Broadband Long Wavelength Light Promotes Myopic Eye Growth and Alters Retinal Responses to Light Offset in Chick

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Purpose. Prolonged exposure to broadband light with a short-wavelength (blue) or long-wavelength (orange/red) bias is known to impact eye growth and refraction, but the mechanisms underlying this response are unknown. Thus, the present study investigated the effects of broadband blue and orange lights with well-differentiated spectrums on refractive development and global flash electroretinography (gfERG) measures of retinal function in the chick myopia model.

Methods. Chicks were raised for 4 days with monocular negative lenses, or no lens, under blue, orange, or white light. Chick weight, eye dimensions, and refraction were measured at the conclusion of rearing. In a separate cohort of chicks, the effect of 4 days of colored light rearing on retinal responses to orange, blue, or white light flashes was assessed using gfERG.

Results. Chicks reared under orange light for 4 days exhibited a significantly larger myopic shift in response to negative lenses compared to those reared under blue light. Orange light rearing for 4 days increased the gfERG d-wave amplitude and implicit time in response to orange light flashes but did not alter responses to white or blue flashes. Blue and white light rearing did not affect the retina's response to light flashes of any color.

Conclusions. Orange light rearing exacerbated defocus-induced myopia relative to blue light rearing. The gfERG recordings revealed that prolonged orange light exposure increased retinal responsivity to the offset of long wavelength light flashes, suggesting a potential role for ON/OFF pathway balance in generating the refractive response that requires further electrophysiological and molecular investigation.

Keywords: myopia, wavelength, chromatic, refractive error, electroretinogram (ERG), chick

Myopia (short-sightedness) occurs when the eye grows excessively, causing light to focus in front of the retina. It is the most common ocular disorder[1] and, in many regions, myopia prevalence and severity are increasing (e.g. see Refs. 2–5) such that by 2050 approximately half of the world's population is expected to be myopic.[5] Although myopic refractive errors can typically be optically corrected with spectacles or contact lenses, the morphological changes associated with excessive eye growth predispose a range of complications, such as glaucoma, retinal detachment, and maculopathy later in life.[6] Therefore, interventions to regulate eye growth and prevent sight-threatening secondary complications are urgently needed.[7,8] Recent research has explored whether the cue provided by longitudinal chromatic aberration (LCA), where short wavelength blue light is refracted more strongly by the cornea and lens than long wavelength red light, can influence eye growth.[9,10]

Animal studies have demonstrated that eye growth and refractive development can be altered by rearing under different colored lights that would be expected to affect LCA-based emmetropization mechanisms. In fish,[11] chicks,[9,12–15] mice,[16] and guinea pigs,[17–23] shorter wavelength violet-blue light decreases eye growth, promoting hyperopic shifts in refraction, whereas longer wavelength green-red lights have the opposite effect. Violet-blue light can also suppress the development of defocusing lens and occluder-induced myopia in these species.[15,16,19,24–26] On the other hand, as reviewed previously,[27,28] not all species respond to colored lights in this manner, with tree shrew and rhesus monkey studies showing more variable results, such that red light generally promotes a hyperopic shift and blue light causes variable responses depending on the developmental stage of the animals.

Chromatic cues also appear to influence refractive development in humans, with acute exposure to narrowband blue light in humans associated with axial shortening relative to red light,[29] although short red light exposures have also been shown to cause axial shortening relative to pre-exposure baseline measures.[30] Acute simulation of chromatic defocus can also alter choroidal thickness in emmetropic (but

not myopic) individuals.[31] In longer-term studies, violet light emitting eyeglasses have shown efficacy against myopia progression in some groups of children.[32,33] Repeated low red light therapy in children has also shown efficacy for myopia control.[34,35] However, spectacle lenses that block short wavelength light were reported to not significantly affect myopia development,[36] with the lack of the hypothesized effect attributed to scattered broadband light entering around the frames.[37]

Within this context, recent research has focused on understanding how broadband lighting with a short-, or long-wavelength bias affects eye growth and refraction (as opposed to narrow band red or blue/violet lights that stimulate only one cone subtype).[37–45] Such investigations are relevant to the development of myopia-control interventions (e.g. tinted spectacle lenses) where less stringent wavelength filtering is necessary to improve tolerability and ensure adequate illumination. They may also assist in understanding whether incidental wavelength biases in existing light sources (e.g. screen filters and room lighting) have the potential to alter refractive development. Filters that limit a broad spectrum light source to the short-wavelength (blue) range inhibit axial eye growth in response to negative lenses,[25] consistent with narrow-band blue light research. Similarly, blue-enriched white light has been shown to suppress axial eye growth in the chick form deprivation model,[40] although another study found no significant effect of blue-enriched light on eye growth and refraction during a shorter period of form deprivation.[41] In monkeys[39] and chicks,[44] rearing with long-wavelength (red) pass filters in front of the eye resulted in hyperopia. This finding in chicks was unexpected as they generally become more myopic in narrowband red light (for review see Ref. 28). Previous research has demonstrated that red light can limit weight gain and increase activity levels in young chicks, with associated effects on eye growth measures.[12,25] Thus, further research is required to investigate the interaction of local ocular and systemic light wavelength effects over time, particularly for broadband long wavelength light sources.

In an effort to understand the biological underpinnings of light wavelength-induced eye growth changes, many studies have focussed on the role of visual and non-visual light-sensitive opsins, and the associated ratio of short (S), medium (M), and long (L) wavelength sensitive cones. Such studies have shown that the abundance of visual opsins in animal models is affected by rearing under spectrally biased light that also promotes myopia or hyperopia.[44,46,47] This suggests that prolonged colored light rearing may induce adaptations to functional responses to spectrally biased light. The global flash electroretinogram (gfERG) technique has previously identified changes to photoreceptor function and ON/OFF pathway balance in animal myopia models and human myopes,[48–53] although its use to assess functional changes in wavelength-induced eye growth models has been limited. One study measuring gfERGs in mice reared under green light for 12 weeks to induce myopia observed no functional effects.[47] However, this study used short duration white light ERG flashes only in a dichromatic nocturnal species.[54] Chromatic gfERGs that are known to preferentially stimulate different populations of opsins and associated photoreceptors[55] may provide a more useful measure of retinal function in studies of wavelength-induced eye growth changes. Moreover, it is of interest to examine longer duration ERG flash stimuli that allow separate examination of responses to light onset and offset because shifts in the balance of retinal ON and OFF pathway signaling have previously been linked to myopia development (e.g. see Refs. 51, 56–59).

The present study aimed to assess the impact of broadband blue and orange light rearing on negative lens induced myopia development and retinal function in chicks, a diurnal species with a cone-rich retina and spectral sensitivity from the UVA range through to 700 nm.[60,61] The gfERG setup for retinal function measures was tailored to enable recording of responses to the onset and offset of blue, orange and white light flashes with a similar spectrum range as the lights used in the rearing environment. It was hypothesized that orange and blue light rearing would exacerbate and inhibit the development of lens-induced myopia, respectively, and that prolonged exposure to blue or orange light would alter the functional sensitivity of the retina to light of the same spectrum as measured using the gfERG.

Methods

Animals

Male White Leghorn/New Hampshire chicks (Gallus domesticus, $N = 195$) were obtained from a commercial supplier on the morning of hatching, and housed in light-tight rearing boxes (internal dimensions 900 mm long × 620 mm wide × 525 mm high; N per box = 15–32 prior to experimentation and 6–16 during experimentation). The tray in the bottom of the box was filled with sawdust bedding. Box temperature was maintained at 30°C ± 2. Water and food (Chick Starter; Barastoc Poultry, Victoria, Australia) were freely available, except immediately prior to anesthesia. A 12-hour day/night light cycle was maintained throughout the experiment. All procedures involving animals were approved by the La Trobe University Animal Ethics Committee (Application ID AEC21024) 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. The methodological reporting herein adheres to the ARRIVE Essential 10 guidelines.[62]

Lens Conditions

Chicks were assigned to a "no lens" condition to assess the effects of filter rearing on normal development and emmetropization, or a monocular –10 diopter (D) "negative lens" condition to assess the effects of filter rearing on myopia induction. An independent no lens group was used in preference to contralateral eyes of the lensed animals to avoid confounding yoking effects in which refractive error in the lens-wearing eye affects the refractive state of the fellow no lens eye.[63] Lens goggles were made from modified human polymethyl methacrylate (PMMA) contacts (8.1 mm diameter; Australian Custom Lenses, Victoria, Australia) affixed to a 22 mm Velcro loop fastener ring. The PMMA lens material transmits light uniformly across the visible spectrum (Supplementary Fig. S1). Goggles were attached to the complementary Velcro hook fastener ring glued to the periocular feathers of the right eye. The orientation of male chicks in the embryo leads to developmental asymmetries within the left and right visual pathways post-hatch.[64–66] To avoid introducing this confound into the experimental design, lenses were attached to the right eye only (rather than counterbalancing between the eyes).

Light Conditions

Within each lens condition, chicks were assigned to one of three light conditions: blue light, orange light, or white light. Each rearing box was lit with a 150 W Eye Color Arc 6500 K metal halide bulb (MT150FD; Iwasaki, Tokyo, Japan) mounted on the roof of the enclosure, providing broad spectrum lighting with a similar distribution to natural sunlight. The three light conditions were achieved by fitting glass filter shades over the light source (blue light = B410 filter, orange light = O54 filter, and white light = HA50 filter; manufactured by Hoya Candeo Optronics Corporation). The transmittance of each filter is shown in Supplementary Figure S2. Illuminance across conditions was matched using a Protech 400 K Lux Meter (QM1584), in accordance with previous research indicating that the standard CIE photopic function is appropriate for photometric calculations in chickens.[67] Chicken-adjusted illuminance measures are for each light and are provided in a Supplementary Table S3. Illuminance varied from 137 to 351 lux across the box floor (with the mean of measurement from 40 locations being 229 lux). The difference in illuminance between colored light conditions was limited to ±7 lux by adding neutral density filters (Hoya Candeo Optronics Corporation) to the light shade. Emission characteristics of the combined metal halide bulb, neutral density filters, and spectrum filters of interest were determined using an Ocean Optics Red Tide USB-650 Series Spectrometer. Figure 1A illustrates the resulting spectral emission curves in relation to the relative sensitivity of chick photoreceptors,[9] and Figures 1B and 1C estimate retinal photopigment absorbance values for each light condition.

The gfERGs were used to determine the retina's response to blue, white, and orange light rearing. For this procedure, the broadband ganzfeld light source (formed by an array of 4 white LEDs) was shaded with the same filters as used in the rearing boxes to achieve square-wave blue, white, and orange light flashes with a similar wavelength range to that in the rearing environment (Fig. 2). Note that it was not possible to use a metal halide broadband light source in the ERG setup due to its temporal properties (i.e. inability to flash on and off), so whereas the wavelength range of the ERG light flashes was matched to the rearing environment, the overall spectrum was not identical. Moreover, as illuminance was not matched across different ERG colored light flashes, responses were statistically compared within each light flash condition only (e.g. responses to blue light flashes at baseline and following blue, orange, and white rearing were compared).

Experiment Protocol

The experiment was conducted in two steps. An initial "timepoint study," henceforth called study 1, was conducted to examine how broadband orange light affects myopia induction and overall health and development (as indicated by chick weight) over time. The results of this study were used to determine the optimal rearing duration for subsequent examination of wavelength effects on myopia without the confounding influence of systemic weight gain changes. The main study in the experimental series (study 2) was then conducted to determine the effects of colored light rearing on ocular biometrics (eye growth and refraction) and retinal function during normal development and myopia induction by negative lenses. Both studies used a between-subjects design, with analyses representing the data for different groups of chicks for each relevant comparison.

In both studies, the chicks were raised under white light until day 5 post-hatch when weight and ocular biometric measures were collected while they were under light anesthesia (ketamine 20 mg/kg, xylazine 2 mg/kg, intramuscularly [IM]). Chicks were then randomly assigned to experimental lens and lighting conditions using the random number function in Microsoft Excel. For the duration of experimentation (i.e. the point of lens application until the completion of final biometric measures), all husbandry and data collection tasks were completed under ambient lighting matched to that in the rearing box. Experimenters were blind to group allocation during baseline measurements; however, blinding was not possible after the beginning of experimentation due to the visible nature of the colored lighting in the rearing boxes and procedure room.

Study 1. This study compared orange and white light conditions across 2-, 4- and 7-day timepoints in chicks wearing negative lenses. These timepoints were chosen based on the timeline of refractive compensation to –10 D lenses in chicks,[68] and previous studies in chick identifying wavelength effects following 2 to 7 days[15,25,69] that diminish[25] or do not get larger[12] with further rearing, possibly due to systemic effects associated with long wavelength light exposure.[12,25,70] Thus, following baseline biometric data collection, –10 D lenses were attached to the right eye as described above and chicks were raised for a further 2, 4, or 7 days under orange or white light. At the end of the rearing period, chicks were anesthetized (ketamine 45 mg/kg and xylazine 4.5 mg/kg, IM), refracted by retinoscopy (18240 Streak Retinoscope, Welch Allyn), 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 Escalon). 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.

Power analysis (G-Power version 3.1.9.6) determined that a minimum sample size of 70 was required to achieve 80% power to detect a medium-large effect (based on Riddell et al. [25]) using a 2-way ANOVA design. A total of 94 chicks were raised in 3 batches. No animals were excluded based on a priori criteria (animal unwell, displaying ocular complications or problematic lens attachment or cleanliness). Table 1 lists the final number of chicks included in each experimental group.

Study 2. On the basis of the refractive change statistical effect sizes and weight differences observed in study 1, 4 days of lens rearing was chosen as the optimal timepoint for the main experiment. This experiment examined ocular biometrics and retinal function in chicks reared with no lens or negative lenses under blue, white, or orange light.

Biometrics. As with study 1, power analysis indicated that a minimum sample size of 70 animals was required for biometric assessment. A total of 81 chicks were raised in 4 batches. As per a priori criteria noted above, two unwell chicks and one that developed a problem with the defocusing lens attachment were excluded from the analysis. See Table 2 for the final number of chicks included in each experimental group. Following baseline biometric measures on day 5 post-hatch, chicks were assigned to a lens (–10 D or no lens) and light (blue, orange, and white) condition and

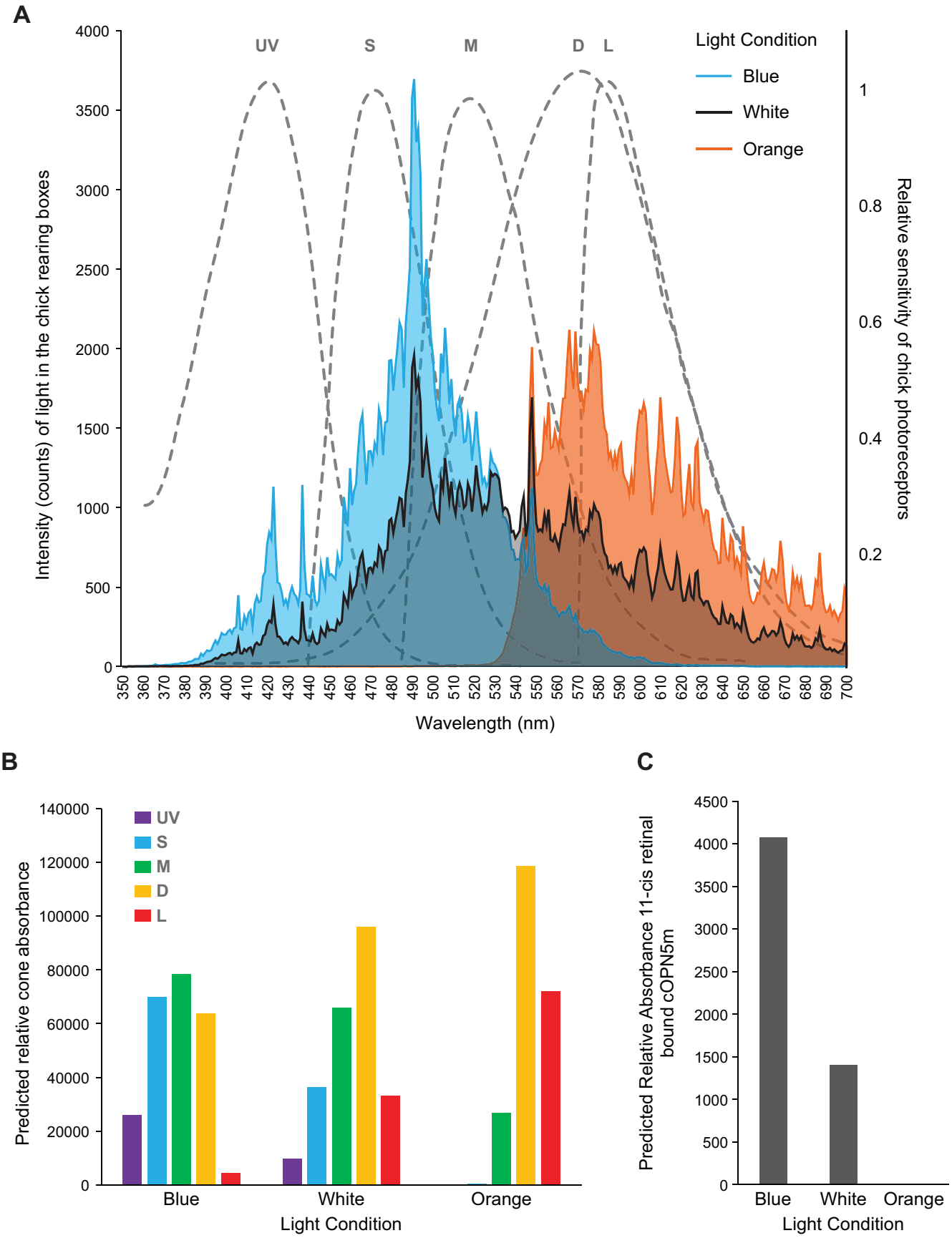

Figure 1. Spectrum of experimental light conditions relative to the sensitivity of chick retinal opsins. (**A**) Spectral emission curves of light in the rearing boxes for each light condition. Emission curves were measured with an Ocean Optics Red Tide USB-650 Series Spectrometer with illuminance matched across conditions. Chick ultraviolet (UV), short (S), medium (M), and long (L) wavelength and double

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(D) cone sensitivity data from Rucker and Wallman[9] are shown as *grey dotted lines*. Bottom graphs show predicted relative absorbance for (**B**) each cone photoreceptor type and (**C**) 11-cis retinal bound cOPN5m calculated from the spectra in panel **A** and chick sensitivity data from Rucker and Wallman[9] and Yamashita et al.[71] Note that additional non-visual opsins expressed in the inner retina are not shown,[72] and that values do not account for filtering by the ocular media.[73,74]

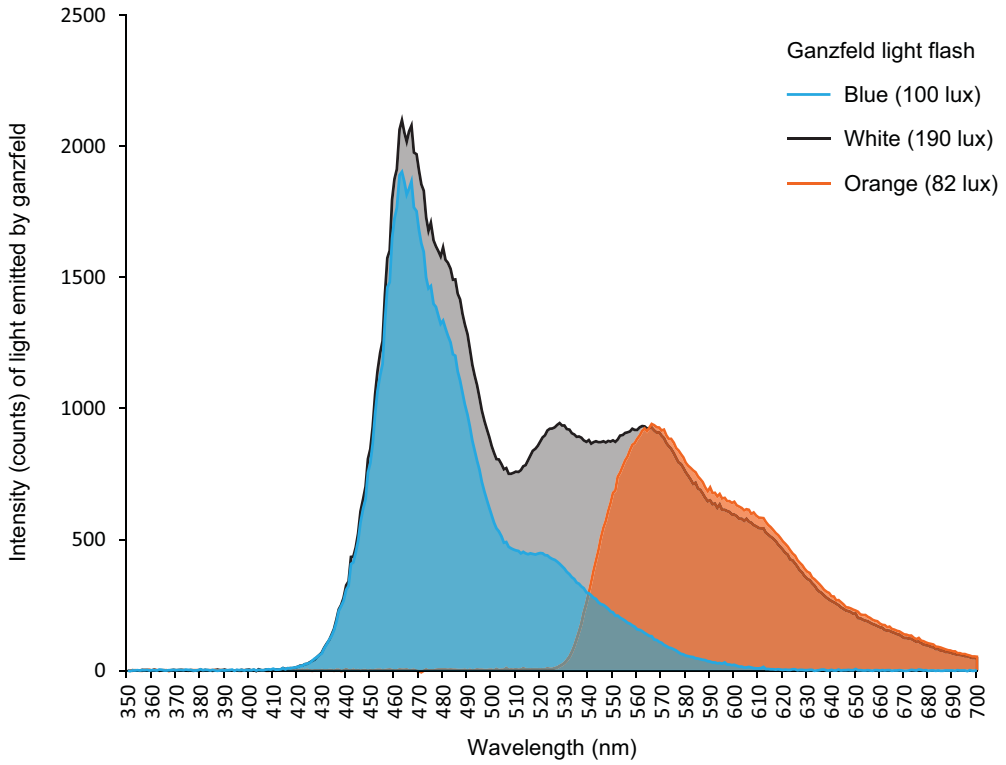

Figure 2. Spectral emission curves of light emitted by the gfERG ganzfeld stimulator. The ganzfeld light source was shaded with filters to limit the wavelengths emitted to a similar range as in the rearing boxes (note that differences in overall spectrum exist due to the necessity of using different light sources in the rearing boxes and ERG ganzfeld). Emission curves were measured with an Ocean Optics Red Tide USB-650 Series Spectrometer.

Table 1. Number of Chicks Per Group in Study 1

	Negative Lens	
Timepoint	**White Light**	**Orange Light**
2 d	15	15
4 d	16	16
7 d	16	16

Table 2. Number of Chicks Per Group for Biometric Assessment in Study 2

Light	**Negative Lens**	**No Lens**
Blue	12	12
White	13	15
Orange	13	13

raised for a further 4 days under filtered light. At the end of the rearing period, ocular axial dimension and refraction measures were collected, as described above.

Electroretinograms. Retinal function was assessed in an additional 20 animals using gfERGs before and after 4 days rearing in light of different wavelengths. Baseline testing of 4 chicks occurred on day 5 post-hatch, and experimental testing occurred following 4 days of rearing with no lenses under blue ($n = 5$), white ($n = 5$), or orange light ($n = 6$). Following induction of surgical anesthesia (ketamine 45 mg/kg and xylazine 4.5 mg/kg, IM), chicks were placed on Small Animal Temperature Controller (ATC2000, WPI) and mounted in a custom stereotaxis. A silver chloride electrode was inserted into the vitreous chamber using a catheter placement unit (24G $\frac{3}{4}$" Terumo Surflo I.V.). A silver chloride reference electrode was inserted behind the sclera, and a ground electrode was placed in the skin of the neck. The retina was stimulated using a 500 ms onset, 2000 ms offset squarewave light flash generated by a 150 mm Ganzfeld stimulator. Broadband ERGs (0.5–500 hertz [Hz] band pass filter) were recorded with a Powerlab data acquisition system (ADInstruments Pty Ltd., New South Wales, Australia) and LabChart software (version 8). A minimum of 50 responses to blue, orange, and white light flashes were recorded for each animal.

Data Analysis

Biometric Data. Biometric data were analyzed using IBM SPSS Statistics (version 28). Analysis of baseline values

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confirmed that there were no differences between groups at the start of experimentation. The end point to baseline difference values were calculated for experimental (right) eye refraction and axial dimensions, and chick weight. Data were screened for statistical outliers (via examination of boxplots and standardized residuals[75]), and 1 and 3 cases were removed from the study 1 and study 2 datasets, respectively. Missing values were handled by pairwise deletion. Data were normally distributed (skewness and kurtosis z-scores between –3.29 and 3.29). Two-way ANOVAs were used to examine the effects of induction time and light condition (study 1) or lens and filter condition (study 2) on chick weight and ocular biometric outcomes. The Tukey HSD post hoc tests were used as required. Where the assumption of heterogeneity was not met (Levene's test $P < 0.05$), the Games Howell post hoc tests were used. Raw biometric data are available in Supplementary Datasets 1 and 2. See Supplementary Figures S4 and S5 for left eye data. Figures illustrating biometric data were created using GraphPad Prism (version 9).

Electrophysiology Data. Raw ERG waves were screened for outlier traces in LabChart. Extracted text files were imported into RStudio (version 2022.07.0) where a custom script (provided in Supplementary Materials S6) was used to extract 1 second epochs around each stimulus flash (–100 ms to 900 ms) and normalize to baseline using the 100 ms epoch before flash onset. Baseline normalized data were then exported to Excel for calculation of grand mean and standard error waves, and peak data extraction using MIN, MAX and MATCH formulas. Wave amplitude was defined as the distance from waveform trough to peak, whereas implicit time was defined as the time between the flash onset and the wave peak.[76] One-way ANOVAs in IBM SPSS Statistics (version 28) were used to compare the effect of rearing conditions (baseline, and 4 days of orange, blue, and white light rearing) on the amplitude and implicit time of a-wave, b-wave, and d-wave responses to each type of colored light flash. Ratios of the a-wave to b-wave and b-wave to d-wave were also assessed, as they can assist in evaluating the neural source of differences in recorded responses. The a- to b-wave ratio indicates whether there is an abnormality in signal transmission from the photoreceptors to the bipolar cells of the inner retina,[77,78] whereas the b-wave to d-wave ratio reflects the balance between retinal ON- and OFF-pathway responses (e.g. see Refs. 79, 80). ANOVA assumptions were tested as described above for the biometric data analysis. Average waves with dynamic standard error bars were plotted using Igor Pro (version 9) and collated in Adobe Illustrator (version 2024). Raw ERG traces for each animal are available in Supplementary Dataset S3.

Results

Study 1

Prolonged Exposure to Orange Light Disrupts Weight Gain. Weight gain following negative lens attachment and rearing under orange or white light was assessed to broadly indicate how the light conditions affected systemic physiological and behavioral processes (e.g. see Re. 81). There was a significant interaction between the effects of filter rearing and induction time ($F_{2,87} = 3.644$, $P = 0.030$, $\eta_p^2 = 0.077$), such that chicks in the white light condition had gained significantly more weight than those in the orange light condition at 7 days, but not at earlier timepoints ($F_{1,30} = 4.900$, $P = 0.035$, $\eta_p^2 = 0.140$; Fig. 3).

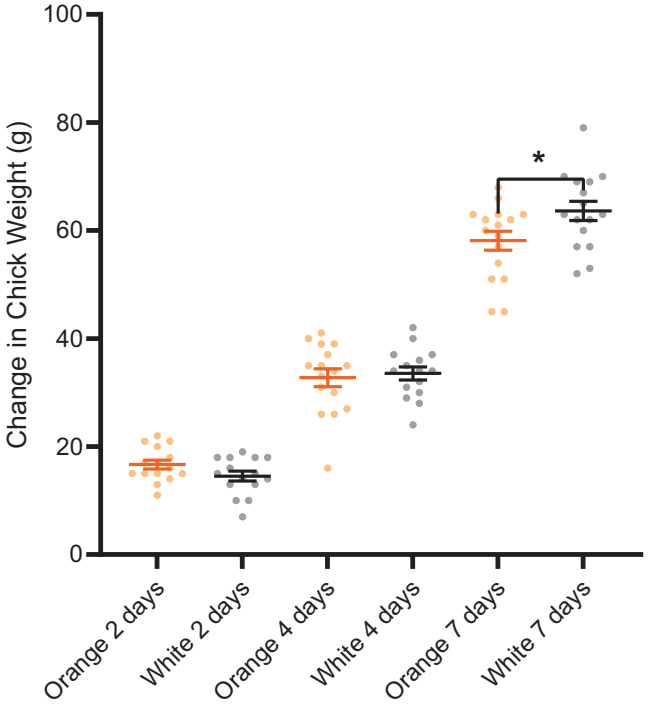

Figure 3. Mean (± SE) change in chick body weight (end point – baseline) at 2, 4, and 7 days. Individual cases are shown as dots. Chicks in the white light condition had gained significantly more weight than those in the orange light condition at 7 days ($P = 0.035$), as indicated by the *asterisks*.

Orange Light Induces an Exaggerated Lens-Induced Myopic Shift Relative to White Light. Refraction and ocular axial dimension measurements are illustrated in Figure 3. Myopic shifts in response to the –10 D lens occurred rapidly in both light groups, with refractive compensation (or overcompensation) evident by 4 days. A 2-way ANOVA identified a statistically significant main effect of filter rearing on the change in experimental eye refraction ($F_{1,87} = 11.989$, $P < 0.001$, $\eta_p^2 = 0.121$), such that chicks reared under orange light displayed a larger myopic shift than those reared under white light (Fig. 4) with large effect sizes at 4 and 7 days (η_p^2 2 days = 0.080, η_p^2 4 days = 0.138, and η_p^2 7 days = 0.154).[82] Although the average axial growth was greater in the orange condition than the white condition at all the timepoints (consistent with refraction data), the effect size was small ($\eta_p^2 = 0.012$). The main effect of filter rearing on axial growth, vitreous chamber growth, and anterior chamber growth in negative lens-wearing eyes was not statistically significant, and no significant interactions between filter rearing and induction time were observed (see Fig. 3).

Study 2

Study 1 demonstrated that the effects of the light intervention on myopic growth were most pronounced at 4 and 7 days, and that differences in weight gain (indicative of a systemic effect of the light intervention) occur at 7 days, but not at earlier timepoints. Previous studies have shown that such systemic effects can counter-

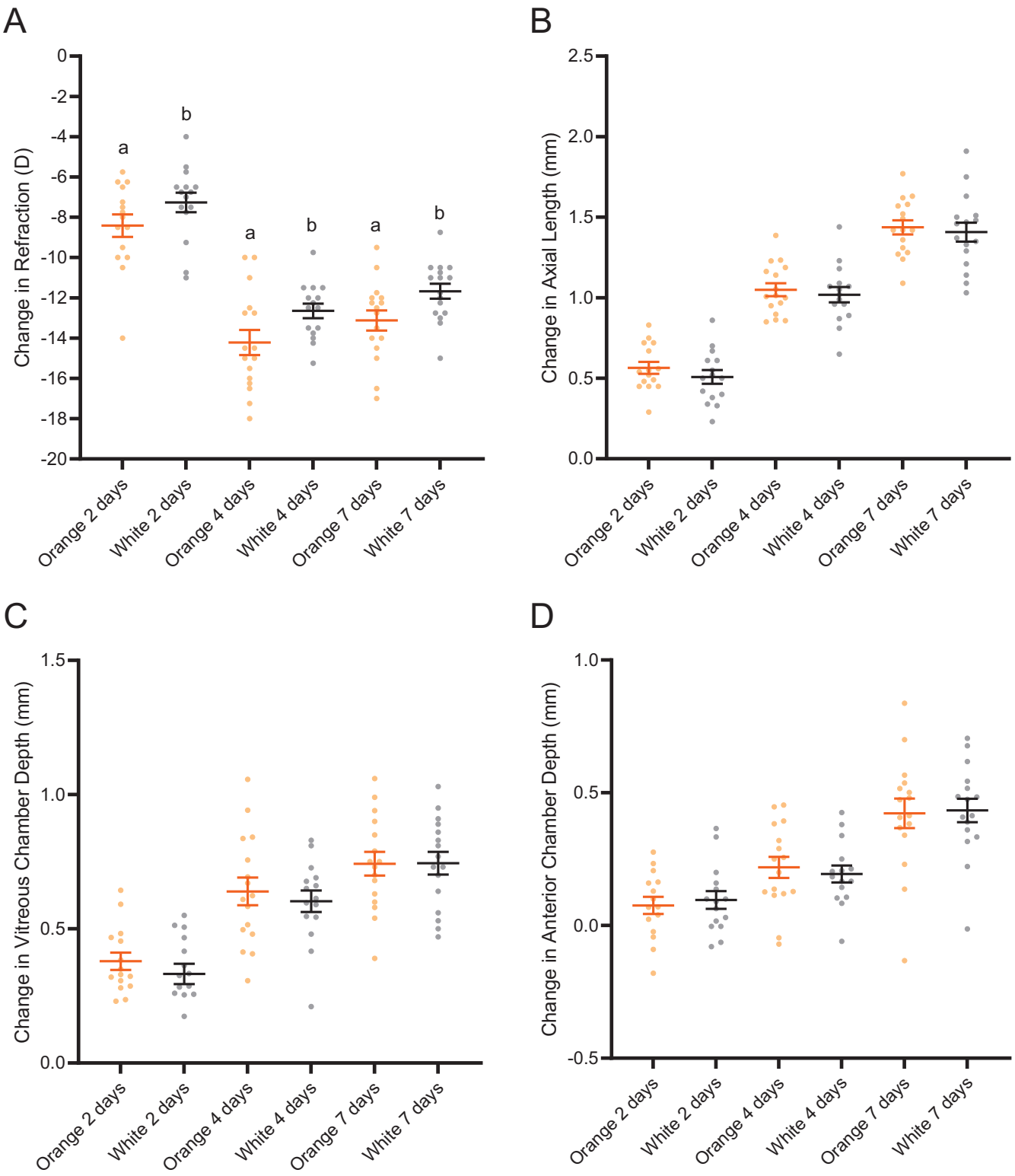

Figure 4. Mean (± SE) change in (A) refraction, (B) axial length, (C) vitreous chamber depth, and (D) anterior chamber depth following 2, 4, and 7 days of rearing with negative lenses under colored light. Individual cases are shown as *dots*. Chicks reared under orange light displayed a larger myopic shift than those reared under white light across timepoints ($P < 0.001$), as indicated by the annotations "a" and "b" in panel **A**.

act local wavelength-dependent effects and complicate the interpretation of findings,[12,25] a finding consistent with the pattern of mean axial length and vitreous chamber depths at 4 and 7 days in the present investigation. Thus, 4 day's induction was chosen as the timepoint of interest for study 2.

In study 2, following baseline measures, a –10 D lens or no lens was attached to the right eye and chicks were reared

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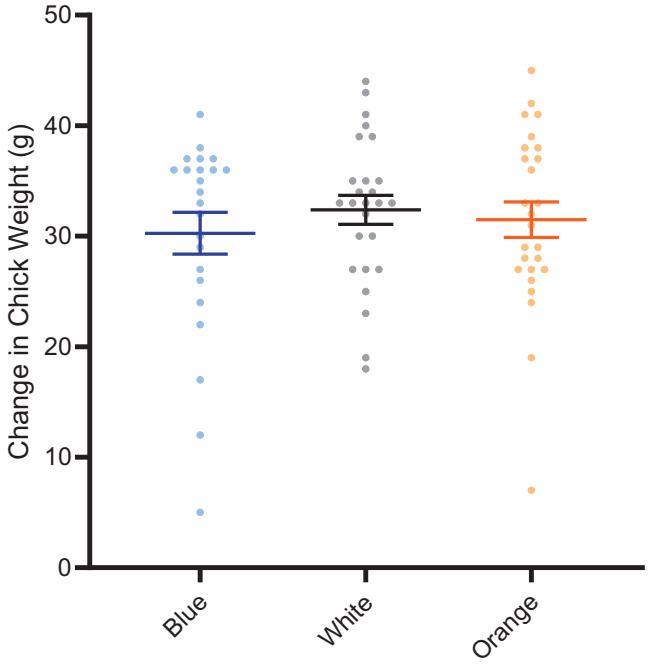

Figure 5. Mean (± SE) change in chick body weight (end point – baseline) at 4 days. Individual cases are shown as *dots*.

for a further 4 days under a blue, white, or orange light. As illustrated in Figure 5, chicks continued to gain weight during the experiment, with no differences in weight gain between groups ($P > 0.05$) as expected based on the study 1 findings.

Blue Light Suppresses Lens-Induced Myopia Relative to Orange Light. As illustrated in Figure 6, refractive compensation to negative lenses was rapid, with a myopic shift occurring in all light conditions. A 2-way ANOVA identified a statistically significant interaction between the effects of filter rearing and lens-wear on the change in experimental eye refraction at 4 days ($F_{2,69} = 4.417$, $P = 0.016$, $\eta_p^2 = 0.113$). Simple main effects analysis showed that filter rearing significantly affected the change in experimental eye refraction in the negative lens condition ($F_{2,34} = 4.683$, $P = 0.016$, $\eta_p^2 = 0.216$), but not in the no lens condition ($F_{2,35} = 0.235$, $P = 0.792$, $\eta_p^2 = 0.013$). Post hoc tests revealed that chicks reared under blue light showed a significantly smaller myopic shift in response to negative lenses than those reared under orange light ($P = 0.044$; see Fig. 6).

There were strong negative correlations between the shift in experimental eye refraction and the shift in experimental eye axial length ($r = -0.861$, $P < 0.001$) and vitreous chamber depth ($r = -0.800$, $P < 0.001$) across lens conditions at 4 days. Although effect sizes were small to medium, the main effects of filter rearing on the change in ocular axial dimensions (axial length, vitreous chamber depth, and anterior chamber depth) were not statistically significant ($P > 0.05$, η_p^2 VCD = 0.021, and η_p^2 AL = 0.066), and there were no statistically significant interactions between the effects of the lens-wear and filter rearing ($P > 0.05$, η_p^2 VCD = 0.068, and η_p^2 AL = 0.030).

Orange Light Rearing Alters gfERG Measures of Retinal Function. An additional 20 chicks were assessed electrophysiologically at baseline (day 5 post-hatch), and following 4 days of rearing under blue, white, or orange light without any lens defocus. Figure 7 illustrates the retina's functional response to blue, white, and orange squarewave light flashes for chicks in each of the light rearing conditions. One-way ANOVAs revealed a significant difference between rearing conditions in the d-wave amplitude ($F_{3,16} = 10.610$, $P < 0.001$, and $\eta^2 = 0.665$) and implicit time ($F_{3,16} = 7.238$, $P = 0.003$, and $\eta^2 = 0.576$) of responses to orange light flashes. Chicks reared under orange light for 4 days displayed a significantly greater d-wave amplitude, with longer implicit time, in response to orange light flashes than chicks at baseline (amplitude $P = 0.010$ and implicit time $P = 0.021$) and those reared under blue (amplitude $P < 0.001$ and implicit time $P = 0.006$) or white (amplitude $P = 0.020$ and implicit time $P = 0.009$) light (see Figs. 7A, 7B). The b/d-wave ratio was also significantly altered in response to orange light flashes ($F_{3,16} = 4.858$, $P = 0.014$, and $\eta^2 = 0.477$) such that chicks reared under orange light displayed a smaller b-wave to d-wave ratio than those reared under blue light ($P = 0.013$), consistent with the d-wave amplitude findings for this flash condition. This resulted in an average b/d-wave ratio in response to the orange flash following orange light rearing that was similar to those observed in response to blue and white flashes following rearing in all light conditions (Table 3). Orange light rearing did not alter functional responses to broadband white or blue flashes, and white and blue light rearing did not affect the retina's response to light flashes of any color (see Figs. 7C, 7D).

Discussion

The present research investigated the effects of orange and blue light rearing on refractive development and retinal function in the chick model of lens-induced myopia. As expected, we found that broadband orange light exacerbated myopia induction with negative lenses relative to broadband blue and white light. Changes in ocular axial dimensions consistent with the refractive effect were observed, although axial eye growth was not significantly different between groups. Furthermore, electroretinography indicated that orange light rearing caused a change in retinal sensitivity to the offset of orange light flashes (but not blue or white light flashes), whereas neither blue nor white light rearing significantly affected the retina's response to light of any color as measured using the gfERG.

Effects of Blue and Orange Light on Eye Growth and Refraction

The findings of this study are consistent with the majority of past research showing that chicks reared under light profiles dominated by longer wavelengths develop larger eyes and more myopic refractions than those reared under light profiles dominated by shorter wavelengths.[9,12–15,25] Consideration of the pattern of responses across previous studies suggests that, as expected, broadband short- or long-wavelength stimuli tend to have a lesser effect on refractive development and ocular axial dimensions than their narrowband counterparts (e.g. see Refs. 13, 25, 41, 43). For example, Foulds et al.[13] reported a 4.68 D difference between chicks reared with no lens under narrowband blue versus red light for 14 days, relative to the 2.65 D difference in refractive compensation to negative lenses reported following broadband blue and orange light rearing in the present

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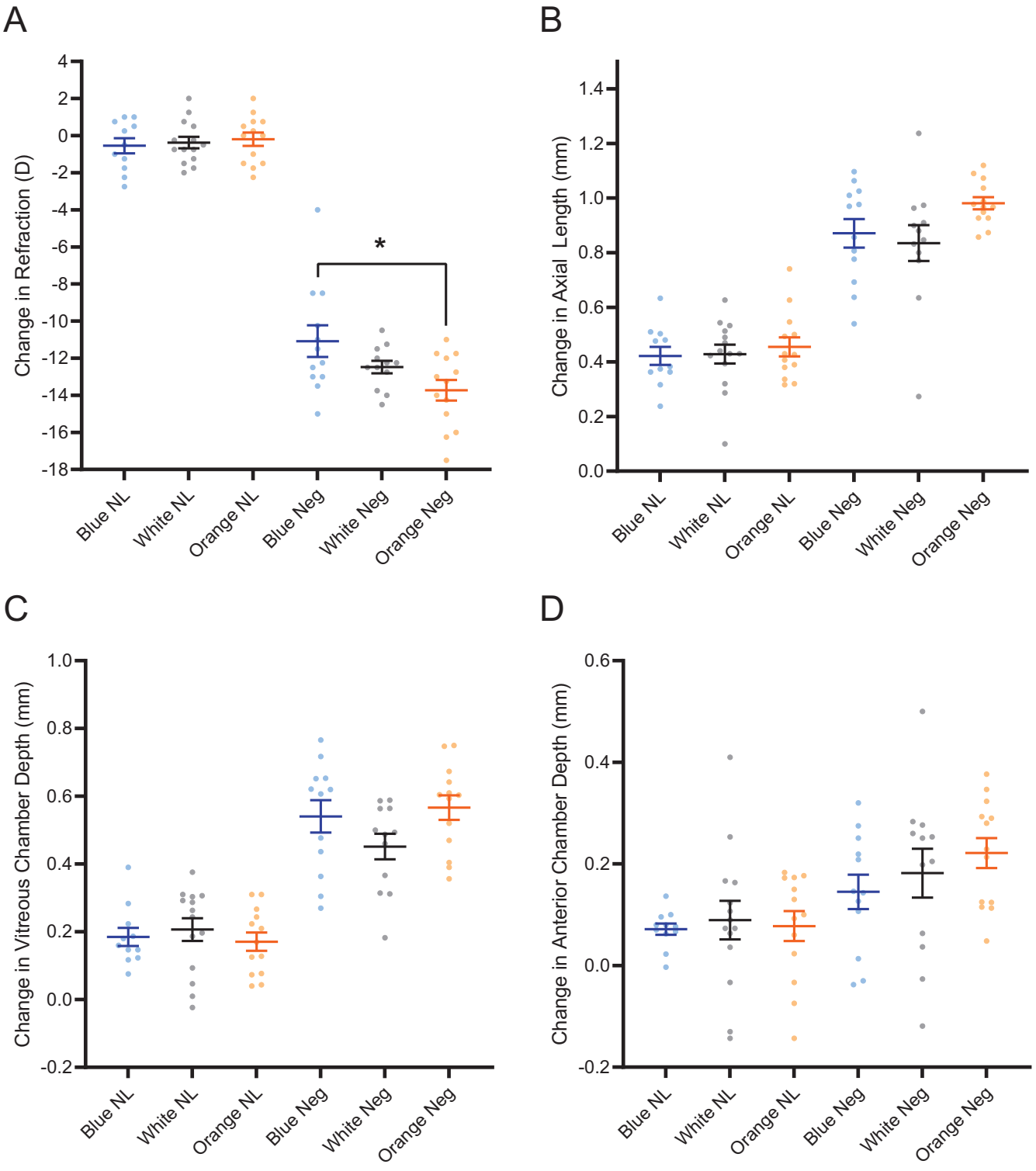

Figure 6. Mean (± SE) change in (A) refraction, (B) axial length, (C) vitreous chamber depth, and (D) anterior chamber depth following 4 days of rearing with negative lenses (Neg) or no lens (NL) under colored light. Individual cases are shown as *dots*. Chicks reared under blue light displayed a smaller myopic shift in response to negative lenses than those reared under orange light ($P = 0.044$), as indicated by the *asterisks*.

study. Despite this difference in magnitude, both responses are greater than that expected based on longitudinal chromatic aberration where a chromatic defocus of about 1.95 D is expected between 400 and 700 nm for the chick eye.[83–85] The narrowband blue conditions tested in previous chick studies, including that by Foulds et al., are typically generated using LEDs with a peak of approximately 450 to 480 nm and a narrow range, and compared to red light conditions

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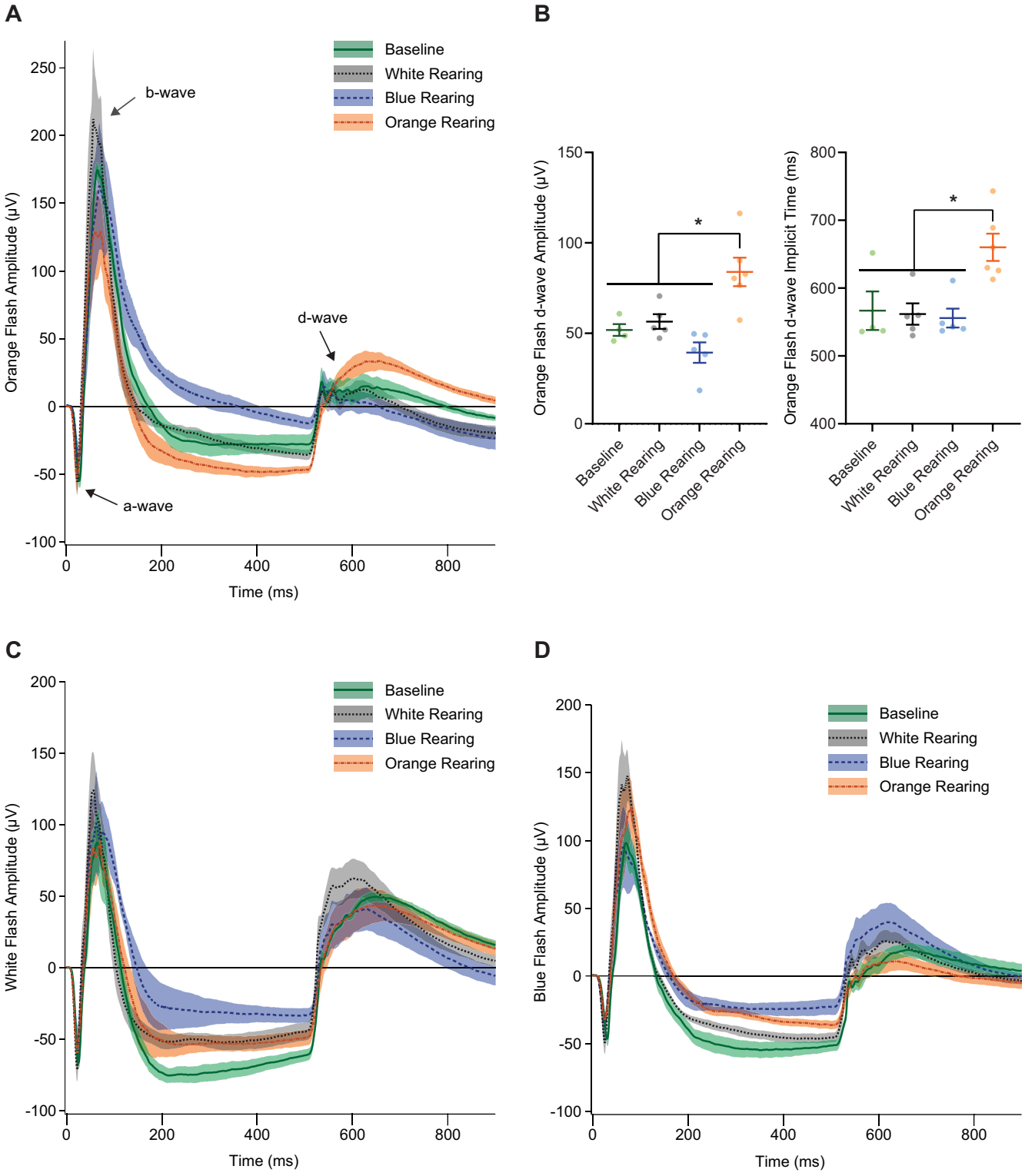

Figure 7. Retinal function as operationalized by gfERG responses to colored light flashes. (**A**) Mean (± SE) ERG responses to orange light flashes at baseline and following 4 days of rearing with no lens under white, blue, or orange light. (**B**) Mean (± SE) d-wave amplitude and d-wave implicit time extracted from the orange light response waveforms in panel **A**. (**C**) Mean (± SE) ERG responses to white light flashes at baseline and following 4 days of rearing with no lens under white, blue, or orange light. (**D**) Mean (± SE) ERG responses to blue light flashes at baseline and following 4 days of rearing with no lens under white, blue, or orange light. Chicks reared under orange light displayed larger orange flash d-wave amplitudes and longer d-wave implicit times relative to those in other conditions, as indicated by the *asterisks*.

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Table 3. Mean (± SE) B-Wave to D-Wave Ratio Across Rearing Conditions for Blue, White, and Orange gfERG Flashes

	Flash Condition		
Rearing Condition	**Blue**	**White**	**Orange**
Baseline	2.21 (0.47)	1.50 (0.25)	4.74 (0.23)
Orange	3.82 (0.78)	2.03 (0.23)	2.57 (0.37)
Blue	2.47 (0.28)	2.70 (0.42)	5.85 (0.86)
White	2.94 (0.25)	2.02 (0.33)	5.23 (0.95)

Note: As shown in Supplementary Figure S3, flash conditions are not illuminance-matched.

with a peak of approximately 620 to 640 nm (e.g. see Refs. 12, 13, 15, 86). Such blue and red lights are expected to activate UV+S and D+L cones, respectively, in chicks with relatively little activation of other cone types.[87–89] By contrast, the present study, and a previous similar study in our laboratory,[25] have filtered light sources with a continuous spectrum to produce short-wavelength and long-wavelength biased lights with a broad spectrum and peaks that are closer together (blue = 491 nm and orange = 566 nm in the present study). The main difference, in terms of cone activation, for these broadband light conditions relative to narrow-band studies is that M and D cones are predicted to be more strongly activated by both lights.

Although orange light promoted lens-induced myopia development in the negative lens wearing animals in our study, it did not significantly affect refractive development in no lens animals over 4 days. This is consistent with our previous findings in plano lens animals at 7 days,[25] and suggests that the effects of broadband orange and blue lights on refractive development are more pronounced in the presence of negative lens optical defocus (relative to normal emmetropization conditions) for short exposure times. This is consistent with previous research showing the effects of wavelength manipulations on eye growth and refraction are larger in the presence of defocusing lenses and occluder stimuli. For example, Chun et al.[86] noted a potential "synergistic" effect between blue light and optical defocus in producing a hyperopic shift, and Najjar et al.[40] reported a greater effect of blue-enriched white light (versus white light) in preventing axial eye growth in form deprivation versus contralateral control eyes. Interestingly, reports in humans also indicate that emmetropic and myopic eyes respond differently to longitudinal chromatic aberration cues.[31,90]

The 2-, 4-, and 7-day timepoints in our initial study allowed us to observe how light wavelength and negative defocusing lenses interact to affect ocular development over time. If orange light accelerated lens-induced myopia development (but did not alter the final "set point" of refraction), then an effect may not be seen at 7 days when white and orange light groups had ample time to compensate to the –10 D lenses.[68] However, if an orange light altered the "set point" of refraction, then the effect may increase or accumulate with time. Our findings are consistent with the latter interpretation; the effect of orange light on myopia development was smallest at 2 days and largest at 7 days. However, as outlined below, the effect of light on overall chick weight gain became evident at this later timepoint and may indicate a counter-effect that limits the impact of light on refractive development in prolonged studies (at least in chicks).

Colored light has a range of systemic effects such as on circadian entrainment of behavior and physiological processes,[91,92] stress and the hypothalamic-pituitary-adrenal (HPA) axis[70,93] and, in poultry, reproductive maturation.[94–96] In the present study, orange light inhibited weight gain relative to white light at 7 days (but not at the 2- and 4-day timepoints). Our previous research similarly identified decreased weight gain in chicks exposed to orange light for 10 days, an effect that appeared to counteract significant axial eye growth effects present at earlier timepoints.[25] Lin and colleagues also found that ocular axial length and vitreous chamber depth differences observed at 10 days were no longer significant following 17 days of rearing under red versus blue light, and that red light increased activity levels (without affecting weight gain). These findings are consistent with a range of studies outside of the myopia literature showing decreased weight gain[70,97,98] and higher activity and stress levels[70,96,98,99] in chicks reared under long- versus short-wavelength light, and further suggest that systemic effects may interact with local effects in the eye to determine the impact of light wavelength on eye growth and refraction over time. Notably, Gisbert et al.[44] have previously reported a hyperopia-inducing effect of broadband long wavelength light at 7 days, an effect they suggested could potentially reflect a difference between narrow-band and broadband red light responses in chicks. Our findings do not support this conclusion, although, it seems possible based on our findings that systemic growth effects of a red light could have contributed to this seemingly discrepant ocular biometric response.

Effects of Colored Light Rearing on Retinal Function

In line with the lack of biometric effect, blue light rearing in the present study did not alter retinal gfERG responses relative to baseline and white light control conditions. However, in the orange light rearing condition that promoted lens-induced myopia, we observed an increase in the amplitude and implicit time of the gfERG d-wave response to orange light offset. This functional change was limited to responses to the long-wavelength light in which the animals were reared, with orange-reared chicks displaying "normal" responses to blue and white light flashes. This pattern of results is suggestive of a specific functional adaptation to the altered light profile, similar to previous studies showing selective changes in the abundance of cone opsins during wavelength-induced refractive change.[44,47] Within this context, it is notable that the chick cone oil droplets are not fully matured until approximately 2-weeks post-hatch,[100] and the contribution of this continued development (if any) to the refractive and ERG responses measured in the present study is unknown.

The ERG d-wave is a corneal positive response with complex cellular origins, recorded at the offset of the ERG light when the stimulus is sufficiently long.[101] The initial rapid rising phase of the d-wave has been suggested to primarily reflect the activity of OFF-center (hyperpolarizing) bipolar cells,[102,103] with the remainder of the wave primarily reflecting a slower contribution made by photoreceptor depolarization at light offset.[101,102] Due to the "push-pull" nature of the ON and OFF retinal pathways,[104] hyperpolarization of ON-center bipolar cells plays a role in shaping the response, and inner retinal neurons also make a small contribution.[102,103] Examination of the d-wave shape and time-course suggests that the adaptation observed in our ERGs primarily reflects a change in the slow compo-

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nent of the wave. However, given the complex composition of the d-wave further research is needed to fully characterize the cellular origins of this response to orange light rearing.

Long wavelength flash stimuli elicit a low d-wave ERG amplitude, and consequently a high b-wave to d-wave ratio.[105] This phenomenon was observed in the present study such that the b/d-wave ratio was higher in response to orange light flashes than white and blue flashes at baseline. Due to the change in the d-wave amplitude, the b-wave to d-wave ratio in response to orange light flashes was decreased following orange light rearing such that the ratio was similar to that exhibited for other flash colors (across all rearing conditions). Like the d-wave, the b-wave has complex origins,[103,104] although, it primarily reflects the depolarization of ON-center bipolar cells in response to light onset. Thus, the ERG waveform change occurring following 4 days orange light rearing may represent a functional adaptation that assisted in "normalizing" the balance of ON and OFF pathway signaling (measured with the temporal stimulus) under orange light conditions. Similar shifts in ON/OFF pathway balance that result in relatively greater OFF pathway signaling have been linked to myopia development in many previous studies (as reviewed by Ref. 28). In humans, retinal OFF pathway stimulation with a spatiotemporal stimuli enhances accommodation-induced choroidal thinning.[106] Moreover, Poudel et al. have recently demonstrated a reduction in the ON pathway ERG response to mid-contrast temporal stimuli in human myopes.[51] In the present study, ERGs were recorded in the "no lens" animals following exposure to different light wavelength rearing conditions. Given Poundel et al.'s[51] findings, it would be of interest to explore the combined effects of light wavelength and defocus-induced myopia on the gfERG in the future.

Limitations and Future Directions

The illuminance of the lights used in our study was low, relative to some previous work (e.g. see Refs. 12, 43, 107). Yoon et al. demonstrated that a hyperopic shift occurred in response to a broadband "equal" light condition at 985 lux, but not at lower illuminances (70 and 680 lux) in chicks. The authors suggested that a "threshold" level of cone contrast may be needed to generate a wavelength defocus signal and a molecular response.[43,107] Our results demonstrated a refractive impact of short- versus long-wavelength biased light at 229 lux, demonstrating that the "threshold" necessary to generate a response was met for our light conditions. It would be of interest to investigate in the future whether the size of this effect is altered at higher illuminances. Indeed, because matching the intensity of light conditions requires a trade-off of different biological considerations, it would be optimal to compare different spectrums at a range of light intensities in the future. Our light sources were well matched based on human lux measures, and also appeared relatively well matched when considering lux measures that were adjusted (using psychophysical and electrophysiological data) to account for differences in the relative sensitivity of chicks to light across the visible spectrum. However, they varied in power, because short wavelength light must be a higher irradiance than long wavelength light to achieve the same intensity in lux. High intensity short wavelength light can damage ocular tissues[108,109] and excite a range of non-visual opsins[72] (e.g. our blue light was expected to strongly stimulate OPN5, with the orange light providing minimal stimulation for this opsin). These biological effects of energy at the blue end of the spectrum have been implicated previously in ocular growth regulation[24,110] and warrant further investigation.

We were unable to precisely match the spectrum or illuminance of the orange, blue, and white light ERG flashes with that of the light in the chick rearing environment. Typically, gfERG investigations of retinal function use a white light stimulus with a fast onset/offset profile.[76] In this study, we used the same wavelength filters from the chick rearing environment to limit the wavelength range of the broadband white gfERG ganzfeld light stimulus, producing orange, white, and blue light flashes with a square-wave temporal ON/OFF profile. This novel approach identified changes in retinal function not apparent in a previous ERG investigation of wavelength-induced myopia, which used broad spectrum white light flashes that were too short to separately assess responses to stimulus onset and offset.[47] Given the limited understanding of wavelength-dependent refractive responses across species, our findings highlight the need for further electrophysiological studies evaluating functional ON and OFF pathway responses across the visible spectrum in animal models. As the present research identified a functional ERG adaptation to orange light within 4 days in chicks, how the ERG response is affected by longer-term orange light exposure is an important question for future research with potential implications for the continued efficacy of wavelength-biased light sources in clinical myopia-control settings. As our ganzfeld ERG stimulus assessed temporal ON/OFF responses only, future assessments of how light wavelength manipulations affect spatiotemporal responses (as have been associated with myopia in other contexts, e.g. see Ref. 106) would also aid in further understanding the parameters of ON/OFF pathway functional impacts.

Conclusions

As expected, we found that orange light rearing exacerbated defocus-induced myopia relative to blue and white light rearing. Effect sizes were largest at 4 and 7 days, with overcompensation to the lenses at these times suggesting that the light source altered the "set point" of refraction in the model rather than the speed of refractive compensation to lenses. ERG recordings demonstrated that, in addition to promoting myopia, orange light rearing increased the sensitivity of the d-wave response to orange light offset, suggesting a potential role for ON/OFF pathway balance in generating the refractive response that requires further electrophysiological and molecular investigation.

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References

1. Modjtahedi BS, Ferris FL, Hunter DG, Fong DS. Public health burden and potential interventions for myopia. *Ophthalmology*. 2018;125:628–630.
2. Seet B, Wong TY, Tan DT, et al. Myopia in Singapore: taking a public health approach. *Br J Ophthalmol*. 2001;85:521–526.
3. Lee YY, Lo CT, Sheu SJ, Lin JL. What factors are associated with myopia in young adults? A survey study in Taiwan military conscripts. *Invest Ophthalmol Vis Sci*. 2013;54:1026–1033.
4. Tsai TH, Liu YL, Ma IH, et al. Evolution of the prevalence of myopia among Taiwanese schoolchildren a review of survey data from 1983 through 2017. *Ophthalmology*. 2021;128:290–301.
5. Holden BA, Fricke TR, Wilson DA, et al. Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. *Ophthalmology*. 2016;123:1036–1042.
6. Flitcroft D. The complex interactions of retinal, optical and environmental factors in myopia aetiology. *Prog Retin Eye Res*. 2012;31:622–660.
7. Modjtahedi BS, Abbott RL, Fong DS, Lum F, Tan D, Myopia TF. Reducing the global burden of myopia by delaying the onset of myopia and reducing myopic progression in children the academy's task force on myopia. *Ophthalmology*. 2021;128:816–826.
8. Bullimore MA, Brennan NA. Myopia control: why each diopter matters. *Optom Vis Sci*. 2019;96:463–465.
9. Rucker FJ, Wallman J. Cone signals for spectacle-lens compensation: differential responses to short and long wavelengths. *Vision Res*. 2008;48:1980–1991.
10. Rucker FJ, Kruger PB. Accommodation responses to stimuli in cone contrast space. *Vision Res*. 2004;44:2931–2944.
11. Kröger R, Wagner H-J. 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.
12. Lin G, Taylor C, Rucker F. Effect of duration, and temporal modulation, of monochromatic light on emmetropization in chicks. *Vision Res*. 2020;166:12–19.
13. 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.
14. Seidemann A, Schaeffel F. Effects of longitudinal chromatic aberration on accommodation and emmetropization. *Vision Res*. 2002;42:2409–2417.
15. Wang M, Schaeffel F, Jiang B, Feldkaemper M. Effects of light of different spectral composition on refractive development and retinal dopamine in chicks. *Invest Ophthalmol Vis Sci*. 2018;59:4413–4424.
16. Strickland R, Landis EG, Pardue MT. Short-wavelength (violet) light protects mice from myopia through cone signaling. *Invest Ophthalmol Vis Sci*. 2020;61:13.
17. 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.
18. Liu R, Qian Y-F, 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.
19. 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.
20. Zou L, Zhu X, Liu R, et al. Effect of altered retinal cones/opsins on refractive development under monochromatic lights in guinea pigs. *J Ophthalmol*. 2018;2018:9197631.
21. Qian YF, Liu R, Dai JH, Chen MJ, Zhou XT, Chu RY. Transfer from blue light or green light to white light partially reverses changes in ocular refraction and anatomy of developing guinea pigs. *J Vis*. 2013;13:16.
22. Tian T, Zou L, Wu S, Liu H, Liu R. Wavelength defocus and temporal sensitivity affect refractive development in guinea pigs. *Invest Ophthalmol Vis Sci*. 2019;60:2173–2180.
23. Wen Y, Dai B, Zhang X, et al. Retinal transcriptomics analysis reveals the underlying mechanism of disturbed emmetropization induced by wavelength defocus. *Curr Eye Res*. 2022;47:908–917.
24. Jiang X, Pardue MT, Mori K, et al. Violet light suppresses lens-induced myopia via neuropsin (OPN5) in mice. *Proc Natl Acad Sci USA*. 2021;118:e2018840118.
25. Riddell N, Crewther SG, Murphy MJ, Tani Y. Long-wavelength-filtered light transiently inhibits negative lens-induced axial eye growth in the chick myopia model. *Transl Vis Sci Technol*. 2021;10:38.
26. Jeong H, Kurihara T, Jiang X, et al. Suppressive effects of violet light transmission on myopia progression in a mouse model of lens-induced myopia. *Exp Eye Res*. 2023;228:109414.
27. Rucker F. Monochromatic and white light and the regulation of eye growth. *Exp Eye Res*. 2019;184:172–182.
28. Schaeffel F, Swiatczak B. Mechanisms of emmetropization and what might go wrong in myopia. *Vision Res*. 2024;220:108402.
29. Thakur S, Dhakal R, Verkicharla PK. Short-term exposure to blue light shows an inhibitory effect on axial elongation in human eyes independent of defocus. *Invest Ophthalmol Vis Sci*. 2021;62:22.
30. Swiatczak B, Schaeffel F. Effects of short-term exposure to red or near-infrared light on axial length in young human subjects. *Ophthalmic Physiol Opt*. 2024;44:954–962.
31. Swiatczak B, Schaeffel F. Myopia: why the retina stops inhibiting eye growth. *Sci Rep*. 2022;12:21704.
32. Mori K, Torii H, Hara Y, et al. Effect of violet light-transmitting eyeglasses on axial elongation in myopic children: a randomized controlled trial. *J Clin Med*. 2021;10:5462.
33. Torii H, Mori K, Okano T, et al. Short-term exposure to violet light emitted from eyeglass frames in myopic children: a randomized pilot clinical trial. *J Clin Med*. 2022;11:6000.
34. Tang J, Liao Y, Yan N, et al. Efficacy of repeated low-level red-light therapy for slowing the progression of childhood myopia: a systematic review and meta-analysis. *Am J Ophthalmol*. 2023;252:153–163.
35. Zhou W, Liao Y, Wang W, et al. Efficacy of different powers of low-level red light in children for myopia control. *Ophthalmology*. 2024;131:48–57.
36. Zhao H-L, Jiang J, Yu J, Xu H-M. Role of short-wavelength filtering lenses in delaying myopia progression and amelioration of asthenopia in juveniles. *Int J Ophthalmol*. 2017;10:1261–1267.
37. Gawne TJ, Samal AV, She Z. The effects of intensity, spectral purity and duty cycle on red light-induced hyperopia in tree shrews. *Ophthalmic Physiol Opt*. 2023;43:1419–1426.
38. Khanal S, Norton TT, Gawne TJ. Amber light treatment produces hyperopia in tree shrews. *Ophthalmic Physiol Opt*. 2021;41:1076–1086.
39. Smith EL, Hung L-F, 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.

40. Najjar RP, Chao De La Barca JM, Barathi VA, et al. Ocular growth and metabolomics are dependent upon the spectral content of ambient white light. *Sci Rep*. 2021;11:7586.
41. Gisbert S, Wahl S, Schaeffel F. Impact of cone abundancy ratios and light spectra on emmetropization in chickens. *Exp Eye Res*. 2022;219:109086.
42. Muralidharan AR, Low SW, Lee YC, et al. Recovery from form-deprivation myopia in chicks is dependent upon the fullness and correlated color temperature of the light spectrum. *Invest Ophthalmol Vis Sci*. 2022;63:16.
43. Yoon H, Taylor CP, Rucker F. Spectral composition of artificial illuminants and their effect on eye growth in chicks. *Exp Eye Res*. 2021;207:108602.
44. Gisbert S, Feldkaemper M, Wahl S, Schaeffel F. Interactions of cone abundancies, opsin expression, and environmental lighting with emmetropization in chickens. *Exp Eye Res*. 2020;200:108205.
45. Li WT, Lan WZ, Yang SQ, et al. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. *Invest Ophthalmol Vis Sci*. 2014;55:6324–6332.
46. Hu M, Hu Z, Xue L, et al. Guinea pigs reared in a monochromatic environment exhibit changes in cone density and opsin expression. *Exp Eye Res*. 2011;93:804–809.
47. Ji S, Mao X, Zhang Y, Ye L, Dai J. Contribution of M-opsin-based color vision to refractive development in mice. *Exp Eye Res*. 2021;209:108669.
48. Westbrook AM, Crewther DP, Crewther SG. Cone receptor sensitivity is altered in form deprivation myopia in the chicken. *Optom Vis Sci*. 1999;76:326–338.
49. Zahra S, Murphy MJ, Crewther SG, Riddell N. Flash electroretinography as a measure of retinal function in myopia and hyperopia: a systematic review. *Vision*. 2023;7:15.
50. Gupta SK, Chakraborty R, Verkicharla PK. Electroretinogram responses in myopia: a review. *Doc Ophthalmol*. 2022;145:77–95.
51. Poudel S, Jin J, Rahimi-Nasrabadi H, et al. Contrast sensitivity of ON and OFF human retinal pathways in myopia. *J Neurosci*. 2024;44:e1487232023.
52. Riddell N, Murphy MJ, Crewther SG. Electroretinography and gene expression measures implicate phototransduction and metabolic shifts in chick myopia and hyperopia models. *Life*. 2021;11:501.
53. Fujikado T, Hosohata J, Omoto T. ERG of form deprivation myopia and drug induced ametropia in chicks. *Curr Eye Res*. 1996;15:79–86.
54. Joesch M, Meister M. A neuronal circuit for colour vision based on rod–cone opponency. *Nature*. 2016;532:236–239.
55. Pasmanter N, Occelli LM, Komaromy AM, Petersen-Jones SM. Use of extended protocols with nonstandard stimuli to characterize rod and cone contributions to the canine electroretinogram. *Doc Ophthalmol*. 2022;144:81–97.
56. Chakraborty R, Na Park H, Hanif AM, Sidhu CS, Iuvone PM, Pardue MT. ON pathway mutations increase susceptibility to form-deprivation myopia. *Exp Eye Res*. 2015;137:79–83.
57. Wang M, Aleman AC, Schaeffel F. Probing the potency of artificial dynamic ON or OFF stimuli to inhibit myopia development. *Invest Ophthalmol Vis Sci*. 2019;60:2599–2611.
58. Crewther S, Crewther DP. Inhibition of retinal ON/OFF systems differentially affects refractive compensation to defocus. *Neuroreport*. 2003;14:1233–1237.
59. Crewther D, Crewther S, Xie R. Changes in eye growth produced by drugs which affect retinal ON or OFF responses to light. *J Ocul Pharmacol Ther*. 1996;12:193–208.
60. Wisely CE, Sayed JA, Tamez H, et al. The chick eye in vision research: an excellent model for the study of ocular disease. *Prog Retin Eye Res*. 2017;61:72–97.
61. Wortel J, Rugenbrink H, Nuboer J. The photopic spectral sensitivity of the dorsal and ventral retinae of the chicken. *J Comp Physiol A*. 1987;160:151–154.
62. Percie du Sert N, Ahluwalia A, Alam S, et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol*. 2020;18:e3000411.
63. Wildsoet C, Wallman J. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Res*. 1995;35:1175–1194.
64. Rogers LJ, Bolden SW. Light-dependent development and asymmetry of visual projections. *Neurosci Lett*. 1991;121:63–67.
65. Rogers LJ. Light input and the reversal of functional lateralization in the chicken brain. *Behav Brain Res*. 1990;38:211–221.
66. Rogers LJ, Sink HS. Transient asymmetry in the projections of the rostral thalamus to the visual hyperstriatum of the chicken, and reversal of its direction by light exposure. *Exp Brain Res*. 1988;70:378–384.
67. Saunders JE, Jarvis JR, Wathes CM. Calculating luminous flux and lighting levels for domesticated mammals and birds. *Animal*. 2008;2:921–932.
68. Riddell N, Giummarra L, Hall NE, Crewther SG. Bidirectional expression of metabolic, structural, and immune pathways in early myopia and hyperopia. *Front Neurosci*. 2016;10:390.
69. Hammond DS, Wildsoet CF. Compensation to positive as well as negative lenses can occur in chicks reared in bright UV lighting. *Vision Res*. 2012;67:44–50.
70. Archer GS. Color temperature of light-emitting diode lighting matters for optimum growth and welfare of broiler chickens. *Animal*. 2018;12:1015–1021.
71. Yamashita T, Ohuchi H, Tomonari S, Ikeda K, Sakai K, Shichida Y. Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. *P Natl Acad Sci USA*. 2010;107:22084–22089.
72. Guido ME, Marchese NA, Rios MN, et al. Non-visual opsins and novel photo-detectors in the vertebrate inner retina mediate light responses within the blue spectrum region. *Cell Mol Neurobiol*. 2020;42:59–83.
73. Lind O, Kelber A. Avian colour vision: effects of variation in receptor sensitivity and noise data on model predictions as compared to behavioural results. *Vision Res*. 2009;49:1939–1947.
74. Lind O, Mitkus M, Olsson P, Kelber A. Ultraviolet vision in birds: the importance of transparent eye media. *Proc Biol Sci*. 2014;281:20132209.
75. Tabachnick BG, Fidell LS, Ullman JB. *Using multivariate statistics*. Boston, MA: Pearson; 2007.
76. Robson AG, Frishman LJ, Grigg J, et al. ISCEV Standard for full-field clinical electroretinography (2022 update). *Doc Ophthalmol*. 2022;144:165–177.
77. Perlman I. Relationship between the amplitudes of the b wave and the a wave as a useful index for evaluating the electroretinogram. *Br J Ophthalmol*. 1983;67:443–448.
78. Perlman I, Meyer E, Haim T, Zonis S. Retinal function in high refractive error assessed electroretinographically. *Br J Ophthalmol*. 1984;68:79–84.
79. Popova E, Kupenova P. Effects of dopamine receptor blockade on the intensity-response function of ERG b- and d-waves in dark adapted eyes. *Vision Res*. 2013;88:22–29.
80. Yamamoto S, Hayashi M, Tsuruoka M, et al. Selective reduction of S-cone response and on-response in the cone electroretinograms of patients with X-linked retinoschisis. *Graefes Arch Clin Exp Ophthalmol*. 2002;240:457–460.

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81. Prayitno DS, Phillips CJ, Stokes DK. The effects of color and intensity of light on behavior and leg disorders in broiler chickens. *Poult Sci*. 1997;76:1674–1681.
82. Richardson JTE. Eta squared and partial eta squared as measures of effect size in educational research. *Educ Res Rev Neth*. 2011;6:135–147.
83. Mandelman T, Sivak JG. Longitudinal chromatic aberration of the vertebrate eye. *Vision Res*. 1983;23:1555–1559.
84. Rohrer B, Schaeffel F, Zrenner E. Longitudinal chromatic aberration and emmetropization: results from the chicken eye. *J Physiol*. 1992;449:363–376.
85. Wildsoet CF, Howland HC, Falconer S, Dick K. Chromatic aberration and accommodation: their role in emmetropization in the chick. *Vision Res*. 1993;33:1593–1603.
86. Chun RK, Choy KY, Li KK, Lam TC, Tse DY, To CH. Additive effects of narrowband light and optical defocus on chick eye growth and refraction. *Eye Vis (Lond)*. 2023;10:15.
87. Bowmaker J, Knowles A. The visual pigments and oil droplets of the chicken retina. *Vision Res*. 1977;17:755–764.
88. Bowmaker JK, Heath L, Wilkie S, Hunt D. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res*. 1997;37:2183–2194.
89. Bowmaker JK, Hunt DM. Evolution of vertebrate visual pigments. *Curr Biol*. 2006;16:R484–R489.
90. Swiatczak B. Inhibiting myopia development by the digital chromatic filters. *Acta Ophthalmol*. 2024;102:16413.
91. Walmsley L, Hanna L, Mouland J, et al. Colour as a signal for entraining the mammalian circadian clock. *PLoS Biol*. 2015;13:e1002127.
92. Spitschan M, Lucas RJ, Brown TM. Chromatic clocks: color opponency in non-image-forming visual function. *Neurosci Biobehav Rev*. 2017;78:24–33.
93. Robertson-Dixon I, Murphy MJ, Crewther SG, Riddell N. The influence of light wavelength on human HPA axis rhythms: a systematic review. *Life*. 2023;13:1968.
94. Baxter M, Joseph N, Osborne V, Bedecarrats G. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poult Sci*. 2014;93:1289–1297.
95. Harrison PC, Latshaw JD, Casey JM, McGinnis J. Influence of decreased length of different spectral photoperiods on testis development of domestic fowl. *J Reprod Fertil*. 1970;22:269–275.
96. Lewis P, Morris T. Poultry and coloured light. *Worlds Poult Sci J*. 2000;56:189–207.
97. Wabeck C, Skoglund W. Influence of radiant energy from fluorescent light sources on growth, mortality, and feed conversion of broilers. *Poult Sci*. 1974;53:2055–2059.
98. Prayitno D, Phillips C, Omed H. The effects of color of lighting on the behavior and production of meat chickens. *Poult Sci*. 1997;76:452–457.
99. Remonato Franco B, Shynkaruk T, Crowe T, et al. Light color and the commercial broiler: effect on behavior, fear, and stress. *Poult Sci*. 2022;101:102052.
100. López R, López-Gallardo M, Busturia I, Anezary L, Prada C. Spatial and temporal patterns of growth and differentiation of cone oil droplets in the chick retina. *J Neurosci Res*. 2005;79:401–411.
101. Sustar M, Holder GE, Kremers J, et al. ISCEV extended protocol for the photopic On-Off ERG. *Doc Ophthalmol*. 2018;136:199–206.
102. Ueno S, Kondo M, Ueno M, Miyata K, Terasaki H, Miyake Y. Contribution of retinal neurons to d-wave of primate photopic electroretinograms. *Vision Res*. 2006;46:658–664.
103. Khan NW, Kondo M, Hiriyanna KT, Jamison JA, Bush RA, Sieving PA. Primate retinal signaling pathways: suppressing ON-pathway activity in monkey with glutamate analogues mimics human CSNB1-NYX genetic night blindness. *J Neurophysiol*. 2005;93:481–492.
104. Sieving PA, Murayama K, Naarendorp F. Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. *Vis Neurosci*. 1994;11:519–532.
105. Sustar M, Hawlina M, Brecelj J. ON-and OFF-response of the photopic electroretinogram in relation to stimulus characteristics. *Doc Ophthalmol*. 2006;113:43–52.
106. Hoseini-Yazdi H, Read SA, Alonso-Caneiro D, Collins MJ. Retinal OFF-pathway overstimulation leads to greater accommodation-induced choroidal thinning. *Invest Ophthalmol Vis Sci*. 2021;62:5.
107. Rucker FJ, Eskew RT, Jr., Taylor C. Signals for defocus arise from longitudinal chromatic aberration in chick. *Exp Eye Res*. 2020;198:108126.
108. Tao J-X, Zhou W-C, Zhu X-G. Mitochondria as potential targets and initiators of the blue light hazard to the retina. *Oxid Med Cell Longev*. 2019;2019:6435364.
109. Ouyang X, Yang J, Hong Z, Wu Y, Xie Y, Wang G. Mechanisms of blue light-induced eye hazard and protective measures: a review. *Biomed Pharmacother*. 2020;130:110577.
110. Jiang X, Pardue MT, Mori K, et al. Violet light suppresses lens-induced myopia via neuropsin (OPN5) in mice. *Proceedings of the National Academy of Sciences*. 2021;118(22):e2018840118.

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