rapid change in ocular refraction during the recovery from a monochromatic SL environment. In fact, it is still unknown whether changing in vitreous chamber depth is due to short-term choroidal thickness changes or long-term scleral remodeling. Our experimental period might be too short to see the actual change.

Our study had a technical limitation. Our use of low-frequency/low-resolution ultrasonography, which measures eye length from the cornea to the vitreoretinal junction instead of the sclera, did not include choroidal thickness. The border between the anterior segment and vitreous chamber was somewhat blurred, and this may have increased the standard deviations of
measurements, making it difficult to identify more subtle changes.

**Conclusion**

Our study of developing guinea pigs indicated that ML promoted axial eye growth and led to myopia and that SL slowed axial eye growth and led to hyperopia. The alterations in ocular refraction and vitreous length induced by ML and SL were rapid, and the process of recovery occurred upon transfer to BL. However, only partial recuperation occurred after 10 weeks of BL. This may be because the eyes had overcompensated or had insufficient developmental plasticity at the time of transfer to BL. These experiments represent the initial stages of our study of the complex feedback mechanisms underlying developmental emmetropization.

**Keywords:** emmetropization, longitudinal chromatic aberration, ocular refraction, vitreous length, monochromatic lighting, guinea pig

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**References**


Rucker, F. J. (2013). The role of luminance and


