Blue Light Protects Against Temporal Frequency Sensitive Refractive Changes

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Purpose. Time spent outdoors is protective against myopia. The outdoors allows exposure to short-wavelength (blue light) rich sunlight, while indoor illuminants can be deficient at short-wavelengths. In the current experiment, we investigate the role of blue light, and temporal sensitivity, in the emmetropization response.

Methods. Five-day-old chicks were exposed to sinusoidal luminance modulation of white light (with blue; $N = 82$) or yellow light (without blue; $N = 83$) at 80% contrast, at one of six temporal frequencies: 0, 0.2, 1, 2, 5, 10 Hz daily for 3 days. Mean illumination was 680 lux. Changes in ocular components and corneal curvature were measured.

Results. Refraction, eye length, and choroidal changes were dependent on the presence of blue light ($P < 0.03$, all) and on temporal frequency ($P < 0.03$, all). In the presence of blue light, refraction did not change across frequencies (mean change −0.24 [diopters] D), while in the absence of blue light, we observed a hyperopic shift (>1 D) at high frequencies, and a myopic shift (>−0.6 D) at low frequencies. With blue light there was little difference in eye growth across frequencies (77 μm), while in the absence of blue light, eyes grew more at low temporal frequencies and less at high temporal frequencies (10 vs. 0.2 Hz: 145 μm; $P < 0.003$). Overall, neonatal astigmatism was reduced with blue light.

Conclusions. Illuminants rich in blue light can protect against myopic eye growth when the eye is exposed to slow changes in luminance contrast as might occur with near work.

Keywords: myopia, blue cones, temporal frequency

Several studies demonstrate a link between time spent outdoors during childhood and a reduced prevalence of myopia.[1–6] The lower prevalence of myopia was not related to a reduced amount of near work,[7] nor to levels of physical activity engaged in while outdoors.[8] Simply put, the amount of outdoor activity was the strongest predictor of myopia, irrespective of time spent on near work.[7] Because these studies indicate that time spent outdoors may have beneficial effects, the goal of our study was to examine how short-wavelength light, more prevalent in sunlight than in many indoor illuminants, and its interaction with the temporal sensitivity of the visual system, affects the development of myopia.

The sun is a natural source of radiant energy, and its spectral radiant power distribution depends on whether the light is direct or reflected, its position in the sky, and the conditions of the atmosphere.[9] As a result, sunlight is redder at dawn and dusk than in the middle of the day, with roughly equal amounts of red, green, and blue at noon and under cloudy conditions. Sunlight is much richer in short-wavelength light than most artificial illuminants, such as tungsten and fluorescent lights, and the correlated color temperature can range from 5000 to 7000 K (sunlight incident on a horizontal surface regardless of cloud cover) to 40,000 K (clear, north sky). In Figure 1, sunlight is represented by the International Commission on Illumination (CIE) Standard Illuminant D_{65}, which has a color temperature of 6504 K. For the purpose of allowing comparison between illuminants, the intensity of the D_{65} light source has been normalized to the other illuminants at 555 nm. Figure 1 illustrates that tungsten lights are especially low in short wavelength light and rich in long wavelength light (2850–3100 K), while fluorescent lights have energy-rich bands distributed throughout the visible spectrum, which are dependent on the phosphors and activators present. Fluorescent lighting has been associated with increased prevalence of hyperopia[10] and astigmatism[11] and the preponderance of longer wavelengths in artificial illuminants has been proposed as a potential cause of myopia,[12] though others have not found any effect.[13] Because neither tungsten nor fluorescent lights replicate the spectral output or intensity of the sun, short-wavelength sensitive cone stimulation may be compromised at normal indoor illumination levels, particularly by tungsten bulbs.

The spectral sensitivity of the human eye to changes in brightness is described by the 1924 CIE Vλ function, a psychophysical function that is based on the brightness or luminance sensitivity of the long- and middle-wavelength sensitive cones.[14] Sensitivity of the Vλ function peaks at approximately 555 nm, and then falls off at either end of the visible spectrum. As a result, much greater levels of short-wavelength illumination are required to stimulate the sensation of brightness than are required at longer wavelengths. In 1951, the standard CIE Vλ function was modified to include more sensitivity to short-wavelength light, after it became clear that the standard function was too insensitive at the blue end of the light spectrum.[15] Yet, only around 7% of the cone excitation attributed to the Vλ function is from wavelengths shorter than 500 nm. Our hypothesis is that this lack of luminance sensitivity to short wavelengths may affect emmetropization in artificially illuminated environments with low energy at short wavelengths.

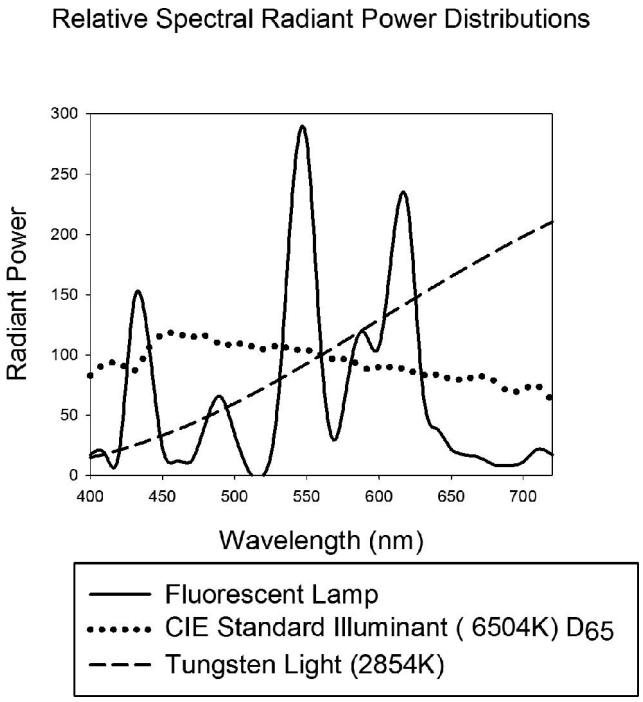

Figure 1. Relative spectral power distributions of CIE illuminant D65, tungsten, and fluorescent lamps. Energy levels have been normalized to 555 nm to allow comparison of the spectral output in the three different types of illuminant. The spectral output of the sun is represented by the output of the CIE illuminant D65.

In humans, cone signals pass through the retina-striate system via one of three temporally sensitive visual pathways traversing the parvocellular, koniocellular, and magnocellular layers of the lateral geniculate nucleus. First, the parvocellular pathway forms the physiological substrate of the red/green color pathway, responding in a sustained manner to high contrast stimuli with low pass temporal frequency characteristics. The parvocellular pathway responds to stimulation of ON and OFF bipolar cells and opponent midget retinal ganglion cells, by long-wavelength sensitive cones (L-cones) and middle-wavelength sensitive cones (M-cones).[16–23] Second, the koniocellular pathway forms the physiological substrate of the blue/yellow color pathway, with anatomically distinct short-wavelength cone (S-cone) ON and OFF pathways.[24] The S-cone ON pathway responds in a sustained manner to stimulation of both large and small bistratified retinal ganglion cells.[25–27] S-cone input to these ganglion cells is carried by blue ON-bipolar cells[28,29] with antagonistic input from L-cones and M-cones, carried by OFF-bipolar cells.[24–26] S-cone OFF pathway signals may be carried by midget bipolar cells,[30,31] and large monostratified ganglion cells.[32] Lastly, the magnocellular pathway forms the physiological substrate of the luminance visual pathway with a spectral sensitivity that corresponds to the Vλ function. The magnocellular pathway responds to low-contrast stimuli in a transient manner, with bandpass high temporal frequency response characteristics, and in response to stimulation of opponent retinal ganglion cells with additive input from both L- and M-cones[16,17,19–23,33] but not S-cones. Because these opponent visual pathways show temporal sensitivity, we hypothesized that the luminance-sensitive emmetropization mechanism would also evince temporal frequency sensitivity.

A signal from cone contrast is a more reliable measure for guiding emmetropization than cone excitation as it is independent of the color of the illuminant (or reflected light). A cone contrast signal differs from a measure of cone excitation in that it represents how cone excitation at edges differs from the mean illumination level. Cone contrast also takes into account the level of adaptation of the cones. If the mean cone excitation level is low, then cone contrast will be higher. Cone contrast is therefore an indicator of the sensitivity of the cones to changes in cone excitation across a boundary and an indicator of the input to the three visual pathways.

A theoretical analysis of the change in cone contrast with defocus by Rucker and Wallman[34] indicated that changes in color and luminance contrast can predict the sign of defocus. As the eye grows the typical postnatal hyperopic defocus decreases. With the decrease in hyperopic defocus, S-cone contrast decreases, while L- and M-cone contrast increases, introducing changes in blue/yellow color contrast. If the eye continues to grow, and myopic defocus increases, contrast of all three cone types decreases, without color changes, decreasing luminance contrast of the retinal image. Rucker and Wallman[34] confirmed that the emmetropization mechanism uses these color and luminance contrast cues to identify the sign of defocus; eyes exposed to changes in luminance contrast became hyperopic, while eyes exposed to changes in color contrast became more myopic.

While longitudinal chromatic aberration is instrumental in creating the more myopic defocus of blue light, the minimal contribution of S-cones to luminance sensitive visual pathways[35,36] would predict that they would not contribute to emmetropization through changes in luminance contrast. Nevertheless, S-cones have been shown to contribute to accommodation responses.[37,38] Our hypothesis is that S-cones contribute to emmetropization, but that in environments illuminated with low levels of blue light (tungsten bulbs), the paucity of S-cones and the insensitivity of the luminance contrast pathway to S-cone stimulation, may mean that the S-cone contrast signal is below threshold for emmetropization. With higher levels of S-cone stimulation in sunlight, the more myopic defocus of blue light may provide a protective effect against the development of myopia.

Because the chick has a broad spectral sensitivity, and possesses a temporal sensitivity qualitatively similar to that of humans, we considered it the ideal choice for myopia research. The spectral sensitivity functions of the five cone types and their associated oil droplets are described in detail in Rucker and Wallman.[39] In chicks, these cone signals are carried to the brain by four visual pathways: one luminance and three color.[40] Indeed, chicks have red/green, blue/yellow, and UV/blue color discrimination in addition to luminance contrast discrimination.[41] With regard to temporal processing, chicks' temporal sensitivity shows many qualitative similarities to that of humans,[42] albeit with somewhat reduced peak temporal sensitivity around 15 Hz, a low frequency fall off, and at high luminance levels, slightly higher critical flicker fusion values. Thus, chicks are an excellent model for investigating the interaction of spectral sensitivity with temporal sensitivity in the development of myopia.

To investigate whether the light spectrum and/or temporal properties of the light source affect eye growth during development in chicks, we assessed the effects of temporal changes in light, either with or without a blue light component, on eye development. The results of this study are important in understanding the environmental factors that drive myopia development.

Methods

Animals

One hundred sixty-five mixed sex white leghorn chicks (*Gallus gallus domesticus*, Cornell K strain; Cornell University, Ithaca, NY, USA) were randomly selected for use in this

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experiment. After hatching, the chicks were raised in 12-hour cycles of light and dark, with a continuous supply of food and water. Illumination levels ranged from 50 to 350 lux, depending on the chick's location in the cage. Care and use of the animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Measurements

Refraction measurements were taken at the start and end of each exposure period. For the duration of the measurements, performed with the use of a Hartinger Refractometer (Zeiss, Jena, Germany) the birds were anesthetized with 1.5% isofluorane in oxygen.[43] Isofluorane relaxes the accommodation response in chicks. On each occasion, three measurements were made in the horizontal (180) and vertical (90) meridians. Lid retractors were used to hold the eyelids open.

Measurements of the ocular components were made with a noncontact ocular biometer (Lenstar LS 900; Haag-Streit AG, Konig, Switzerland) at the start and end of each exposure period using a low-coherence reflectometry technique. Unanesthetized chicks were held in the dark in a specially designed holder. The holder allows movement in three planes to allow orientation of the head and alignment of the pupil with the circular pattern of lights produced by the Lenstar. Lid retractors were not necessary, as the chicks were awake and their eyes open. A total of six measurements were made per eye, with each measurement recorded on the machine, averaging 16 measurements taken by the Lenstar. The location of each component is seen as a peak in the A-scan produced by the Lenstar. Unlike humans, the majority of chicks have a distinct peak that corresponds to the posterior choroid. To avoid potential effects of diurnal variation on choroidal thickness,[44] measurements were made between 10 AM and 2 PM, and each chick was measured at approximately the same time of day before and after the experiment.

Measurements of corneal curvature were made using images of the Lenstar alignment rings, taken during the measurements of the ocular components. The images collected show the circular alignment mires positioned directly in the center of the pupil. Next, these were uploaded into ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). A rectangular outline was drawn around the mires, and the dimensions of the rectangle used to determine the radius of curvature in the horizontal and vertical meridians. Corneal curvature was calibrated using ball bearings of known radius, and the refractive index of 1.33749. These calibrations were used to convert the dimensions of the rectangle into corneal radii and corneal power measures.[45] Finally, corneal power was described by Fourier decomposition (rectangular form) with spherical power component M and power vectors J_0 (cosine Jackson cross cyl [JCC]) and J_{45} (sine JCC) as described by Thibos et al.[46] This method of analysis enabled us to compare changes in meridional power among birds. Changes in corneal power (not refractive correction) along these meridians were calculated as the difference in the pre- and postmeasures of corneal power.

Light Source

Lighting conditions were produced with light emitting diodes (LED) that consist of independently controlled red, green, and blue components (Atlas Light Engine; peak wavelengths: 619 nm, 515 nm, 460 nm; Lamina Ceramics, Westhampton, NJ, USA) with a beam spread of 36°. The illuminants used were Lamina Titans RGB LEDs driven by an eight channel, 12-bit Access I/O, USB-DA12-8A digital to analog converter with waveform generator functionality connected to BuckPucks (LuxDrive: 3021 D-E-500; LEDdynamics, Randolph, VT, USA) to provide a linear current output over a range of 1.6 to 4.3 V. Light output was calibrated and a sinusoidal output was produced digitally using lookup tables, and confirmed by recording illuminance output (Newport Model 818-SL serial number: 6915; Newport, Franklin, MA, USA). Because we were interested in the role of blue light, which does not contribute significantly to luminance measures, the irradiance of the light source was used to equate the lighting components.

Chicks have different wavelength sensitivities than humans, so we refer to illuminance (E_c) corrected for the chick photopic spectral sensitivity function (V_c)[47] as "chick lux,"[39] which differs from "human lux" as a function of wavelength. Chick lux can be calculated from the spectral emission curve of a source P_λ according to the following equation. Thus, if P_λ is in watts per unit wavelength per meter^2, chick lux, E_c, in photopic lumens per unit area (lux) is described by:

$$E_c = k \int_0^\infty P_\lambda V_c d\lambda, \quad (1)$$

where $k = 683$ lumens W^{-1}.

The mean irradiances were 50 μW/cm^2 for the red, green, and blue components of each light source, which is equivalent to 214 "chick lux" for red, 191 "chick lux" for green, and 64 "chick lux" for blue. Small differences in illuminance between the "with blue" and "without blue" conditions were controlled for with neutral density filters to maintain a mean illuminance equivalent to 680 human lux in both conditions.

The illuminants produced 80% contrast in the red, green, and blue components of the illuminants. Contrast was calculated as Michelson contrast (% Modulation = [(Amplitude/Mean)] ×100]), which takes the mean level and the effects of adaptation into account. In this study, contrast and mean illumination levels were kept constant.

Illumination and Frequency Conditions

Chicks were exposed to one of six temporal frequency conditions 0, 0.2, 1, 2, 5, and 10 Hz. Ten hertz is well within the range of flicker sensitivity for chicks.[42,48] Bird numbers in each experimental group are shown in Table 1. Each frequency was presented in two illumination conditions:

1) With blue condition was produced with in-phase sinusoidal modulation of the red (615 nm, half-bandwidth 20 nm), green (515 nm, half-bandwidth 35 nm), and blue (465 nm, half-bandwidth 25 nm) LEDs.
2) Without blue condition was produced with in-phase sinusoidal modulation of the red (615 nm, half-bandwidth 20 nm) and green (515 nm, half-bandwidth 35 nm) LEDs.

Procedure

Chicks were 5- to 7-days old at the start of the experiment. Neither eye was fitted with a lens. During the experiment, chicks were free-roaming in a 32 × 20 inch wire cage for 8 hours a day from 9 AM to 5 PM (2 PM to 5 PM on day 1) for 3 days. Cages were illuminated with the modulated light sources, placed on top of the cage, and both eyes were simultaneously exposed. Both illumination conditions were run simultaneously, using chicks from the same batch, on multiple occasions. Experiments were performed in designated areas, screened by custom-made black-out curtains, and separated from office areas by a double door system. Chicks were otherwise kept in the dark in a sound and light-proof chamber.

Table 1. Numbers of Chicks in Each Illumination and Temporal Frequency Condition

Illumination Condition	Temporal Frequency					
	0 Hz	0.2 Hz	1 Hz	2 Hz	5 Hz	10 Hz
With blue	15	13	13	17	13	11
Without blue	15	13	14	12	15	14

Analysis

The effects of illumination conditions on each of the ocular components (anterior chamber depth, vitreous, choroidal thickness, eye length) and refraction were calculated as the change between pre- and postmeasurements in both eyes of all birds averaging right and left eyes for each chick.[49] Eye length was calculated as the distance from the anterior cornea to the posterior sclera. Changes in the ocular components during the exposure period in the same birds were compared using Student's *t*-tests for repeated measures.

Changes in ocular dimensions and refractions were compared with regard to frequency (0, 0.2, 1, 5, 10 Hz) and illumination condition (with and without blue light), and for the interaction between frequency and illumination using a two-way ANOVA. If the ANOVA was significant, conditions were compared using the more conservative Tukey HSD or the Scheffé unpaired, two-tailed, post hoc *t*-tests for multiple comparisons. Student's *t*-tests were used for simple comparisons at 0 Hz.

Regressions were performed in SigmaPlot using a least-squares approach. The SigmaPlot curve fitter uses the Marquardt-Levenberg algorithm to find the values of the parameters that minimize the sum of the squared differences between the values of the observed and predicted values of the dependent variable.

Results

Data is shown in Table 2.

As shown in Figure 2, inclusion of blue light protected the eye against temporal frequency sensitive changes in refraction. Refraction data showed an interaction between frequency and illumination condition (ANOVA: $P = 0.012$). At the beginning of the experiment, chicks were hyperopic (with blue: 0.23 ± 0.11 [diopter] D; without blue: 0.37 ± 0.15 D). After exposure to the "with blue" illumination condition, refraction showed a mean change of –0.24 ± 0.30 D across the frequency range. In contrast, exposure to the "without blue" illumination condition resulted in a marked swing in refraction with frequency, a hyperopic shift at 5 Hz (1.1 ± 0.44 D) and at 10 Hz (1.0 ± 0.45 D), contrasting to a myopic shift at 0.2 Hz (–0.93 ± 0.37 D; $P < 0.05$ both; Tukey HSD), 1 Hz (–0.67 ± 0.44 D), and 2 Hz (–0.59 ± 0.39 D). Thus, the absence of blue light causes temporal frequency sensitive changes in refraction.

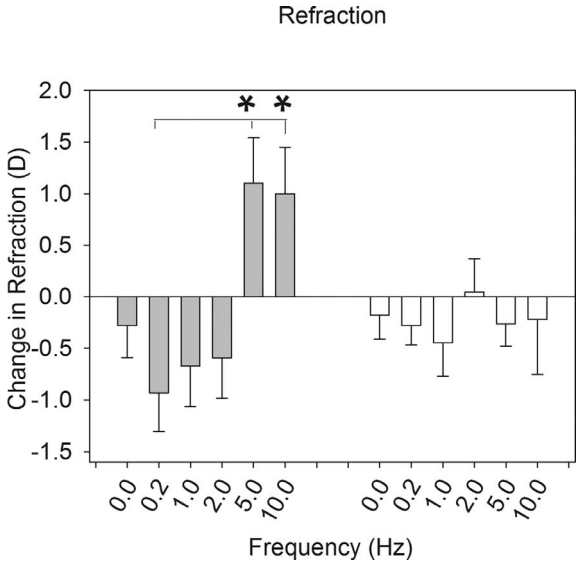

Figure 2. The change in refraction that occurs when eyes are exposed to flicker at a range of different temporal frequencies, for an illuminant with (*white bars*) or without (*gray bars*) blue light. Without blue light, the refraction was more hyperopic at high temporal frequencies than at low temporal frequencies. The statistical difference is indicated by * for *P* values less than 0.05. *Bars* indicate comparisons of the refraction at 10 and 5 Hz with the refraction at 0.2 Hz. *Standard error bars* are shown.

It has been proposed that changing the color of the illuminant will affect refraction. To examine the effect of changing the color temperature of the broad spectrum illuminant, we compared the change in refraction in the two illumination conditions with steady light (0 Hz). There was no difference in refraction change with (–0.18 ± 0.23 D) or without (–0.28 ± 0.31 D) blue light in steady light ($P = 0.8$;

Table 2. Mean Changes (in mm) in Ocular Components and Refraction (in D) During Exposure to Flickering Light With (RGB) and Without (RG) Blue at Each Temporal Frequency

Frequency, Hz	Illumination	Δ Ant. Chamber Mean, mm	Δ Lens Mean, mm	Δ Choroid Mean, mm	Δ Vitreous Mean, mm	Δ Eye Length Mean, mm	Δ Refraction Mean, D
0	RGB	0.037 ± 0.009	0.140 ± 0.006	−0.046 ± 0.006	0.130 ± 0.012	0.269 ± 0.016	−0.18 ± 0.23
0.2	RGB	0.040 ± 0.006	0.131 ± 0.007	−0.039 ± 0.008	0.146 ± 0.019	0.284 ± 0.020	−0.28 ± 0.19
1	RGB	0.048 ± 0.004	0.130 ± 0.005	−0.030 ± 0.008	0.140 ± 0.016	0.289 ± 0.017	−0.45 ± 0.32
2	RGB	0.040 ± 0.008	0.115 ± 0.007	−0.026 ± 0.007	0.134 ± 0.014	0.267 ± 0.011	0.05 ± 0.32
5	RGB	0.030 ± 0.007	0.117 ± 0.009	−0.026 ± 0.006	0.118 ± 0.020	0.241 ± 0.022	−0.26 ± 0.21
10	RGB	0.015 ± 0.006	0.114 ± 0.006	−0.070 ± 0.010	0.146 ± 0.024	0.207 ± 0.025	−0.22 ± 0.53
0	RG	0.050 ± 0.004	0.135 ± 0.006	−0.033 ± 0.009	0.141 ± 0.013	0.336 ± 0.031	−0.28 ± 0.31
0.2	RG	0.057 ± 0.012	0.123 ± 0.009	−0.048 ± 0.008	0.148 ± 0.024	0.343 ± 0.041	−0.93 ± 0.37
1	RG	0.049 ± 0.006	0.120 ± 0.008	−0.056 ± 0.008	0.120 ± 0.014	0.239 ± 0.022	−0.67 ± 0.40
2	RG	0.048 ± 0.008	0.127 ± 0.006	−0.032 ± 0.007	0.108 ± 0.016	0.256 ± 0.021	−0.59 ± 0.39
5	RG	0.053 ± 0.004	0.098 ± 0.006	−0.073 ± 0.006	0.123 ± 0.014	0.196 ± 0.019	1.10 ± 0.44
10	RG	0.020 ± 0.006	0.116 ± 0.007	−0.053 ± 0.010	0.121 ± 0.018	0.198 ± 0.024	1.00 ± 0.45

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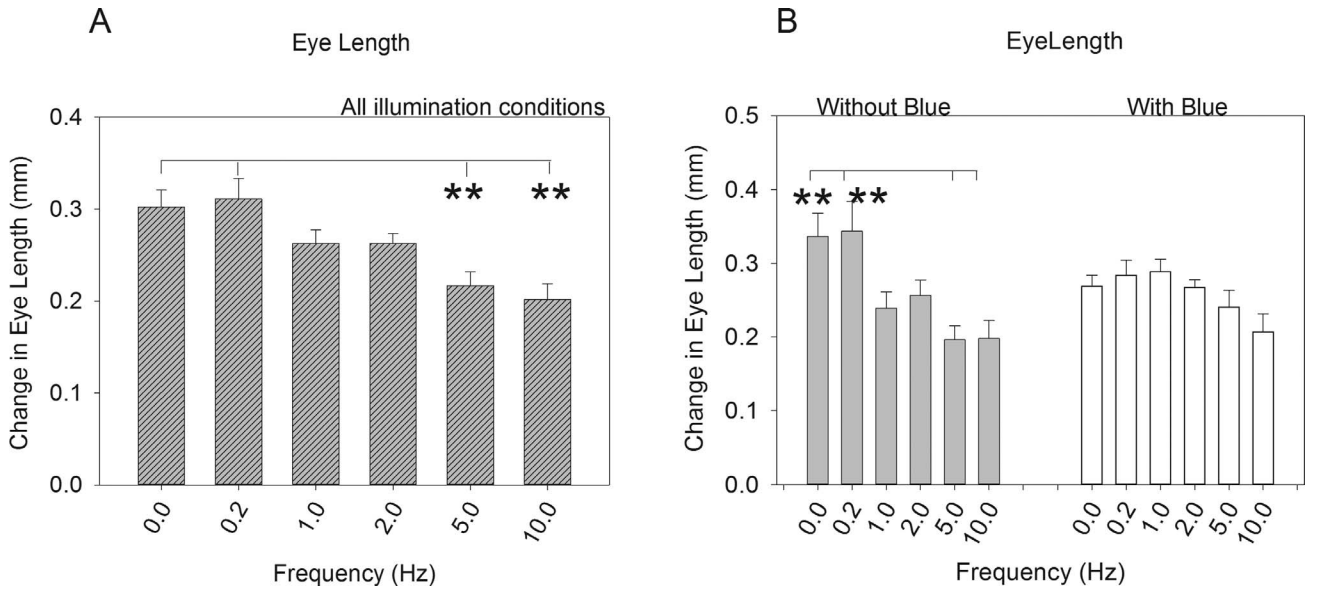

Figure 3. (**A**) Change in eye length during the 3-day exposure over a range of temporal frequencies when the data for both illumination conditions are pooled. Eye growth is dependent on the temporal frequency of the illuminant. (**B**) Change in eye length during the 3-day exposure when the two illumination conditions are considered separately. *P* values less than 0.05 and 0.01 are shown as * and **, respectively. Without blue light the eyes show a temporal frequency dependent change in refraction.

Student's *t*-test). In other words, whether the light is white or yellow, incandescent or tungsten, refractive effects are only observed when the light is modulated.

Changes in eye length are shown in Figure 3A. Analysis of pooled data from both illumination conditions revealed that eye length changes were affected by temporal frequency (ANOVA: $P = 0.0001$). Eye growth measured at 5 and 10 Hz was 217 ± 0.15 μm and 201 ± 17 μm, respectively, while eye growth at 0 and 0.2 Hz was 302 ± 28 and 311 ± 22 μm, respectively. Temporal frequencies of 5 and 10 Hz produced significantly shorter eye lengths than lower temporal frequencies of 0 and 0.2 Hz ($PV < 0.01$ both; Tukey HSD). Higher temporal frequencies caused a reduction in eye growth, in line with the hyperopic shift in refractions, while lower temporal frequencies caused an increase in eye growth.

The illumination condition, however, does play an important role. Eye length data showed an interaction between frequency and lighting condition (Fig. 3B: ANOVA: $P = 0.034$). After exposure to the "with blue" light illumination condition, the difference between eye growth at 10 Hz and at lower frequencies was small; eyes grew only 77 μm ($P = 0.44$) and 62 μm ($P = 0.65$) less at 10 than at 0.2 and 0 Hz, respectively. In contrast exposure to the "without blue" condition resulted in 145 μm more eye growth at 0.2 than at 10 Hz ($P < 0.01$; Tukey HSD) and 138 μm more at 0 than at 10 Hz ($P < 0.01$; Tukey HSD). Blue light prevented temporal frequency sensitive changes in eye growth.

To examine the effect of illuminant color temperature on eye length changes, we looked at changes in eye growth with steady light (0 Hz) in the two illumination conditions. There was no significant difference in mean eye length in steady light with (269 ± 16 μm) or without (336 ± 31 μm) blue light ($P = 0.06$; Student's *t*-test), though on average eyes were 67 μm shorter in the "with blue" illumination condition. In line with our refraction data, the color temperature of the illuminant (white or yellow) did not affect eye growth significantly when chicks were exposed in a restricted cage environment where the only changes in temporal frequency are those resulting from head and eye movements.

Interestingly, the vitreous chamber depth showed no change with illumination condition (ANOVA: $P = 0.21$) or frequency (ANOVA: $P = 0.54$), and no interaction between frequency and illumination condition was observed (ANOVA: $P = 0.66$). Analysis of pooled frequency conditions comparing only lighting condition revealed similar changes in mean vitreous chamber depth in the "with blue" and "without blue" conditions (136 and 124 μm). Similarly, analysis of frequency for pooled illumination conditions revealed no significant differences in mean vitreous chamber depth between 10 (128 ± 14) and 0.2 Hz (146 ± 14 μm). The vitreous chamber did not change with frequency or illumination condition because compensatory choroidal changes partially negated the eye length changes.

Choroidal thickness changed with frequency (ANOVA: $P = 0.03$), with choroids thinning 60 ± 9 μm at 10 Hz, compared with 28 ± 6 μm at 2 Hz (10 vs. 2 Hz: $P < 0.05$; Scheffé) when the data for the illumination conditions was pooled. As seen in Figure 4, choroids also showed an interaction between frequency and illumination condition (ANOVA: $P = 0.007$). Without blue light choroids thinned at intermediate temporal frequencies (5 Hz: $P = 0.02$; 1 Hz: $P = 0.053$; 2 Hz: $P = 0.01$; Student's *t*-test), thinning 73 ± 6 μm at 5 Hz, 56 ± 8 μm at 1 Hz, and 32 ± 7 μm at 2 Hz. Choroids thinned more without blue light at 5 Hz than they did with blue light at 5 and 2 Hz (Scheffé). Choroids thinned more at high temporal frequencies, while there was more choroidal thinning at intermediate temporal frequencies without blue light.

Choroidal thinning has been associated with a subsequent increase in eye length. However, our data suggest that that choroidal thinning was correlated with a reduction in eye length (Fig. 5). Without blue light, thinner choroids, and shorter eyes are seen at the highest temporal frequencies, and thicker choroids and longer eye lengths are seen at lower temporal frequencies ($R^2 = 0.4$). With blue light, choroidal thinning was fairly constant across frequency with a decrease at the highest temporal frequency. The data can be fit with a polynomial ($R^2 = 0.8$). Without blue light, the compensatory changes in choroidal thickness are insufficient to compensate for the associated eye length changes and changes in refraction ensue.

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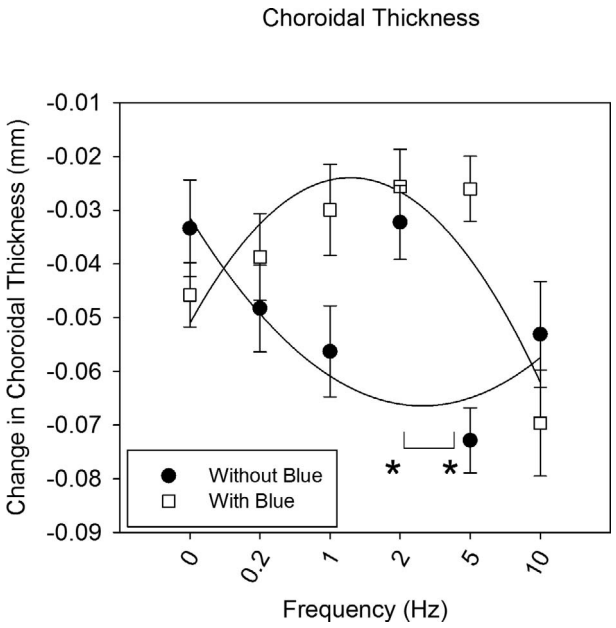

Figure 4. Change in choroidal thickness during the 3-day exposure to flicker with (*white squares*) or without (*black circles*) blue light at a range of temporal frequencies. Choroidal changes at 5 Hz without blue light were significantly different to choroidal changes at 5 and 2 Hz with blue light. *Bars* and * show *P* values less than 0.05. *Standard error bars* are shown.

Changes in anterior chamber depth showed a dependence on blue light (ANOVA: $P = 0.006$) and frequency (ANOVA: $P = 0.003$). Changes in anterior chamber depth were small, but exposure to blue light produced shallower anterior chambers (with blue: 35 ± 6 μm; without blue: 46 ± 13 μm). Anterior chamber depth was also significantly shallower at 10 Hz compared with all other frequencies (24–30 μm; $P < 0.05$). To summarize, blue light caused a reduction in anterior chamber depth that would be expected to decrease the power of the eye, causing a hyperopic shift, particularly at higher temporal frequencies.

Corneal astigmatism is reduced during emmetropization in the presence of blue light (Fig. 6), but the effect is not temporal frequency sensitive. In the presence of blue light, J0 was reduced by −1.51 ± 0.09 D, producing a significant reduction in neonatal astigmatism similar to that which occurred in steady light. In contrast, only small changes in J0 (–0.39 ± 0.17 D) were observed in the absence of blue light. The axis of astigmatism did not change over the duration of the experiment and was predominantly around 180° making J45 close to zero. Changes in J0, with and without blue light, were significantly different (ANOVA: $P < 0.001$) but there was no frequency effect (ANOVA: $P = 0.436$). The spherical corneal power (M) component was similar with blue light (–5.27 ± 0.45 D) and without blue light (–4.53 ± 0.48 D). These results indicate that blue light contributes to a reduction in neonatal astigmatism during emmetropization.

Low frequency flicker induced greater lens thickening (ANOVA: $P = 0.001$). Combining data from the two illumination conditions, the lens increased by 137 μm at 0 Hz, but only by 115 μm at 10 Hz, and 108 μm at 5 Hz. Only the change at 5 Hz was significantly different to that at 0 Hz ($P = 0.003$). The lens

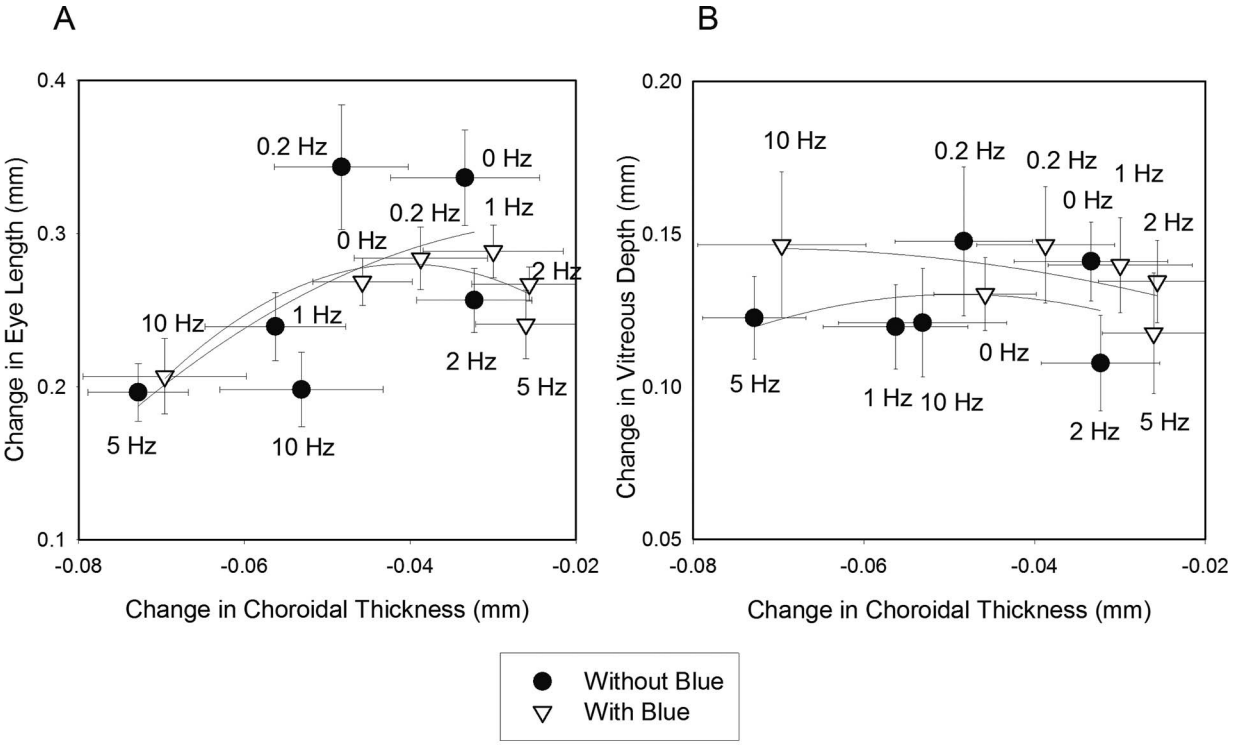

Figure 5. Eye length (**A**) and vitreous (**B**) change during the experiment is plotted against choroidal changes for the with blue (*white square*) and without blue (*black circle*) illumination conditions. Each *symbol* represents a particular temporal frequency as indicated on the graph. Data for each illumination condition was fit with a polynomial function as shown by the *solid lines*.

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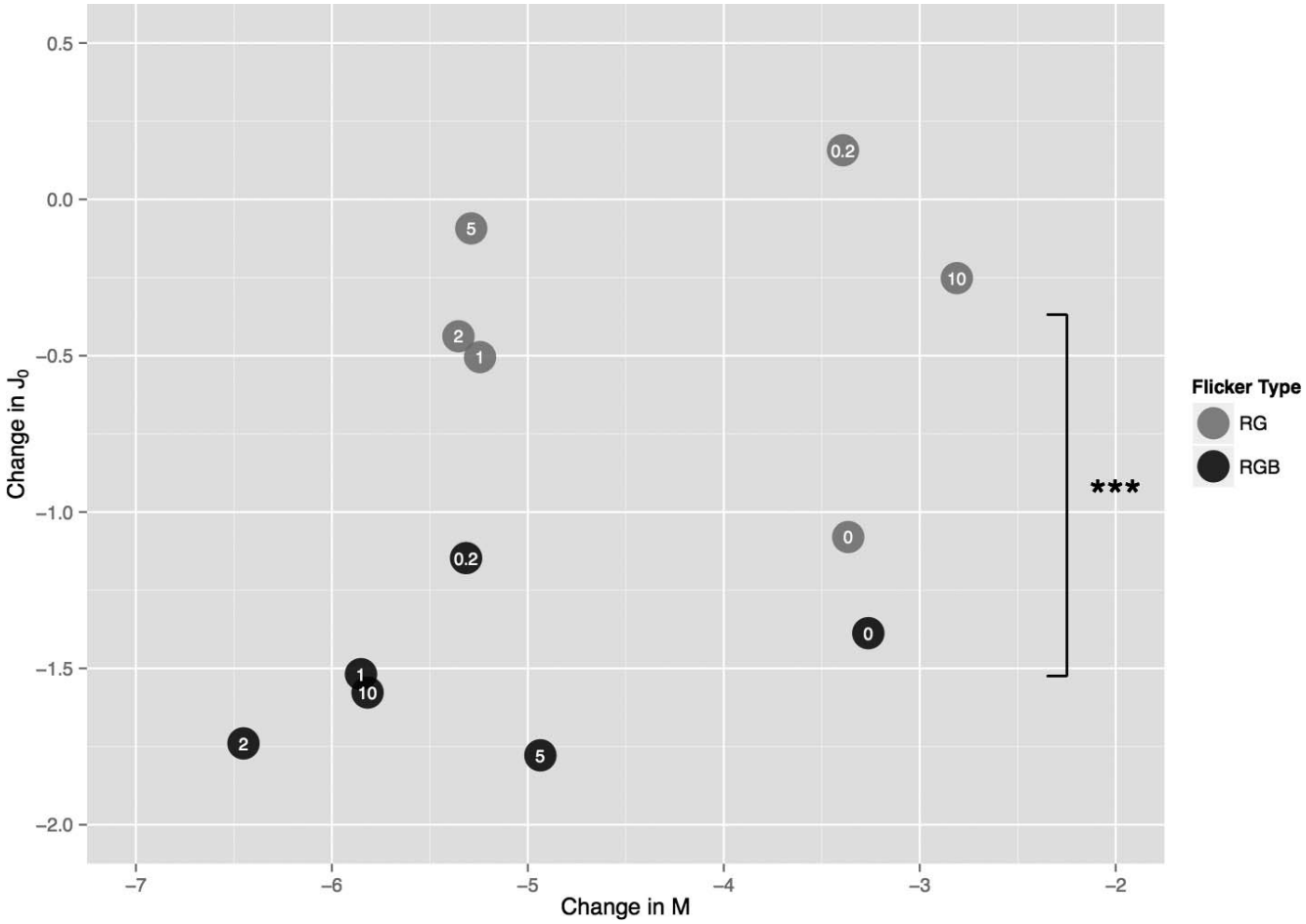

Figure 6. Graph shows the change in corneal power (not spectacle correction) along J0 during the experiment while exposed to modulated light, with (*black symbols*) and without (*gray symbols*) a blue light component. Individual frequency conditions are indicated on the graph. *Bars* and *** indicate that the change in corneal power along J0, with and without blue, were significantly different at the *P* less than 0.001 level.

thinning at 5 Hz may have contributed to the hyperopia found at higher frequencies.

Discussion

Blue Light and Its Interaction With Temporal Frequency

In this study, we have shown for the first time that blue light and temporal contrast changes play an important role in emmetropization. In the absence of blue light, high temporal frequencies, which favor the luminance sensitive visual mechanism, drive a reduction in eye length and a hyperopic shift in refraction. In contrast, low temporal frequencies, which favor the color sensitive visual mechanism, drive an increase in eye length and a more myopic shift. However, we showed that the presence of blue light prevented these hyperopic and myopic shifts. Blue light was also essential for the reduction in astigmatism during development. Therefore, an environment that deprives the eye of blue light stimulation, either as a result of the type of illuminant (tungsten bulbs), intensity (dim light), blue light reducing filters on spectacles, or reduced exposure to outdoor illumination, will tend to make the eye vulnerable to temporal frequency sensitive changes in refraction, particularly myopia at low temporal frequencies.

Temporal Frequency Effects

In an indoor environment, especially when focused on near work or computer use, the eye is exposed to very different temporal stimulation than in an outdoor environment. In the outdoor environment the scene is typically dynamic, with fast, fluttering movement of leaves, ripples on water, motion of people or cars. In the indoor environment, the scene is typically static, the walls, fixtures and fittings, do not move. While doing near work indoors, the central visual field is stimulated by changing characters, while the peripheral visual field is static, apart from slow changes associated with head and eye movements. The results of this experiment suggest that a static visual field and low temporal frequency movements are likely to induce myopic increases in eye growth in the absence of blue light stimulation.

High temporal frequencies caused reduced growth, thinner choroids, and thinner anterior chambers regardless of the illumination condition. High temporal frequency flicker has been linked to retinal dopamine release from the dopaminergic retinal ganglion cells and a reduction in eye length.[50,51] In addition, choroidal thinning has been observed with administration of intravitreal apomorphine (a dopamine agonist) in chicks exposed to flicker.[52] Correspondingly, the results of our study suggest that choroidal thinning and reduction in anterior chamber depth might also be associated with increased dopamine release.

An Explanation for the Blue Light Effect

This study showed increased eye length at low temporal frequencies compared with high temporal frequencies in the "without blue" condition. Previous studies have suggested that the more myopic defocus of blue light provides a myopically defocused stimulus resulting in reduced eye growth.[53,54] At lower temporal frequencies the visual system would have had time to recognize this myopically defocused blue light and respond with slower growth in the "with blue" condition. We can conclude that at low temporal frequencies the eyes responded to the focal planes of the illuminant growing less when blue light was present and more when blue was absent.

At high temporal frequencies the differences in eye length between the "with blue" and "without blue" conditions were not as evident, but the difference in refraction was marked. The less hyperopic refractions in the "with blue" condition may be derived from the failure to detect the more myopically defocused blue light or from the introduction of a color signal as a result of slower processing of S-cone signals than L- and M-cone signals.[55–62] This delay in the S-cone signal means that the visual system sees the red and green components (yellow) before the blue component, which introduces a blue/yellow color signal. Consequently, we might expect to see a relative decrease in hyperopia at higher temporal frequencies in the "with blue" condition, as the delayed blue signal introduces a color component that signals hyperopic defocus to the emmetropization mechanism.

Comparison With Previous Experiments

Hyperopic shifts in refraction with high frequency flicker have also been seen in earlier experiments.[34,63–65] Rucker and Wallman[34] found that refraction remained hyperopic in response to 2-Hz luminance flicker. This experiment extends these findings to show that when frequency is increased further, to 5 or 10 Hz, a greater hyperopic shift is found. Other examples of hyperopic shifts with high frequency flicker include studies showing that high contrast, stroboscopic flicker, around 10 to 15 Hz, reduces deprivation myopia[63–65] and produces hyperopia in the control eye of form deprived or negative lens-wearing chicks.[63] In studies performed by Schwahn and Schaeffel,[63] flicker was produced with a 150-W xenon lamp, a light source that is rich in blue light. The authors found both a reduction in eye length and less myopic refraction in response to form deprivation with a 50% duty cycle at both 6 and 12 Hz. It has been suggested that high contrast, high temporal frequency changes may mimic signals the eye receives from objects that are in focus,[51] thus resulting in slowed eye growth to prevent further refractive change in response to such conditions.

Other experiments have shown a myopic shift in refraction at low temporal frequencies including experiments done on chicks,[66] mice,[67] and guinea pigs.[68] Crewther et al.,[66] measured refractions in chicks that were exposed to luminance flicker with a slow on/fast off profile. Similar to the results in the current study, chicks wearing a plano lens became more myopic, close to −5 D at 1 and 2 Hz. However, no change in refraction was observed in chicks without lenses. The authors used a halogen light source, which provided little illumination at the blue end of the spectrum, and low illuminance levels (1.5–180 lux).[66] It is likely that these factors reduced the transmission of short wavelengths below a required blue light threshold in the plano lens condition, analogous to our findings from the "without blue" condition in the current study.

One of the many factors that differ between indoor and outdoor environments is the illuminance level. The illuminance of the outdoor environment is much greater than that of the indoor environment, with levels of between approximately 30,000 and 130,000 lux on a sunny day compared with approximately 300 lux or less indoors. The effect of these higher illuminance levels on myopia development in chicks, monkeys, and tree shrews has been studied in several experiments.[69–73] Results showed that high intensity illuminants (30,000 lux) retarded the development of myopia in diffuser wearing chicks.[70] However, in negative lens-wearing chicks, bright light did not translate into less myopia at the end of the experiment.[71] A similar result was found in lid-sutured Rhesus monkeys (18,000–28,000 lux) and in tree shrews at more moderate light levels.[73] High light intensities have therefore been associated with slower rates of myopia progression.

In the current experiment, mean light levels of 680 lux were used to illuminate the cages. These light levels (680 lux) were similar to light levels found in brightly lit office environments, but much lower than the light levels used in the above mentioned light intensity experiments. Light levels of 680 lux were also higher than those used in some experiments on eye growth in monochromatic light that have shown more myopic eye growth in red light in chicks, guinea pigs, fish, and mice (Refs. 12, 15, 73–77 and Siegwart JT Jr, et al. *IOVS*. 2012;53:ARVO E-Abstract 3457), though it is difficult to compare the different measurement units between experiments. In contrast, marmosets and tree shrews (Ref. 78 and Smith EL, et al. *IOVS*. 2013;54:ARVO E-Abstract 4039) have shown hyperopic growth in red monochromatic light at mean levels of 50 and 325 lux (with a combination of slow and fast flicker), respectively. While Liu et al.[74] found normal emmetropization in rhesus monkeys in red light at 200 lux. Given the results of this experiment it is possible that the temporal aspects of the monkey's visual environment played a role in controlling refraction in above mentioned monochromatic light experiments.

Safe Levels of Blue Light

As the energy of light increases at short wavelengths, so does its potential to cause damage to tissues, and care must be taken in selecting artificial lighting that is high in blue energy in order to avoid adverse effects. In contrast to sunlight, indoor illuminants typically have much less energy. Illuminance levels on a bright sunny day are around 130,000 lux. In contrast, most indoor illuminants, such as fluorescent office lighting, produce much lower illuminance levels of between approximately 300 and 700 lux at desk level, as did the bulbs used in this experiment (680 lux, RGB Titans: Lamina Ceramics). In 2010, French Agency for Food, Environmental and Occupational Health & Safety (ANSES) performed a quantitative analysis on a selected a range of commercially available LEDs[79] indicating that all of the tested illuminants were safe when the light source was maintained at more than 2.1-m distance from the observer. None of the multiple die LEDs, or LED arrays (warm or cold white), ranging from 7,000 to 10,000 lumen, exceeded the permissible safe exposure times. However, in an effort to protect children's eyes from light damage,[80] ANSES discourages the use of LED light sources in places frequented by children, the population who are most vulnerable to myopia progression.[79]

Conclusions

A simple solution to the problem of temporal frequency sensitive changes in eye length and refraction is to use a broad-

spectrum light source with a strong blue light component for indoor lighting. The commercially sold "cold white" LED bulbs (7,000–10,000 lumens) that provide illumination conforming to the CIE standard illuminant D_{65} (6504 K) may provide sufficient blue light when used at a distance of 2.1 m, as recommended by ANSES, though brightness levels for controlling myopia have not yet been determined.

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