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Interactions of chromatic and lens-induced defocus during visual control of eye growth in guinea pigs (*Cavia porcellus*)

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ABSTRACT

It was recently demonstrated that chromaticity could affect eye growth and refractive development in guinea pigs but it remained unclear whether correction with spectacle lenses could balance these effects and how retinal responses change with different spectral compositions of light. Three illumination conditions were tested: blue, red and white light. Animals were raised without or with monocular spectacle lenses from three to seven weeks of age. Luminance electroretinograms (ERGs) were recorded to explore retinal responses with the different spectral compositions. In our special colony of pigmented guinea pigs, characterized by residual hyperopia, spontaneous myopia and poor emmetropization, red light induced early thinning of the choroid and relative myopia, compared to white light. Effects of red light could not be suppressed if positive spectacle lenses were worn. ERGs showed that red light failed to elicit robust retinal responses. Blue light inhibited axial eye growth, even when animals were reared with negative lenses. Intensity-matched blue and white light elicited similar a-waves but different b-waves, suggesting that the wavelength of light affects visual control of eye growth through different processing in the inner retina. We hypothesize that blue light might stimulate preferentially the ON pathway to inhibit myopia induced by negative lenses, at least in guinea pigs.

1. Introduction

Visual control of eye growth has been extensively investigated and abundant evidence shows that the axial eye growth and refractive state are altered not only by visual deprivation and lenses rearing but also by the alteration of specific visual cues, such as spatial frequency composition (Schmid & Wildsoet, 1997), ambient illuminance (Ashby & Schaeffel, 2010), and spectral composition of light (Kroger & Wagner, 1996; Rucker & Wallman, 2008; Seidemann & Schaeffel, 2002). As a result of the longitudinal chromatic aberration (LCA), light with shorter wavelengths is focused more anteriorly compared to light with longer wavelengths. As a consequence, eyes of African cichlid fishes that were raised under red light were larger than those raised under blue light (Kroger & Wagner, 1996); in chicks, the eyes compensated for chromatic defocus imposed by LCA (Rucker & Wallman, 2009) and among mammals, guinea pigs were found to become more myopic when they were reared under red (769 nm) or green light (530 nm), compared to those raised under white light or blue light (430 nm). However, illuminance was not controlled in those studies (Liu et al., 2011; Long, Chen, & Chu, 2009).

Kroger and Binder (2000) proposed that children could become less myopic if they read in blue light or from paper that reflects preferentially at short wavelengths. However, a more recent study (Graef & Schaeffel, 2012) found that over-accommodation occurs in deep blue light below 430 nm. Furthermore, defocus imposed by the LCA affects accommodation. At shorter wavelengths, the accommodation response is reduced compared to longer wavelengths (Kruger et al., 1995; Seidemann & Schaeffel, 2002).

A striking observation by Rucker and Wallman (2008) in chicks was that cones sensitive to short wavelengths guide lenses compensation preferentially by modulating scleral growth, whereas cones sensitive to long wavelengths modulate choroidal thickness. That different fundal tissues are targets for emmetropization was an unexpected observation. But there is still little known as to how LCA affects the underlying retinal processing.

Beyond LCA and accommodation, an interaction has been reported between the ON and OFF retinal responses and refractive compensation in chicks (Crewther & Crewther, 2002). It is possible

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that ON and OFF channels are differentially affected by the spectral composition of light. Under photopic conditions, the b-wave is dominated by the ON pathway (Stockton & Slaughter, 1989). Therefore, we have recorded the luminance electroretinogram (L-ERG) b-wave to explore the possible mechanisms under monochromatic light stimulation.

Until now, there have been no experiments in guinea pigs to describe eye growth in blue light above 430 nm. In the current study, we selected a blue light source, LEDs with a narrow emission spectrum (λ_{max} = 470 ± 5 nm), and compared it to the effects of red light (λ_{max} = 600 ± 5 nm). Furthermore, a white light source was used, without or with monocular spectacle lenses treatment. Although guinea pig retinas contain rods with peak sensitivity around 494 nm and two classes of cones with peak sensitivities at 429 nm and 529 nm (Jacobs & Deegan, 1994), we used also red light to learn whether the retina can still respond to LCA using the broad band absorption of their 529 nm cone.

Different from chickens which have powerful accommodation, guinea pigs (at least in our colony) do not seem to accommodate at all since they never changed their refractions when accommodation targets, like a pencil, were presented in front of their eyes (Jiang et al., 2009). Also when the experimentator moved a fellow animal towards them, they never accommodated. Accommodation was monitored by eccentric infrared photoretinoscopy, as it was previously done in the chicken (Schaeffel, Howland, & Farkas, 1986). Frozen sections of the eyes show that the crystalline lens is thick and large (Howlett & McFadden, 2007; Fig. 7), making it unlikely that their small ciliary muscle could significantly deform or move it.

Guinea pigs, like most non-primate mammaliam species, have dichromatic color vision (Parry & Bowmaker, 2002). The current study was undertaken to provide a better understanding of the effects of LCA on eye growth in a dichromatic mammalian model.

2. Methods

2.1. Animals

Three-week-old pigmented guinea pigs (*Cavia porcellus*, the English short hair stock, n = 81) were involved in this study. Animals were maintained in temperature-controlled rooms in the animal facilities at the Wenzhou Medical School. All guinea pigs had free access to standard food and water, and fresh vegetables were provided twice a day. The procedures used were approved by the Institutional Animal Care and Ethics Committee at Wenzhou Medical College, Wenzhou, China, and were in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Experimental design

All guinea pigs were kept in cages measuring 65 × 45 × 23 cm. Each cage accomodated up to five animals. Daylight was simulated by fluorescent light (36 W, PHILIPS lifemax TLD, Shenzhen, China) on the ceiling. Lamps were operating at a 12–12-h light–dark cycle. Illuminance at cage floor was about 300 lux. Experimental groups were raised under illumination by colored LEDs (Dianfei Ltd., Shenzhen, China), fixed on the inside top of the cages. The cages were covered inside by silvered paper to ensure homogenous illumination.

2.2.1. Experiment A

From three weeks of age, guinea pigs were raised for four weeks with unobstructed vision under either red light (RL, λ_{max} = 600 ± 5 nm; n = 13) or blue light (BL, λ_{max} = 470 ± 5 nm; n = 13) using LEDs (Dianfei Ltd., Shenzhen, China), or white light (WL, fluorescent lamp, color temperature 6500 K, n = 14) as a control. The illuminance at cage level was 50 (human) lux under BL, 300 (human) lux under RL, and 350 (human) lux under WL on the cage floor respectively. Refractive error, lens thickness, vitreous chamber depth, and axial length were measured on days 0, 6, 14, and 28, and choroid thickness was measured on days 0, 1, 2, 4, 6, 10, 14, and 28 of treatment.

2.2.2. Experiment B

Three-week-old guinea pigs were monocularly treated with either both kind of lenses (+4.0 D or −4.0 D) under RL (+4.0 D lenses: n = 10; −4.0 D lenses: n = 11) or minus lenseses under BL (−4.0 D, n = 7) for 4 weeks. No positive lenses were tested in blue light because calculations showed that the focal plane would then be even further in front of the retina than with blue light alone. Guinea pigs with plus lenses (+4.0 D, n = 7) or minus lenses (−4.0 D, n = 6) under white ambient light served as control. Lens-rearing was continued for four weeks. Lenses were attached via a facemask with two hole openings for the eyes as described earlier (Lu et al., 2009). In short, lenses, made of polymethylmethacrylate were attached to the right side hole of the facemask, with the distance from the cornea to the lenses apex of about 3 mm. The left eye served as control. Lenses were cleaned daily. Measurements of the ocular parameters were performed one day before the lens-wearing began, and on days 14 and 28 while wearing the lenses.

2.3. Calculations of imposed defocus by LCA and by the spectacle lenses, including the effects of the small eye artifact

Based on the dispersion of the ocular media and the schematic eye model of the guinea pig (Howlett & McFadden, 2007; detailed in Table 1a), ZEMAX (EE version February 3, 2005, ZEMAX Development Corporation) was used to evaluate the paraxial defocus of guinea pig eyes at different wavelengths. No adjustments were made for the spectral sensitivity function. Calculated refractions were normalized to the refraction at 530 nm. The distance in micrometers between the photoreceptor layer and the focal plane with different spectacle lenses in front of the eye was also calculated.

Table 1
(a) Detailed parameters of the schematic eye and (b) detailed parameters of the attached lenses.

Parameters of the guinea pig schematic eye	
Cornea front surface radii	3.28 mm
Cornea thickness	0.25 mm
Cornea refractive index	1.376
Cornea back surface radii	3.28 mm
Anterior chamber depth	0.90 mm
Aqueous humor refractive index	1.335
Lens front surface radii	2.94 mm
Lens thickness	3.50 mm
Lens refractive index	1.539
Lens back surface radii	−2.18 mm
Vitreous refractive index	1.335
Vitreous chamber depth	3.15 mm
Retina refractive index	1.357
Retina thickness	0.129 mm
Parameters of attached lenses	
−4.0 D lens front surface radii	18.60 mm
−4.0 D lens thickness	0.18 mm
−4.0 D lens back surface radii	16.00 mm
+4.0 D lens front surface radii	10.98 mm
+4.0 D lens thickness	0.30 mm
+4.0 D lens back surface radii	12.00 mm
The lens-to corneal vertex distance	3 mm
Material of lens	PMMA

The small eye artifact was calculated by small · eye · artifact $= \frac{N_v \times T_{ret}}{P' \times AL \times (AL - T_{ret})}$ (Norton & McBrien, 1992) for the three week old guinea pig, where N_v = refractive index of vitreous chamber (1.335); T_{ret} = thickness of retina which is 0.129 mm in three week old animals (Lu et al., 2009); P' = posterior focal length (6.16 mm at 530 nm), and AL = axial length (the distance from cornea to the photoreceptor layer, 7.88 mm).

2.4. Measurements of refractive state and ocular biometry

Refractive error was measured by eccentric infrared photorefraction, as previously described for the mouse (Schaeffel et al., 2004). The photorefractor was calibrated for guinea pig eyes using a set of trial lenses as previously described (Jiang et al., 2009). Alert guinea pigs are inherently cooperative and it was easy to align their heads by hand until the pupil was visible in the video frame and the pupil axis aligned with the optical axis of the camera.

Corneal curvature was measured in alert guinea pigs with a modified keratometer (Zhou et al., 2006). Anterior chamber depth, lens thickness, vitreous chamber depth, and axial length were measured in alert animals by an A-scan ultrasound device (11 Hz, AVISO Echograph Class I-Type Bat, Quantel Medical, Clermont-Ferrand, France). The cornea was topically anesthetized with one drop of 0.5% proparacaine hydrochloride (Alcon, Puurs, Belgium) before the transducer was gently placed on the cornea. Velocities of sound were assumed to be 1557.5 m/s for the aqueous humor, 1723.3 m/s for the lens, and 1540 m/s for the vitreous humor (Zhou et al., 2006).

Choroidal thickness was measured with a Stratus OCT3 (Carl Zeiss Meditec, Dublin, CA, USA) (Lu et al., 2009). In brief, alert animals were held by an assistant on an examination table and their eyes were aligned until the pupil was centered and the optic disc was visualized in the OCT scan. When the scan area was focused, linear scanning was followed with the scan length of 3 mm at horizontal and vertical directions. Images were collected and analyzed by the analysis protocols provided with the Stratus OCT3.

Three images were obtained from each eye, and three locations of each image were selected to generate typical ScanProfile Charts. Borders between the different tissues layers were marked by cursors based on their reflectivity in the false color images of the OCT B-scans, as detailed in Fig. 1.

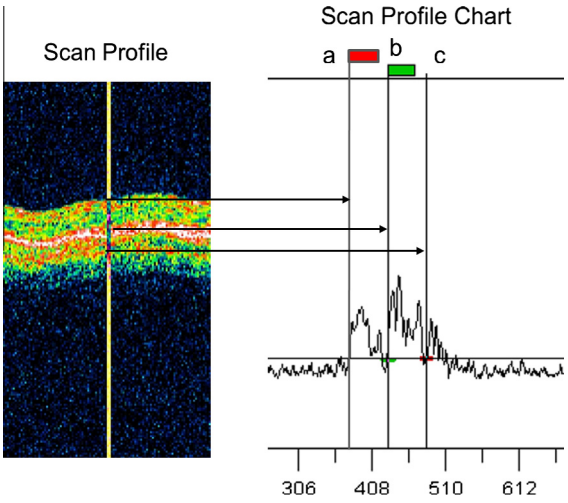

Fig. 1. Measurement of OCT images: ScanProfile (left panel) and its analysis (right panel): "a" denotes the front surface of the retina, "b" the boundary between retina and choroid (the RPE layer), and "c" refers to the boundary between choroid and sclera.

2.5. Electroretinograms

Ten untreated three-week-old animals were randomly selected before any treatment had occurred to record L-ERGs and match the brightness of light stimuli at different wavelengths. Full-field ERGs were recorded with a custom-built Ganzfeld dome connected to a commercially available ERG system (Q450SC UV; Roland, Wiesbaden, Germany). The white (color temperature 6700 K), red (625 ± 5 nm), or blue LEDs (470 ± 5 nm) were used as light sources for recording the photopic all cone ERG, or the mid and short wavelength cone photopic ERG, respectively. Because guinea pig eyes are 0.35 log units more sensitive to blue than to red light (Jacobs & Deegan, 1994; Fig. 2; upper curve used), brightness matching required that the intensity of the red light was about 1.33 log units higher than of the blue light. Thus, −15 dB (−1.02 log cd/m^2) were used for the blue light and the 0 dB (0.48 log cd/m^2) for the red while light intensity in the white was used not attenuated (0 dB; 0.48 log cd/m^2). Flicker frequency was maintained for all conditions at 0.9 Hz, with flicker pulse duration of 500 ms and a background intensity of 25 cd/m^2.

ERG testing was performed in a temperature-controlled, electrically isolated chamber. Guinea pigs were anesthetized with ketamine (72 mg/kg) and xylazine (4 mg/kg). Body temperature was maintained by placing the animals on a 37 °C warming pad. Corneas were anesthetized with a drop of 0.5% proparacaine hydrochloride, and pupils dilated using 0.5% tropicamide and 0.5% phenylephrine hydrochloride. A small amount of 2.5% methylcellulose gel was applied to the eye and a gold wire loop electrode was placed over the cornea to record the ERG. Needle reference and ground electrodes were inserted into the cheek and buttock,

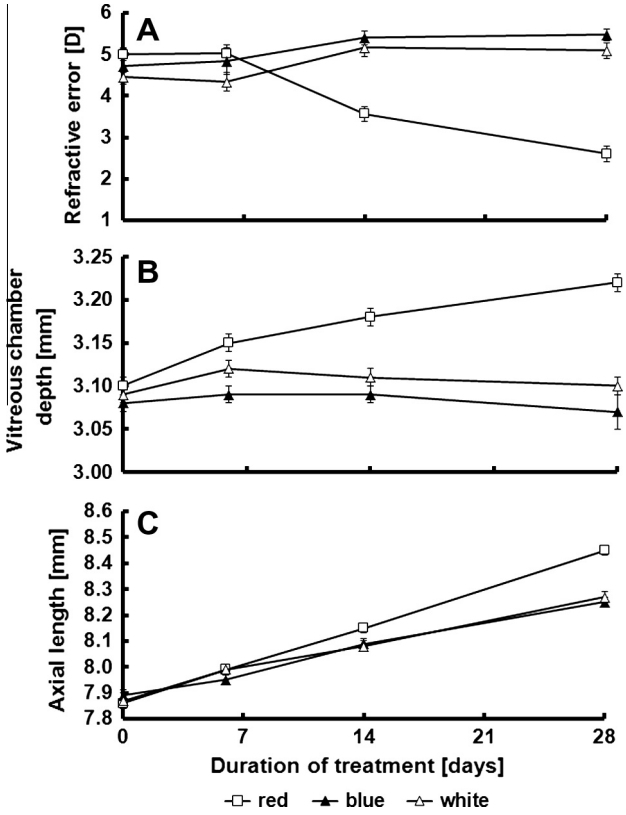

Fig. 2. Development of refractive state (A) and eye growth (B and C) in the three groups of guinea pigs raised under red light (□, $n = 13$), blue light (▲, $n = 13$), and white light exposure (△, $n = 14$), plotted as a function of the duration of the experiment. Error bars denote standard errors.

respectively. Recordings were started 10 min after beginning of the anesthesia.

2.6. Statistics

All data were reported as the mean ± standard error. Both eyes of the guinea pigs in experiment A were measured at each time point, but only the data of the right eyes were analyzed, using repeated measures analysis. *Post hoc* tests were corrected for multiple comparisons among the different ages and two-way ANOVA was used when comparisons were performed among different light environments. The longitudinal comparison of each group and analysis of effects of different color light on eye growth at each time point were performed using a one-way ANOVA.

In experiment B, the comparison between lenses wearing eyes and contralateral eyes as well as the comparisons between lens-treated eyes among the various experimental groups were made using two-way ANOVA. Longitudinal comparisons were made using one-way ANOVA.

ERG waveforms were analyzed for the amplitudes of the a-wave and the b-wave (Racine et al., 2005), and the significances of the differences in amplitudes recorded at different wavelengths were determined by one-way ANOVA. Comparisons between intensity-matched blue and red light were performed using paired *t*-tests.

3. Results

3.1. Baseline data

Baseline data refer to the refractions and other ocular parameters of the guinea pigs before the experiments at three weeks of age. Baseline refractions were +5.11 ± 0.10 D ($n = 81$). In none of the groups was there any statistically significant difference between the right and left eyes as compared using paired *t*-test ($p > 0.05$), nor any such differences among the different groups (one-way ANOVA: in experiment A: $F(2,37) = 1.153$, $p = 0.327$; in experiment B: $F(4,36) = 1.139$, $p = 0.354$).

Choroidal thickness (Mean ± SE, $n = 40$), measured by OCT at baseline was 120.98 ± 3.69 μm which is similar to the one measured by ultrasonography (Howlett & McFadden, 2007) and to the one measured by the same machine in a previous study (Lu et al., 2009).

3.2. Calculated differences in refractions and positions of the focal plane at different wavelengths during lens-wearing, and the small eye artifact

Based on the new Descartes rule, light of 470 nm is focused in front of the photoreceptor layer by 43.87 μm while light of 600 nm is focused behind the photoreceptor layer at 25.24 μm. The calculated difference in refraction of the guinea pig eye is 3.35 D. With spectacle lenses, the focal planes shifted accordingly. As expected, plus lenses induced a myopic shift and minus lenses a hyperopic defocus relative to the photoreceptor layer (magnitudes detailed in Table 2).

The calculated small eye artifact (Glickstein & Millodot, 1970) is +3.65 D in three week old guinea pigs. Accordingly, the "true" refraction of the animals was 1.46 D of hyperopia (measured refraction of 5.11 D, minus the small eye artifact of 3.65 D = 1.46 D). Using the refraction at 530 nm as a reference, blue light was focused in front of the retina by 1.86 D (change in focal plane position −38.17 μm). Adding a −4.0 D lenses, the focal plane was moved behind the photoreceptor plane by 119.58 μm (only 0.71 D hyperopic defocus shift) while it was moved in front of the retina by −119.79 μm (about 1.99 D myopic defocus shift) when a +4 D lenses was worn. In fact, with 3 mm the lenses-to corneal vertex distances the positive lenses has more effective powers and less the negative lenses has, which contribute to the shift of focal plane (detailed in Table 2).

Table 2
The calculated results of focal planes (μm), principal planes (μm) and diopters (D) under a 470 nm/600 nm wavelength light with or without +4.0/−4 D lenses, 530 nm as referenced wavelength.

Wavelength (nm)	Lens power (D)	Focal plane[a] (μm)	Equivalent optical power[b] (D)
470	–	−43.87	218.87
470	+4.0	−163.66	216.88
470	−4.0	+75.71	218.16
530	–	−5.70	217.01
530	+4.0	−126.08	215.08
530	−4.0	+114.59	216.28
600	–	+25.24	215.52
600	+4.0	−95.62	213.65
600	−4.0	+146.11	214.78

[a] Calculated linear distances of the focal plane from the photoreceptor plane at different wavelengths, without and with the spectacle lenses. All data in image space, and negative numbers indicate that the focal plane was in front of the photoreceptor layer while positive numbers indicate that the focal plane was behind the photoreceptor layer.
[b] Calculated total refractive power of the eyes at different wavelengths without or with lenses.

In summary, red light generated more hyperopic refractions (calculated after correction for the small eye artifact: +2.95 D) than blue (calculated refraction 1.46–1.86 D = −0.4 D of myopia). Adding a +4.0 D lenses in red light moves the refraction to −1.05 D, but adding a −4.0 D lenses renders the eye 7.95 D hyperopic (focal point 146.11 + 25.24 = 171.35 μm behind the retina). Experiments in red light were performed with both plus and minus lenses.

3.3. Effects of spectral composition of light on eye growth and refractive state

In experiment A, we found that spectral composition had a significant impact on eye development (two-way ANOVA: $F(2,37) = 10.553$, $p < 0.0001$). Guinea pigs exposed to red light developed relative myopia (one-way ANOVA, $F(3,48) = 41.723$, $p < 0.0001$). A significant myopic shift was not observed before 14 days of exposure, compared to the base line refractions (−1.44 ± 0.53 D, $p < 0.01$, one-way ANOVA, *post hoc* Bonferroni test). The relative myopic shift increased further and was −2.40 ± 0.82 D at day 28 ($p < 0.01$, one-way ANOVA, *post hoc* Bonferroni test). Refractive development was significantly different in animals raised in white or blue light (one-way ANOVA: 14 days, $F(2,37) = 25.876$, $p < 0.0001$; 28 days, $F(2,37) = 74.865$, $p < 0.0001$). No significant difference was found in the refractions of animals raised in white or blue light (two-way ANOVA: $F(1,25) = 2.056$, $p = 0.164$). Results are illustrated in Fig. 2A, with the refractive error plotted as a function of light exposure.

Under red light, vitreous chamber depth (VCD) increased already before the myopic shift could be measured (one-way ANOVA, $F(3,48) = 37.9$, $p < 0.0001$: six days versus baseline, $p = 0.001$; 14 days versus six days, $p = 0.017$; 28 days versus 14 days, $p = 0.024$, *post hoc* multiple comparisons). Vitreous chamber depth was also larger than in eyes of animals in white or blue light (two-way ANOVA, $F(2,37) = 21.398$, $p < 0.001$). There were no significant differences in ocular parameters at any time point between the white and the blue light group (one-way ANOVA, $F(3,48) = 0.467$, $p = 0.706$; and one-way ANOVA, $F(3,52) = 1.833$, $p = 0.153$), as shown in detail in Fig. 2B.

As shown in Fig. 2C, axial length had increased in the red light group compared to the other two groups in white or blue light (two-way ANOVA, $F(2,37) = 4.436$, $p = 0.019$), again with no

difference between the blue and white light groups (two-way ANOVA, $F(1,25) = 0.046$, $p = 0.832$). No changes were observed in corneal curvature (two-way ANOVA, $F(2,37) = 1.535$, $p = 0.229$), and lens thickness (two-way ANOVA, $F(2,37) = 1.104$, $p = 0.342$) among the three groups.

In summary, our data show that exposure to red light induced more myopic refractions over time, deeper vitreous chambers and increased axial lengths, compared to blue and white light exposure.

Effects of light with different spectral composition on choroidal thickness were also studied. Fig. 3 summarizes the findings. During the first two weeks, the changes in choroidal thickness were variable but they became more consistent between treatment days 14–28. Under blue light, the choroid thickened and, under red light it became temporarily thinner but finally returned to a similar thickness as in age-matched controls raised in white light (one-way ANOVA) at day two, $F(2,37) = 6.787$, $p = 0.003$; at day four, $F(2,37) = 22.061$, $p < 0.001$; at day six, $F(2,37) = 4.169$, $p = 0.023$; at day 14, $F(2,37) = 2.577$, $p = 0.09$; at day 28, $F(2,37) = 1.287$, $p = 0.288$. In summary, the data suggest that the choroid responds differently to light with different spectral composition.

3.4. Effects of light with different spectral composition on lens-induced changes in refractive state and eye growth

To find out whether chromatic defocus, induced by LCA, and lenses defocus that affects light at all visible wavelengths in a similar way have similar effects on eye growth, we examined the effects of lenses correction to blue or red light defocus in experiment B (data are detailed in Table 3).

Under white light, −4.0 D lenses induced significant myopia compared with the fellow eyes (two-way ANOVA, $F(1,10) = 12.597$, $p = 0.005$) which was due to vitreous chamber elongation (two-way ANOVA, $F(1,10) = 47.037$, $p < 0.001$). By contrast, +4.0 D lenses had no detectable effect, compared with the fellow eyes (two-way ANOVA: refraction, $F(1,12) = 1.733$, $p = 0.222$; VCD, $F(1,12) = 0.251$, $p = 0.782$) (see Fig. 4).

It was calculated above that, in blue light, the −4.0 D lenses moved the focal plane behind photoreceptor layer by 75.71 μm, imposing significant hyperopia of 0.71 D. But the eyes failed to develop significant myopia (one-way ANOVA: refraction, $F(2,18) = 0.6$, $p = 0.559$; VCD, $F(2,18) = 2.989$, $p = 0.076$) compared to their fellow eyes without lenses (two-way ANOVA: refraction, $F(1,12) = 2.934$, $p = 0.112$; VCD, $F(1,12) = 2.171$, $p = 0.166$). Compared to white light, blue light suppressed the effects of −4.0 D lenses on refraction and eye growth (two-way ANOVA: refraction, $F(1,11) = 26.696$, $p < 0.001$; VCD, $F(1,11) = 145.365$, $p < 0.001$) (see Fig. 5).

In red light, there was no significant effect of either lenses on refraction and eye growth, compared to the fellow eyes

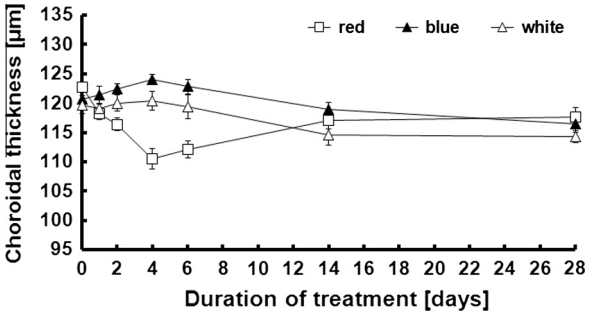

Fig. 3. Choroidal thickness at different time points. The choroid responded to red light (□, $n = 13$) and blue light (▲, $n = 13$) differently, compared to the white light (△, $n = 14$), but only in the first week of the treatment.

Table 3
Biometric parameters (mean ± SE) of different groups in experiment B. Including the +4 D lenses group (RL+, $n = 10$) and red −4 D lenses group (RL−, $n = 11$) under red light, −4 D lenses group (BL−, $n = 7$) under blue light, +4 D lenses group (WL+, $n = 7$) and −4 D lenses group (WL−, $n = 6$) under white light.

GROUP	Refractive error (diopter)			Vitreous chamber depth (mm)			Axial length (mm)			Corneal radius of curvature (mm)		
	0w	2w	4w	0w	2w	4w	0w	2w	4w	0w	2w	4w
RL+ treated eye	5.14 ± 0.30	4.31 ± 0.23	3.40 ± 0.27	3.10 ± 0.01	3.14 ± 0.01	3.21 ± 0.01	7.84 ± 0.02	8.16 ± 0.02	8.36 ± 0.02	3.30 ± 0.01	3.42 ± 0.01	3.51 ± 0.01
Fellow eye	5.01 ± 0.27	3.70 ± 0.23	3.10 ± 0.30	3.12 ± 0.02	3.14 ± 0.01	3.23 ± 0.01	7.86 ± 0.03	8.14 ± 0.01	8.42 ± 0.02	3.28 ± 0.01	3.41 ± 0.02	3.54 ± 0.01
RL− treated eye	5.66 ± 0.21	3.94 ± 0.30	2.49 ± 0.27	3.08 ± 0.02	3.18 ± 0.02	3.28 ± 0.01	7.84 ± 0.03	8.18 ± 0.02	8.47 ± 0.02	3.29 ± 0.01	3.40 ± 0.02	3.52 ± 0.03
Fellow eye	5.40 ± 0.42	3.75 ± 0.44	2.89 ± 0.20	3.08 ± 0.02	3.16 ± 0.01	3.24 ± 0.02	7.82 ± 0.03	8.15 ± 0.02	8.47 ± 0.01	3.32 ± 0.02	3.44 ± 0.02	3.53 ± 0.02
BL− treated eye	5.39 ± 0.42	5.32 ± 0.15	5.01 ± 0.26	3.10 ± 0.02	3.09 ± 0.01	3.06 ± 0.02	7.90 ± 0.02	8.11 ± 0.02	8.23 ± 0.02	3.33 ± 0.01	3.44 ± 0.02	3.54 ± 0.02
Fellow eye	5.31 ± 0.17	5.61 ± 0.24	5.95 ± 0.27	3.08 ± 0.02	3.07 ± 0.02	3.05 ± 0.02	7.92 ± 0.02	8.09 ± 0.02	8.29 ± 0.02	3.30 ± 0.01	3.38 ± 0.01	3.53 ± 0.01
WL+ treated eye	5.79 ± 0.25	5.29 ± 0.24	5.11 ± 0.21	3.11 ± 0.01	3.09 ± 0.03	3.09 ± 0.03	7.86 ± 0.06	8.08 ± 0.03	8.24 ± 0.01	3.31 ± 0.01	3.42 ± 0.02	3.52 ± 0.02
Fellow eye	5.31 ± 0.18	5.25 ± 0.12	4.89 ± 0.30	3.11 ± 0.01	3.10 ± 0.01	3.09 ± 0.02	7.86 ± 0.05	8.08 ± 0.02	8.26 ± 0.02	3.34 ± 0.01	3.42 ± 0.02	3.55 ± 0.02
WL− treated eye	5.57 ± 0.23	3.65 ± 0.32	2.01 ± 0.42	3.13 ± 0.01	3.20 ± 0.01	3.28 ± 0.02	7.83 ± 0.04	8.19 ± 0.03	8.45 ± 0.02	3.28 ± 0.01	3.37 ± 0.01	3.51 ± 0.01
Fellow eye	5.12 ± 0.47	5.27 ± 0.30	4.88 ± 0.20	3.11 ± 0.02	3.09 ± 0.02	3.08 ± 0.02	7.87 ± 0.05	8.06 ± 0.02	8.27 ± 0.02	3.31 ± 0.01	3.40 ± 0.03	3.51 ± 0.02

+ indicates +4.0 D lenses; − indicates −4.0 D lenses; RL, red light; BL, blue light; WL, white light. 0w, before the lens treatment; 2w, after two weeks of lens treatment; 4w, after four weeks of lens treatment.

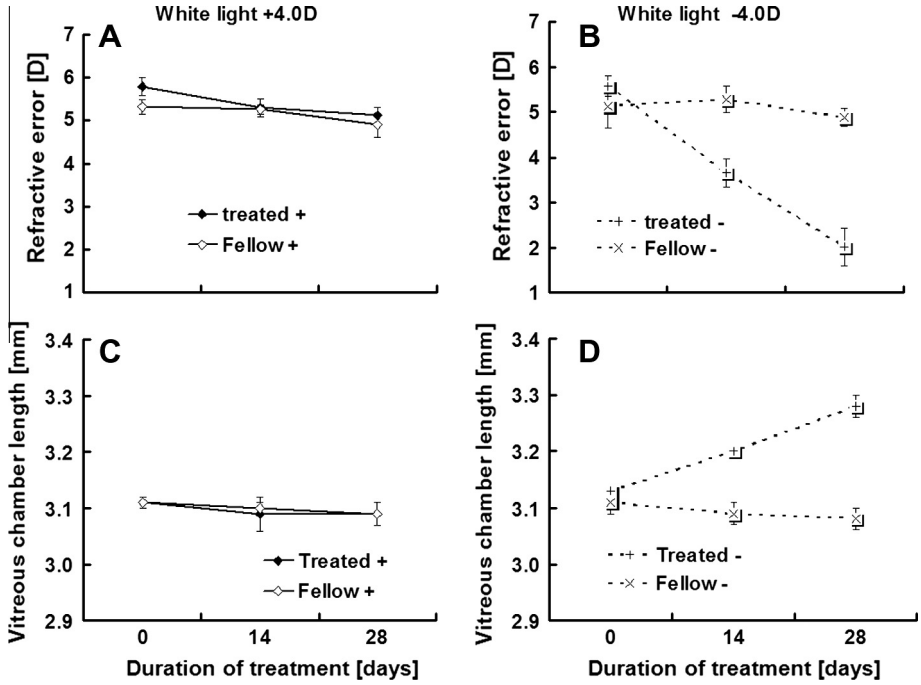

Fig. 4. Eye growth in white light with monocular +4.0 D lens treatment (A: changes in refraction; C: changes in vitreous chamber depth), and with monocular −4.0 D lens treatment (B: changed in refraction; D: changes in vitreous chamber depth). Error bars denote standard errors.

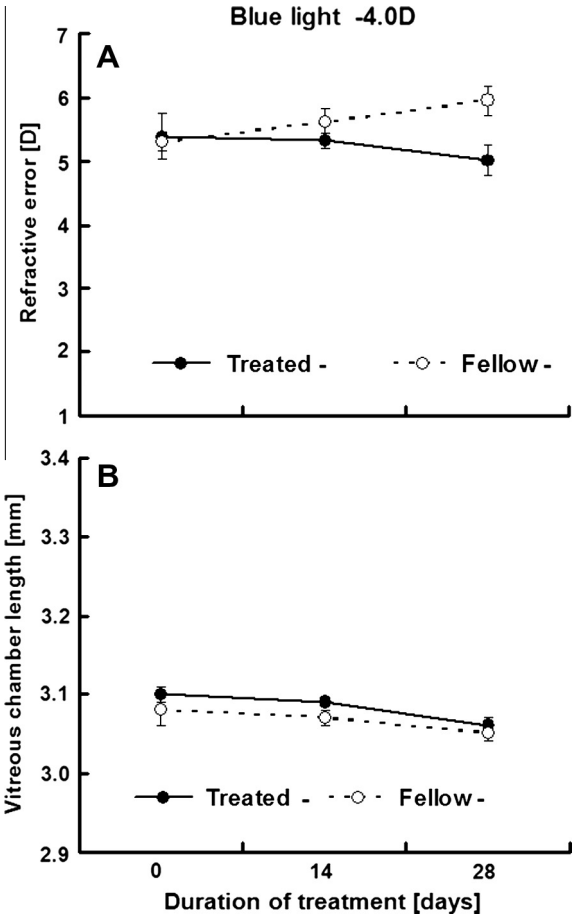

Fig. 5. Eye growth in blue light with monocular −4.0 D lens treatment (A: changes in refraction; B: changes in vitreous chamber depth). Error bars denote standard errors.

(two-way ANOVA: refraction, $F(3,38) = 0.493$, $p = 0.689$; VCD, $F(3,38) = 2.499$, $p = 0.074$). In particular, it was calculated above that +4.0 D lenses should have largely balanced the animals hyperopic refractions and place the focal plane on to the photoreceptor layer but the animals still developed significant myopia compared to animals in which +4 D lenses were attached to eyes under white light (two-way ANOVA: refraction, $F(1,15) = 17.846$, $p < 0.001$; VCD, $F(1,15) = 10.197$, $p = 0.006$) (see Fig. 6).

Even though axial lengths were significantly different in the three groups following treatment with positive lenses (two-way ANOVA, $F(3,30) = 3.467$, $p = 0.028$), there was no significant difference measured in axial lengths between the different groups. Corneal curvature was also not different among groups (two-way ANOVA, $F(3,30) = 1.345$, $p = 0.279$).

There were significant differences among groups treated with negative lenses (two-way ANOVA, $F(5,44) = 6.146$, $p < 0.001$), but only the axial length of lenses wearing eyes under red light was significantly longer than that under blue light ($p = 0.005$, *post hoc* Bonferroni test). Corneal curvature was not changing under any of the conditions (*post hoc* Bonferroni test).

In summary, plus lenses did not inhibit axial eye growth under red light, and minus lenses did not promote eye growth under blue light.

3.5. Effects of composition of light on the flash ERG

The photopic flash ERG was recorded at different wavelengths. After background light adaptation under the dome, a- and b-waves were recorded at different intensities.

The ERG a-wave is a photoreceptor-related response while the b-wave is sum potential of various sources in the inner retina, including light-evoked depolarization of the ON bipolar neurons (Stockton & Slaughter, 1989). Fig. 7 shows example ERG traces from the various stimulation protocols. Matched blue (−15 dB) and red (0 dB) light intensities elicited similar a-wave amplitudes (BL versus RL: 2.78 ± 0.50 μV versus 2.12 ± 0.30 μV, paired *t*-test,

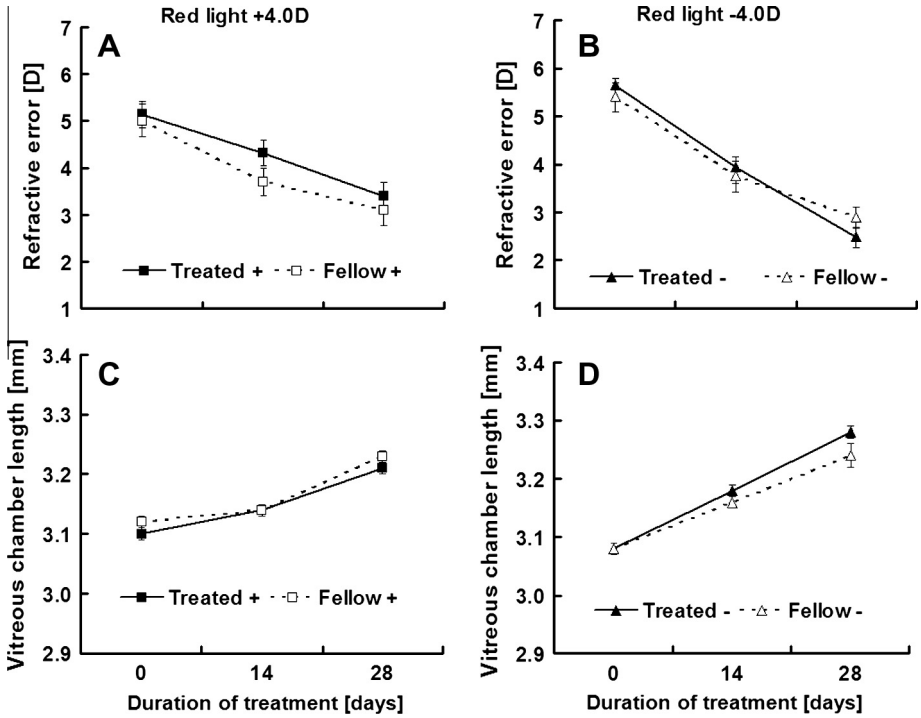

Fig. 6. Eye growth during red light exposure with monocular +4.0 D lens treatment (A: changes in refraction; C: changes in vitreous chamber depth), and with monocular −4.0 D lens treatment (B: changes in refraction; D: changes in vitreous chamber depth). Error bars denote standard errors.

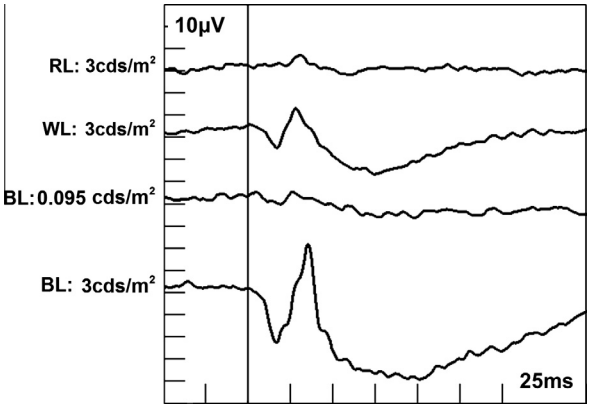

Fig. 7. Sample ERG traces from the various stimulation protocols.

$p = 0.18$, Fig. 8A), but different b-wave amplitudes (BL versus RL: 10.30 ± 2.07 μV versus 6.72 ± 1.49 μV, paired t-test, $p = 0.003$, Fig. 8B). The matched intensities of red, white, and blue light flashes (0 dB) elicited quite different shapes of the ERG traces (Fig. 8C and D): red light generated a series of noisy waves compared to white and blue light and blue light stimulated significantly larger a-wave amplitudes compared to white light (BL versus WL: 25.65 ± 3.37 μV versus 11.29 ± 1.22 μV, paired t-test, $p < 0.001$) and b-wave amplitudes (BL versus WL: 73.24 ± 10.81 μV versus 41.56 ± 6.64 μV, paired t-test, $p < 0.001$).

4. Discussion

In the current study, the refractions of the guinea pigs were about +5 D at the start of the study at three weeks of age. The hyperopia can be largely attributed to the small eye artifact (Glickstein & Millodot, 1970) but it is also possible that our strain is in fact slightly hyperopic since it was previously found to have unusual emmetropization (Jiang et al., 2009). With the small eye artifact considered, the animals were still 1.46 D hyperopic.

The principal finding of the current study was that the short wavelength light ($\lambda_{max} = 470$ nm) thickens the choroid and, consequently, makes the vitreous chamber more shallow. In contrast, long wavelength ($\lambda_{max} = 600$ nm) initially thinned the choroid but later thickened it again to approach finally the thickness of the controls. Interestingly, blue light inhibited negative-lens-induced axial myopia and slowed axial eye growth down. Plus lenses could no longer induce hyperopia under red light.

One study in humans (Kroger & Binder, 2000) found that there is less accommodation during reading in blue light which might reduce myopia progression. Experiments in chickens (Seidemann & Schaeffel, 2002) showed that a change in accommodation response in blue light is followed by a shift in the refractive state. It remains unclear how refractive state of the eye can change after accommodation had already compensated for the chromatic defocus. Apparently, blue light can stimulate accommodation and emmetropization at the same time in this animal model. In the current study we used three-week-old guinea pigs when emmetropization has begun to slow down (Howlett & McFadden, 2007; Zhou et al., 2006). We believe that the inhibition of eye growth that we observed in our animals under blue light is not mediated by changes in accommodation tonus but rather by a direct effect of retinal processing on axial eye growth.

Previous studies on lens compensation in chicks under monochromatic light showed that longitudinal chromatic aberration has subtle effects on eye growth (Rohrer, Schaeffel, & Zrenner, 1992; Rucker & Wallman, 2009). Furthermore, more complete lens compensation was found in chicks under white light than under monochromatic light. These results suggest that the LCA may provide a signal used as a cue for detecting the sign of defocus (Rucker & Wallman, 2008). Also in guinea pigs, we observed better compensation of lens defocus under white light. It is surprising that chromatic hyperopic defocus in red light induced still myopia even

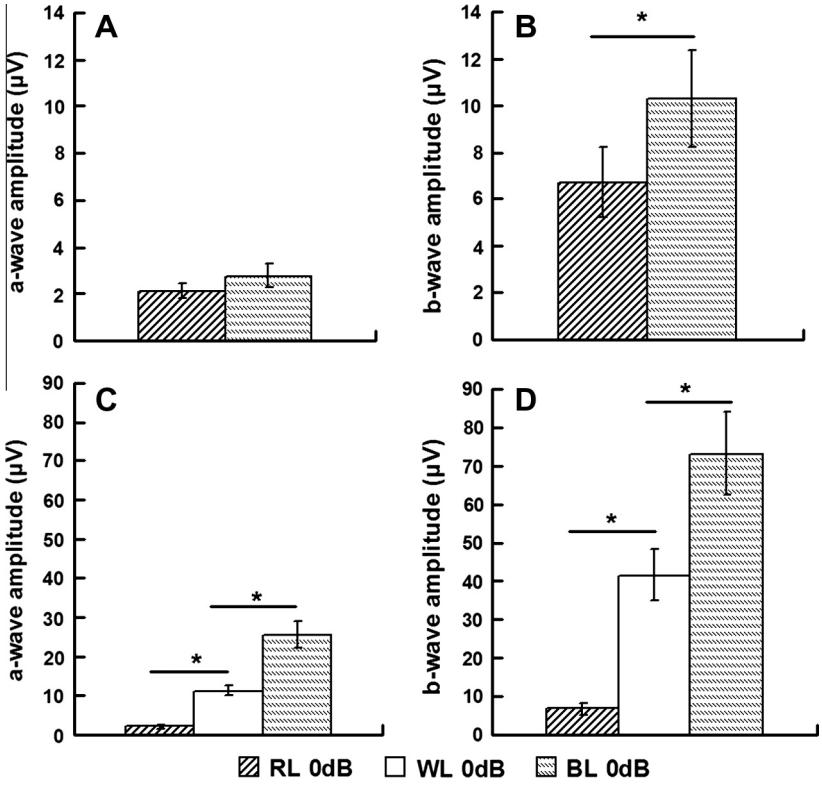

Fig. 8. Photopic ERG from the right eyes of three-week-old guinea pigs (n = 10), elecited by different light stimulations: a-wave (A) and b-wave (B) amplitudes with red and blue light stimulation of matched intensity for the guinea pig. (C) a-wave and (D) b-wave amplitudes with light stimulation of the same intensity. Data is shown as means ± SE. Guinea pigs are around 1.33 log cds/m^2 more sensitive to blue light than to red light, so that −15 dB (−1.02 log cds/m^2) attenuation of blue light is assumed to match the sensitivity of the animals in the red (0.48 log cds/m^2). RL: red light, BL: blue light, WL: white light. Single asterisk indicates $p < 0.01$ and double asterisks $p < 0.001$ (paired t-test).

after the hyperopia was corrected by +4.0 D lenses. In contrast, chromatic myopic defocus induced by blue light in combination with negative lenses (−4.0 D) induced no significant myopia. Possible explanations are that the lens powers did not exactly match the chromatic defocus so that some residual focus error signal remained, or that chromatic defocus is not fully equivalent to lens defocus perhaps because other factors related to the biochemistry of the different stimulated photopigments trigger different second-order signalling pathways in the retina.

The ERG data could explain to some extent the effects of chromaticity on eye growth. In agreement with Jacobs and Deegan (1994), our ERG results showed that red light stimulation (λ_{max} = 600 nm) under photopic conditions produced a poor signal-to-noise ratio due to the low sensitivity of the animals in the long wavelengths range. Thinning of the choroid, increase in vitreous chamber depth, and myopic shift under red light, despite treatment with positive lenses may be indicators of poor sensitivity to defocus under red light. In other cases of low cone activity, such as in darkness or scotopic conditions, chickens (Gottlieb, Fugate-Wentzek, & Wallman, 1987; Lauber & Kinnear, 1979; Yinon & Koslowe, 1986), fish (Kroger & Fernald, 1994), tree shrew (Norton, Amedo, & Siegwart, 2006), and primate eyes (Guyton, Greene, & Scholz, 1989; Raviola & Wiesel, 1978) became also larger and displayed higher variability in size and refractions than in white light (Cohen et al., 2008; Smith et al., 2003).

A possible limitation of the current study was that we used a different light source for white light, but measurement of the light spectrum using Maya2000 Pro Spectrometer (Ocean Optics, USA) showed that its energy was mainly emitted between 500 nm and 600 nm with a peak wavelength around 545 nm, near the peak sensitivity of the guinea pig (529 nm, Jacobs & Deegan, 1994). As shown in Fig. 8, we tried to match the intensities (3 cds/m^2) of white and blue light but still recorded significantly higher a- and b-waves in blue light. When the brightness of the light source was adjusted until similar a-waves were recorded (n = 3, blue light versus white light: 34.8 ± 10.1 μV versus 32.5 ± 7.9 μV, paired t-test, n.s.), the b-waves were still different (n = 3, blue light versus white light: 100 ± 35.1 μV versus 81.9 ± 29.3 μV, paired t-test, p = 0.03) (unpublished data). This could indicate that the photoreceptor signals were differently reflected in the inner retina for white and blue light.

Crewther and Crewther (2003) hypothesized that the ON and OFF systems are separately involved in the mechanisms of refractive control, and stimuli that produce a robust ON response can reduce eye growth and possibly prevent myopia (Crewther DP, et al., IOVS 2011; 52: ARVO E-Abstract 6317). Our results support this idea. Guinea pigs lack a substantial phase of OFF-component with white light stimulation (Lei, 2003). They are sensitive to blue light. The presence of blue-ON-responses (Dacey & Packer, 2003) let us speculate about a possible relationship between the blue light stimulation and the eye growth. Under the same reference light and the same stimulus intensity (0.48 log cds/m^2), the blue light stimulus raised b-wave amplitudes significantly above those seen under white light. The red light stimulus (which induced axial myopia) did not have this effect on the ERG. Given there was no defined visual exposure around animals in cage in the current study, the enhanced b-wave amplitude elicited by the short wavelength light used in these experiments gave hint that the blue cone-mediated ON pathways might contribute to the observed effects on eye growth. We speculate that shorter wavelength light might affect the ON pathway to inhibit eye growth, whereas guinea pigs lack the long wavelength type cone and the accelerating eye growth

under red light could be the effects of darkness, at least in guinea pigs. The guinea pig is dichromatic and its second sensitivity peak is around 530 nm (Jacobs & Deegan, 1994). In future studies, we plan to investigate whether light of longer wavelength can elicit green-OFF responses and lead to different degrees of the stimulation or inhibition of the eye growth to lenses rearing.

5. Conclusions

In our colony of guinea pigs, animals responded differently to lens treatment in blue and red light. Blue light inhibited negative-lens-induced axial myopia and slowed axial eye growth down. Positive lenses no longer induced hyperopia under red light. Furthermore, red light induced early thinning of the choroid and axial myopia in guinea pigs not wearing lenses and positive spectacle lenses could not suppress this myopia even though they corrected for the imposed chromatic defocus. The low ERG responses in the red light suggest low sensitivity of the animals in the red, and that eye growth may be more similar as in darkness.

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