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Juvenile Tree Shrews Do Not Maintain Emmetropia in Narrow-Band Blue Light

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Abstract

Significance: In spectrally broad-band light, an emmetropization mechanism in post-natal eyes uses visual cues to modulate the growth of the eye to achieve and maintain near-emmetropia. When we restricted available wavelengths to narrow-band blue light, juvenile tree shrews (diurnal dichromatic mammals closely related to primates) developed substantial refractive errors suggesting that feedback from defocus-related changes in the relative activation of long- (LWS) and short- (SWS) wavelength-sensitive cones are essential to maintain emmetropia.

Purpose: To examine the effects of narrow-band ambient blue light on refractive state in juvenile tree shrews that had completed initial emmetropization (decrease from hyperopia toward emmetropia).

Methods: Animals were raised in fluorescent colony lighting until they began blue-light treatment at 24 days of visual experience (DVE) at which age they had achieved age-normal low hyperopia (mean±SEM refractive error 1.2±0.5 D). Arrays of LEDs, placed atop the cage, produced wavelengths of 457 nm (5 animals) or 464 nm (5 animals), flickered in a pseudo-random pattern (temporally broad-band). A third group of 5 animals was exposed to steady 464 nm blue light. Illuminance on the floor of the cage was 300 to 500 human lux. Non-cycloplegic autorefractor measures were made daily for a minimum of 11 days and up to 32 days. Seven age-matched animals were raised in colony light.

Results: The refractive state of all blue-treated animals moved outside the 95% confidence limits of the colony-light animals' refractions. Most first moved toward hyperopia; then refractive state decreased monotonically and, in some animals, passing through emmetropia, becoming myopic.

Conclusions: From the tree shrew cone absorbance spectra, the narrow-band blue light stimulated both LWS and SWS cones, but the relative activation would not change with the refractive state. This removed feedback from longitudinal chromatic aberration that may be essential to maintain emmetropia. (292 words)

Keywords

emmetropization; myopia; animal models; refraction; wavelength; longitudinal chromatic aberration

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It is well established that, in many species (including humans, monkeys, tree shrews, and chicks), an emmetropization mechanism operates in postnatal eyes, using refractive-error cues to dynamically adjust the eye's elongation rate to match the retinal location to that eye's focal plane.[1–5] By fine-tuning the eye's growth, the emmetropization mechanism achieves *and actively maintains* approximate emmetropia (typically, a slight hyperopia) as the eye continues to grow throughout the postnatal period and into adulthood.[1,2,6–10] It is a failure of this mechanism to use visual cues to correctly guide the continued eye growth that is responsible for most human myopia. Therefore, understanding what these visual cues are, and how the emmetropization mechanism interprets them, is of high clinical relevance.

Animal experiments have determined that the emmetropization mechanism can respond appropriately not just to the presence of refractive error, but also to its sign.[1,4,6,9,11–21] Hyperopia (focal plane behind the retina), either naturally occurring or induced by placing a minus-power lens in front of the eye, causes the emmetropization mechanism to increase the axial (vitreous chamber) elongation rate. This moves the retina outwards to the focal plane and reduces the hyperopia so the eye becomes nearly emmetropic while wearing the lens, ("lens compensation"). Myopia (focal plane in front of the retina), induced by placing a positive (plus-power) lens in front of the eye, produces slowed axial elongation if applied early in the postnatal period.[20, 22, 23] The slowed axial elongation, coupled with maturation of the optics, gradually moves the retina to the focal plane so that the eye becomes emmetropic while wearing the lens and hyperopic with the plus lens removed.

Although the emmetropization mechanism can determine the sign of refractive error, the visual cues used to determine this are still poorly understood. In addition to optical defocus, many have suggested that longitudinal chromatic aberration could be used to determine the sign of refractive error because long-wavelength ("red") light is focused farther from the cornea than is short-wavelength ("blue") light.[25–30] In most vertebrates, across the range of visible wavelengths, the short (blue) wavelengths focus two to three diopters closer to the cornea than do long (red) wavelengths,[32] suggesting that longitudinal chromatic aberration would have a plausibly large magnitude to be useful as a cue to the emmetropization mechanism. In a visual environment containing a broad spectrum of wavelengths, if an eye is hyperopic, the blue wavelengths within an image will be in better focus than the red wavelengths and the image contrast of the blue wavelengths will be greater than the image contrast of the red. This will be reversed if an eye is myopic. If the emmetropization mechanism uses this information as a *signal* to adjust the axial elongation rate to reduce refractive error, the image contrast will change so that, at emmetropia, a balance of image contrast at short versus long wavelengths will be achieved. Advantages of using longitudinal chromatic aberration as a cue are that the amount of longitudinal chromatic aberration is relatively constant across the surface of the retina (from center to periphery) and that its magnitude is relatively constant across both individuals and species. There is evidence that the emmetropization mechanism in chicks can make use of longitudinal chromatic aberration cues: One classic experiment using chicks showed that the eyes responded to simulations of longitudinal chromatic aberration-induced defocus as would be expected if longitudinal chromatic aberration was being used as a cue for emmetropization.[33]

In an environment that contains wavelengths across the visible spectrum (as in colony fluorescent lighting, Figure 1A), refractive error could be signaled by optical defocus, higher-order aberrations (such as spherical aberration and off-axis astigmatism), longitudinal chromatic aberration, and possibly other cues, all providing information to the emmetropization mechanism that will change over time as refractive error is reduced. In the case of longitudinal chromatic aberration, ambient light containing a range of wavelengths will produce altered image contrast at long and short wavelengths as refractive error changes over time.

However, if only a narrow band of wavelengths is present, longitudinal chromatic aberration-related feedback will be absent (or reduced to a negligible level); no matter how refractive error changes, the relative effect of the narrow band of light on the short-, middle, and long-wavelength sensitive cones will remain essentially the same. In such a condition, it is possible that the emmetropization mechanism could use other refractive error cues to achieve and maintain emmetropia.[34] However, it also is possible that if longitudinal chromatic aberration feedback is absent, that the other refractive error cues become ineffective – that longitudinal chromatic aberration feedback is an *essential* cue.

We have previously found evidence suggesting that, without longitudinal chromatic aberration feedback, other refractive error cues become ineffective.[31, 35] When infant tree shrews (dichromatic mammals closely related to primates,[36] were housed in an environment containing only narrow-band red light (Figure 1B), which stimulates the long-wavelength sensitive cones almost exclusively, the normal decrease in refraction from hyperopia to emmetropia did not occur. The growth of the eyes (primarily, vitreous chamber) was slowed and the refractions remained substantially hyperopic. When juvenile and adolescent tree shrews, that had achieved a nearly emmetropic refractive state, were exposed only to the red light, the eyes also slowed their axial elongation and became significantly hyperopic. In this condition, hyperopic defocus was ineffective as a cue. However, when returned to colony fluorescent lighting containing a wide range of wavelengths (Figure 1A), the hyperopia is quickly reduced by increased axial elongation.[31, 35] Similar results have been found in infant monkeys.[37]

If only narrow-band blue light is present, image contrast exists only for blue. However, in dichromatic mammals, the long-wavelength sensitive cone pigment absorbs the short-wavelength light (albeit less effectively (Figures 1B, 1C)), so no blue wavelength can activate the short wavelength sensitive cones without also significantly activating the long-wavelength sensitive cones (and, in tri-chromatic species, the middle wavelength sensitive cones as well). However, in narrow-band blue light, if refractive error changes over time, the ratio of excitation of all cones types remains constant, removing longitudinal chromatic aberration feedback.

In addition, in many species including humans,[38] monkeys,[39] and tree shrews,[24] the density of short wavelength sensitive cones is sparse relative to that of longer wavelength cones (in tree shrews short wavelength sensitive cones appear to be between 4 and 10% of the cone population, depending on location in the retina[40]) which likely would make it difficult to detect image defocus using the short wavelength sensitive cones. We had previously

speculated that the emmetropization mechanism might use temporal flicker as a proxy for image sharpness in short wavelengths, signaling the emmetropization mechanism that the eye is too short and triggering axial elongation that would produce myopia. We found, in infant tree shrews, that flickering blue light created significant myopia.[31] However, in steady blue light, eyes appeared to emmetropize normally.

Because the refractive state in the infant animals studied previously was changing rapidly (as in Figure 2A, leftmost data points), decreasing from hyperopia toward emmetropia, we were concerned that the shifting normal baseline refractive state and normal variability might have made it difficult to accurately assess the effect of narrow-band blue light. Thus, in the present study, we re-examined the effect of flickering, and steady, narrow-band blue light on the refractive state of juvenile tree shrews that had completed the initial phase of emmetropization, providing a more stable baseline against which to judge the effects of the blue light.

METHODS

Subjects and Experimental Groups

The tree shrews used in this study were raised by their mothers in the University of Alabama at Birmingham (UAB) Tree Shrew Core until the time of the pedestal surgery (see below) and the initiation of the experiment. This is approximately six weeks of age, which is generally the age of weaning. The colony is maintained on a 14-hour light on/10 hour light off cycle.[41–43] Fluorescent lighting (F34CW RS WM ECO) containing a wide range of wavelengths (Figure 1A) provided illuminance of 100 to 300 lux on the floor of the cages. All procedures complied with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and visual research and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Tree shrews are born with their eyes closed. It is presumed that the emmetropization mechanism does not start functioning until the eyes open and retinal image formation is possible. The first day that both eyes are open, which typically occurs about three weeks after birth, was designated as the first day of visual experience (days of visual experience). Eye opening status was checked daily, and all of our experimental manipulations were synchronized to this developmental time point. This is in accord with all previous published work from this site.

There were three blue-light treated groups (n = 5 per group) exposed to: flickering 457 ± 10 nm narrow-band light, flickering 464 ± 10 nm narrow-band light, and steady 464 ± 10 nm light. These were compared with a group (n=7) of “normal” animals raised in the fluorescent colony light, data from which was reported in previous studies.[31] All groups were balanced to include both males and females and there were no siblings in any group. All four groups were maintained on the same 14-hour light/10-hour darkness schedule.

Blue light exposure was started at 24 days of visual experience, an age when tree shrews have completed their initial steep descent from hyperopia towards emmetropization. At this

point the refractive state of colony-raised animals is relatively stable, typically around 1.2 diopters hyperopic and is very slowly trending towards emmetropia over time. All blue-treated animals had blue light exposure for a minimum of 11 days, but blue exposure was continued longer for 13 of the animals because it became apparent that a longer exposure was needed to achieve a clear understanding of the direction and magnitude of the refractive changes. Many were followed until 35 days of visual experience and one animal continued in blue light until 66 days of visual experience.

Blue Light Stimulators

Figure 1A illustrates the spectrum of the colony fluorescent lighting in which all animals were initially raised and in which our normal animals remained. It has numerous sharp peaks, but nonetheless spans the range of visible light and overlaps the absorbance spectra of both tree shrew cone types (Figure 1B). Two different blue LED arrays were used. One provided flickering blue light at a peak emission of 457 nm (Figure 1C). It consisted of an array of 5 “Royal Blue” LXML-PR02-A900 LEDs (Luxeon Star LEDS, Brantford, Ontario, Canada), that were focused by a Carclo 44° Circular beam optics onto a standard 60 cm × 60 cm lenticular diffuser mounted in a commercial metal fluorescent light fixture. This was placed on top of the cage (which was a cube 60 cm on a side). The LEDs were powered by a 3023-D-E-700, 700 mA controlled current “BuckPuck DC Driver,” itself controlled by an Arduino microcontroller.

The other LED array provided steady, or flickering, light at a peak emission of 464 nm (Figure 1C). It was comprised of six 50-cm long strips of NFLS-RGBX2 high power RGB flexible light strips (Superbright, St. Louis, Missouri), with only the blue LEDs activated. The strips were affixed to a white-painted piece of 60 cm × 60 cm plywood, placed atop the cage. The LEDs were powered by a custom-designed MOSFET circuit controlled by an Arduino microprocessor. This array was used in our previous study.[35]

In the two flickering light groups (one at each blue wavelength), the LEDs were flickered around a mean luminance in a pseudo-random temporal pattern meant to mimic the natural time-course of luminance on the retina as an animal moves around. This flicker is spectrally broad-band, containing all frequencies less than 50 Hz; i.e., it was not a single frequency. This temporal flicker pattern has been described previously, and was used because in infant tree shrews in our previous study there appeared to be a difference between steady and flickering blue light.[31]

The spectrum of the two blue wavelengths (Figure 1C) was measured with a PhotoResearch LS670 spectrophotometer. The illuminance of the light at the cage floor was measured with a LX1330B digital illuminance meter (Hisgadget, Inc.), and ranged from a mean of 300 to 500 human lux at the bottom center of the cage. The blue lights had a total irradiance power on the order of 2 watts/m2, compared to direct sunlight at the Earth’s surface which is typically on the order of 1,000 watts/m2. In the blue part of the visible spectrum (400 – 500 nm), the blue lights were more than two orders of magnitude less intense than sunlight. It therefore was unlikely that the blue lights could be damaging to the retina.

In Figure 1D, the relative absorbance by the short wavelength sensitive and long-wavelength sensitive cones is shown. This was calculated by first multiplying the power at each wavelength by the wavelength, to convert from power to photon count. Then, the dot-product was taken with the cone absorption curve. The wavelengths emitted by the 464 nm blue and the 457 nm blue both are absorbed by the long-wavelength sensitive cones as well as the short wavelength sensitive cones. However, the relative activation of the long-wavelength sensitive cones by the 457 nm LED is slightly less than that produced by the 464 nm LEDs. During blue-light treatment, the 457 nm or the 464 nm light was the sole illuminant present during the 14 h lights-on period. Thus, the *relative* activation of the long-wavelength sensitive vs. the short wavelength sensitive cones would have been essentially constant over this time (our light stimuli were not truly monochromatic but were narrow band), as would the image contrast as sensed by these cones, regardless of changes in refractive state. This would prevent any substantial feedback from longitudinal chromatic aberration.

During blue-light exposure, the animals in all three groups were free to move about their cage, and were provided with unrestricted access to food, water, and a dark gray plastic nest tube with one open end, as were the normal colony-light animals. The amount of time that the animals spent in the nest tube was not recorded but did not appear to differ across groups. The cages also contained an elevated white shelf about half-way up from the bottom of the cage floor; light levels were typically about 40% higher on the shelf than on the cage floor. The cages had solid sides and backs, but the front and top were mesh. The tree shrews could look out the front of the cages at the room walls, typically 2–3 meters away. Because the lighting arrays atop the cages were the only source of light in the room, this distant view was relatively dim. The floors of the cages were covered with newspaper. When the investigators entered the room, the animals were often found resting on the shelf, but also sometimes were on the floor of the cage or inside their nest tube. No differences in behavior were noted across the blue-light and colony light conditions.

Pedestal Installation

In order to consistently align the animals for awake refractive and axial component measures, a dental acrylic pedestal was installed on the skull of the experimental animals two to three days before treatment began at 24 days of visual experience, following procedures described previously.[44] The pedestal in the normal group was installed at 10 days of visual experience. In brief, the animals were removed from their maternal home cage and anesthetized i.m. with 100 mg/kg ketamine, 7 mg/kg xylazine. After initial anesthesia, but before the procedure began, they were given atropine i.p. 0.27 mg/kg, buprenorphine i.m. 0.02 mg/kg, and carprofen s.q. 5 mg/kg. Anesthesia was supplemented with 0.5–2.0% isoflurane as needed. After recovery from anesthesia, the animals were weaned and housed singly, or in pairs, in colony lighting until the start of blue light treatment.

Refractive and Axial Measures

The refractive measures were made in awake, gently restrained animals with a Nidek ARK-700A infrared autorefractor (Marco Ophthalmic, Jacksonville, FL) using the pedestal for alignment, viewed on a video monitor.[45] As in previous studies, non-cycloplegic

measures were made because atropine may interfere with emmetropization[46, 47] and because non-cycloplegic measures have been shown to provide a valid estimate of a wide range of refractive states in tree shrews. Measured in the same animals, cycloplegic refractions for untreated and for myopic eyes were approximately 0.8 D hyperopic in comparison to non-cycloplegic refractions (Norton TT, et al. IOVS 2000;41:ARVO E-Abstract 563)[48] indicating the presence of a small tonic accommodation in treated and control eyes. All refractive values were corrected for the "small eye artifact"[49] previously shown to be about +4 D in tree shrews.[45]

Refractive state was measured daily during treatment shortly after 9 AM, approximately 30 minutes after the lights were turned on. Animals were kept in darkness while being transported between their treatment cage and the measurement room. While measurements were made, the room was dimly illuminated only with blue LEDs similar to those in the treatment cage so that the animals did not have a period of visual stimulation that was different from the treatment conditions. The internal incandescent target light of the autorefractor was disabled to further avoid spurious visual stimulation.

Axial component dimensions were measured in all animals immediately before the start of treatment at 24 days of visual experience, after 11 days of treatment (35 days of visual experience) and at or near the end of blue-light treatment. Measures were made in awake gently restrained animals with a LenStar LS-900 optical biometer (Haag-Streit, Mason, OH USA). This system uses optical low-coherence optical interferometry, and has been found to typically give similar dimensions as other techniques such as ultrasonography, but with better repeatability.[50] The LenStar provides and stores waveforms with peaks that correspond to the front and back of the cornea, front and back of the lens, front and back of the retina, and the front and back of the choroid.[51] Off-line, the retinal cursors were manually moved to provide measures of vitreous chamber depth and choroidal thickness. All axial components (including choroid) were summed to provide a measure of axial length.

Statistical Analysis

Refractive and axial component measures were summarized in Excel spreadsheets. For all animals, left and right eyes generally had similar refractions. Therefore the data from the two eyes were averaged for each animal and treated as a single data point.[52] Refractive measures were plotted as a function of time (days of visual experience). Comparisons were made between the normal animals and the three blue groups using a two-way ANOVA ($P < 0.05$) with repeated measures using IBM SPSS Statistics 24. We also performed independent one-way ANOVAs on the refractive data both just before the start of treatment (24 days of visual experience) and after 11 days of treatment (35 days of visual experience), two time-points when all animals were measured. To explore the variability of the refractive state over time, we first calculated the mean refraction over time for the seven normal animals. We then calculated the 95% confidence interval around this mean as simply +/− two standard deviations. It is important to remember that this is not the confidence interval for the mean: this is the area in which one would expect 95% of all individual data points to fall.

In most blue-treated animals, the refractive state change over time eventually became nearly linear. The linear region was determined by eye for each animal, and the slope of the

refractive change was calculated with the Excel regression function using all data points between the start and end of this linear region.

RESULTS

Refractive Measures

The refractive measures over time for the individual animals in the colony light and blue-treatment groups are shown in Figure 2. As found previously, the refractions of the colony-reared animals were hyperopic and variable when measurements began at 11 days of visual experience.[53,54] From 11 to 24 days of visual experience, the refractions decreased toward emmetropia and became less variable. From 24 to 60 days of visual experience, the refractive state of each animal slowly declined toward emmetropia and the variability across animals continued to decrease. At 35 days of visual experience the range of the colony-light refractions (maximum individual animal refraction minus minimum refraction) was 1.2 D. By 60 days of visual experience, the range of refractive states across animals was 0.7 D. As shown in Figure 2A, the refractions of the seven colony-light animals were within the 95% confidence intervals for the group at all measurements after 24 days of visual experience.

When blue-light exposure began at 24 days of visual experience, there was no significant difference in refraction across the four groups (one-way ANOVA, $F(3,18) = 0.29$, $P = 0.833$). However, during blue-light treatment, the refractions of all animals in the three groups diverged from those of the colony-light animals. Figures 2B − D show the individual animals of each blue treatment group and the 95% confidence interval of the colony-light group. The refractive state of many (13 of 15) of the blue-light animals moved in the hyperopic direction within 1 – 4 days after the onset of treatment. The duration and magnitude of the hyperopic shift was highly variable; for some animals, it lasted only 1 day, in others, it continued for up to 10 days. For the animals that showed a brief hyperopic shift, the refractive state initially remained within the 95% confidence intervals. For those in which the hyperopic shift continued for more than 3 days, the refractive state eventually became more hyperopic than the 95% confidence intervals by 1 − 6 D.

After a variable time period, the refractions of all blue-treated animals began to decrease in the direction of myopia; when this occurred varied from animal to animal. For some, this occurred within 1 − 4 days after the onset of blue-light exposure. For others, the decrease did not begin until up to 10 days. This includes two animals in the 457 nm blue flicker group that developed substantial hyperopia and were only followed for 11 days. Refractions peaked after 7 and 10 days. For the remaining 13 animals, during the period of myopic drift, the slope of the daily decrease in refractive state (from maximum refraction to last measure) was (mean ± SEM, −0.3 ± 0.1 D/day). In six animals, members of all three blue-treatment groups, the refractions first became more hyperopic than the 95% confidence intervals and then more myopic, passing through emmetropia without an apparent change in slope. Two additional animals' refractions were decreasing slowly but were still hyperopic when treatment ended at 50 and 66 days of visual experience.

All animals were exposed to blue light for at least 11 days (until 35 days of visual experience). At this time-point the refractive state of the four groups were significantly

different from each other (one-way ANOVA, F(3,18) = 9.31 P=0.0006). The mean refraction for the normal group was 1.0±0.2 D (SEM), for the flickering 464 nm group it was −2.6±1.2 D, for the flickering 457 nm group it was 3.4±0.9 D, and the steady 464 nm group it was 0.7±0.8 D. However, the group refractive states at this time point fails to convey the diversity of the response trajectories of the individual animals in blue light. It is the increased variability and departure from the colony-light group that was the most prominent result.

Generally, during blue-light treatment, the two eyes followed the same trajectory. However, at the end of blue treatment (mean 49 days of visual experience) the absolute difference between the right and left eyes for the 15 blue-treated animals was 1.2 ± 0.2 D. This was significantly greater than the interocular difference of the colony animals at 39 days of visual experience (0.4 ± 0.1 D) or at 52 days of visual experience (0.4 ± 0.1 D) (independent t-test, $P < 0.05$) and suggests that guidance, needed to coordinate the refraction of the two eyes, was absent.

Ocular Component Dimensions

Axial component dimensions were measured on all animals at or near the last day of blue light exposure (which varied from 35 to 66 days of visual experience, see Methods). Figure 3 shows that the vitreous chamber depth was correlated with refractive state. Eyes with a larger vitreous chamber were all myopic. Vitreous chamber depth was smaller in eyes with less myopia and smaller still in the four animals that were hyperopic at the end of their (short) blue-light treatment period. This correlation had an R^2 of 0.60 and a statistically significant ($P < 0.05$) slope of −0.015 mm per diopter. None of the other ocular component dimensions showed a significant relationship with refractive state.

DISCUSSION

In Narrow-band Blue Light, Juvenile Tree Shrew Eyes Did Not Maintain Emmetropia

In the present study, the refractive state of the juvenile tree shrews, whose eyes were nearly emmetropic at the start of treatment, became unstable with continued exposure to blue light – whether flickering or steady. The refractions of all 15 blue-treated animals moved outside the 95% confidence intervals of the colony-light animals. Other cues for refractive error, such as optical defocus, did not allow the emmetropization mechanism to keep eyes within the normal range of refractive state. The refractive state of most animals in all three blue-treatment groups first moved in the hyperopic direction by varying amounts in the first few days after the onset of blue treatment. After a variable period of time, the refractive state moved toward myopia. In all three groups, some eyes which had become hyperopic then became myopic, passing through emmetropia. For those eyes optical, or other, cues were insufficient to signal the emmetropization mechanism to stop the decrease in refractive state so that it remained at emmetropia. It appears that in the absence of a wide range of wavelengths, that can provide substantial differential wavelength (longitudinal chromatic aberration) cues, juvenile tree shrew eyes are unable to maintain emmetropia.

In the present study, we found that both flickering and steady narrow-band blue light had very similar effects. In our previous study using in infant tree shrews[31] we found that

flickering blue light produced myopia while steady blue light allowed normal emmetropization (although with increased variability). Although it is possible that the effects of steady and flickering blue light changes with age, it seems more likely that by studying older animals in which the refractive state was relatively stable and less variable, we were able in the present study to more accurately assess the effect of flickering and steady blue light and found that there was little difference in the refractive responses to these conditions.

Types of Instability of Refractive State in Blue Light

The three salient effects of blue-light exposure in this study are: the instability of the refractions, the variable initial hyperopic shift that occurred in most animals, and the eventual myopic shift in refractive state. In the tree shrews raised in colony light, like others observed in past studies,[35, 53] the emmetropization mechanism produced a highly consistent refractive state that was maintained within a narrow range. The instability of the refractions in the blue light groups suggests that the feedback (error signals) normally used by the emmetropization mechanism to maintain emmetropia was greatly reduced or set to zero, allowing the eyes to drift away from age-appropriate refractions. The divergence of the refractions of the right and left eyes in the blue-treated animals (relative to normals) is also consistent with the notion that feedback to the emmetropization mechanism was reduced or absent.

The reason for the initial hyperopic shift observed in many animals is unknown. However, we speculate that differences in illuminance between the colony light and the blue light may have been a factor. The colony light, measured with as 100 − 300 lux with a meter calibrated for humans, contained light at many wavelengths. The blue light, measured as 300 − 500 lux with the same meter, may have seemed brighter to the emmetropization mechanism (especially if this mechanism heavily weights luminance using the blue-sensitive intrinsically photosensitive ganglion cells (ipRGCs), shown to be present in tree shrew retinas[55]). This may have had a similar effect as did increasing the illuminance of colony lighting to approximately 975 lux in a previous study, which also produced a small transient hyperopic shift.[35] Increasing the colony illuminance to approximately 15,000 lux also produced a hyperopic shift in a group of colony-raised juvenile animals (unpublished data). Why the hyperopic shift was so variable across animals is undetermined.

A myopic drift in the refractions was the dominant long-term effect. If observations in blue light had continued longer, it seems likely that all animals would have become myopic. The myopic drift in blue light resembles the myopic effect of dark treatment in juvenile tree shrews that had experienced colony lighting until they were a similar age to the animals in the present study.[54] During 10 days in complete darkness, where no visual guidance was available, the refractive state of all eyes shifted in the myopic direction by variable amounts. The slope of the refractive shift was −0.4 ± 0.1 D/day, slightly greater than the −0.3 ± 0.1 D/day observed in the blue-light animals. Also, the absolute difference in refraction between the right and left eyes increased during dark treatment. The similar myopic drift and reduced interocular coordination in the blue-light and dark-treated animals suggests that feedback to guide the emmetropization mechanism may have been reduced or absent during exposure to

the blue light, despite the presence of other refractive error cues. Both results also demonstrate that the maintenance of emmetropia in juvenile eyes depends on the continued functioning of the emmetropization mechanism.

The variable trajectories of the refractions in this study may help to explain the reports of the variable effect of blue light in other species. Guinea pigs have been reported to become consistently hyperopic in blue light. [56, 57] Some studies have suggested that chickens do not require wavelength cues for emmetropization, and will emmetropize normally under blue light [25, 30] or that blue light tends to produce hyperopia.[29] In the present study, most animals were maintained in blue light until the refractive trajectory was clear. Importantly, refractive state was measured daily, rather than just at the start and end of treatment. If we had compared the refractive state of the animals only at the start and end of a fixed, short period, such as the 11 days that was originally intended (before we realized how unstable the refractions were over time), we would have reported increased variability, but would have missed the overall myopic effect. If we had chosen even shorter treatment periods, we would have reported that blue light produced a hyperopic shift in refraction. We suggest that, when exploring the effects of new types of visual stimuli on refractive state, it is critical to not use fixed, short treatment period, comparing just the starting and ending refractions, but rather to follow the refractive development of experimental animals with multiple measures over a sufficient time period to determine the overall effect.

Effect of Limited Wavelength Cues at Short and Long Wavelengths

In addition to examining the effect of narrow-band blue light, we also have studied how exposure to narrow-band long wavelength (red) light affects emmetropization.[31,35,58] The effect of the red light is both similar to, and different from, the effect of the blue. Both are similar in that the eyes do not remain emmetropic if only a restricted band of wavelengths (±10 nm) is present. In both conditions, refractive error develops that is not corrected by other defocus cues. It appears that the presence of a range of wavelengths, that permit feedback from longitudinal chromatic aberration cues, may be essential for the emmetropization mechanism to perform normally. The most evident difference is that, in narrow-band red light, infant, juvenile, and adolescent tree shrews became significantly hyperopic compared to colony-light animals.[35] In contrast, the blue light produced an eventual myopic drift.

We have speculated that the very consistent red-induced hyperopia occurred because the red light only stimulates long-wavelength sensitive cones. Figure 1C shows the illuminance spectrum provided by red LEDs that were used in previous studies.[31, 35, 58] The red wavelengths were very far removed from the short wavelength sensitive cone absorption curve for tree shrews[24]; the effect on the short wavelength sensitive cones is between 5 and 6 log units less than on the long-wavelength sensitive cones (Figure 1D). Thus, the red light almost exclusively activated the long-wavelength sensitive cones and the post-receptoral retinal circuitry with long-wavelength sensitive -cone input. This may have caused the emmetropization mechanism to signal that the eye was too long for its optics because image contrast at red was present, but image contrast at the blue end of the spectrum was not detected, a condition that would be associated with the blue wavelengths being very far out-

of-focus. It would follow that the emmetropization mechanism would slow the rate of axial elongation, producing eyes that are hyperopic. It may be that the narrow-band blue light, because it stimulates the short wavelength sensitive as well as the long-wavelength sensitive cones, cancels the hyperopic effect of long-wavelength sensitive cone activation.

Although exposure to an environment that only contains a narrow band of long or short wavelengths appears to reduce the ability of other visual cues to provide effective input to the emmetropization mechanism, these cues can still affect the emmetropization mechanism. We have previously shown that monocular form deprivation (FD) in red light produces myopia as does minus lens wear, although the amount of lens-induced myopia was less than in colony light (Ward, AH, et al. IOVS 2017;58:ARVO E-Abstract 449). Thus, the ability of other cues is not completely disrupted in narrow-band light.

Accommodation in Narrow-band Light

Accommodation and emmetropization both have the same basic task: to evaluate focus and adjust the optics appropriately to maintain good focus (although on very different time scales). One might therefore expect that these two systems would use the same optical cues. In general, human subjects can correctly accommodate to scenes lit by narrow-band light of different wavelengths. Because longer wavelengths focus farther back than shorter wavelengths, there is more accommodation needed to clear the image if only long wavelengths are present, compared with the amount of accommodation needed in short wavelengths, so that the image is optimally focused.[29] However, accommodation in narrow-band light is slower and the “gain” of accommodation is less than for images viewed in broad-band illumination.[59] Also, there is a paradoxical effect for wavelengths below 430 nm, where human subjects start to accommodate more rather than less.[60] Thus, the accommodation mechanisms *can* use wavelength cues,[61] but it does not appear that, under most conditions, wavelength cues are absolutely required for accommodation in humans. (We have not, however, measured accommodation in tree shrews, which is a limitation of this study).

However, accommodation makes use of central processing mechanisms and can integrate information about depth from stereopsis, motion parallax and vergence angle etc. In addition, accommodation could potentially use a ‘hunting’ strategy, wherein if changing accommodation in one direction makes focus worse, it need only go the other way. In contrast, the emmetropization mechanism probably functions primarily within the eye using local retinal processing. This may explain why both accommodation and emmetropization may be able to use wavelength (longitudinal chromatic aberration) cues and, for accommodation these are optional, but for emmetropization, they are essential.

Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs)

Tree shrew retinas have been found to have melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs).[55] These cells receive input from photoreceptors via bipolar cells, but they are also directly activated by light via the blue-sensitive photopigment, melanopsin. They appear to be responsible for many non-image forming functions of the visual system such as the pupillary light reflex and entraining

circadian rhythms to the pattern of light and dark.[62] Because the photopigment is distributed widely in dendrites, the receptive fields of these cells are large, so their activity cannot be involved in the detection of image focus. However, these cells could be involved in regulating the state of light adaptation within the retina, which could possibly affect emmetropization. The narrow-band blue light presumably could activate the ipRGCs, and perhaps affect emmetropization, but any effect is unknown at this time.

Tree Shrews as a Model for Human Emmetropization

Although tree shrews are a dichromatic species and most humans are trichromatic, we suggest that the results in tree shrews are likely to provide useful information for human emmetropization. Tree shrews are diurnal mammals, with good visual acuity, that are very closely related to primates.[36] Because emmetropization is a fundamental issue for any animal with a camera-type eye that needs good visual acuity, one might expect this mechanism to be evolutionarily ancient and conserved across species with substantial retinal cones. Indeed, trichromacy evolved only recently in the primate lineage; dichromacy has been suggested to be the baseline state of color vision for all mammals including primates.[63] Dichromacy is adequate for emmetropization, both in dichromatic species and in humans who are red/green color blind, and thus have only the same basic long- versus short-wavelength color vision system as tree shrews; there is no evidence that these individuals emmetropize and differently from trichromatic humans. One paper suggested that some forms of human red/green color blindness are actually protective against myopia.[64] It seems that having two cone types with wavelength absorption profiles that can utilize information provided by longitudinal chromatic aberration, is essential for normal emmetropization in mammals. Further experiments in tree shrews and monkeys will either strengthen or weaken this hypothesis.

Relationship to Clinical Studies Using Blue Light

Blue light has been much in the news lately, most notably as regards its possible effects on circadian rhythms and sleep quality.[65] However, blue light has also been suggested to be important for emmetropization. One group claims that lenses and windows that block blue light of a wavelength < 400 nm can be myopiagenic,[66] although this is currently quite controversial.[67] There are also many commercially available blue-light blocking glasses, and many claims are made for these, but there is hardly any peer-reviewed literature on this topic. The only relationship of these studies and products with this study is the use of the word “blue.” The only clear clinical prediction to be derived from our results is that it would probably be unwise to raise children in a constant narrow-band blue light environment. However, our findings suggest that wavelength cues are essential for emmetropization in tree shrews, and, possibly, also in humans. Narrow-band light radically interferes with emmetropization. The question then arises: could artificial ambient light that is not strictly narrow-band, but that is still limited in the range of wavelengths compared to natural lighting, interfere with emmetropization in a way that is less extreme than found here but still clinically significant? Further research will be needed to answer this question.

CONCLUSIONS

The results of the present study using narrow-band blue light, taken with our previous results with narrow-band red light, have established that an environment containing only a narrow band of wavelengths is insufficient to establish and maintain emmetropia in tree shrews. In both red and blue, it appears that other visual cues, such as optical defocus, cannot provide information to the emmetropization that is sufficient for normal functioning. Rather, the presence of a wide range of wavelengths may be *essential* to maintain emmetropia. The studies in red and blue light also have shown that future studies into the nature of the visual cues that guide emmetropization would benefit following refractive development over time with frequent measures, rather than just at arbitrarily-defined start and stop points.

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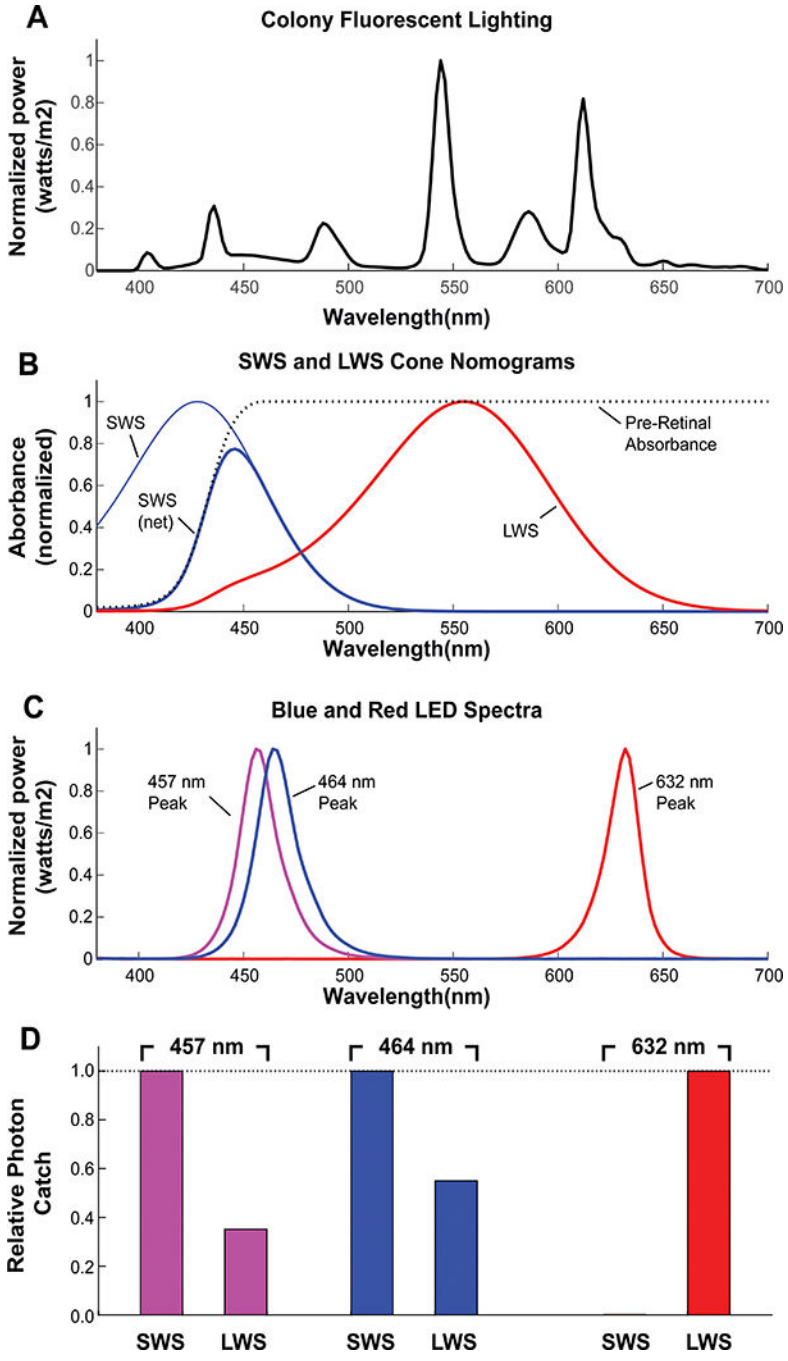

Figure 1.

(A) Normalized spectra of the fluorescent lights used for normal (colony raised) animals. **(B)** Normalized tree shrew cone absorbance (data from Petry and Harosi 1990)[24] "Net" is adjusted for pre-retinal absorbance. **(C)** Normalized spectra of the LEDs used in these experiments: 464 nm peak = "standard blue" and 457 nm peak = "Royal Blue." Also shown is a typical spectrum of a red LED used in previous experiment (see Gawne et al. 2017)[31] **(D)** Relative photon catch for the short wavelength sensitive (SWS) and long wavelength

sensitive (LWS) cones. The blue LEDs significantly affected both the SWS and LWS cones, whereas the red LEDs stimulated the LWS cones almost exclusively.

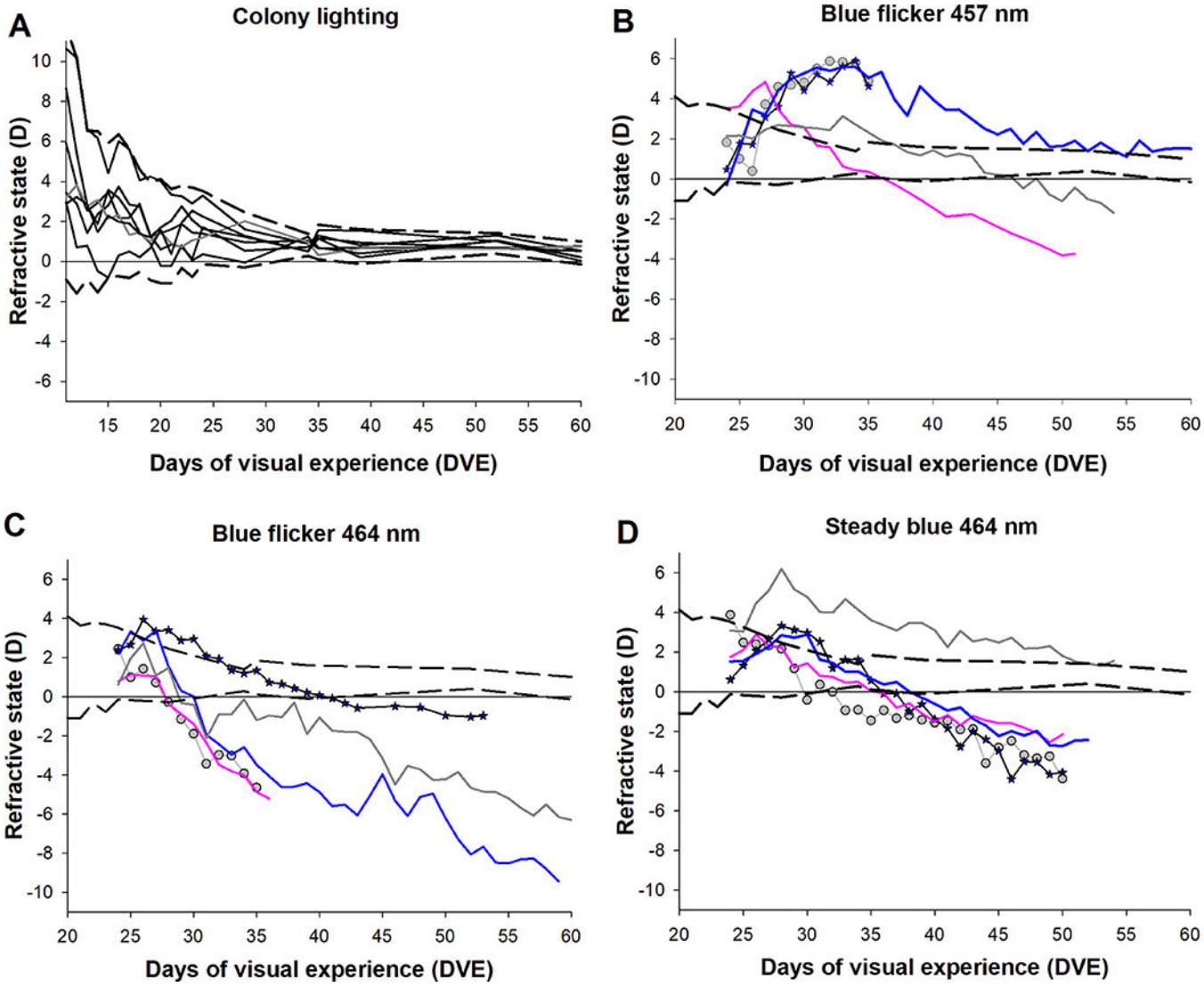

Figure 2.

Refractive state as a function of time. **(A)** fluorescent-light colony-raised tree shrews. **(B)** Animals exposed to ambient flickering short-wavelength (blue) light at 457 nm. **(C)** flickering blue at 464 nm. **(D)** steady blue at 464 nm. Each line plots the refractive state for one animal (average refraction of the left and right eyes). Blue-light treatment began at 24 days of visual experience and continued for at least 11 days. Dashed lines in each panel show the 95% confidence intervals for the colony-light animals. Different lines and symbols are used as an aid to following the changes in refractive state of individual animals.

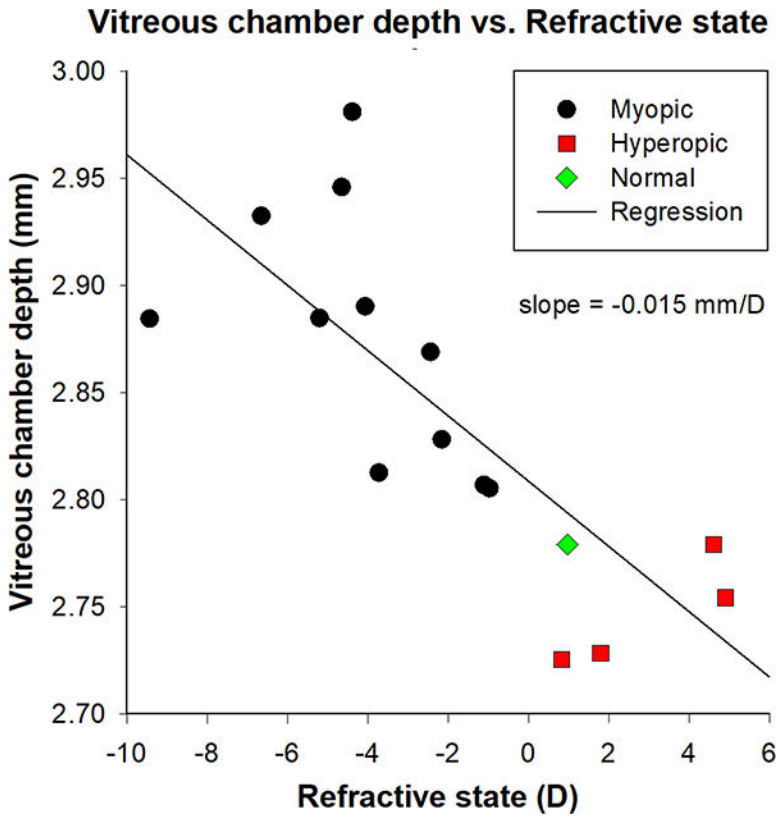

Figure 3.

Vitreous chamber depth vs. refractive state, measured at the end of blue treatment. Myopic eyes had longer vitreous chamber depths. Eyes that were hyperopic had shorter vitreous chambers. The slope of the regression was −0.015 mm/D. The diamond symbol represents the mean refraction and vitreous chamber from the “normal” (colony-light group, control), measured at 35 days of visual experience. $R^2 = 0.60$, $p < 0.01$.