

# Color and Temporal Frequency Sensitive Eye Growth in Chick

Frances Rucker, Stephanie Britton, and Christopher Taylor

New England College of Optometry, Department of Biomedical Science and Disease, Boston, Massachusetts, United States

Correspondence: Frances Rucker, New England College of Optometry, Department of Biomedical Science and Disease, 424 Beacon Street, Boston, MA 02115, USA; ruckerf@neco.edu.

Submitted: July 18, 2018

Accepted: November 3, 2018

Citation: Rucker F, Britton S, Taylor C. Color and temporal frequency sensitive eye growth in chick. *Invest Ophthalmol Vis Sci.* 2018;59:6003-6013. <https://doi.org/10.1167/iovs.18-25322>

**PURPOSE.** Longitudinal chromatic aberration can provide luminance and chromatic signals for emmetropization. A previous experiment examined the role of temporal sensitivity to luminance flicker in the emmetropization response. In the current experiment, we investigate the role of temporal sensitivity to color flicker.

**METHODS.** Five-day-old chicks were exposed to sinusoidal color modulation of blue/yellow ( $N = 73$ ) or red/green LEDs ( $N = 84$ ) at 80% contrast for 3 days. The modulation frequencies used were as follows: 0, 0.2, 1, 2, 5, and 10 Hz. There were 5 to 16 chicks per condition. Mean illumination was 680 lux. Changes in ocular components were measured using Lenstar, and refraction was measured with a Hartinger refractometer.

**RESULTS.** Eyes grew less when exposed to high temporal frequencies and more at low temporal frequencies. With blue/yellow modulation, the temporal variation was small; eyes grew  $268 \pm 15 \mu\text{m}$  at 0 Hz and  $224 \pm 12 \mu\text{m}$  at 10 Hz, representing a 16.4% growth reduction. With red/green modulation, eyes grew  $336 \pm 31 \mu\text{m}$  at 0 Hz and  $218 \pm 20 \mu\text{m}$  at 10 Hz, representing a 35% growth reduction. Choroidal and anterior chamber changes compensated for eye growth, reducing refractive effects; blue/yellow refraction changes ranged from  $-0.63$  to  $1.04$  diopters.

**CONCLUSIONS.** At high temporal frequencies, color is not a factor, but at low temporal frequencies, red/green modulation produced maximal growth. The pattern of changes observed in each ocular component with changes in the temporal frequency and/or the color of the stimulus was consistent with the idea that the natural sunlight spectrum may be optimal for emmetropization.

**Keywords:** myopia, emmetropization, temporal sensitivity, blue light sensitivity, refractive error

Time spent outdoors during childhood is positively associated with reduced myopia prevalence.<sup>1-6</sup> This positive association is not affected by near work<sup>7</sup> nor levels of physical activity engaged in while outdoors.<sup>8</sup> With this in mind, a previous study using the chick model<sup>9</sup> examined how short-wavelength light interacts with the temporal sensitivity of the visual system during emmetropization. The results showed that when a temporally modulated light source lacked a blue component, the eye grew more when presented with low temporal frequencies than high temporal frequencies. Thus, the presence of blue light prevented temporal frequency dependent increases in eye growth. Given that typical indoor illuminants have lower energy at the blue end of the spectrum than the red, it was inferred that this paucity of short-wavelength light in indoor illumination may increase the likelihood of human childhood myopia development.

## LCA as a Signal for Defocus

The hypothesis driving the experiments reported in this paper is that the shorter focal length of blue light, produced as a result of the aberration called longitudinal aberration (LCA), may provide signals for emmetropization.<sup>10-12</sup> LCA produces a difference in the focal planes at different wavelengths and introduces a nonsigned signal at each wavelength for defocus

that the emmetropization process can use to maximize the clarity of the retinal image at each wavelength.

Many previous studies have suggested that the more myopic defocus of blue light produces a stimulus that results in reduced eye growth in a large number of different species including chick, Cichlid fish, guinea pig, rhesus monkeys, and humans (UV).<sup>13-22</sup> Also, long-wavelength light tends to generally increase eye growth; however, there are exceptions, such as the tree shrew and rhesus monkey, which, when reared in monochromatic red light for prolonged periods (tree shrew: 13 days; rhesus monkey: 121 days), show less eye growth than normal.<sup>23-26</sup>

In addition to an unsigned signal from LCA, a signed chromatic signal arises from the relative clarity of the different wavelengths. This signed signal provides information on the direction and amplitude of defocus irrespective of the color of the illuminant with a broadband light source.<sup>27</sup> For example, when long wavelengths are in focus and the retinal image contrast is greater for red than for blue, then the eye responds by slowing its growth.

## Spatial Contrast Signals From LCA

Rucker and Wallman<sup>12</sup> demonstrated that the emmetropization system uses predictable differences in the relative clarity of

different wavelength components of the retinal image to identify the sign of defocus. Chick eyes were alternately exposed to printed patterns of two or five cycles/degree sine-wave gratings that simulated myopic defocus (that occurs when the eye is too big for its optics) or hyperopic defocus (when the eye is too small), whereas the other eye was patched. Early eye growth was monitored through measurement of proteoglycan (GAG) synthesis in the sclera. Chick eyes showed greater scleral proteoglycan synthesis, a measure of growth, when the blue component of the printed pattern was clearest and less when the red component was clearest. The results indicated that the chick eyes were able to infer whether they were hyperopically or myopically defocused using the relative chromatic signal arising from LCA.

### Temporal Contrast Signals From LCA

An analysis of how blurred the retinal image becomes with defocus<sup>12,27</sup> showed that, whereas changes in myopic defocus affected the clarity, or luminance contrast, of the retinal image, it was only when the eye was exposed to changes in hyperopic defocus that the color of the retinal image was affected. To test this hypothesis, eyes were exposed to 2-Hz temporal simulation of myopic defocus via exposure to luminance modulation and to 2-Hz temporal simulations of hyperopic defocus via color modulation. Temporal modulation ensures that the chick eye is stimulated continuously with a constant level of contrast. Temporal contrast modulation has the benefit that, unlike spatial contrast, it is not dependent on the chick's gaze or attention to a spatial stimulus (e.g., patterned wallpaper) nor is it affected by defocus.<sup>28</sup> The results showed that luminance modulation slowed eye growth, whereas color modulation increased eye growth. These results were taken to be evidence that the emmetropization mechanism uses the presence of luminance and color temporal modulations to determine the direction of defocus.

### Temporal Frequency Dependence of the Signal From LCA

The ability to detect color and luminance modulation is dependent on the temporal frequency of the light source (the ability to detect red/green or blue/yellow color modulation disappears at high temporal frequencies). The macaque and human eyes are most sensitive to luminance modulation at high temporal frequencies and to color modulation at low temporal frequencies.<sup>29,30</sup> Because of the dependence on temporal frequency sensitivity, we hypothesized that the combination of color and temporal frequency could influence the emmetropization process.

Examining the temporal sensitivity of the emmetropization response, Rucker et al.<sup>9</sup> exposed chicks to high contrast (80%) luminance flicker, in white or yellow light conditions, for 3 days, at a range of temporal frequencies from 0 to 10 Hz. The white and yellow conditions tested the effect of adding modulated blue light to an equally modulated yellow base. Chicks that were reared in yellow showed a difference in growth of 145  $\mu\text{m}$  across frequencies and a refractive change of more than 1.6 diopters (D). Those that were kept in white showed only a 77- $\mu\text{m}$  difference in growth across frequencies and minimal refractive change ( $-0.24$  D). In both white and yellow light, eyes grew less when presented with high temporal frequencies (i.e., those that favor the detection of luminance contrast rather than color contrast), supporting the hypothesis that detection of changes in luminance contrast indicates that the eye is too small. In yellow, eyes grew more than in white when presented with low temporal frequencies that favor detection of color, supporting the hypothesis that

the eye can detect and respond to the shorter focal length of blue light at low temporal frequencies. The protective effects of white light disappeared with contrast levels below 80%.<sup>31</sup>

### Association of Temporal Modulation and Refraction

Hyperopic shifts in refraction have been observed in chick with high-frequency luminance flicker, including stroboscopic flicker, at approximately 10 to 15 Hz. Flicker was found to reduce deprivation myopia (or negative lens response) in the experimental eye<sup>32-34</sup> and induce hyperopia in the control eye.<sup>33</sup>

Myopic shifts in refraction have been found at low temporal frequencies in other luminance flicker experiments in chicks,<sup>35</sup> mice,<sup>36</sup> and guinea pigs.<sup>37</sup> Myopic shifts in refraction have also been seen with low temporal frequency (2 Hz) red/green color flicker in chick.<sup>10</sup>

In this experiment, our aim is to test how temporal frequency affects emmetropization when chicks are exposed to color flicker and compare this with the results of a previous study that tested the effects of luminance flicker. As in the earlier experiment, we will test stimuli with and without blue light, exposing them to red/green and blue/yellow color modulated light, thereby testing the role of color and blue light in the emmetropization response.

## METHODS

### Animals

One hundred and fifty-seven, mixed-sex White Leghorn chicks (*Gallus gallus domesticus*, Cornell K strain; Cornell University, Ithaca, NY, USA) were randomly selected for this experiment. Five to 16 chicks were used in each condition (Table), and chicks were exposed to only one illumination/frequency condition. After hatching, the chicks were raised in 12-hour cycles of light and dark until they were 5 to 6 days old when they were placed in the illumination condition for 3 days. They had a continuous supply of food and water during all stages of the experiment. Illumination levels the chicks experienced during the preliminary period ranged from 50 to 350 lux (the exact value depended on the location of the chick within the cage at any moment). 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 made with a Hartinger Refractometer (Zeiss, Jena, Germany). Measurements were made before and after exposure, in the horizontal (180°) and vertical (90°) meridians. Lid retractors were used to hold the eyelids open during measurement. Chicks were anesthetized with 1.5% isoflurane in oxygen,<sup>38</sup> which causes cycloplegia.

Measurements of the ocular components were made with a low-coherence reflectometry technique with a noncontact ocular biometer (Lenstar LS 900; Haag-Streit AG, Konig, Switzerland). A total of six measurements were made per eye (16 recordings were averaged to give a single measurement). A peak in the A-scan trace corresponds to each of the eye's components. The chicks were awake and held in a specially designed holder without lid retractors (to prevent corneal warping). 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. Measurements were made between 10:00 AM and 2:00 PM to avoid

TABLE. Mean Changes (mm) in Ocular Components

Freq.	N	EL	EL_SE	Vit	Vit_SE	Chor	Chor_SE	Lens	Lens_SE	CAC	CAC_SE	RE	RE_SE
Blue/yellow													
0	15	0.269	0.016	0.130	0.012	-0.046	0.007	0.140	0.006	0.037	0.009	-0.18	0.236
0.2	13	0.176	0.022	0.068	0.020	-0.058	0.009	0.149	0.010	0.019	0.007	-0.63	0.325
1	14	0.240	0.018	0.094	0.014	-0.035	0.009	0.135	0.011	0.037	0.008	0.36	0.340
2	13	0.212	0.015	0.081	0.020	-0.048	0.015	0.131	0.011	0.029	0.011	0.13	0.363
5	13	0.266	0.020	0.124	0.014	-0.010	0.012	0.106	0.008	0.045	0.009	1.03	0.388
10	5	0.224	0.012	0.149	0.018	-0.077	0.016	0.126	0.009	0.021	0.008	0.52	0.597
Red/green													
0	15	0.336	0.031	0.130	0.012	-0.033	0.010	0.140	0.006	0.050	0.005	-0.28	0.311
0.2	11	0.328	0.045	0.159	0.037	-0.019	0.024	0.129	0.012	0.062	0.011	0.14	0.332
1	15	0.214	0.019	0.100	0.018	-0.051	0.009	0.129	0.006	0.044	0.006	-0.18	0.457
2	11	0.259	0.023	0.136	0.009	-0.065	0.020	0.124	0.005	0.057	0.007	-0.23	0.374
5	16	0.245	0.020	0.161	0.026	-0.069	0.013	0.096	0.006	0.074	0.006	0.33	0.311
10	16	0.218	0.020	0.083	0.013	-0.039	0.010	0.121	0.006	0.048	0.007	0.14	0.448

SEs are shown for each component. Also shown are the number of birds in each condition (N). EL, eye length; Vit, vitreous chamber depth; Chor, choroid; CAC, anterior chamber depth; RE, refraction (in diopters); Freq, temporal frequency of the flicker.

diurnal variation,<sup>39</sup> and each chick was measured at approximately the same time of day before and after the experiment.

### Light Source

As in Rucker et al., lighting conditions were produced with LEDs that consist of independently controlled red, green, and blue components (Lamina Ceramics, Westhampton, NJ, USA; Atlas Light Engine; peak wavelengths: 619 ± 20, 515 ± 35, and 460 ± 35 nm). The lights were placed above the center of the cage and had a beam spread of 36°. 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; LED Dynamics, Randolph, VT, USA) were used to create the stimuli.

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 Corp., Irvine, CA, 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. The red, green, and blue components of each light source had a mean irradiance of 50 μW/cm<sup>2</sup>. Small adjustments were made to the mean illuminance for both conditions using neutral density filters so that they were equivalent to 680 human lux. In a previous study,<sup>9,11</sup> a unit of illuminance “chick lux” (E<sub>c</sub>) was calculated as the irradiance of the light source multiplied by the chick photopic spectral sensitivity function (V<sub>c</sub>).<sup>40</sup> The irradiance value above is equivalent to 214 chick lux for red, 191 chick lux for green, and 64 chick lux for blue.

The modulated lights produced 80% contrast in the red, green, and blue components of the illuminants. Contrast was calculated as Michelson contrast: Contrast % = (L<sub>max</sub> - L<sub>min</sub>) / (L<sub>max</sub> + L<sub>min</sub>) × 100. For the experiments reported in this paper, the relative contrast of the RGB components and mean illumination levels were kept constant in all conditions to test the hypothesis that a color signal was temporal frequency dependent. Temporal contrast is unaffected by defocus, and a temporal stimulus does not affect the modulation transfer function of the eye with regard to spatial frequency.<sup>28</sup>

### Illumination and Frequency Conditions

There were two illumination conditions (blue/yellow or red/green) and six temporal frequency conditions (0, 0.2, 1, 2, 5,

and 10 Hz). The highest frequency used, 10 Hz, falls within the range of flicker sensitivity for chicks.<sup>41,42</sup> The 0-Hz conditions produce steady white and yellow light. Bird numbers in each experimental group are shown in the Table.

1. Blue/yellow condition (B/Y) was produced with equal amplitude, counter-phase, sinusoidal modulation of the red (615 nm, half-bandwidth 20 nm) and green (515 nm, half-bandwidth 35 nm) with blue (465 nm, half-bandwidth 25 nm) LEDs to produce 80% blue/yellow color contrast.
2. Red/green condition (R/G) was produced with equal amplitude, counter-phase, sinusoidal modulation of the red (615 nm, half-bandwidth 20 nm) and green (515 nm, half-bandwidth 35 nm) LEDs to produce 80% red/green color contrast.

### Procedure

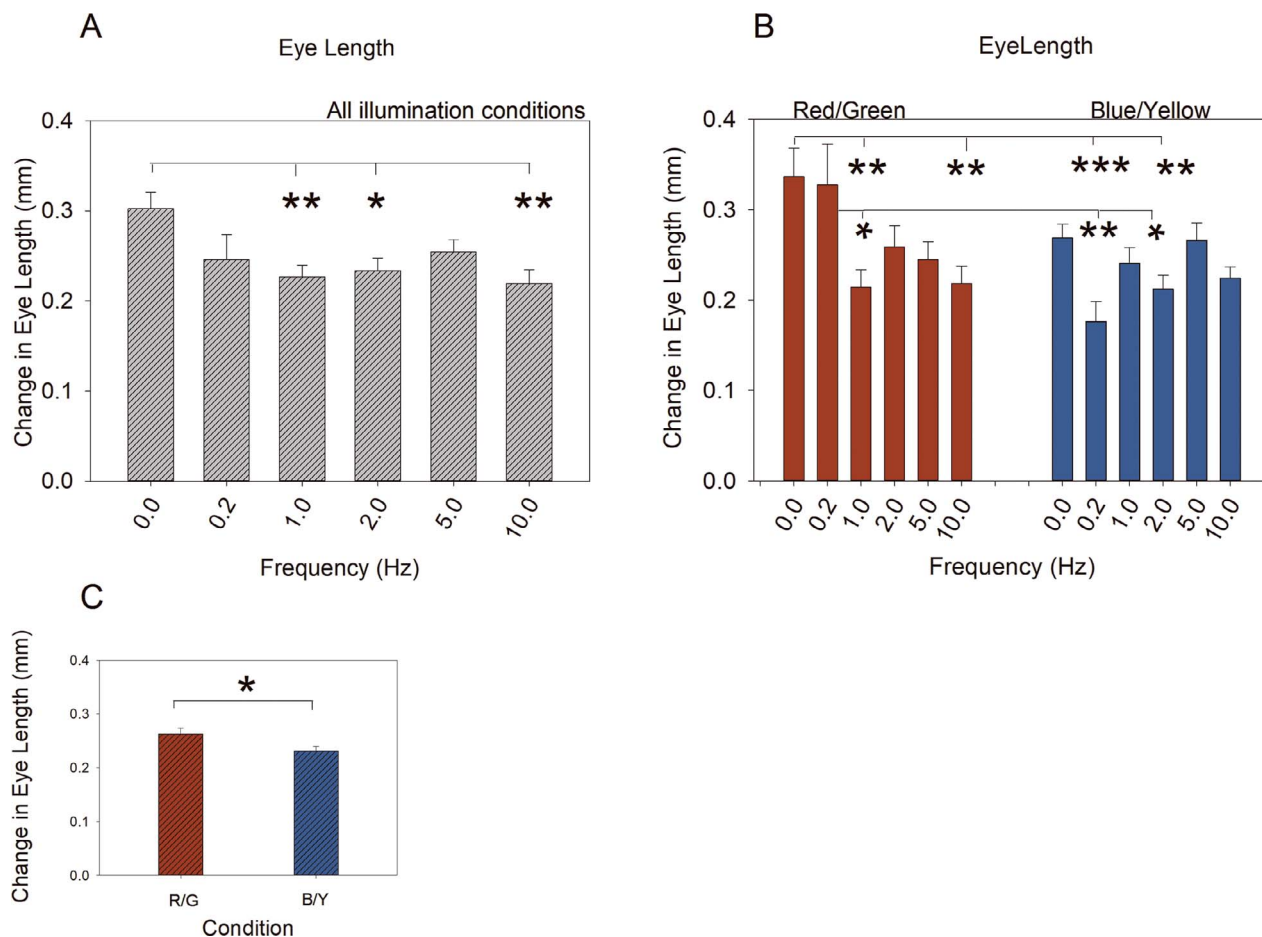
Five- to 8-day-old chicks were randomly selected and assigned to one of the lighting conditions (R/G or B/Y) at one temporal frequency (0 to 10 Hz). Chicks were kept free-roaming in a 32 × 20-inch wire cage for 8 hours/day from 9:00 AM to 5:00 PM. On day 1, measurements were made between 12:00 PM and 2:00 PM and again at the same time on day 4 (3-day exposure). The light sources were located on the top of the cage. Both eyes were exposed to the lighting condition and lenses were not used. Two illumination conditions were run concurrently in two separate cages. Batches of birds were run in blocks in the two cages, until each condition reached a cutoff number for birds in that condition. The conditions were randomly assigned within blocks. During the day, the chicks were exposed only to the experimental light, whereas at night, the chicks were kept in the dark in a sound- and light-proof chamber.

### Analysis

The change in ocular components between pre- and post-measurements was calculated. The average of the two eyes was used in the analysis as recommended by Armstrong.<sup>43</sup> Eye length was calculated as the distance from the anterior cornea to the posterior sclera.

A 2-way ANOVA (on frequency and illumination condition) was performed followed by post hoc tests using the Tukey's honestly significant difference (HSD).





**FIGURE 1.** (A) Mean changes in eye length during the 3-day exposure over a range of temporal frequencies when the data for both illumination conditions (R/G and B/Y) are pooled. The increase in eye length decreases with increasing temporal frequency. (B) Mean change in eye length after 3 days of exposure to R/G and B/Y. In R/G, the eye shows a greater temporal frequency-dependent increase in eye length than in B/Y. (C) Changes in eye length during the 3-day exposure in the two illumination conditions when frequency conditions are pooled. \* $P < 0.05$  and \*\* $P < 0.01$ . Error bars denote SEM.

To determine the correlation between choroidal changes and eye length changes, correlations were performed in SigmaPlot using a least-squares approach. For Figure 7, we used a second-order polynomial fit that includes a linear term; the full model is  $y = ax^2 + bx + c$ . This model generally fits a parabola; however, if the weight for the second-order term approaches zero, the fit will appear to be a linear function.

**RESULTS**

The Table shows results from both illumination conditions, B/Y and R/G, at the six temporal frequencies used. The number of birds in each condition is shown (*N*). The mean changes and SE of the ocular components and refraction are shown for eye length, vitreous chamber depth, choroid, lens, anterior chamber depth, and refraction.

**Eye Length**

Figure 1A shows that the mean increase in eye length was reduced when the birds experienced high temporal frequency stimuli (ANOVA,  $P = 0.003$ ). Eye growth at 10 Hz was only  $220 \pm 0.16 \mu\text{m}$ , whereas at 0-Hz growth was  $302 \pm 28 \mu\text{m}$ . Temporal frequencies of 10, 2, and 1 Hz produced significantly less eye growth than 0 Hz (10 and 1 Hz:  $P < 0.01$ ; 2 Hz:  $P < 0.05$ ; Tukey's HSD).

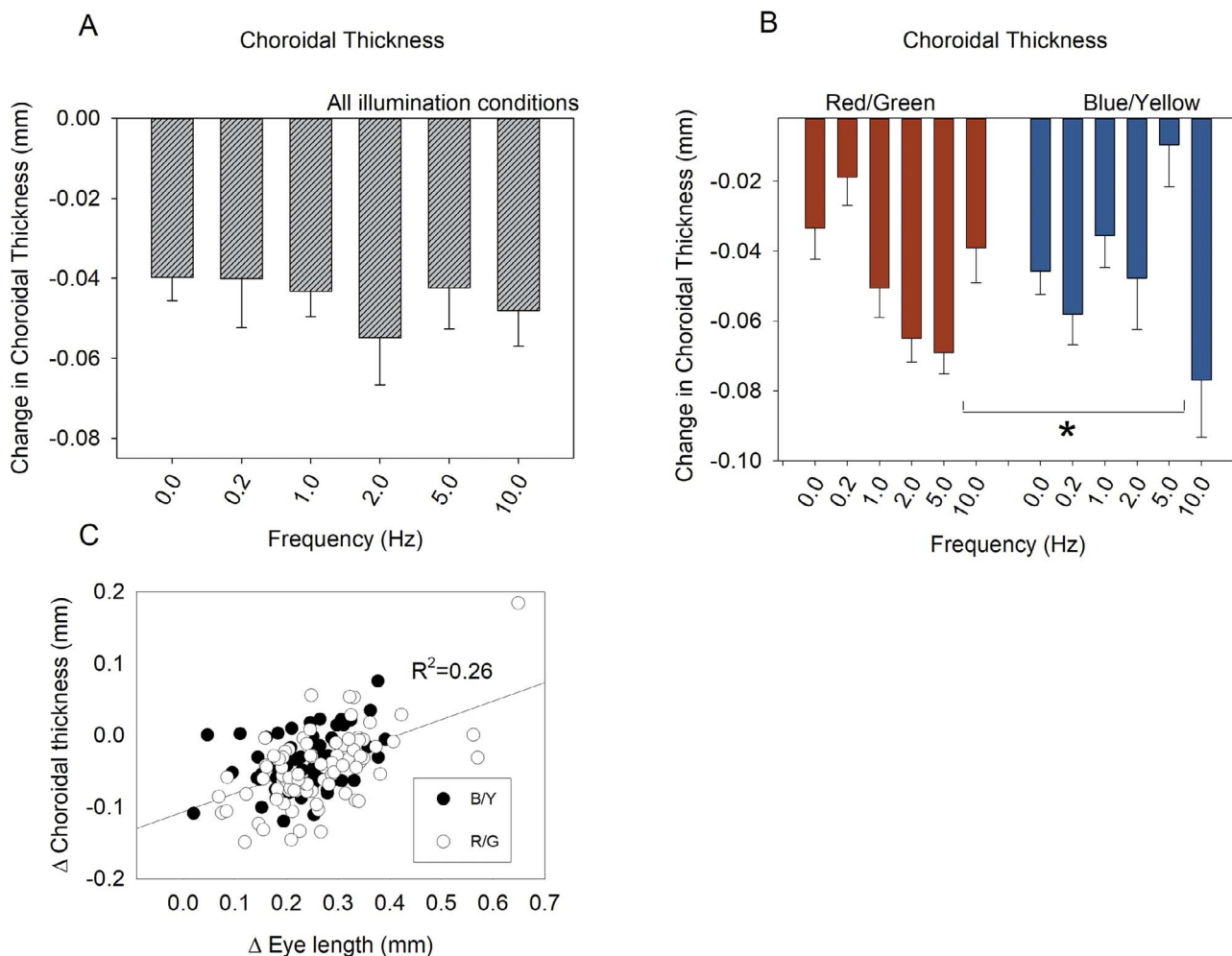
Figure 1C shows that when data were pooled across the six frequency conditions, mean change in eye length was reduced in the B/Y condition compared with the R/G condition (ANOVA,  $P = 0.03$ ). Eyes exposed to B/Y only grew  $232 \pm 8 \mu\text{m}$ , whereas those exposed to R/G grew  $263 \pm 12 \mu\text{m}$ . The blue component in the light source reduced eye growth.

There was an interaction effect between frequency and illumination for eye length (Fig. 1B; ANOVA,  $P = 0.002$ ). With exposure to B/Y, eye length did not change significantly across frequency; eyes grew  $269 \pm 16 \mu\text{m}$  at 0 Hz and  $224 \pm 12 \mu\text{m}$  at 10 Hz. However, exposure to R/G produced greater growth at 0 Hz than at 10 or 1 Hz (both  $P < 0.01$ ; Tukey's HSD). At 0 Hz, eyes grew  $336 \pm 31 \mu\text{m}$ , whereas at 10 Hz, eyes only grew  $218 \pm 20 \mu\text{m}$ . Results were similar for comparisons of 10 Hz with 0.2 Hz.

B/Y exposure produced less growth at low temporal frequencies than R/G exposure when the light was modulated. Eyes grew more when presented with 0 or 0.2 Hz in R/G than they did at 0.2 or 2 Hz B/Y (Tukey's HSD,  $P < 0.01$  all).

**Choroidal Thickness**

When the illumination conditions were pooled, there was little to no evidence of differences in choroidal thinning across frequencies (Fig. 2A). However, changes in choroidal thickness



**FIGURE 2.** (A) Mean change in choroid thickness during the 3-day exposure over a range of temporal frequencies when the data for both illumination conditions are pooled. Choroidal thinning was not dependent on temporal frequency. (B) Mean change in choroidal thickness when the two illumination conditions, R/G (red) and B/Y (blue), are considered separately. \* $P < 0.05$ . Choroids thinned more in birds exposed to intermediate frequencies in R/G than in B/Y. (C) Changes in eye length and choroidal thickness in individual eyes in R/G (white circles) and B/Y (black circles). Error bars denote SEM.

showed an interaction between condition and frequency (ANOVA,  $P = 0.001$ ; Fig. 2B).

In general, relative thinning of the choroidal tissue compensated for smaller changes in eye length (Fig. 2C;  $R^2 = 0.26$ ). Figure 2B shows that choroids thinned in all conditions but significantly more at intermediate temporal frequencies in R/G, thinning  $69 \pm 13 \mu\text{m}$  at 5 Hz,  $51 \pm 9 \mu\text{m}$  at 1 Hz, and  $65 \pm 20 \mu\text{m}$  at 2 Hz (5 Hz:  $P = 0.02$ ; 1 Hz:  $P = 0.053$ ; 2 Hz:  $P = 0.01$ ;  $t$ -tests with  $H_0 = 0$  or no thinning). Choroids thinned more at 5 Hz in R/G ( $69 \pm 13 \mu\text{m}$ ) than in B/Y ( $10 \pm 12 \mu\text{m}$ ;  $P < 0.03$ ).

### Vitreous Chamber

The change in vitreous chamber depth showed an interaction between the frequency and illumination conditions (ANOVA,  $P = 0.02$ ), but main effects of illumination (ANOVA,  $P = 0.07$ ) or frequency (ANOVA,  $P = 0.13$ ) were marginal. When data were pooled, the variation in vitreous chamber across frequency was only  $26 \mu\text{m}$  (Fig. 3A).

Figure 3B shows the changes in mean vitreous chamber depth for each illumination condition. There was an increase in vitreous chamber depth with B/Y from 0.2 to 10 Hz, but the

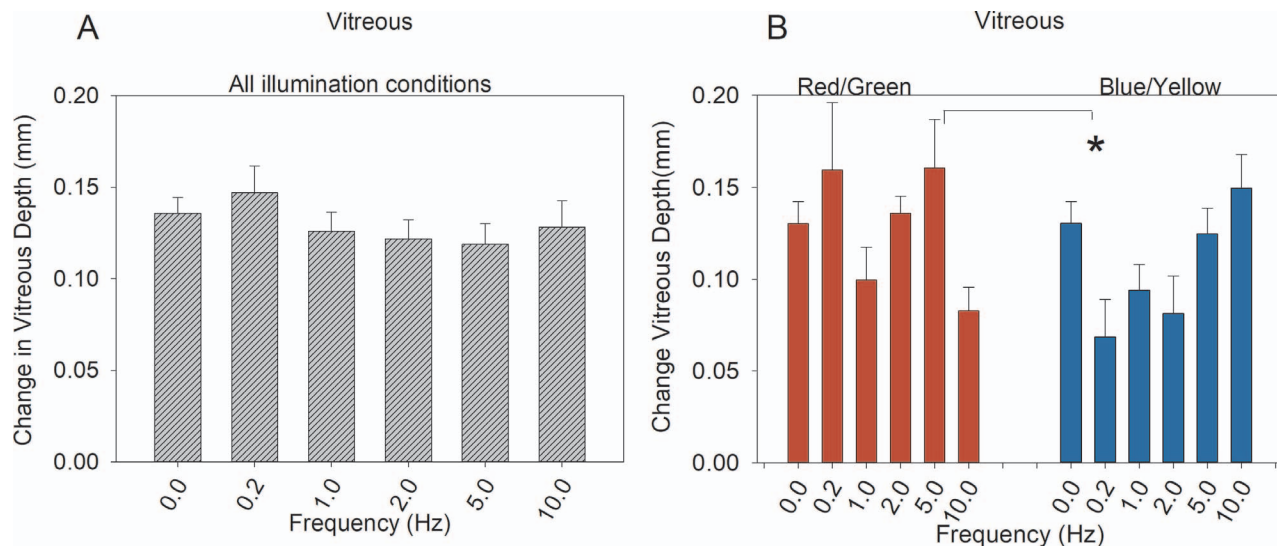
increase in depth was more variable with R/G and decreased at high temporal frequencies. Exposure to B/Y increased vitreous chamber depth at 10 Hz to  $149 \pm 18 \mu\text{m}$  from  $68 \pm 20 \mu\text{m}$  at 0.2 Hz. Conversely, exposure to R/G increased vitreous chamber depth at 10 Hz to only  $83 \pm 13 \mu\text{m}$  compared with  $159 \pm 37 \mu\text{m}$  at 0.2 Hz. There was significantly less change in vitreous chamber depth at 0.2 Hz B/Y than in R/G at 5 Hz (Tukey's HSD,  $P < 0.05$ ) and marginally less at 0.2 Hz ( $P = 0.08$ ).

### Lens Thickness and Anterior Chamber Depth

Overall, the lens thickened more at low temporal frequencies (ANOVA,  $P < 0.001$ ; Fig. 4A). Combining data from the two illumination conditions, the lens increased by  $140 \mu\text{m}$  at 0 Hz but only by  $110 \mu\text{m}$  at 5 Hz. Low (0, 0.2 Hz) and intermediate temporal frequencies (1, 2 Hz) induced greater lens thickening than 5 Hz (Tukey's HSD,  $P < 0.001$  and  $P < 0.02$ , respectively).

Overall, the lens thickened less with R/G than B/Y (ANOVA,  $P = 0.02$ ). The lens thickened  $132 \pm 4 \mu\text{m}$  in B/Y but only  $121 \pm 3 \mu\text{m}$  in R/G.

As a result of greater lens thickening in B/Y there was a relative decrease in the anterior chamber depth compared to



**FIGURE 3.** (A) Mean change in vitreous chamber depth during the 3-day exposure over a range of temporal frequencies when the data for both illumination conditions (R/G and B/Y) are pooled. There was no overall effect. (B) Mean change in vitreous chamber depth when the R/G and B/Y conditions are considered separately. Vitreous chamber depth depends on an interaction between illumination condition and temporal frequency. \* $P < 0.05$ . Error bars denote SEM.

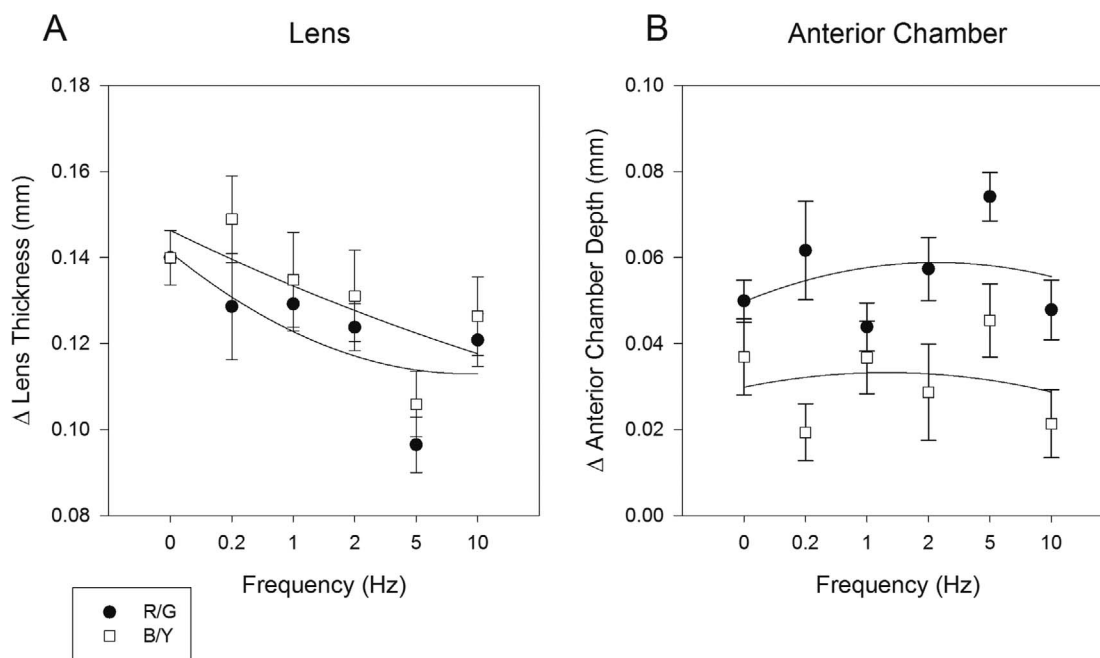
R/G. When the data is pooled, exposure to B/Y produced smaller increases in anterior chamber depth than R/G (mean: B/Y:  $31 \pm 4 \mu\text{m}$ ; R/G:  $56 \pm 4 \mu\text{m}$ ).

Changes in anterior chamber depth varied with frequency (ANOVA,  $P = 0.02$ ) particularly in R/G (ANOVA,  $P < 0.001$ ; Fig. 4B). Differences were most marked when comparing low frequency B/Y to low/intermediate frequencies R/G (B/Y 0, 0.2, or 2 Hz versus R/G 5 Hz; Tukey's HSD,  $P < 0.05$ ).

Changes in anterior chamber depth showed a strong positive correlation with change in vitreous chamber depth

in R/G (Fig. 5A). In the R/G condition, 72% of the variance in the depth of the anterior chamber was dependent on the depth of the vitreous chamber (Fig. 5A). However, in B/Y, only 7% of the variance in the depth of the anterior chamber was dependent on the depth of the vitreous chamber.

In both conditions, the increase in anterior chamber depth was correlated with a more hyperopic change in refraction (Fig. 5B). In R/G, anterior changes accounted for 43% of the variance in refraction, whereas in B/Y, anterior changes accounted for 59% of the variance in the change in refraction.



**FIGURE 4.** Mean changes in lens thickness and anterior chamber depth with frequency during the 3-day exposure to a range of temporal frequencies. (A) Birds exposed to R/G (black symbols) show less lens thickening than those exposed to B/Y (white symbols). The lens thickens more at low temporal frequencies in both illumination conditions. (B) Anterior chamber depth increased more in R/G than in B/Y. Error bars denote SEM.

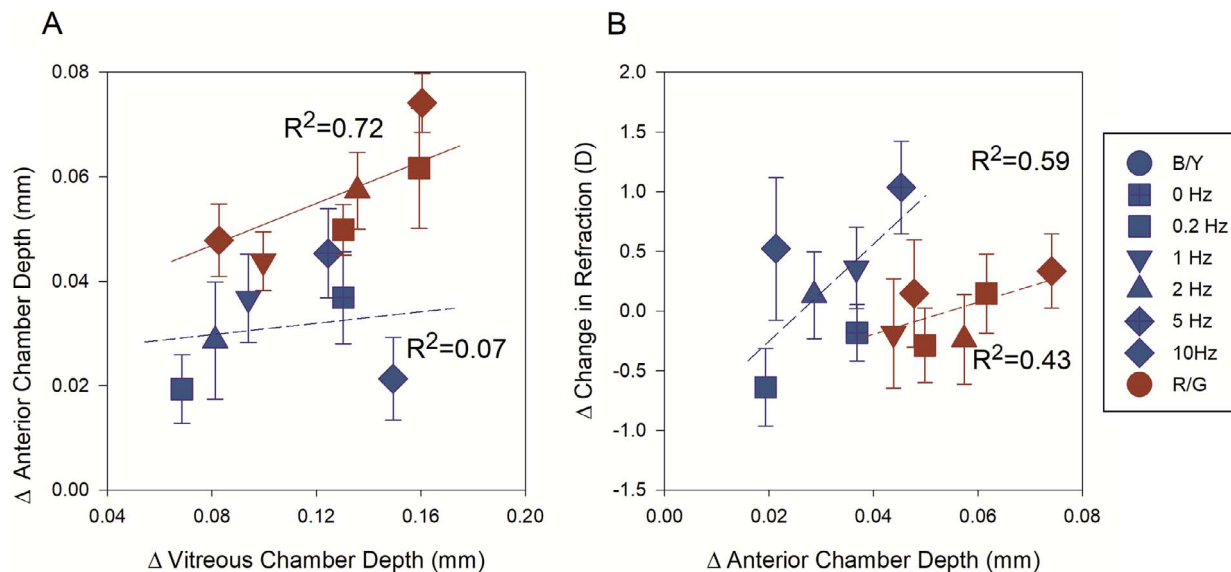


FIGURE 5. (A) Relationship between changes in vitreous chamber depth and anterior chamber depth. In R/G, an increase in vitreous chamber depth was associated with an increase in anterior chamber depth. (B) Relationship between changes in anterior chamber depth and refractive error. The increase in anterior chamber depth resulted in more hyperopic refractive changes in both conditions.

From a refractive perspective, an increase in anterior chamber depth counteracts the effect of an increase in vitreous chamber depth.

### Refraction

Figure 6A shows that when data were pooled, refraction did not change with temporal frequency and/or the color of the illuminant. There was no effect of illumination condition on refraction (ANOVA: Cond,  $P = 0.42$ ) and the effect of frequency was marginal (Freq,  $P = 0.09$ ). There was no evidence of an interaction between frequency and illumination condition (Fig. 6B; ANOVA,  $P = 0.48$ ).

Increases in choroidal thickness and anterior chamber depth compensated for increases in eye length and vitreous chamber depth and resulted in small refractive changes. As shown in Figure 6C, there was a positive correlation between the change in vitreous chamber depth and the change in refraction in B/Y ( $R^2 = 0.60$ ) but not in R/G ( $R^2 = 0.08$ ). Relative choroidal thickening in B/Y was associated with more hyperopic refractions (Fig. 6D;  $R^2 = 0.24$ ) but was not associated in R/G ( $R^2 = 0.001$ ). In both conditions, increases in anterior chamber depth were associated with more hyperopic refractions (Fig. 5B).

## DISCUSSION

### Temporal Effects of Color Flicker on Eye Growth

In this experiment, animals exposed to high temporal frequencies showed less eye growth, regardless of the color condition. One possible explanation is that high temporal frequency visual stimulation favors the detection of luminance contrast for the emmetropization system for both color and luminance modulated light. At high temporal frequencies, the emmetropization mechanism, like perceptual mechanisms, may be unable to distinguish between the R/G and B/Y color conditions.<sup>44-51</sup> At low temporal frequencies, which could favor the use of color for the emmetropizing eye, eyes showed increased growth compared with eyes exposed to high

temporal frequencies, particularly in the R/G, blue-deficient condition.

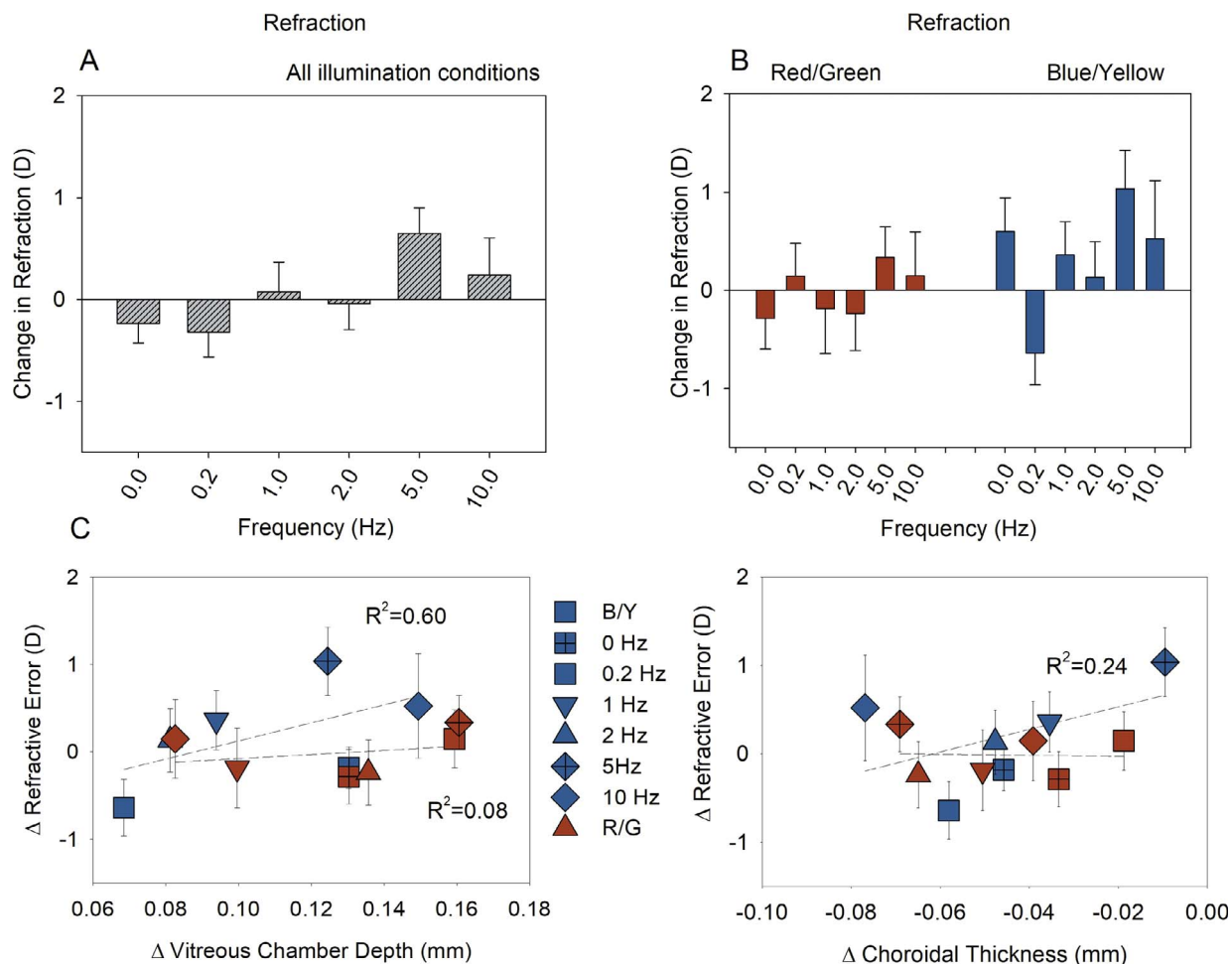
### Relationship Between Changes in Ocular Components and Refraction

Vitreous chamber depth typically mirrors the changes in eye length. Smaller changes in eye length are usually associated with smaller changes in the vitreous chamber. However, the stimuli used in this experiment modified this association. In both conditions, choroidal thickening compensated for an increase in eye length reducing the vitreal changes. However, only in B/Y did choroidal changes account for a proportion of the variance in the association between refractive error and frequency.

Consistent with the idea of a compensatory response in the anterior chamber, we observed that the changes in anterior chamber depth accounted for 43% to 59% of the variance in refractive error in the R/G and B/Y conditions, respectively. Thus, increases in anterior chamber depth counteracted refractive changes due to increases in vitreous chamber depth in R/G. Increases in anterior chamber depth have also been observed in lens induced myopia or form deprivation myopia in chick<sup>52</sup> and monkey.<sup>53</sup> This compensatory activity among ocular components remains speculative given our data collection time points, but nevertheless demonstrates the resilience of the emmetropization mechanism.

The positive association of choroidal thinning with a decrease in eye growth has been a persistent finding across many flicker experiments.<sup>9,10,12,31</sup> The current belief is that choroidal thinning is a precursor to eye growth,<sup>54,55</sup> but these results suggest the choroid can respond to defocus produced by eye growth (as with lens induced defocus) to improve clarity of the image. However, because we only measured two time points, there is possibility that the choroid changed appropriately during the experimental period and then rebounded. Disassociations between eye length and choroidal thickness have also been demonstrated by several others<sup>56-60</sup> as discussed in Rucker and Wallman.<sup>10</sup> This does not deny the fact that choroids thicken in response to myopic lens-induced defocus





**FIGURE 6.** Mean changes in refraction when eyes are exposed to color flicker at a range of different temporal frequencies in B/Y (blue bars) or R/G (red bars) either pooled over illumination condition (A) or separated by illumination condition (B). (C) Only B/Y showed an association between the mean change in refraction and vitreous chamber depth. Temporal frequency is indicated as low (square), medium (triangles), and high (diamond) in the illumination conditions R/G (red) and B/Y (blue). SE bars are shown. (D) Mean changes in choroidal thickness with mean changes in refraction in R/G (red) and B/Y (blue) at low (squares), intermediate (triangles), and high (diamonds) temporal frequencies.

and thin in response to lens-induced hyperopic defocus, only that we propose that the choroidal mechanism of emmetropization could be compensating for eye growth rather than being the precursor, and care should be taken in using choroidal changes as predictors of eye growth in untreated eyes.

**Comparison of the Temporal Effects of Luminance and Color Flicker**

We proposed<sup>9</sup> that the spectrum of light, particularly the exposure to broad band light with a strong short-wavelength light component, and temporal frequency content play an important role in emmetropization, and we were interested in whether the type of modulation (color or luminance) was important. Figure 7 compares the changes in the ocular components and refraction with both color (R/G and B/Y) and luminance flicker (white [RGB] and yellow [RG]) using data from Rucker et al.<sup>9</sup>

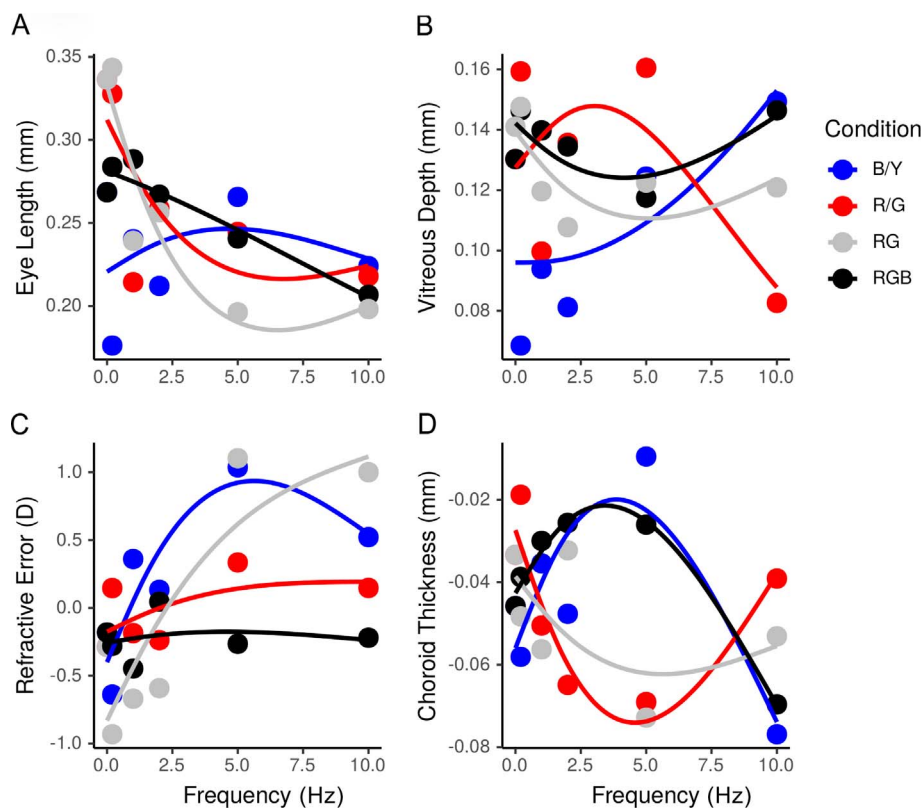
Temporal frequency has a dominant effect on eye growth, especially when there is no blue component. Figure 7A shows the reduction in eye length that is seen in all conditions at high temporal frequencies and the increase in eye length at low temporal frequencies (except B/Y) with associated changes in refractive trends (Fig. 7C). At low temporal frequencies, the

increase in eye length is smallest with B/Y and greatest when there is no blue light component (RG and R/G).

The type of modulation, whether it is luminance or color, is important in the emmetropization mechanism along with the spectral content. With low temporal frequencies, the increase in vitreous chamber depth (Fig. 7B) is smallest with exposure to B/Y color flicker (increasing with temporal frequency) and greatest with exposure to R/G color flicker (decreasing with temporal frequency). In general, the vitreous chamber changes over a range of 0.077 to 0.80 mm with color modulation (R/G and B/Y), whereas with luminance modulation, the vitreous chamber only changes over a range of 0.029 to 0.039 mm (two-tailed *t*-test, *P* = 0.20). Thus, color modulation has a greater effect on vitreous chamber depth than luminance modulation.

The temporal and spectral qualities of the light affect the choroid. Figure 7D shows that choroidal thinning is most pronounced at intermediate temporal frequencies and is defined by the presence or absence of blue light. With blue light (RGB and B/Y), the choroid thickens more at intermediate frequencies; without blue light, the choroid thins (RG and R/G). The absence of a blue light component in RG has an effect on refraction as choroidal thinning causes an increase in vitreous chamber depth, in an elongated eye, and ultimately makes the eye around 0.5 to 1 D more myopic below 5 Hz (Fig.





**FIGURE 7.** Comparison of the mean changes in (A) eye length, (B) vitreous chamber depth, (C) refractive error, and (D) choroidal thickness in response to luminance and color flicker. Flicker frequency is indicated on the x-axis, 0 Hz is steady light illumination conditions included thicker with a blue light component (RGB [black] and B/Y [blue]) and without a blue light component (RG [gray] and R/G [red]). RGB is a white light luminance flicker illumination condition, and RG is a yellow light luminance flicker illumination condition described in Rucker et al.,<sup>9</sup> whereas R/G and B/Y were the color flicker illumination conditions examined in this experiment.

7C). The choroidal thickening at 5 Hz in the presence of blue light (RGB and B/Y) promotes a 1-D hyperopic change in refraction. Although the changes in refraction were not significant, a 2-D range of refractive effect in non-lens-treated eyes over 3 days is a considerable amount of visually induced refractive manipulation in chick.

In the previous manuscript,<sup>9</sup> it was suggested that the dependence of eye growth on the presence of detectable blue light and low temporal frequencies was because the emmetropization mechanism can detect the presence of the more myopically defocused blue light. In this series of experiments, we showed that the ability to detect the blue component was affected by whether the blue component was modulated at a frequency that could bias it to produce either luminance or color contrast. Because there is only a minimal contribution of S-cones to luminance sensitive visual pathways,<sup>61,62</sup> we expect the S-cone response to be reduced with luminance flicker. Thus, the pattern of modulation is important (i.e., luminance or color). Detectable blue modulation modifies the eye growth-related emmetropization response at low temporal frequencies and more so with color stimulation than luminance stimulation.

The results of this experiment agree with many previous studies that have suggested that the myopic defocus of blue light from longitudinal chromatic aberration results in reduced eye growth. This has been observed in a number of different species including chick, Cichlid fish, guinea pig, rhesus monkeys, and humans.<sup>13–22</sup> However, the results of other experiments complicate a straightforward explanation for short-wavelength light driving the slowing of eye growth. For example, the eyes of tree shrews and rhesus monkeys kept in

monochromatic red light for prolonged periods (tree shrew: 13 days; rhesus monkey: 121 days) grow less than normal.<sup>23–26</sup> Tree shrews reared in monochromatic blue light show an initial hyperopic shift during the exposure period around 26 to 33 days of visual experience (DVE; ~2 months old) followed by a myopic shift in refraction.<sup>63</sup> These differences in refraction may arise from species differences, but the influence of the spectral content of the illuminant on the circadian system<sup>64–68</sup> and hormonal influences from sexual maturation (sexual maturity in a tree shrew is around 2 months) may complicate any simple species-based wavelength explanation. Short-duration experiments in immature animals are needed to eliminate these effects.

To summarize, we found that changes in eye length, choroid, vitreous, anterior chamber depth, and lens are affected by temporal frequency and/or the color of the stimulus. As mentioned above, at low temporal frequencies (0.2 Hz), the ability to detect color contrast is optimized, and eye length changes depend on the color of the stimulus (range: 0.176 to 0.343 mm). At intermediate temporal frequencies (5 Hz), changes in choroidal thickness were dependent on the presence of blue light (B/Y and RGB) or its absence (RG and R/G). Changes in the choroid and the effect on vitreous chamber depth were correlated with refractive changes in B/Y. At high temporal frequencies (10 Hz), the ability to detect luminance contrast is optimized, and changes in eye length are reduced in all conditions. At this temporal frequency, the color of the stimulus is not distinguishable, and all stimuli produce similar changes in eye length (range: 0.198 to 0.224 mm). The color of the stimulus also affects the anterior chamber depth and is moderately correlated with changes in refraction in both

conditions. These results are consistent with the idea that moving the illuminant away from the natural sunlight spectrum can have significant effects on emmetropization.

### Myopiagenic Visual Environments

As far as protecting the human eye from becoming myopic, speculation is required to relate these experiments performed with the chick to human myopia development. In the chick model, the most myopiagenic light stimulation comes from low temporal frequency modulation of a yellow light. This indicates that broad spectrum lighting with sufficient blue light may be important in indoor lighting. However, the precise light intensity and time of exposure needed to protect against myopia are still under investigation. Low temporal frequency stimulation of the eye may mimic signals the eye receives from objects that are out of focus<sup>69</sup> and resemble the visual input that the observer experiences when sedentary. Conversely, it has been suggested that high contrast, high temporal frequency modulation may mimic signals the eye receives from objects that are in focus<sup>69</sup> and provide a signal to slow eye growth to prevent refractive changes away from emmetropia. High temporal frequency stimulation of the eye is more likely to occur when moving through an environment, being exposed to moving objects, or during saccadic eye movements, mirroring situations when an organism is active in a natural environment. Thus, one possible solution to prevent exposure to myopiagenic low temporal frequency stimulation is to ensure that, during development, the eye is exposed to broad-spectrum light sources such as those that occur outdoors when navigating a naturalistic (i.e., not indoor) environment.

### Acknowledgments

The authors thank Jane Gwiazda for review of the manuscript, Li Deng for review of the statistical design, and Molly Fellows for assistance with data collection.

Supported by National Eye Institute of the National Institutes of Health Award R01EY023281. SB was sponsored by National Institutes of Health T35 Program Grant T35 EY007149.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosure: **F. Rucker**, P; **S. Britton**, None; **C. Taylor**, P

### References

- Rose KA, Morgan IG, Ip J, et al. Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology*. 2008;115:1279-1285.
- Jones LA, Sinnott LT, Mutti DO, Mitchell GL, Moeschberger ML, Zadnik K. Parental history of myopia, sports and outdoor activities, and future myopia. *Invest Ophthalmol Vis Sci*. 2007;48:3524-3532.
- Onal S, Toker E, Akingol Z, et al. Refractive errors of medical students in Turkey: one year follow-up of refraction and biometry. *Optom Vis Sci*. 2007;84:175-180.
- Dirani M, Tong L, Gazzard G, et al. Outdoor activity and myopia in Singapore teenage children. *Br J Ophthalmol*. 2009;93:997-1000.
- Parssinen O, Lyyra AL. Myopia and myopic progression among schoolchildren: a three-year follow-up study. *Invest Ophthalmol Vis Sci*. 1993;34:2794-2802.
- Deng L, Gwiazda J, Thorn F. Children's refractions and visual activities in the school year and summer. *Optom Vis Sci*. 2010;87:406-413.
- Rose KA, Morgan IG, Smith W, Burlutsky G, Mitchell P, Saw SM. Myopia, lifestyle, and schooling in students of Chinese ethnicity in Singapore and Sydney. *Arch Ophthalmol*. 2008;126:527-530.
- Guggenheim JA, Northstone K, McMahon G, et al. Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. *Invest Ophthalmol Vis Sci*. 2012;53:2856-2865.
- Rucker F, Britton S, Spatcher M, Hanowsky S. Blue light protects against temporal frequency sensitive refractive changes. *Invest Ophthalmol Vis Sci*. 2015;56:6121-6131.
- Rucker F, Wallman J. Chicks use changes in luminance and chromatic contrast as indicators of the sign of defocus. *J Vis*. 2012;12(6):23.
- Rucker FJ, Wallman J. Cone signals for spectacle-lens compensation: differential responses to short and long wavelengths. *Vision Res*. 2008;48:1980-1991.
- Rucker FJ, Wallman J. Chick eyes compensate for chromatic simulations of hyperopic and myopic defocus: evidence that the eye uses longitudinal chromatic aberration to guide eye-growth. *Vision Res*. 2009;49:1775-1783.
- Kröger RH, Wagner HJ. The eye of the blue acara (*Aequidens pulcher*, Cichlidae) grows to compensate for defocus due to chromatic aberration. *J Comp Physiol A*. 1996;179:837-842.
- Seidemann A, Schaeffel F. Effects of longitudinal chromatic aberration on accommodation and emmetropization. *Vision Res*. 2002;42:2409-2417.
- Long Q, Chen D, Chu R. Illumination with monochromatic long-wavelength light promotes myopic shift and ocular elongation in newborn pigmented guinea pigs. *Cutan Ocul Toxicol*. 2009;28:176-180.
- Liu R, Qian YE, He JC, et al. Effects of different monochromatic lights on refractive development and eye growth in guinea pigs. *Exp Eye Res*. 2011;92:447-453.
- Wang F, Zhou J, Lu Y, Chu R. Effects of 530 nm green light on refractive status, melatonin, MT1 receptor, and melanopsin in the guinea pig. *Curr Eye Res*. 2011;36:103-111.
- Foulds WS, Barathi VA, Luu CD. Progressive myopia or hyperopia can be induced in chicks and reversed by manipulation of the chromaticity of ambient light. *Invest Ophthalmol Vis Sci*. 2013;54:8004-8012.
- Liu R, Hu M, He JC, et al. The effects of monochromatic illumination on early eye development in rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2014;55:1901-1909.
- Torii H, Ohnuma K, Kurihara T, Tsubota K, Negishi K. Violet light transmission is related to myopia progression in adult high myopia. *Sci Rep*. 2017;7:14523.
- Torii H, Kurihara T, Negishi K, et al. Violet light exposure can be a preventive strategy against myopia progression. *EBio-Medicine*. 2017;15:210-219.
- Jiang L, Zhang S, Schaeffel F, et al. Interactions of chromatic and lens-induced defocus during visual control of eye growth in guinea pigs (*Cavia porcellus*). *Vision Res*. 2014;94:24-32.
- Smith EL III, Hung LF, Arumugam B, Holden BA, Neitz M, Neitz J. Effects of long-wavelength lighting on refractive development in infant rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2015;56:6490-6500.
- Gawne TJ, Ward AH, Norton TT. Long-wavelength (red) light produces hyperopia in juvenile and adolescent tree shrews. *Vision Res*. 2017;140:55-65.
- Gawne TJ, Siegwart JT Jr, Ward AH, Norton TT. The wavelength composition and temporal modulation of ambient lighting strongly affect refractive development in young tree shrews. *Exp Eye Res*. 2016;155:75-84.
- Hung LF, Arumugam B, She Z, Ostrin L, Smith EL III. Narrow-band, long-wavelength lighting promotes hyperopia and

- retards vision-induced myopia in infant rhesus monkeys. *Exp Eye Res.* 2018;176:147-160.
27. Rucker FJ. The role of luminance and chromatic cues in emmetropisation. *Ophthalmic Physiol Opt.* 2013;33:196-214.
  28. Flitcroft DI, Judge SJ. The effect of stimulus chromaticity on ocular accommodation in the monkey. *J Physiol.* 1988;398:36P.
  29. Lee BB, Pokorny J, Smith VC, Martin PR, Valberg A. Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. *J Opt Soc Am A.* 1990;7:2223-2236.
  30. Lee BB, Martin PR, Valberg A. Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. *J Physiol.* 1989;414:223-243.
  31. Rucker F, Henriksen M, Yanase T, Taylor C. The role of temporal contrast and blue light in emmetropization. *Vision Res.* 2018; 151:78-87.
  32. Gottlieb MD, Wallman J. Retinal activity modulates eye growth: Evidence from rearing in stroboscopic illumination. *Soc Neurosci Abstr.* 1987;13:1297.
  33. Schwahn HN, Schaeffel F. Flicker parameters are different for suppression of myopia and hyperopia. *Vision Res.* 1997;37: 2661-2673.
  34. Kee CS, Marzani D, Wallman J. Differences in time course and visual requirements of ocular responses to lenses and diffusers. *Invest Ophthalmol Vis Sci.* 2001;42:575-583.
  35. Crewther SG, Barutcu A, Murphy MJ, Crewther DP. Low frequency temporal modulation of light promotes a myopic shift in refractive compensation to all spectacle lenses. *Exp Eye Res.* 2006;83:322-328.
  36. Yu Y, Chen H, Tuo J, Zhu Y. Effects of flickering light on refraction and changes in eye axial length of C57BL/6 mice. *Ophthalmic Res.* 2011;46:80-87.
  37. Zhi Z, Pan M, Xie R, Xiong S, Zhou X, Qu J. The effect of temporal and spatial stimuli on the refractive status of guinea pigs following natural emmetropization. *Invest Ophthalmol Vis Sci.* 2013;54:890-897.
  38. Wallman J, Adams JI. Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res.* 1987;27:1139-1163.
  39. Nickla DL, Wildsoet C, Wallman J. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. *Exp Eye Res.* 1998;66:163-181.
  40. Chen DM, Goldsmith TH. Appearance of a Purkinje shift in the developing retina of the chick. *J Exp Zool.* 1984;229:265-271.
  41. Jarvis JR, Taylor NR, Prescott NB, Meeks I, Wathes CM. Measuring and modelling the photopic flicker sensitivity of the chicken (*Gallus g. domesticus*). *Vision Res.* 2002;42:99-106.
  42. Jarvis JR, Prescott NB, Wathes CM. A mechanistic interspecies comparison of flicker sensitivity. *Vision Res.* 2003;43: 1723-1734.
  43. Armstrong RA. Statistical guidelines for the analysis of data obtained from one or both eyes. *Ophthalmic Physiol Opt.* 2013;33:7-14.
  44. Stromeyer CF III, Eskew RT Jr, Kronauer RE, Spillmann L. Temporal phase response of the short-wave cone signal for color and luminance. *Vision Res.* 1991;31:787-803.
  45. McKeefry DJ, Parry NR, Murray IJ. Simple reaction times in color space: the influence of chromaticity, contrast, and cone opponency. *Invest Ophthalmol Vis Sci.* 2003;44:2267-2276.
  46. Smithson HE, Mollon JD. Is the S-opponent chromatic subsystem sluggish? *Vision Res.* 2004;44:2919-2929.
  47. Rabin J, Switkes E, Crognale M, Schneck ME, Adams AJ. Visual evoked potentials in three-dimensional color space: correlates of spatio-chromatic processing. *Vision Res.* 1994;34:2657-2671.
  48. Robson AG, Kulikowski JJ. Objective specification of tritanopic confusion lines using visual evoked potentials. *Vision Res.* 1998;38:3499-3503.
  49. Lee RJ, Mollon JD, Zaidi Q, Smithson HE. Latency characteristics of the short-wavelength-sensitive cones and their associated pathways. *J Vis.* 2009;9(12):5.
  50. Lee BB, Sun H. Chromatic input to cells of the magnocellular pathway: mean chromaticity and the relative phase of modulated lights. *Vis Neurosci.* 2004;21:309-314.
  51. Stockman A, MacLeod DI, DePriest DD. The temporal properties of the human short-wave photoreceptors and their associated pathways. *Vision Res.* 1991;31:189-208.
  52. Schmid K, Wildsoet C. Breed- and gender-dependent differences in eye growth and form deprivation responses in chick. *J Comp Physiol A.* 1996;178:551-561.
  53. Qiao-Grider Y, Hung LF, Kee CS, Ramamirtham R, Smith EL III. Nature of the refractive errors in rhesus monkeys (*Macaca mulatta*) with experimentally induced ametropias. *Vision Res.* 2010;50:1867-1881.
  54. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res.* 1995;35: 37-50.
  55. Wildsoet C, Wallman J. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Res.* 1995;35:1175-1194.
  56. Nickla DL, Totonelly K. Choroidal thickness predicts ocular growth in normal chicks but not in eyes with experimentally altered growth. *Clin Exp Optom.* 2015;98:564-570.
  57. Winawer J, Wallman J. Temporal constraints on lens compensation in chicks. *Vision Res.* 2002;42:2651-2668.
  58. Park TW, Winawer J, Wallman J. Further evidence that chick eyes use the sign of blur in spectacle lens compensation. *Vision Res.* 2003;43:1519-1531.
  59. McLean RC, Wallman J. Severe astigmatic blur does not interfere with spectacle lens compensation. *Invest Ophthalmol Vis Sci.* 2003;44:449-457.
  60. Wildsoet C. Neural pathways subserving negative lens-induced emmetropization in chicks: insights from selective lesions of the optic nerve and ciliary nerve. *Curr Eye Res.* 2003;27:371-385.
  61. Eisner A, MacLeod DI. Blue-sensitive cones do not contribute to luminance. *J Opt Soc Am.* 1980;70:121-123.
  62. Cavanagh P, MacLeod DI, and Anstis SM. Equiluminance: spatial and temporal factors and the contribution of blue-sensitive cones. *J Opt Soc Am A.* 1987;4:1428-1438.
  63. Gawne TJ, Ward AH, Norton TT. Juvenile tree shrews do not maintain emmetropia in narrow-band blue light. *Optom Vis Sci.* 2018;95:911-920.
  64. Chakraborty R, Ostrin LA, Nickla DL, Iuvone PM, Pardue MT, Stone RA. Circadian rhythms, refractive development, and myopia. *Ophthalmic Physiol Opt.* 2018;38:217-245.
  65. Nickla DL, Wildsoet C, Wallman J. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. *Exp Eye Res.* 1998;66:163-181.
  66. Nickla DL. Ocular diurnal rhythms and eye growth regulation: where we are 50 years after Lauber. *Exp Eye Res.* 2013;114:25-34.
  67. Nickla DL. The phase relationships between the diurnal rhythms in axial length and choroidal thickness and the association with ocular growth rate in chicks. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol.* 2006;192:399-407.
  68. Nickla DL, Wildsoet CF, Troilo D. Diurnal rhythms in intraocular pressure, axial length, and choroidal thickness in a primate model of eye growth, the common marmoset. *Invest Ophthalmol Vis Sci.* 2002;43:2519-2528.
  69. Rohrer B, Iuvone PM, Stell WK. Stimulation of dopaminergic amacrine cells by stroboscopic illumination or fibroblast growth factor (bFGF, FGF-2) injections: possible roles in prevention of form-deprivation myopia in the chick. *Brain Res.* 1995;686:169-181.