Wavelength Defocus and Temporal Sensitivity Affect Refractive Development in Guinea Pigs

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Purpose. Environmental light plays an important role in the process of emmetropization. This study investigated how the retina integrates wavelength and temporal signals to regulate eye emmetropization.

Methods. Guinea pigs ($n = 220$) were randomly divided into 11 groups ($n = 20$/group) that received different environmental lighting (12:12 light cycle) for 8 weeks: white, green, or blue light at steady, 0.5 or 20 Hz. White-steady group was repeated for each wavelength. Refraction, axial length, and corneal curvature were measured using streak retinoscopy, A-scan ultrasonography, and keratometry, respectively, every 2 weeks.

Results. (1) In white light, the white-0.5 Hz group was more myopic than the white-steady group or the white-20 Hz group (both $P < 0.0001$), with a longer axial length (both $P < 0.0001$). White-20 Hz did not significantly differ from white-steady. (2) At low temporal frequencies (0 and 0.5 Hz), green-steady ($P = 0.0008$) and green-0.5 Hz ($P < 0.0001$), were more myopic than the white-steady group, with longer axial lengths (both $P < 0.0001$). No significant difference was found between green-0.5 Hz and green-steady. Blue-steady and blue-0.5 Hz were more hyperopic than white-steady (both $P < 0.0001$), with shorter axial lengths (both $P < 0.0001$). Blue-0.5 Hz showed no significant difference from blue-steady. (3) At high temporal frequencies (20 Hz), green-20 Hz, was more hyperopic than green-steady or green-0.5 Hz (both $P < 0.0001$) and had a shorter axial length (both $P < 0.0001$). Green-20 Hz showed a 1.10 D hyperopic shift compared to green-steady. Blue-20 Hz was less hyperopic than blue-steady ($P < 0.0001$) or blue-0.5 Hz ($P = 0.0012$), with a longer axial length (both $P < 0.0001$). Blue-20 Hz showed a 1.18 D myopic shift compared to blue-steady.

Conclusions. Eyes use both wavelength and temporal frequency of light to regulate emmetropization. Their interactions provide different cues to control eye growth. At low temporal frequencies, the eye can use wavelength defocus to guide eye growth. This signal is weakened at high temporal frequencies.

Keywords: emmetropization, wavelength defocus, temporal sensitivity, guinea pigs

Neonates are typically born with hyperopia, after which the eye can use visual cues to identify signs of defocus and eliminate the refractive error to achieve emmetropia during the developmental period. Abnormal visual environments can disrupt the emmetropization process and introduce ametropia, allowing the eyes to grow longer (myopia) or shorter (hyperopia) than normal in adolescence. The basic question in the field of emmetropization is how the eye encodes visual inputs to modulate eye growth in order to reduce refractive error and achieve in-focus images (emmetropia).

Several studies have examined a number of aspects of ambient lighting as a visual cue during the course of emmetropization. Among these studies, there have been many epidemiological experiments examining the effects of outdoor light intensity and spectrum on refractive development. Rose et al.[1] reported in 2008 that increased time spent on outdoor activities was associated with a reduced prevalence of myopia. Since then, an increasing number of epidemiological studies have shown that children who spend more time outdoors have lower risk of developing myopia than those who spend less time outdoors.[1–7] These findings were shown in Asians,[2–4] Australians,[1,5] Europeans, and Americans.[6,7] The mechanisms underlying the protective effect associated with outdoor activities include the intensity and spectral composition of sunlight compared with indoor artificial lighting. Evidence from animal studies has shown that high light intensity, in the form of either artificial light or natural daylight, significantly reduced experimental myopia in chicks,[8–11] guinea pigs,[12,13] monkeys,[14,15] and tree shrews (Siegwart JT, et al. *IOVS* 2012;53:ARVO E-Abstract 3457). Cohen et al.[8] found that chickens raised under high-intensity light of 10,000 lux with either light-dark cycles or continuous light demonstrated significant hyperopia (+1.1 D∼+3.9 D) compared to those exposed to normal 500-lux illumination. Studies suggested that these effects of high light intensity on refraction might be related to the retinal dopaminergic system.[12,16–24]

The spectral composition of ambient light is one of several important cues influencing refractive development. Studies on fish,[25,26] chicks,[27–29] guinea pigs,[30–33] tree shrews,[34,35] and monkeys[36,37] demonstrated that the eye could use wavelength

defocus produced by longitudinal chromatic aberration (LCA) to regulate refractive development. In our previous study, guinea pigs became more myopic in green light and more hyperopic in blue light.[30] The effect was reversible when the light conditions were switched.[31,32] Similar findings were reported in fish[25,26] and chicks.[27–29] These findings may be used to interpret the potentially protective effects of outdoor environments, since sunlight is much richer in short-wavelength light.[38,39]

While the findings mentioned above were true for short-term experiments, the magnitude of the refractive changes under longer duration monochromatic light was greater than predicted by LCA.[27,30,32] For instance, guinea pigs have approximately 1.5 D of LCA between the middle-wavelength (530 nm) and short-wavelength (430 nm) focal planes, but the mean refractive difference between middle and short wavelengths reached as high as 4.5 D after 12 weeks of observation, which was three times the measured LCA.[30] This phenomenon indicates that LCA-based principles are not always sufficient to explain refractive development and that anomalous visual inputs derived from monochromatic lights are involved in the emmetropization process.

In contrast to our previous study in rhesus monkeys and other studies on fish, chickens, and guinea pigs, in which long-wavelength light was a risk factor for myopia development,[25–33,37] Smith et al.[36] found that monkeys reared in environments dominated by long-wavelength lights exhibited a hyperopic shift compared with monkeys reared in normal environments. Similar results were obtained in tree shrews, with hyperopic shifts in red light and myopic shifts in flickering blue light.[34,35] These differences might be due to methodological differences in duration and temporal patterns of stimulation.

However, studies on the interaction between wavelength and temporal frequency are limited. Rucker and Wallman[28] (2012) suggested that emmetropization may depend on changes in color and luminance contrast that are dependent on the spectral and temporal properties of the stimuli. Eyes exposed to changes in luminance contrast became more hyperopic at high temporal frequencies and more myopic at low temporal frequencies. Eyes exposed to changes in color contrast showed little change in refraction across frequencies despite an increase in eye length at low temporal frequencies.[28] Previous studies on the temporal characteristics of emmetropization have revealed that exposure to high temporal frequency illumination prevents myopic shifts in form-deprived chicks,[40–42] while exposure to low temporal frequency illumination leads to a myopic shift in chicks and guinea pigs.[43–45] These results may occur because the high temporal frequencies stimulate the luminance-sensitive neural mechanism and low temporal frequencies stimulate the color-sensitive mechanism.[28] Visual information in humans is conveyed from the retina to the cortex by at least three major pathways: parvocellular, magnocellular, and koniocellular. External visual inputs are integrated by these visual pathways, forming our perception of objects with color, form, motion, and depth.[46,47] The stimulation of different visual pathways under diverse illumination might contribute to the regulation of emmetropization.

It is still incompletely understood how the eye integrates both wavelength and temporal signals to detect the signs of defocus and affect eye growth. At high temporal frequencies, refractions were more hyperopic in yellow light (without blue) than in white light (with blue) in chicks.[39,48] This may be because blue light stimulates the koniocellular color pathway in addition to intrinsically photosensitive retinal ganglion cells.[49] In contrast, at low temporal frequencies, chicks demonstrated a more myopic shift in yellow light compared with white light.[39] On the other hand, in tree shrews, blue monochromatic light combined with flickering acts as a cue to increase eye growth and induce myopia[34]; red light with or without flickering induced a hyperopic shift.[34]

Experiments on guinea pigs have demonstrated significant myopic shifts with exposure to low temporal frequency illumination (1 Hz),[44,45] but not high temporal frequency illumination (20 Hz).[45] Guinea pigs raised in 0.5 Hz flickering white light (color temperature 2850 K) expressed the greatest myopic shift (–5.5 ± 1.5 D) with 5, 1, 0.5, 0.25, and 0.1 Hz stimuli.[44] Based on these findings, we chose 0.5 and 20 Hz as the two extremes of low and high frequencies, respectively. To investigate how interactions between wavelength and temporal cues from ambient light affect emmetropization, we assessed refractive development in guinea pigs raised in white, green, or blue light at steady, low (0.5 Hz), or high (20 Hz) flickering frequencies.

Methods

Animals

Guinea pigs (English short-hair stock, tricolor strain) were obtained from the laboratory of Fudan University. The treatment and care of the animals were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

A total of 220 guinea pigs (2 weeks old) were randomly divided into 11 groups: white-steady, white-0.5 Hz and white-20 Hz; white-steady, green-steady, green-0.5 Hz and green-20 Hz; white-steady, blue-steady, blue-0.5 Hz and blue-20 Hz, with 20 animals in each group. The cabinet was divided into separate compartments. Each compartment was 65 cm long, 55 cm wide, and 60 cm high. Each compartment had a 60-cm long, 50-cm wide, and 40-cm high cage, ensuring that the light could not affect other cages. Stainless steel trays were placed under the cages in each compartment to collect the excrement from the animals. Animals were provided with a continuous supply of food and water.

Light Stimulation

The peripheral walls and the ceiling were installed with light emitting diode (LED) tubes. There were three types of LED tubes: white (color temperature 5000 K), green (peak value 530 nm, half bandwidth 30 nm), and blue (peak value 430 nm, half bandwidth 20 nm). The frequency was controlled via temporal luminance modulation produced by function generators (Yinuo Automation Co., LTD, Changsha, China; 0.5 Hz: 1 second bright and 1 second dark; 20 Hz: 0.025 seconds bright and 0.025 seconds dark). The wave form was square-wave. The intensity of the illumination was tested using a luminometer (SMART-SENSOR AR823, China). The average intensity of illumination in all the groups was controlled at 500 lux. Illumination followed a 12-hour:12-hour light-dark cycle (on at 6 AM and off at 6 PM).

Optical Measurements

All measurements were performed by an optometrist with the help of an assistant, and data from the two eyes were collected. All measurements were performed without anesthetizing the guinea pigs because the measurements taken should be in line with the normal physiological state of the animals. The refractive errors were examined with a streak retinoscope at a working distance of 0.65 m in a dark environment. One percent cyclopentolate hydrochloride (ALCON, Puurs, Belgium) was topically administered to dilate the pupils and relax

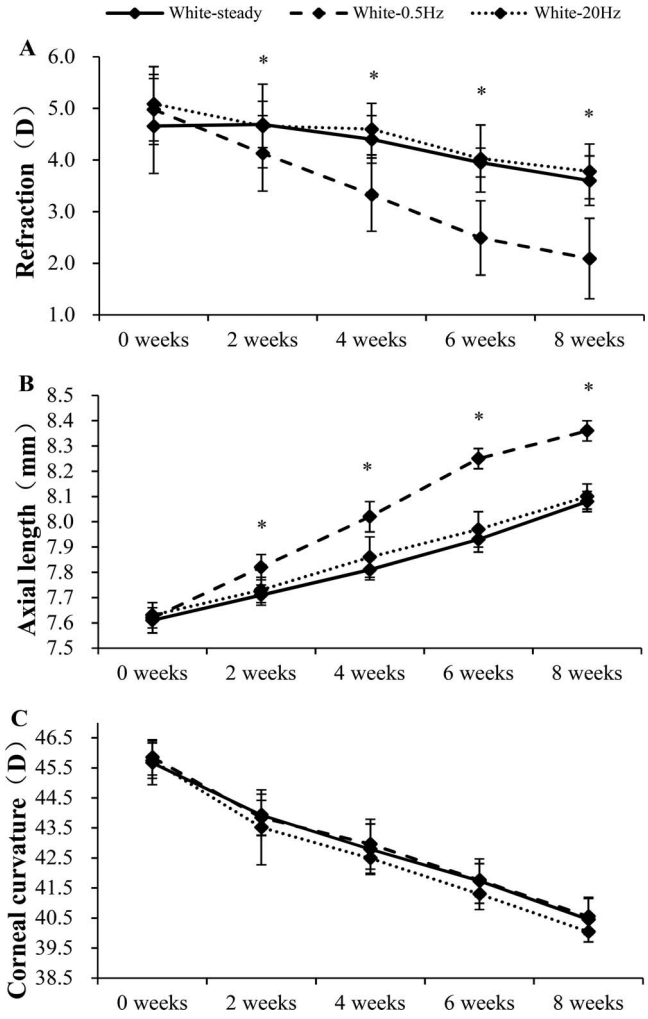

FIGURE 1. Developmental changes under white light (measured at 0, 2, 4, 6, and 8 weeks). (**A**) Refraction changes under white light. The refraction was more myopic at white-0.5 Hz than at white-steady and white-20 Hz from weeks 2 to 8 (*). (**B**) Axial length changes under white light. The axial length was longer at white-0.5 Hz than at white-steady and white-20 Hz from weeks 2 to 8 (*). (**C**) Corneal curvature changes under white light. There were no intergroup differences at any time point. The significant difference is indicated by * for $P < 0.05$. Standard error bars are shown.

the ciliary muscle before examination. 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 was in direct contact with the corneal surface to provide measurements of the depth of anterior chamber, thickness of the crystalline lens, and axial length. The axial length measurements were made from the anterior cornea to posterior sclera. The corneal curvature was measured using a keratometer (Topcon OM-4, Tokyo, Japan) with a +8.0 D spherical lens on the anterior surface. The measurements were performed at the center of the cornea.[42]

Statistical Analysis

In this study, data from both eyes were collected from each animal. We presented the data as the average of right and left eyes for each animal. For statistical analysis, the data of the white-steady group was an average of white-steady in the three experiments. The biometric measurements are summarized as the mean ± standard deviation (SD). The repeated measurements were analyzed by the mixed-effect model (PROC MIXED in SAS), followed by Bonferroni's test for post-hoc analyses. Statistical analyses were performed with SAS version 9.4 for Windows (SAS Institute, Cary, NC, USA). A two-tailed P value < 0.05 indicated statistical significance.

RESULTS

Before the experiments, there were no significant differences among all the groups in refraction or any ocular component (Bonferroni, $P > 0.05$, all). After exposure to different light conditions, both wavelength and temporal components of stimuli showed significant effects on emmetropization in guinea pigs. The interaction effects on refraction and axial length were also found between these two components ($F_{4,167} = 12.16$, $P < 0.0001$ for refraction; $F_{4,167} = 81.50$, $P < 0.0001$ for axial length).

Developmental Changes Under White Light

Refraction Under White light. Figure 1A shows the mean spherical equivalent refraction of each group over 8 weeks under white light. The mean ocular refraction in the white-0.5 Hz group showed a significant myopic difference from that of the white-steady and white-20 Hz groups starting at week 2 (0.5 Hz versus steady $t = 3.89$, $P = 0.0001$; 0.5 Hz versus 20 Hz $t = -2.14$, $P = 0.0342$). At week 8, ocular refraction decreased to 2.09 ± 0.78 D in the white-0.5 Hz group, a refraction that was more myopic than 3.60 ± 0.48 D in the white-steady group and 3.78 ± 0.53 D in the white-20 Hz group (0.5 Hz versus steady $t = 7.21$, $P < 0.0001$; 0.5 Hz versus 20 Hz $t = -6.05$, $P < 0.0001$). However, there was no significant difference between the white-steady and white-20 Hz groups ($t = -0.38$, $P = 0.7068$).

Axial Length Under White Light. Figure 1B shows the mean axial length of each group over 8 weeks under white light. The mean axial length of the white-0.5 Hz group was significantly longer than that of the white-steady and white-20 Hz groups starting at week 2 (0.5 Hz versus steady $t = -7.12$, $P < 0.0001$; 0.5 Hz versus 20 Hz $t = 5.09$, $P < 0.0001$). At week 8, the mean axial lengths of the white-steady, white-0.5 Hz, and white-20 Hz groups were 8.08 ± 0.04, 8.36 ± 0.04, and 8.10 ± 0.05 mm, respectively. There was no significant difference between the white-steady and white-20 Hz groups ($t = -0.82$, $P = 0.4160$).

The anterior chamber depth and lens thickness were similar in the groups during the 8-week experimental period. There were no intergroup differences at any time point ($P > 0.05$; data not shown).

Corneal Curvature Under White Light. Figure 1C shows the mean corneal curvature of each group over 8 weeks under white light. The mean corneal curvature decreased in a similar manner in all the groups from approximately 45.68 D to approximately 40.25 D during the 8-week experimental period. The mean corneal curvatures of the white-steady, white-0.5 Hz, and white-20 Hz groups reached 40.45 ± 0.74, 40.55 ± 0.60, and 40.04 ± 0.34 D, respectively, at week 8. There were no intergroup differences at any time point.

Developmental Changes Under Green Light

Refraction Under Green Light. Figure 2A shows the mean spherical equivalent refraction of each group over 8 weeks under green light. The mean ocular refraction in the green-steady and green-0.5 Hz groups showed a significant difference from that of the white-steady group starting from

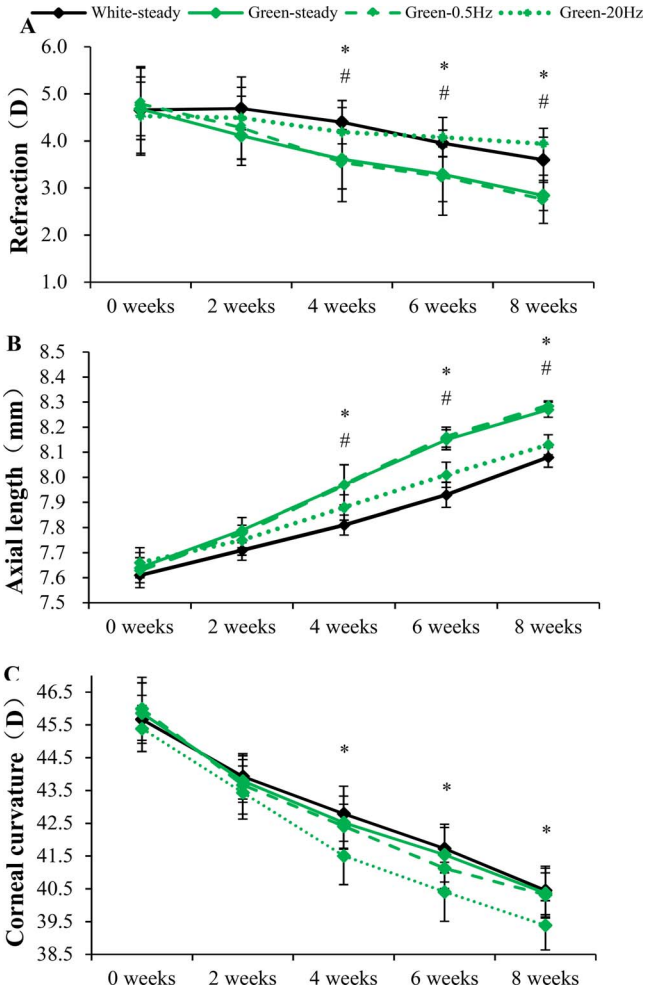

Figure 2. Developmental changes under green light (measured at 0, 2, 4, 6, and 8 weeks). (**A**) Refraction changes under green light. The refractions were more myopic at green-steady and green-0.5 Hz than at white-steady from weeks 4 to 8 (*). The refraction was more hyperopic at green-20 Hz than at green-steady and green-0.5 Hz from weeks 4 to 8 (#). (**B**) Axial length changes under green light. The axial lengths were longer at green-steady and green-0.5 Hz than at white-steady from weeks 4 to 8 (*). The axial length was shorter at green-20 Hz than at green-steady and green-0.5 Hz from weeks 4 to 8 (#). (**C**) Corneal curvature changes under green light. The corneal curvature in green-20 Hz was the lowest among the four groups from weeks 4 to 8 (*). The significant difference is indicated by * or # for $P < 0.05$. Standard error bars are shown.

week 4 (green-steady versus white-steady $t = 3.37$, $P = 0.0009$; green-0.5 Hz versus white-steady $t = 3.91$, $P = 0.0001$). At week 8, ocular refraction decreased to 2.84 ± 0.32 D and 2.76 ± 0.51 D in the green-steady and green-0.5 Hz groups, which were more myopic than 3.60 ± 0.48 D in the white-steady group (both $P < 0.0001$).

From week 4, refraction in the green-20 Hz group became more hyperopic than the green-steady and green-0.5 Hz groups (20 Hz versus steady $t = -2.53$, $P = 0.0123$; 20 Hz versus 0.5 Hz $t = -2.98$, $P = 0.0033$). The green-20 Hz group reached 3.94 ± 0.33 D at week 8 and became more hyperopic than both the green-steady and green-0.5 Hz groups (20 Hz versus steady $t = -5.15$, $P < 0.0001$; 20 Hz versus 0.5 Hz $t = -5.87$, $P < 0.0001$). However, there was no significant difference between the green-steady and green-0.5 Hz groups ($t = 0.34$, $P = 0.7332$).

Axial Length Under Green Light. Figure 2B shows the mean axial length of each group over 8 weeks under green light. The mean axial lengths of the green-steady and green-0.5 Hz groups were significantly longer than the white-steady group starting at week 4 (green-steady versus white-steady $t = -7.70$, $P < 0.0001$; green-0.5 Hz versus white-steady $t = -8.17$, $P < 0.0001$).

The mean axial length of the green-20 Hz group was shorter than the green-steady and green-0.5 Hz groups from week 4 (green-20 Hz versus green-steady $t = 4.27$, $P < 0.0001$; green-20 Hz versus green-0.5 Hz $t = 4.46$, $P < 0.0001$). At week 8, the mean axial lengths of the green-steady and green-0.5 Hz groups were 8.27 ± 0.03 and 8.28 ± 0.02 mm, respectively. There was no significant difference between the green-steady and green-0.5 Hz groups ($t = -0.01$, $P = 0.9959$).

The anterior chamber depth and lens thickness were similar in the groups during the 8-week experimental period. There were no intergroup differences at any time point ($P > 0.05$, data not shown).

Corneal Curvature Under Green Light. Figure 2C shows the mean corneal curvature of each group over 8 weeks under green light. The mean corneal curvatures of the white-steady, green-steady, green-0.5 Hz and green-20 Hz groups reached 40.45 ± 0.74, 40.37 ± 0.76, 40.32 ± 0.67, and 39.39 ± 0.75 D, respectively, at week 8. The mean corneal curvature of the green-20 Hz group was the lowest among the four groups from weeks 4 to 8 ($P < 0.0001$, all). There were no significant differences among the white-steady, green-steady, and green-0.5 Hz groups at any time point.

Developmental Changes Under Blue Light

Refraction Under Blue Light. Figure 3A shows the mean spherical equivalent refraction of each group over 8 weeks under blue light. The mean refractions in the blue-steady, blue-0.5 Hz, and blue-20 Hz groups were more hyperopic than the white-steady group starting at week 4 ($P < 0.0001$, all). The refraction reached 6.90 ± 0.99 D in the blue-steady group, 6.54 ± 0.71 D in the blue-0.5 Hz group, and 5.72 ± 0.70 D in the blue-20 Hz group at week 8, which were much more hyperopic than the 3.60 ± 0.48 D observed in the white-steady group ($P < 0.0001$, all).

Although the mean refraction of the blue-20 Hz group was more hyperopic than that of the white-steady group, it was less hyperopic than blue-steady and blue-0.5 Hz groups from week 6 (20 Hz versus steady $t = 2.71$, $P = 0.0073$; 20 Hz versus 0.5 Hz $t = 2.64$, $P = 0.0091$) to week 8 (20 Hz versus steady $t = 4.70$, $P < 0.0001$; 20 Hz versus 0.5 Hz $t = 3.30$, $P = 0.0012$). There was no significant difference between blue-steady and blue-0.5 Hz groups ($t = 1.47$, $P = 0.1434$).

Axial Length Under Blue Light. Figure 3B shows the mean axial length of each group over 8 weeks under blue light. The mean axial lengths of the blue-steady, blue-0.5 Hz, and blue-20 Hz groups were significantly shorter than the white-steady group starting at week 4 ($P < 0.0001$, all). At week 8, the mean axial lengths of the blue-steady, blue-0.5 Hz, and blue-20 Hz groups were 7.73 ± 0.07, 7.74 ± 0.03, and 7.89 ± 0.06 mm, respectively, which were significantly shorter than the 8.08 ± 0.04 mm observed in the white-steady group ($P < 0.0001$, all).

Compared with blue-steady and blue-0.5 Hz groups, the blue-20 Hz group demonstrated a longer axial length at week 8 (blue-20 Hz versus blue-steady $t = -9.02$, $P < 0.0001$; blue-20 Hz versus blue-0.5 Hz $t = -8.79$, $P < 0.0001$).

The anterior chamber depth and lens thickness were similar in the groups during the 8-week experimental period. There were no intergroup differences at any time point ($P > 0.05$, data not shown).

Corneal Curvature Under Blue Light. Figure 3C shows the mean corneal curvature of each group over 8 weeks under

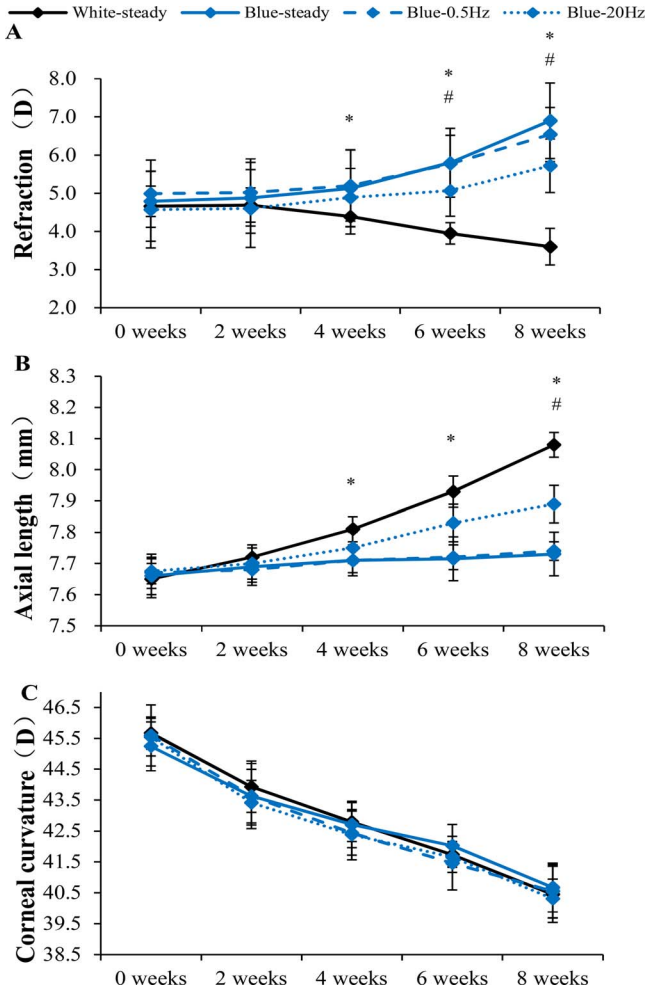

FIGURE 3. Developmental changes under blue light (measured at 0, 2, 4, 6, and 8 weeks). (**A**) Refraction changes under blue light. The refractions were more hyperopic at blue-steady, blue-0.5 Hz, and blue-20 Hz compared with white-steady from weeks 4 to 8 (*). The refraction was less hyperopic at blue-20 Hz compared with blue-steady and blue-0.5 Hz from weeks 6 to 8 (#). (**B**) Axial length changes under blue light. The axial lengths were shorter at blue-steady, blue-0.5 Hz, and blue-20 Hz compared with white-steady from weeks 4 to 8 (*). The axial length was longer at blue-20 Hz compared with blue-steady and blue-0.5 Hz at week 8 (#). (**C**) Corneal curvature changes under blue light. There were no intergroup differences at any time point. The significant difference is indicated by * or # for $P < 0.05$. Standard error bars are shown.

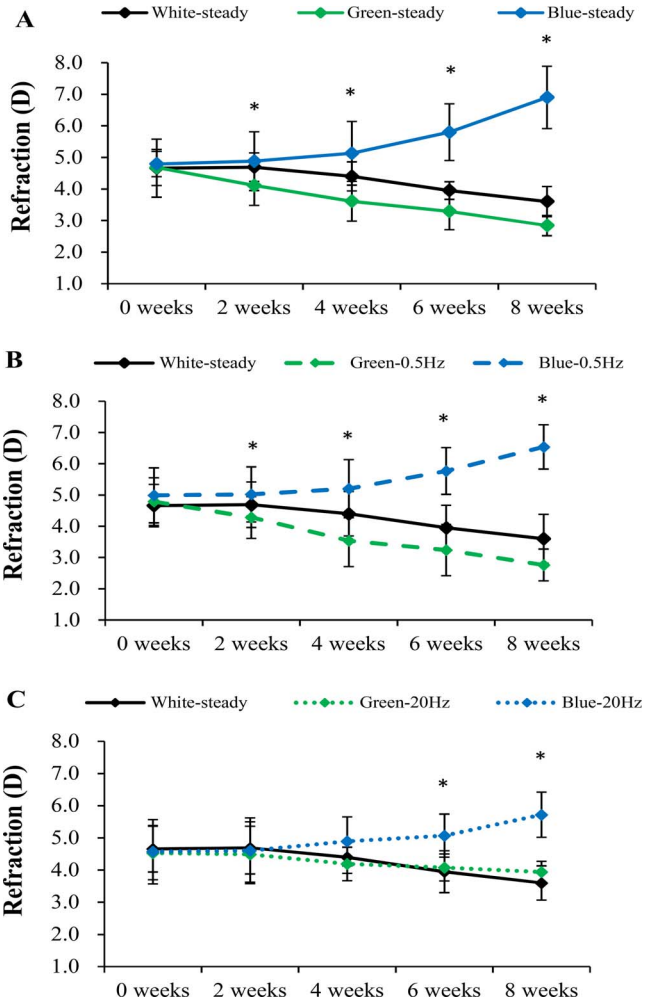

FIGURE 4. Refraction changes under green and blue light at steady (**A**), 0.5 Hz (**B**), and 20 Hz (**C**). The significant difference between green and blue light is indicated by * for $P < 0.05$. Standard error bars are shown.

blue light. The mean corneal curvatures of the white-steady, blue-steady, blue-0.5 Hz, and blue-20 Hz groups reached 40.45 ± 0.74, 40.67 ± 0.79, 40.55 ± 0.86, and 40.31 ± 0.63 D, respectively, at week 8. There were no intergroup differences at any time point.

Refractive Difference Between Green and Blue Light

Figure 4 shows the mean spherical equivalent refraction of green and blue light at steady, 0.5 Hz, and 20 Hz temporal frequencies. The refraction in blue light was more hyperopic than that in green light at all temporal frequencies. From week 2, refraction between blue-steady and green-steady (Fig. 4A) showed a significant difference ($t = -2.73$, $P = 0.0069$) up until week 8 ($t = -17.42$, $P < 0.0001$). The refractive differences between blue-steady and green-steady light reached 4.06 D at week 8. The refraction between blue-0.5 Hz and green-0.5 Hz (Fig. 4B) showed a significant difference from week 2 ($t = -2.91$, $P = 0.0041$) to week 8 ($t = -17.29$, $P < 0.0001$). The refractive differences between blue-0.5 Hz and green-0.5 Hz light reached 3.78 D at week 8. The refraction between blue-20 Hz and green-20 Hz (Fig. 4C) showed no significant difference at weeks 2 and 4 ($t = -0.33$, $P = 0.7434$ for week 2; $t = -1.96$, $P = 0.0516$ for week 4). Only from weeks 6 to 8 did the refraction between blue-20 Hz and green-20 Hz show a difference ($t = -4.11$, $P < 0.0001$ for week 6; $t = -7.78$, $P < 0.0001$ for week 8). The refractive differences between blue-20 Hz and green-20 Hz light decreased to 1.74 D at week 8.

Discussion

In this study, guinea pigs were raised under monochromatic lights with different temporal frequencies to determine the effects of wavelength and temporal composition of ambient lighting on emmetropization. The results showed that wavelength and temporal signals interact with each other, providing different signals to control eye growth.

Wavelength Defocus

According to previous experiments in a variety of species, wavelength defocus plays an important role in the regulation of eye growth. The results of the current study were similar to other published literature on guinea pigs,[30–33] fish,[25,26] and chicks.[27–29] Wavelength-defocus cues from LCA promote eye growth in green light and inhibit eye growth in blue light, which were in accordance with the LCA-related differences in the focal plane position. At all temporal frequencies, refractions in blue light were more hyperopic than those in green light.

However, refractive differences in blue and green light were dependent on temporal frequencies. The refractive differences between blue-steady and green-steady groups reached 4.06 D at week 8. The refractive differences between blue-0.5 Hz and green-0.5 Hz groups reached 3.78 D at week 8. However, the refractive differences between blue-20 Hz and green-20 Hz groups were only 1.74 D at week 8. These results suggest that at the low temporal frequency tested, wavelength defocus played a major role in regulating the eye's refractive development. Whereas at the high temporal frequency tested, the effects of wavelength on refractive development were weakened somewhat.

Temporal Frequency

In the broadband white light conditions, animals in white-0.5 Hz were more myopic than those animals in the white-steady, while white-20 Hz showed no significant difference with white-steady. These results were similar to other published literature on guinea pigs,[44,45] cats,[50] and mice.[51] These prior experiments showed that low temporal frequency lighting promotes eye growth, while high temporal frequency lighting may act as an indicator that the eye is in focus,[34,41] slowing eye growth. However, when animals were exposed to monochromatic lights with low temporal frequency, green-0.5 Hz did not aggravate the myopia caused by green-steady. Guinea pigs in green-0.5 Hz had similar refraction to the animals in green-steady. Similarly, blue-0.5 Hz showed no change in their hyperopic state compared to the blue-steady group. Blue-0.5 Hz had a similar refraction to blue-steady. These results indicate that 0.5 Hz and steady light have the same effect on refraction in monochromatic light, but in white light, a cue from LCA may affect the results.

When animals were exposed to monochromatic lights with high temporal frequency, green-20 Hz prevented the myopic shift observed in green-steady or green-0.5 Hz. It is likely that a high temporal frequency signal indicated that the eye was in focus, slowing eye growth. Animals in blue-20 Hz showed less hyperopia than those in blue-steady or blue-0.5 Hz, although significant differences were not found until week 6. This reduced hyperopia may be due to the inability of S-cones to detect blue light modulation at high temporal frequencies.[52–55] These results indicate that high temporal frequencies might reduce the effect of wavelength defocus on refractive development.

Interaction of Wavelength Defocus With Temporal Frequency

In our previous studies on steady lighting, green light induced myopia and blue light induced hyperopia compared with the white-steady group. When monochromatic light was presented at low temporal frequency, no significant changes were found compared with the corresponding steady green and blue light. However, when monochromatic light was presented at high temporal frequency, the green-20 Hz group showed a hyperopic shift (1.10 D) than the green-steady group. The blue-20 Hz group showed a myopic shift (1.18 D) compared with the blue-steady group.

These results indicate that the regulation of refractive development depends not only on the wavelength and temporal frequency of ambient lighting but also on their interactions. At low temporal frequencies, where the visual inputs are dominated by the wavelength-defocus signal, the wavelength defocus is decoded as a target,[34] causing the eye to become hyperopic to match the focal plane when the ambient light has a short wavelength and myopic when the dominant wavelength is long.[34] Consequently, we might expect to see relative hyperopia in blue-0.5 Hz and myopia in green-0.5 Hz. At high temporal frequencies, where the visual inputs are dominated by luminance signals, the wavelength defocus from monochromatic light is weakened. This weakening of the wavelength defocus signal means that the refractions in blue monochromatic light became less hyperopic, and the refractions in green monochromatic light became less myopic than in steady light.

Comparison With Previous Studies

Recently, Rucker et al.[39] studied the role of temporal sensitivity and blue light in emmetropization in chicks. The results revealed that at high temporal frequencies, chicks showed more hyperopia under yellow light (without blue) than under white light (with blue). At low temporal frequencies, chicks demonstrated a more myopic shift under yellow light than under white light.[39] This study was consistent with our findings that at low temporal frequencies, the eye was using wavelength defocus, but at high temporal frequencies, the wavelength-defocus signal was weakened, resulting in a myopic shift in blue-20 Hz compared with blue-steady.

Smith et al.[36] demonstrated a hyperopic shift under red light in monkeys, which was in contrast to our previous work in monkeys[37] and guinea pigs.[30–33] Comparable results can be found in tree shrews, with a hyperopic shift in steady or flickering red light and a myopic shift in flickering blue light.[34,35]

Based on the current experiment, the temporal aspects of the visual environment played an important role in controlling refraction in monochromatic light experiments, potentially accounting for the inconsistent outcomes among these studies. Short- and long-wavelength light have different effects on the response to temporal frequency,[34] and these differences in temporal processing have previously been shown to affect refraction.[52–55] Wavelength and temporal properties of the visual system in different species, plus inconsistencies in environmental stimuli used in different experiments, could lead to opposite refractive modulations among the studies.

Conclusions

The results of the current study suggest that eyes use both wavelength defocus and temporal characteristics as signals to determine the direction of eye growth. Because of the differences in temporal processing ability of the visual pathways for the detection of high temporal frequency of blue and green light, there are two fundamentally different mechanisms that regulate refractive development: (1) At low temporal frequency, wavelength-defocus signals are used as targets, inducing hyperopic shift at short wavelengths and myopic shift at long wavelengths. (2) At high temporal frequency, wavelength-defocus signals are weakened. This weakening of the wavelength-defocus signal induces a myopic refractive shift in guinea pigs exposed to blue light and a

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hyperopic shift in guinea pigs exposed to green light. Furthermore, it is necessary to explore the temporal-frequency thresholds at which the effects of wavelength defocus on emmetropization diminish for long and short wavelengths.

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