Long-Wavelength–Filtered Light Transiently Inhibits Negative Lens-Induced Axial Eye Growth in the Chick Myopia Model

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Purpose: Eye growth and myopia development in chicks, and some other animal models, can be suppressed by rearing under near-monochromatic, short-wavelength blue light. We aimed to determine whether similar effects could be achieved using glass filters that transmit a broader range of short and middle wavelengths.

Methods: On day 6 or 7 post-hatch, 169 chicks were assigned to one of three monocular lens conditions (−10 D, +10 D, plano) and reared for 7 or 10 days under one of four 201-lux lighting conditions: (1) B410 long-wavelength–filtered light, (2) B460 long-wavelength–filtered light, (3) Y48 short-wavelength–filtered light, or (4) HA50 broadband light.

Results: At 7 days, B410 (but not B460) long-wavelength–filtered light had significantly inhibited negative lens induced axial growth relative to Y48 short-wavelength–filtered light (mean difference in experimental eye = −0.249 mm; P = 0.006) and HA50 broadband light (mean difference = −0.139 mm; P = 0.038). B410 filters also inhibited the negative lens-induced increase in vitreous chamber depth relative to all other filter conditions. Corresponding changes in refraction did not occur, and biometric measurements in a separate cohort of chicks suggested that the axial dimension changes were transient and not maintained at 10 days.

Conclusions: Chromatic effects on eye growth can be achieved using filters that transmit a broad range of wavelengths even in the presence of strong cues for myopia development.

Translational Relevance: Broad-wavelength filters that provide a more “naturalistic” visual experience relative to monochromatic light have potential to alter myopia development, although the effects shown here were modest and transient and require exploration in further species.

Introduction

In an emmetropic eye, the optical power of the eye matches its axial dimensions, such that distant images focus on the retina without accommodative effort. Increases or decreases in ocular axial length relative to optical power result in myopic or hyperopic refractive errors, respectively. Myopia affects approximately 1.5 billion people worldwide,¹ some of whom will develop sight-threatening secondary conditions such as retinal detachment and glaucoma later in life.² Myopia is dramatically increasing in prevalence and severity, and the age of onset is decreasing.³–⁶ Although linkage and association studies have identified genetic contributions,⁷ epidemiological studies highlight a crucial role for the environment in the current myopia epidemic. Thus, there is a critical need to develop strategies to control the onset and progression of environmentally driven myopia and to control ocular elongation in particular. As reviewed by Wildsoet et al.,⁸ a number of optical, behavioral, and pharmacological interventions have been developed; however, the efficacy of these treatments is variable, with no one approach
preventing or slowing myopia in all individuals. Thus, further research is needed to understand the underlying mechanisms and explore combined treatments and novel options.

Ocular growth, at least in animals, is known to be controlled locally in the retina by a process that is dependent on visual feedback, although the specific mechanisms controlling refractive and ocular growth are still unclear. The biological underpinnings of this process have been primarily investigated using animal models that first used form deprivation via occlusion and later optically defocusing lenses to alter the visual environment. Occluders reduce spatial contrast and temporal luminance modulation and rapidly induce severe axial myopia. By contrast, defocusing lenses impose blur on the eye and stimulate rapid compensatory axial growth to match the sign and approximate strength of the imposed defocus.

In addition to lens and form-deprivation occluder models, eye growth in normally developing animals can be modulated by environmental lighting manipulations such as rearing under restricted wavelengths. Fish, chicks, and guinea pigs reared in narrow-band, short-wavelength blue light are reported to develop shorter axial dimensions and more hyperopic and less myopic refractions than those reared in middle-wavelength green light or long-wavelength red light. Studies in tree shrew and monkey models have reported more complex effects, generally in the opposite direction. Gawne and colleagues found that tree shrews become hyperopic under long-wavelength red light (624–636 nm). Infant tree shrews displayed normal emmetropization under short-wavelength blue light (464 nm). However, when blue light exposure commenced later in development after the animals had reached a stable refractive state, they first become hyperopic and then displayed a myopic drift in refraction after a variable time period.

In a study of rhesus monkeys, Smith et al. demonstrated that filters that absorbed wavelengths below 570 nm (resulting in red lighting with luminance levels of ~50 lux) induced relative hyperopia and shorter vitreous chamber depths. Conversely, at higher luminance levels, Liu et al. reported that rearing under quasi-monochromatic red light with a peak of 610 nm induced myopia in a small subset of rhesus monkeys, whereas short-wavelength blue light rearing (peak, 455 nm) had little effect on emmetropization. These studies in non-lensed animals indicate that rearing under narrow-band lighting, typically designed to provide strong stimulation for a subset of one or two cone photoreceptor types, can produce marked changes in eye growth and refraction in several species.

Although few studies have investigated the influence of chromatic wavelength manipulations on eye growth and refraction in the presence of strong optical cues for myopic and hyperopic growth (defocusing lenses and occlusion), exploration of this effect is warranted if chromatic manipulations are to be considered as a potential strategy to control environmentally driven myopia in humans. Such manipulations are potentially of translational interest due to their ability to be combined with other optical interventions. Blue light (470 nm) has been shown to suppress the development of defocus-induced axial myopia in guinea pigs. Similarly, ultraviolet (UV) light (375 nm) and blue light (465 nm) suppressed myopia development in the chick form-deprivation occlusion model. Rhesus monkeys reared under long-wavelength red lighting (630 nm) displayed a hyperopic shift in refraction and shorter vitreous chamber depths across positive lens, negative lens, and form-deprivation occlusion conditions relative to animals reared in white fluorescent lighting. Finally, using very low illuminance levels (<1 lux) designed to isolate individual cone types, Rucker and Wallman found that compensation to lenses was less effective under monochromatic red (620 nm) or blue (460 nm) light relative to white light in chicks. These findings demonstrate that quasi-monochromatic or narrow emission spectrum light sources are capable of altering eye growth even in the presence of strong environmental cues for myopia development.

For chromatic wavelength manipulations to be viable in a clinical myopia-control context, tolerability and safety considerations suggest that the use of broader band lighting that provides some stimulation for short-wavelength–sensitive cones (S-cones), medium-wavelength–sensitive cones (M-cones), and long-wavelength–sensitive cones (L-cones) would be necessary to provide a more ecologically naturalistic visual experience. Therefore, to determine whether the chromatic effects discussed above (particularly with respect to modulation of axial eye growth due to its import for secondary pathology risk) could be achieved under more normal environmental-level lighting containing a broader range of wavelengths, we studied the effects of short- and long-wavelength filters on the development of lens defocus-induced myopia and hyperopia in the widely used chick model.

Two long-wavelength–filtered blue/blue–green light conditions were tested: B410 filters and B460 filters. Both conditions provided some stimulation to all chick cone photoreceptors, with a peak illuminance near the maximum sensitivity range for M-cones (B410 peak = 517 nm, B460 peak = 524 nm). The two conditions differed in their relative stimulation of...
S-cones and L-cones; the B410 filter condition provided relatively greater S-cone stimulation than the B460 filter, and the B460 filter provided relatively greater L-cone stimulation than the B410 filter. These blue/blue–green light conditions were compared with two similar yellow light conditions: Y48 filters with a peak intensity of 619 nm and short wavelengths filtered out and a broadband halogen light condition (620 nm peak). We predicted that chicks reared under long-wavelength–filtered blue light (B410 filters) or blue–green light (B460 filters) would display a hyperopic shift with shorter axial dimensions across lens conditions relative to chicks reared under short-wavelength–filtered yellow and broadband light (i.e., Y48 or HA50 broadband filters). Note that reference to the hue of lighting conditions throughout this manuscript refers to their appearance to human observers, as we are uncertain how wavelength manipulations affect a chick’s perception.

Methods

Animals and Rearing

Male hatchling chicks (Leghorn × New Hampshire) were obtained from a local hatchery and housed in light-tight rearing boxes (internal dimensions, 900 mm long × 620 mm wide × 525 mm high) until the beginning of experimentation. The tray in the bottom of the boxes was filled with sawdust bedding, and the chicks had ad libitum access to food and water. To provide accommodative cues incorporating a range of orientations and spatial frequencies, a grayscale Gabor pattern (100-mm high frieze) was lined immediately above the sawdust bedding (Fig. 1). Box temperature was maintained at 30°C for the first 10 days post-hatch and then gradually decreased to 24°C by post-hatch day 17. All procedures involving animals were approved by the La Trobe University Animal Ethics Committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes.

Lens Defocus and Light Filtering

On day 6 or 7 post-hatch, chicks were lightly anesthetized for baseline biometric measures. Following biometric data collection, chicks were assigned to one of three lens conditions: monocular −10 diopter [D] lens, plano (0 D) lens, or +10 D lens. Lens goggles were made from modified human polymethylmethacrylate (PMMA) contacts (8.1-mm diameter; Gelflex, Melbourne, VIC, Australia) affixed to a 22-mm Velcro ring. The PMMA lens material transmits light uniformly across the visible spectrum (Supplementary Fig. S1). Goggles were attached to the complementary side of the Velcro glued to the periocular feathers of the right eye. As the orientation of young male chicks in the embryo leads to developmental asymmetries within the left and right visual pathways post-hatch,43–45 lenses were attached to the right eye only (rather than counterbalancing between left and right eyes) to avoid introducing this confound into the experimental design. The experiment was designed to analyze changes (endpoint – baseline) in experimental eye biometrics, with a separate plano lens control condition rather than fellow eye controls, to avoid confounding yoking effects10 and to enable detection of
defocus-independent shifts in ocular parameters (e.g., Hung et al.39).

**Illumination Conditions**

Within each lens condition, chicks were assigned to one of four light filtering conditions: (1) B410 long-wavelength filter + HA50 heat filter, (2) B460 long-wavelength filter + HA50 heat filter, (3) Y48 short-wavelength filter + HA50 heat filter, or (4) HA50 heat filter only (Hoya Candeo Optronics Corporation, Saitama, Japan). The transmittance of each filter is shown in Supplementary Figure S2a. Rearing boxes were lit with a 12-V/100-W halogen globe (64623 HLX; OSRAM, Munich, Germany) on the roof of the enclosure (12-hour day/night cycle). Glass filter shades were fitted over the globes at the beginning of experimentation. Previous research has determined that the standard International Commission on Illumination (CIE) photopic function is appropriate for photometric calculations in chicken.46 Thus, illuminance across conditions was matched using a 400K Lux Meter (QM1584; Protech International, Shenzhen, China), and mean illuminance (measured at nine positions across the box floor) was maintained at 201 ± 10 lux across all filter conditions by adjusting the power source of the light. HA50 heat-absorbing filters were used in each condition to control for changes in radiant heat profiles at the different light intensities. Emission characteristics of the stacked filters and globe were determined using the Red Tide USB650 Fiber Optic Spectrometer (Ocean Optics, Dunedin, FL). Figure 2 illustrates the resulting spectral emission curves in

![Spectral Emission Curves](image-url)

**Figure 2.** (A) Illuminance-matched comparison of the spectral emission curves in the rearing boxes relative to the sensitivity of chick photoreceptors. Emission curves were measured with an Ocean Optics Red Tide USB650 Fiber Optic Spectrometer with illuminance matched across conditions as described in the text. The spectrometer measures wavelengths from 350 to 1000 nm. Extended emission curves are provided in Supplementary Figure S2. Chick ultraviolet (UV), short (S), medium (M), and long (L) wavelength and double (D) cone sensitivity data from Rucker and Wallman27 are shown as gray dotted lines. Reprinted with permission from Elsevier. (B) Relative absorbance for each cone type (calculated from the spectra and chick cone sensitivity data in A).
relation to the relative sensitivity of chick photoreceptors. Relative cone absorbance (Fig. 2B) was calculated via standard techniques: the integral over the visible wavelength range of the product of the individual cone spectral sensitivity and the spectral intensity distribution of the source (plus the applied filter). Cone absorbance values were normalized to the sum of M- and L-cone excitation, as all conditions were tested at the same illuminance.

### Duration of Rearing

After lensing, chicks were raised for a further 7 days (post-hatch days 6–13) or 10 days (post-hatch days 7–17) under filtered light. These timepoints were chosen to allow sufficient time for an effect of filter rearing on eye growth to accumulate (e.g., Foulds et al.24). The Table outlines the number of chicks in each experimental group. For the duration of experimentation (i.e., immediately prior to lensing until the completion of final biometric measures), all husbandry and data collection tasks were completed under ambient lighting filtered under the same conditions as the rearing box.

### Biometric Analysis

Chicks were anesthetized at the beginning (ketamine 20 mg/kg and xylazine 2 mg/kg, intramuscular injection [i.m.]) and end (ketamine 45 mg/kg and xylazine 4.5 mg/kg, i.m.) of experimentation, refracted by retinoscopy (18240 Streak Retinoscope; Welch Allyn, Chicago, IL), and axial dimensions were then obtained from the average of peak distance measures from at least three A-scan ultrasonography traces (VuPad A/B Portable Scan Tablet; Sonomed Éscalon, New Hyde Park, NY). Each A-scan trace provided peaks indicating the length of the eye (anterior pole of the cornea to the retina), vitreous chamber depth (posterior pole of the lens to the retina), and anterior chamber depth (anterior pole of the cornea to anterior pole of the lens) in millimeters.

### Data Analysis

Biometric data (refraction, axial length, and vitreous and anterior chamber depth) were analyzed at baseline and at the end of experimentation using SPSS Statistics 26 (IBM Corp., Armonk, NY). Analysis of baseline values revealed some differences in refraction among the groups (see Results section). Difference values (end of experiment – baseline) were calculated for each measure to assist with controlling for this individual variation at the beginning of experimentation. The assumption of normality was violated for some groups (z-skewness and/or z-kurtosis > 1.96). Consequently, non-parametric Kruskal–Wallis tests were used to examine the simple main effects of filter rearing on biometrics within each lens condition. Pairwise comparisons with Dunn–Bonferroni adjustment for multiple testing were conducted as required. Raw data are available in Supplementary Dataset S1.

### Results

#### Ocular Biometrics in the 7-Day Induction Group

##### Axial Dimensions

There were no statistically significant differences in experimental eye axial dimensions at day 6 post-hatch (baseline). Graphs showing mean axial dimensions at baseline and the endpoint of rearing are available in the Supplementary Materials (Supplementary Figs. S3, S4). As expected, negative lenses accelerated the rate of post-hatch axial eye growth, whereas positive lenses inhibited growth during the 7-day induction period (i.e., between post-hatch days 6 and 13). Filter rearing significantly affected the rate of axial growth ($H_3 = 12.491, P = 0.006$) (Fig. 3A) and final axial length ($H_3 = 9.452, P = 0.024$) following 7 days in the negative lens condition. Pairwise comparisons showed that the increase in axial length induced by negative lenses was inhibited by rearing under B410 long-wavelength–filtered light relative to both broadband HA50–filtered light ($P = 0.038$) and Y48 short-wavelength–filtered yellow light ($P = 0.006$) (Fig. 3A). Consequently, the final axial lengths of chicks in the B410 negative lens condition were 0.30 mm shorter than those in the Y48 negative lens condition ($P = 0.040$). There were no differences in axial eye growth or final axial length in the plano or positive lens conditions.

Axial growth differences in the negative lens condition appeared to be driven by differences in vitreous chamber expansion (Fig. 3B). Filter rearing significantly affected the change in vitreous chamber depth from baseline ($H_3 = 19.859, P < 0.001$) (Fig. 3B) and final vitreous chamber depth ($H_3 = 16.388, P < 0.001$) in the negative lens condition. Pairwise comparisons showed that the increase in vitreous chamber depth induced by negative lenses was inhibited by rearing...
under B410 long-wavelength–filtered light relative to all other filter conditions (B460, $P = 0.037$; HA50, $P = 0.001$; Y48, $P < 0.001$) (Fig. 3B). Consequently, the final vitreous chamber depth was shorter in the B410 negative lens condition relative to all other conditions (B460, $P = 0.045$; HA50, $P = 0.002$; Y48, $P = 0.004$).

Filter rearing also significantly affected the final vitreous chamber depth ($H_3 = 9.478$, $P = 0.024$) following positive lens wear, resulting in a vitreous chamber depth of chicks in the B410 positive lens condition that was 0.26 mm shorter than the mean depth in the Y48 positive lens condition at 7 days ($P = 0.027$) (Supplementary Fig. S3F). There were no differences in vitreous chamber expansion or final vitreous chamber depth in the plano lens condition, and filter rearing did not affect experimental eye anterior chamber depth (Fig. 3C).

**Refraction**

Chicks were, on average, hyperopic at the day 6 post-hatch baseline timepoint (mean experimental eye refraction, $+1.42$ D). There were significant differences in baseline experimental eye refraction among the filter groups in each lens condition (negative $H_3 = 14.758$, $P = 0.002$; positive $H_3 = 16.085$, $P < 0.001$; plano $H_3 = 17.702$, $P < 0.001$), with chicks in the B460 filter group displaying a more hyperopic baseline refraction than those in the B410 group (negative, $P = 0.002$; positive, $P = 0.001$; plano, $P = 0.001$) and HA50 group (positive, $P = 0.013$) (Fig. 5). In the plano lens condition, chicks in the Y48 filter group were also more hyperopic than those in the B410 group ($P = 0.005$). Despite this initial difference, regression analysis confirmed that baseline refraction did not significantly predict refraction at the end of the experimental period (adjusted $R^2 = 0.007$, $F_{1,167} = 2.175$, $P = 0.142$).

As illustrated in Figure 4, refractive compensation to negative and positive lenses during the experiment was rapid, with $-11.37$ D myopic and $+5.53$ D hyperopic shifts occurring following 7 days of lens wear (i.e., between post-hatch days 6 and 13). The $-1.75$ D myopic shift in the plano lens condition was presumably primarily due to post-hatch emmetropization from a baseline hyperopic state of $+1.39$ D, with chicks in the plano lens groups achieving a mean endpoint refraction of $-0.36$ D. There were strong negative correlations between the shift in experimental eye refraction and the shift in experimental eye axial length ($r = -0.858$, $P < 0.001$) and vitreous chamber depth ($r = -0.821$, $P < 0.001$) across conditions at 7 days.

Following 7 days of lens wear, there were no significant effects of filter rearing on final refraction or refractive shifts in the negative and plano lens groups.
Figure 4. Mean (± SE) experimental eye refraction at baseline and following 7 days of (A) negative (−10 D), (B) positive (+10 D) or (C) plano (0 D) lens wear. (D) Change in experimental eye refraction (end – baseline). Statistically significant differences between groups are indicated with an asterisk.

(Figs. 4A, 4C, 4D). However, filter rearing did significantly affect final refraction ($H_3 = 8.800, P = 0.032$) and refractive shifts ($H_3 = 14.146, P = 0.003$) in the positive lens condition. Pairwise comparisons with adjusted $P$ values demonstrated that chicks in the B410 condition were more hyperopic than those in the B460 condition following 7 days of positive lens wear ($P = 0.030$) (Fig. 4B). This difference was emphasized further when examining the change in experimental eye refraction, where the hyperopic shift induced by positive lenses was significantly greater under B410 long-wavelength–filtered light ($P = 0.002$) and HA50 broadband light ($P = 0.023$) relative to B460 long-wavelength–filtered light (Fig. 4D).

Ocular Biometrics in the 10-Day Induction Group

Axial Dimensions and Refraction

Biometric data were collected from a separate, smaller, cohort of chicks reared for 10 days with monocular negative or positive lenses under B410 or Y48 filtered light. Chicks were, on average, hyperopic at the day 7 post-hatch baseline timepoint (mean experimental eye refraction, +1.15 D). As expected, negative and positive lenses induced myopic and hyperopic shifts in refraction of −12.45D and 6.43D, respectively. There were no significant differences among the filter groups in baseline or final experimental eye axial dimensions or refraction (Supplementary Fig. S4), or in the change in experimental eye biometrics across the 10-day rearing period (i.e., between post-hatch days 7 and 17). Notably, filter rearing did not affect axial growth (negative $H_1 = 0.137, P = 0.711$; positive $H_1 = 1.089, P = 0.297$), vitreous chamber expansion (negative $H_1 = 0.025, P = 0.874$; positive $H_1 = 0.335, P = 0.563$), or refractive compensation (negative $H_1 = 0.026, P = 0.873$; positive $H_1 = 2.272, P = 0.132$) in either lens condition (Fig. 5).

Chick Weight

There were no significant differences in chick weight at baseline. As illustrated in Figure 6, chicks continued
to gain weight under all lighting conditions during the experiment. There were no significant differences in weight gain between filter groups at 7 days. However, by 10 days, chicks reared under B410 filtered light had gained 15.52 g more weight than those reared under Y48 filtered light ($H_1 = 4.273, P = 0.039$) (**Fig. 6B**).
Discussion

The present study investigated the effects of broadband short- and long-wavelength–filtered light on eye growth and refractive compensation to positive, negative, and plano lenses in chicks. We predicted that chicks reared under long-wavelength–filtered blue/blue–green light would display a hyperopic shift in refraction and shorter axial dimensions across lens conditions relative to chicks reared under short-wavelength–filtered yellow and broadband light. This prediction was partially supported, as long-wavelength–filtered blue light (B410 filters) attenuated the increase in axial length and vitreous chamber depth typically induced by negative lenses relative to normal broadband lighting (HA50 filter) and short-wavelength–filtered yellow lighting (Y48 filter) at 7 days. Additionally, following hyperopia induction with positive lenses, chicks in the B410 long-wavelength filter condition displayed shorter vitreous chambers than those in the Y48 short-wavelength filter condition. These results agree with previous restricted wavelength research in the chick form-deprivation occlusion and guinea pig defocus models, demonstrating that short-wavelength blue lighting can inhibit the development of environmentally induced axial myopia in these species. Our biometric measurements in a separate cohort of chicks demonstrated that the differences in axial dimensions following 7 days of rearing under the B410 long-wavelength filter were transient and not maintained at 10 days.

Small but significant differences in baseline refraction between some groups confounded interpretation of refraction data in the present study. However, in general, similar to previous red and blue light rearing studies in chicks, the significant axial dimension changes were not reflected in refraction changes of equivalent magnitude. This apparent disparity remains unexplained but could reflect changes in corneal curvature (which we did not measure) that compensated for alterations in vitreous chamber depth.

Wavelength Parameters

Importantly, inhibition of defocus-induced axial eye growth in the present study was achieved with long-wavelength B410 filters that transmit a broad range of short and middle wavelengths. The B410-filtered blue light in the present study had a peak of 517 nm, a half maximum range of 80 nm, and a range encompassing approximately 360 to 650 nm. Previous studies showing a hyperopic shift in axial dimensions and/or refraction following non-flicker blue light rearing have primarily used light sources with a lower peak around 460 to 470 nm (e.g., 464 nm, 465 nm, 470 nm, and a narrow range (usually < 20 nm). The present study builds on these previous results to demonstrate that defocus-induced axial growth changes in chick can be altered by varying the wavelength composition of a broadband light source, highlighting the potential for clinical application of filters in the control of refractive error development.

In addition to the previous studies using near-monochromatic light sources, others have investigated the effects of varying wavelength composition using broadband light sources. Li et al. found no difference in refractive development and response to negative lenses in guinea pigs raised under fluorescent light without a UVA component versus those raised under broad-spectrum halogen light with a UVA component. Using red, green, and blue light-emitting diodes (LEDs) with peaks of 615 to 619 nm, 515 nm, and 460 to 465 nm and half-bandwidth ≤ 35 nm, Rucker and colleagues reported an interaction among temporal frequency, contrast, and wavelength composition effects (particularly the presence or absence of blue light) for flickering light in non-lensed animals. Most recently, Yoon et al. demonstrated similar interactions between temporal frequency and wavelength composition in non-lensed chicks using LED light sources with a more continuous radiation composition. These studies using light sources like those commercially available for indoor lighting have not shown significant effects of greater short-wavelength blue components on eye growth in non-lensed animals when the light is steady (i.e., not flickering), consistent with our findings using plano lenses.

The second blue–green light condition tested in our study, B460 filters, did not induce a hyperopic shift in axial dimensions, indicating that the spectral range of the stimulus is important in determining growth outcomes. The peak intensity of light in the B460 + HA50 filter condition was 524 nm, with a half-maximum range of 102 nm. This condition provided approximately equal stimulation within the chick S-cone and L-cone sensitivity ranges (Fig. 2). By comparison, the B410 filtered light, which did affect eye growth, provided stronger stimulation within the sensitivity range of S-cones relative to L-cones. This pattern of findings suggests that the relative excitation of different cone types may be important. A contribution from intrinsically photosensitive ganglion cells in the inner retina is also possible. These ganglion cells containing melanopsin are optimally stimulated by blue light and are involved in a wide range of non-image-forming functions of potential relevance to...
ocular growth control including circadian entrainment and the pupil light response.\textsuperscript{55}

In addition to comparing B410 and B460 blue light conditions, the present study compared two similar yellow light conditions: (1) Y48 filters with a peak intensity of 619 nm and short wavelengths filtered out, and (2) HA50 broadband light with a peak of 620 nm containing some short wavelengths. There were no significant differences in biometric outcomes between these two yellow light conditions, suggesting that small differences in the short wavelength range do not affect chick eye growth in stimuli dominated by longer wavelengths.

**Effect Duration**

Our analysis of a small number of chicks following 10 days of B410 + HA50 blue light and Y48 + HA50 yellow light suggested that significant differences in right eye axial dimensions at 7 days were not maintained at this later timepoint. This is consistent with the findings of past investigations suggesting that responses to wavelength manipulations can be transient or non-uniform over time. Indeed, Lin et al.\textsuperscript{23} recently reported that significant differences in eye growth following 10 days of no-lens blue (464 nm) and red (628 nm) light rearing in chicks did not continue to increase with a further 7 days of treatment. They also compared age-matched chicks exposed to these wavelength manipulations for different lengths of time and found that 10-day-old chicks first exposed to the wavelength manipulation on day 7 and reared for 3 days further displayed significantly more hyperopia in the blue relative to red lighting condition. The same but less extreme effect was seen in age-matched 10-day-old chicks exposed to wavelength manipulation from day 1.\textsuperscript{23} In tree shrews, when blue light rearing (464 nm) was started after 11 days of visual experience, emmetropization proceeded as normal.\textsuperscript{34} However, blue light rearing (464 nm) introduced after 24 days of normal visual experience (by which time the tree shrews were nearly emmetropic) induced hyperopia followed by a myopic shift.\textsuperscript{36} These transient and/or age-dependent responses could reflect a critical period of development during which the eye is more responsive to wavelength manipulations, the length of rearing under altered light, and/or the degree of emmetropia (i.e., refractive state of the eye) at the beginning of the wavelength manipulation.

In the present study, chicks reared under B410 long-wavelength–filtered blue light gained significantly more weight at 10 days relative to those reared under Y48 short-wavelength–filtered yellow light. This is consistent with past research showing increased weight gain\textsuperscript{56,57} and lower activity levels\textsuperscript{23,58} for chicks reared under short- versus long-wavelength light. Red light rearing has also been shown to increase behavioral and hormonal markers of stress in chicks.\textsuperscript{56} As the spectral distribution of lighting has similarly been shown to alter behavior in other species,\textsuperscript{59–61} it is possible that changes in activity patterns and physiological stress contribute to the differences in outcome for the shorter versus longer term rearing studies discussed above. This would be consistent with past research associating diurnal and circadian rhythms,\textsuperscript{62} immune responses,\textsuperscript{63–65} and metabolic factors\textsuperscript{63,64,66–69} with ocular growth regulation and refractive error development.

**Biological Mechanisms**

The biological mechanisms underlying the effects of wavelength manipulations on eye growth and refractive compensation in chick and other species remain unresolved. These effects were first proposed to reflect longitudinal chromatic aberration (LCA), in which short wavelengths are focused in front of long wavelengths.\textsuperscript{70,71} This difference in focal plane was suggested to provide the retina with directional cues to guide emmetropization (a wavelength defocus signal present only in broadband lighting conditions and a chromatic signal arising from differential defocus present only in broadband light).\textsuperscript{53} Studies employing monochromatic light have shown that chromatic cues are not necessary for refractive compensation to occur.\textsuperscript{72–74} Moreover, based on studies involving longer rearing times under narrow-band lights in guinea pig and chick,\textsuperscript{24,29,31} it has been argued that the refractive changes induced by short- and long-wavelength light are progressive and/or much larger than those required to compensate for LCA.\textsuperscript{59} Furthermore, although fish, chicks, and guinea pigs respond to wavelength rearing with eye growth changes in the direction expected based on LCA, eye growth and refractive shifts in tree shrews and rhesus monkeys primarily occur in the opposite direction.\textsuperscript{34–37,39} Together, these observations suggest that factors other than, or in addition to, LCA are involved in ocular growth that need to be investigated morphologically and metabolically. The variation in response across species suggests that differences in biology related to age, emmetropization status of the animal, and ecological niche, particularly associated with the relative distribution of rods and cones as a function of diurnality,\textsuperscript{75,76} may be important determinants of effects of wavelength manipulations on eye growth.
Limitations and Future Directions

Chicks have been one of the most widely used animal models for investigating the effects of restricted wavelength rearing on refractive error development, and they have shown consistent biometric responses across studies. \(^{21-27}\) Because of this, they were chosen as a suitable model species for the present study to test whether the near-monochromatic light effects observed previously generalize to manipulations containing a broader range of wavelengths. However, as indicated above, species differ in their biometric responses to wavelength manipulations, and this precludes direct extrapolation of our results to humans. Further studies are needed to explore whether similar broadband-wavelength manipulations also alter eye growth in other species. Studies in humans associating refraction and sensitivity to longer or shorter wavelength stimula\(^{77-79}\) support the notion that future research in this area may have translational benefits.

This study prioritized ocular axial dimensions and refraction as the primary measures of interest when examining how filtered light affects the growth and refractive development of the eye. Collection of additional biometric measures, such as corneal power, choroidal thickness, and cornea to sclera axial length could assist interpretation of these primary measures in future studies (as discussed by Lin et al.\(^{23}\)). Lighting manipulations have been shown to alter corneal curvature in chicks, although Cohen et al.\(^{50}\) reported that this effect was transitory, with changes in axial dimensions being more persistent across the rearing period. Although previous studies in monkey, \(^{37-39}\) tree shrew, \(^{35}\) and guinea pig \(^{29}\) have not found associations between red, green, and/or blue light rearing and corneal changes, several chromatic rearing studies in chick (including our own) \(^{21,24,26}\) have found inconsistencies between significant refractive and axial dimension changes. This suggests that further investigation of corneal contributions to refraction is particularly warranted in this species.

In addition to the wavelength parameters, the power of the light source varied across filter conditions in the present study as was necessary to match illuminance (where blue light must be a higher irradiance than red light to match intensity in lux). We chose to match illuminance, rather than power, as this is the more physiologically relevant measure related to the biological response to light (i.e., photoreceptor activation) and downstream retinal cell activity and perceived brightness. \(^{46}\) This choice is well accepted in the field, with similar methodology being used by past studies in the area. \(^{80}\) Previous studies investigating changes in illuminance levels in the chick model have shown no effect of varying light intensity within the range used in our paper (as opposed to very low or very high intensities, which are known to affect growth and myopia development). For example, Ashby et al.\(^{51}\) found that chicks fitted with occluders and reared under 50-lux lighting for 4 days developed levels of myopia similar to those of chicks reared under 500-lux lighting, with no differences in axial length or corneal radius between the two lighting groups. This experiment used triphosphor fluorescent lamps, with peaks at 530 nm and 620 nm. Similarly, Feldkaemper et al.\(^{82}\) found that, for chicks reared under 550-lux ambient illuminance generated using a xenon lamp, wearing neutral-density filters of 0, 0.5, or 1 log unit attenuation for 7 days did not affect refraction or axial measures. These findings suggest that varying stimulus power within the range represented in the present study for steady broadband light sources or those with strong mid- to long-wavelength components (similar to our Y48 and HA50 conditions) has little effect on refractive parameters. However, a recent study has shown an effect of stimulus power, within specific ranges (spanning 70-985 lux and 49-920 \(\mu W/cm^2\)), on axial dimensions for flickering light with varying short- and long-wavelength composition. \(^{54}\) Thus, the interaction among wavelength, power, and temporal frequency effects is an interesting topic for further investigation.

Finally, our own pilot data and those of previous studies suggest that biometric effects may not be maintained when animals are exposed to restricted wavelengths for a prolonged period. As this is an important determinant of any translational strategies arising from this approach, work is now needed to understand how filter effects change over time (e.g., with continuous exposure versus intermittent exposure that may be less likely to induce rapid adaptation of the system).

Conclusions

Previous research has demonstrated that rearing under near-monochromatic blue light can retard myopia development in chicks. The present study demonstrates that long-wavelength filters that transmit a broad range of short and middle wavelengths can similarly inhibit vitreous chamber growth and axial expansion in the chick negative lens myopia model at 7 days. These effects were not maintained at the follow-up 10-day timepoint and were not accompanied by corresponding changes in refraction. The specific wavelength parameters needed to induce growth changes remain unclear; however, our results
are consistent with the notion that differences in the relative activation of short- and long-wavelength cones may be necessary. Future studies are needed to explore how different filter parameters and induction times affect the magnitude of the biometric response and to elucidate the underlying structural and biochemical processes involved across species.

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