TEMPORAL FREQUENCY RESPONSE OF THE EMMETROPIZATION MECHANISM IN CHICKS TO LUMINANCE FLICKER WITH AND WITHOUT BLUE LIGHT

A thesis presented to the graduate faculty of
New England College of Optometry in partial fulfillment
of the requirements for the degree of Master of Science

Stephanie Britton, OD, FAAO

February 2018

©Stephanie Britton
All rights reserved

The author hereby grants New England College of Optometry permission to reproduce and to distribute publicly paper and electronic copies of the thesis document in whole or in part.

TEMPORAL FREQUENCY SENSITIVITY OF THE EMMETROPIZATION MECHANISM IN CHICKS TO LUMINANCE FLICKER WITH AND WITHOUT BLUE LIGHT

Stephanie Britton, OD, FAAO

This Manuscript has been read and accepted by the
Thesis Committee in satisfaction of the
thesis requirement for the degree of Master of Science

2-2-18
Date

Frances Rucker, MCOptom, MS, PhD, FAAO
Graduate Faculty Advisor

Jane Gwiazda
Jane Gwiazda, Ph.D, FARVO, FAAO
Thesis Committee Member

Ji-Chang He, MS, Ph.D
Thesis Committee Member

Xiaoying Zhu, M.D, O.D, Ph.D
Thesis Committee Member

2-2-18
Date

Jane Gwiazda
Jane Gwiazda, Ph.D, FARVO, FAAO
Director of Graduate Studies

New England College of Optometry

TEMPORAL FREQUENCY RESPONSE OF THE EMMETROPIZATION MECHANISM IN CHICKS TO LUMINANCE FLICKER WITH AND WITHOUT BLUE LIGHT

Stephanie Britton, OD, FAAO

New England College of Optometry, 2018

Abstract

Purpose: To investigate temporal frequency sensitivity and the role of blue light on ocular structures during the emmetropization process in the chick model**.**

Methods*:* 4-5 day old chicks were exposed daily for three days (9 AM to 5 PM) to sinusoidally modulated with blue or without blue light at 80% contrast, at one of six temporal frequencies: 0, 0.2, 1, 2, 5, 10 Hz. Mean illumination was 680 lux. Changes in ocular components were measured before and after the experiment with a non-contact ocular biometer (Lenstar LS 900), and refractive error was measured with a Hartinger Coincidence Refractometer.

Results**:** Refraction, eye length, and choroidal changes were dependent on temporal frequency ($P<0.03$ all) and the presence of blue light ($P<0.03$ all). Without blue, refractions were more hyperopic at higher temporal frequencies, and more myopic at lower temporal frequencies. There was a hyperopic shift (> 1 D) at 5 and 10 Hz, and a myopic shift (> -0.6 D) at 0.2, 1 and 2 Hz. With blue light, refraction remained constant (mean change -0.24 D). Without blue light, there was less eye growth at high temporal frequencies and more at low temporal frequencies. Eyes were 145 μm shorter at 10 Hz than they were at 0.2 Hz ($P<0.003$), while with blue, they were only 77 μm shorter ($P=0.44$).

Conclusion: Blue light was protective against myopic eye growth changes and refractive error. Without blue light, the visual system showed temporal frequency sensitivity during emmetropization, becoming more myopic at low temporal frequencies.

Acknowledgements

I wish to thank my thesis committee members Dr. Jane Gwiazda, Dr. Ji He, and Dr. Xiaoying Zhu for taking the time to read my thesis and offer their feedback.

I would like to express my sincere gratitude to my advisor Dr. Frances Rucker for her continuous support of my Master's Study and research, for her patience, motivation, enthusiasm, knowledge and guidance. As a result of your expertise and guidance, I have a deeper appreciation of vision research for the advancement of our profession. Your involvement has helped me accomplish this thesis, as well as, opportunities for conference presentations and publications. Once again, thank you for your commitment and assistance.

Thank you, Mom and Dad, for your love, unfailing support and continuous encouragement throughout my years of study. This accomplishment would not have been possible without you.

Finally, I would like to dedicate my Master's thesis to my Nonno Giovanni, my biggest supporter throughout my life of my goals and aspirations. I admire your strength, courage, persistence, and determination. Even though you are no longer with us, I know you are looking down smiling with love.

Table of Contents

Abstract	iii
Acknowledgements	iv
Table of Contents	v
List of Figures	vi
Introduction	1
Methods	
Animals and Methods	11
Experimental Procedure	13
Analysis	15
Results	
Effects on Refractive Error	17
Effects on Eye Length	19
Effects on Choroidal Thickness	22
Effects on Anterior Chamber Depth	24
Effects on Lens Thickness	25
Effects on Corneal Power and Astigmatism	25
Discussion	
How could blue light have an effect?	28
Comparison with previous studies- Posterior Segment	29
Comparison with previous studies- Corneal Astigmatism	30
Clinical Relevance	31
Summary and Conclusions	33
Literature Cited	34

List of Figures

Figure 1-Change in Cone Contrast with Defocus 5

Figure 2- Noncontact Ocular Biometer 11

Figure 3- Measurement of corneal curvature 12

Figure 4-Hartinger refractometer 13

Figure 5- Change in Refraction 18

Figure 6 -Change in Eye Length 21

Figure 7- Change in Choroidal Thickness 23

Figure 8- Change in Choroidal Thickness with Eye Length and Vitreous Depth 24

Figure 9- Change in Corneal Astigmatism 27

Introduction

Emmetropization is dependent upon one's visual experience. Individuals are born with refractive errors that signal a compensatory growth signal within the visual system to initiate a process called emmetropization. When an eye is in a state of emmetropia, the eye maintains distant images in focus on the retina. When developing eyes are in a state of defocus, the eyes correct for this defocus to return the eye to an emmetropic state and bring the image closer to the retina (Smith, 1998; Wildsoet, 1998; Wallman & Winawer, 2004). However, before doing so, the visual system must determine whether the refractive error is myopic or hyperopic and act accordingly. This implies that there is visual regulation of growth of the different components of the eye through the visual detection of the sign of defocus.

Visual regulation in emmetropization

Studies have not determined the precise mechanism for emmetropization, but they have shown that the eye responds with a compensatory growth signal through several of its ocular structures. Evidence has shown that chick eyes are able to discern the sign of blur imposed by an optical lens and can grow in the direction that will allow for emmetropization (Schaeffel & Diether, 1999; Park et al., 2003). The eye can adjust its axial growth of the vitreous chamber to bring the image into focus (Troilo, 1991; Park et al., 2003). A hyperopically defocused eye increases its vitreous chamber growth, while a myopically defocused eye would reduce its growth. This change in vitreous chamber depth is typically accompanied by an increase or decrease in eye length. However, the change in the vitreous is not always correlated with the change in eye length as the choroid layer can thicken or thin to adjust for defocus (Nickla et al., 1997). Studies have also investigated lens-induced myopia where chicks wear concave or convex

lenses which alter the focal point of the image to be either in front or behind the retina. In experimental studies in chicks with positive lens-induced blur, the eye responded by thickening its choroid, whereas with negative lens induced blur, the choroid thinned (Wildsoet & Wallman, 1995; Wallman, et al., 1995), similar results were seen in macaque monkeys (Hung, et al., 2000). In addition, the anterior segment can reduce the overall power of the eye by decreasing the curvature of the cornea under constant light (Padmanabhan et al., 2007), and by moving the lens forward, decreasing the depth of the anterior chamber. Crystalline lens thinning will also reduce the power of the crystalline lens, but will have the counteractive effect of increasing the anterior chamber depth (Mutti et al., 2005, Shih et al., 2009). The crystalline lens displays an asymmetric growth pattern as shown in both monkey and humans; with anterior surface curvature decreasing comparably more than the posterior surface curvature (Qiao-Grider et al., 2007).

Results from studies investigating the effects of lighting conditions on the cornea have demonstrated chicks that were raised in constant light expressed a hyperopic refraction within three weeks due to corneal flattening and anterior chamber depth reduction (Lauber et al., 1970, Lauber et al., 1987; Li et al., 1995, Stone et al., 1995, Liu et al., 2004). Interestingly, Lauber's studies measured a myopic refractive status after 6 weeks as axial elongation continued. Lauber's studies used White Leghorn chicks in comparison to the Cornell-K strain used in other studies. Conversely, Li et al., (1995) reported no myopic measurement, but continuous hyperopia under constant light within 1-2 weeks with the Cornell-K strain of chicks as a result of corneal flattening. Li et al., (1995) reported that chicks exposed to constant light had accompanying vitreous elongation. A flatter cornea produces a lower powered cornea, leading to a more hyperopic refractive error, and the growth in vitreous chamber depth was not enough to offset these anterior segment changes. This would suggest that vitreous elongation is compensating for

corneal flattening (Li et al., 1995, Li & Howland, 2003). A disruption to the normal light-dark cycle clearly causes interruption to corneal curvature in addition to axial length changes.

Mechanisms for visual regulation in emmetropization

Regulation of eye growth appears to be mediated by a local mechanism which relies on visual signals to guide growth (Wallman & Winawer, 2004). In studies which physically isolated the retina from the brain, form deprivation myopia still occurred. Form deprivation myopia uses lid suturing or diffusers to obscure the images on the retina, preventing a clear image to be focused. Other experiments that have demonstrated this effect used optic nerve sectioning, or tetrodotoxin to block neural signaling (Troilo et al., 1987; McBrien et al., 1995; Wildsoet & Pettigrew, 1988; Wildsoet & Wallman, 1995). Furthermore, visual deprivation of a specific area of the retina such as with hemi-field lenses or occluders, lead to changes in only that part of the eye, implying local retinal regions are guiding eye growth from visual signals (Hodos & Erichsen, 1990, Miles & Wallman, 1990, Diether & Schaeffel, 1997).

To determine if the eye is able to detect the sign of defocus, studies have induced blur with positive and negative lenses in the chick eye. When a plus lens is used, the image of a distant object is focused in front of the retina (myopic defocus). The eye responds by slowing down its growth rate. When a negative lens is used, the image of a distant object is focused behind the retina (hyperopic defocus). The eye responds by increasing its growth rate (Schaeffel, Glasser, & Howland, 1988; Norton, 1990). These studies clearly demonstrated for the first time that the eye was able to detect the sign of defocus using visual information encoded in blur.

The negative feedback mechanism has been proposed as a way that the eye could determine the sign of defocus. The idea of negative feedback proposes that the eye determines its

refractive state by growing in the direction that maximizes luminance contrast and image quality (Troelstra et al., 1964; Phillips & Stark, 1977; Charman & Tucker, 1978). However, it has been shown that it is unlikely that the eye uses this mechanism during emmetropization since the response to defocus occurs before there is time for a change in growth. In a study by Zhu et al., (2005), the chick eye was able to detect the sign of defocus in only 10 minutes, and compensate in the appropriate direction, while showing little change in refractive error. This result signifies that the eye can rapidly detect the correct sign of defocus without relying on a negative feedback mechanism.

Researchers have investigated the possibility of a color signal from longitudinal chromatic aberration to help the eye to detect defocus (Fincham, 1951, Flitcroft, 1990). Longitudinal chromatic aberration causes dispersion of light such that an object in space creates a series of images within the eye at different focal planes for each wavelength (Wald & Griffin, 1947; Bedford & Wyszecki 1957, Howarth & Bradley, 1986). For an eye that is emmetropic and viewing a distant object, long wavelength (red) light will focus behind the photoreceptors, short wavelength (blue) light will focus in front of the photoreceptors and middle wavelength light will focus near the retina. Therefore, in a myopic eye that is too large, the red component of the retinal image will be in better focus, whereas in a hyperopic eye that is too small, the blue component will have better focus. An improvement in focus increases the sharpness and contrast of the retinal image. In the human eye, 1.75D of defocus occurs between 440 nm and 680 nm due to longitudinal chromatic aberration (Mandelman & Sivak, 1983, Marcos et al., 1999). In the chick eye, 1.25D of longitudinal chromatic aberration has been calculated between 470 nm and 680 nm (Mandelman & Sivak, 1983). It has been extended to include 400 nm to 700 nm, where 1.95 D of longitudinal chromatic aberration was calculated by Wildsoet (2003).

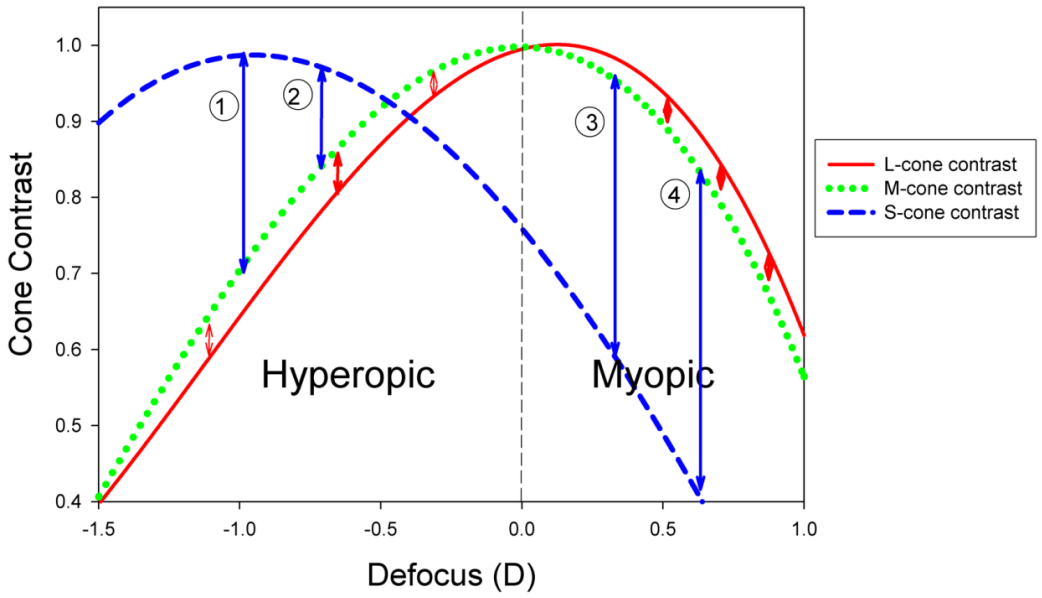


Figure 1: Changes in cone contrast with defocus in an eye with a 3mm pupil viewing a 3 c/d sinusoidal grating. Color contrast changes with defocus in a hyperopic defocused eye. Initially, with hyperopic defocus, blue contrast is higher, but towards emmetropia red contrast is higher. In a myopic defocused eye, color contrast remains constant (red contrast is higher than blue) but contrast for all three cone types decreases with increasing defocus.

Figure 1 shows how Rucker & Wallman (2012) demonstrated that defocus from longitudinal chromatic aberration produces changes in color and luminance contrast that differ with the sign of defocus. Rucker & Wallman proposed that there are three ways the eye can use the effects of longitudinal chromatic aberration to determine whether it is hyperopically or myopically defocused. First, as explained earlier, the eye could maximize luminance contrast and

image quality by adjusting its focus through negative feedback and a trial and error method. Second, the eye could use changes in relative cone contrast that occur with longitudinal chromatic aberration in order to determine the sign of defocus. In a hyperopically defocused eye, blue light is in better focus (Figure 1: Points 1 and 2). As the eye grows the relative contrast of the three cone types change until at some point the contrast for the three cone types is almost equal. Beyond this point, in a myopically defocused eye, the contrast for the long-wavelength cone type will become greater than that of the short-wavelength sensitive cone type (Figure 1: Points 3 and 4). The difference in focus of blue and red light creates a color signal that arises from the difference in cone contrast of the long (L), medium (M), and short (S) wavelength cones and which provides instant recognition of the sign of defocus. Lastly, these cone contrast signals are channeled into the luminance and color neural pathways that carry information through the retina. As such, it is likely that the eye compares the change in luminance and color contrast of the retinal images as the eye grows or changes focus. The presence of changes in color contrast with small changes in focus should indicate that the eye is too small and needs to grow. The lack of color changes, and a steady reduction in contrast of all three cone types, should indicate that the eye needs to slow its growth.

Temporal Sensitivity in Emmetropization

Humans are continuously exposed to a wide range of spatial and temporal frequencies in their daily environments, whether it is in their indoor or outdoor environment (Flitcroft, 2012). Often during indoor settings, humans are exposed to more low temporal frequency stimulation such as is found with uniform walls, furniture and fixtures, which are motionless. When they engage in near work on a uniform background, only the central field is stimulated by changing characters, while the peripheral visual field remains static, aside from small changes in retinal

stimulation associated with head and eye movements. Conversely, the outdoor environmental exposure is more dynamic and contains higher temporal stimulation through objects blowing in the wind, or cars driving down the street, or simply movement through the environment. Understanding the role of temporal frequency during emmetropization can enhance our understanding and help to determine modifications needed in our everyday environment to reduce myopia development.

The chick visual system is sensitive to temporal changes in contrast that are similar to those of humans (Jarvis et al., 2002, Jarvis et al., 2003). In chickens, there is a peak temporal sensitivity of 15 Hz, and a low frequency fall off (Jarvis et al., 2002). The chicks have an overall reduction in sensitivity in comparison to humans except at high temporal frequencies with a slightly higher critical flicker fusion value, specifically in high luminance lighting conditions. Chickens have a critical flicker frequency of 105 Hz (Nuboer et al., 1992) compared to humans, who are sensitive to 58.2 Hz at 500 cd/m^2 (Jarvis et al., 2002). However, it is unknown whether the visual system responds to temporal frequency differences during the emmetropization process.

The temporal response of the human visual system varies depending on the neural pathways that are stimulated. If the luminance of a stimulus is modulated in time, response of the human observer is maximal at approximately 10 Hz. For chromatic modulation between two lights matched in luminance by heterochromatic flicker photometry, response is maximal at low temporal frequencies and falls off at frequencies greater than 5Hz. Such differences in temporal characteristics led to the proposal that separable psychophysical mechanisms are involved in the detection of chromatic and luminance modulations (Kelly & van Noren, 1977).

Earlier results from studies investigating the effects of flickering light in different species are inconsistent, finding either a hyperopic or myopic shift or no change at all. Gottlieb & Wallman (1987) found that an increase in eye growth and myopia, typically found in occluded chick eyes, was prohibited under a 10 Hz stroboscopic flicker but not in a flicker of 100 Hz. Another study by Schwahn & Schaeffel (1997) exposed lens-wearing chicks to 150 W Xenon light pulses, with different duty cycles, at 6 or 12 Hz, and elicited a hyperopic shift in the fellow eye. Other studies have shown a myopic shift at temporal modulation rates of 2 Hz in cats (Cremieux et al., 1989) and in C57BL/6 (B6) mice (Yu et al., 2011). The same temporal frequency of 2 Hz showed no effect on non-lens wearing chicks using a saw-tooth waveform (Crewther et al., (2006). Di et al., (2013) conducted a study in guinea pigs exposing them to various sinusoidal frequencies (5, 1, 0.5, 0.25, and 0.1 Hz), finding 0.5 Hz produced an obvious myopic shift and greater axial elongation. A more recent study, Rucker & Wallman (2012), compared eye growth in chicks in response to sinusoidal temporal changes in color and luminance contrast at 2 Hz. Their results showed a 2 D hyperopic shift in chicks exposed to changes in luminance contrast in comparison to those exposed to change in color contrast. These results demonstrate that temporal stimulation affects emmetropization, though since these studies combine various temporal frequencies, waveforms, photoperiods and contrasts; it makes it difficult to compare results. These results could be better understood if additional temporal frequencies were tested using a consistent waveform.

Neural pathways for emmetropization

The retina uses two types of photoreceptors, rods and cones, to convert the vast range of light intensities in the environment. Rods facilitate vision in dim light, whereas cones facilitate vision in a bright light environment. The color vision system takes photoreceptor input from

three types of cone photoreceptors that have peak spectral sensitivity in the short, medium, and long-wavelength regions of the visible spectrum. A signal from a photoreceptor is relayed to retinal ganglion cells via bipolar cells. The cone signals will then pass through the one of three temporally sensitive pathways consisting of M (magnocellular, or parasol) and P (parvocellular, or midget) pathways, which are anatomically evident in the lateral geniculate nucleus. Experiments have shown that magnocellular cells contribute to the perception of motion, firing action potentials when a stimulus is presented, and fading if the stimulus is not changed. They elicit a transient response to light stimuli and form the physiological substrate of the luminance channel (DeValois et al., 1996, Kaier et al., 1990, Kremers et al., 1992, Lee et al., 1988). Magnocellular cells are more sensitive to luminance contrast compared to P-cells, which prefer color contrast. They also exhibit little color-opponency. On the other hand, parvocellular cells give sustained responses to stimuli, and exhibit a color-opponency response which is dependent on wavelength (Marshak, 2009). Parvocellular cells detect chromatic modulation, in particular at low temporal frequencies (Lee et al., 1989; Lee et al., 1990). A third type of ganglion cell, the small bistratified cells, venture to the koniocellular pathways and mediate blue-yellow color vision (Marshak, 2009).

The parvocellular pathway creates the initial signal of the red/green color pathway, replying to high contrast stimuli with low pass temporal frequency features. The parvocellular pathways react to ON and OFF bipolar cell stimulation, receiving antagonistic input from medium-wavelength sensitive cones (M-cones), and long-wavelength sensitive cones (L-cones) (DeMonasterio et al., 1975; Dreher et al., 1976; Derrington et al., 1984; Derrington et al., 1984; Kaplan & Shapley, 1986; Shapley & Perry, 1986; Lee et al., 1990; Schiller et al., 1990). The blue/yellow ganglion cells project to the koniocellular layer of the LGN, and contains short-

wavelength cone (S-cone) ON and OFF pathways (Dacey et al., 2013). The S-cone ON pathways responds to stimulation of large and small bistratified retinal ganglion cells (Dacey et al., 1994; Dacey, 1996; Dacey et al., 2003). S-cone input to these ganglion cells is through blue ON-bipolar cells, (Mariani, 1984; Kouyama & Marshak, 1992), with antagonistic signals from L-cones and M-cones (Dacey et al., 2013; Dacey, 1996, Dacey et al., 1994). S-cone OFF signals may be carried by midget bipolar cells (Klug et al., 2003; Klug et al., 1993), and large monostratisfied ganglion cells (Dacey & Packer, 2003). Since these opponent visual pathways express temporal sensitivity, we hypothesized that the luminance-sensitive emmetropization mechanism would also show temporal frequency sensitivity.

In this study, chicks are used to examine if emmetropization mechanisms that are sensitive to changes in luminance contrast show sensitivity to a variation in temporal frequencies. We measure how the various temporal frequencies influence the posterior segment—eye length, choroidal thickness, and refractive error—as well as the anterior segment of the eye—anterior chamber depth, and corneal astigmatism. A change in these ocular measurements demonstrates that during emmetropization, luminance-contrast-sensitive visual pathways are utilized and sensitive to temporal frequency.

Methods

Animals and measurements

White leghorn chicks (*Gallus gallus domesticus,* Cornell K strain; Cornell University, Ithaca, NY) were acquired as eggs. After hatching, the chicks were raised in a 12-hour light/12-hour dark cycle under 300 lux fluorescent bulb lighting conditions with continuous food and water access in a free range environment.

Ocular component measurements were conducted before and after the 3 day exposure period with a noncontact ocular biometer (Lenstar LS 900: Haag-Streit AG, Konig, Switzerland), through a low-coherence reflectometry technique. All measurements were started at the same time (9am) to prevent interference from diurnal variations. The unanesthetized chicks were fully awake during measurements and lid retractors were not used (to prevent corneal distortion). The chicks were stabilized in a holder under dim lighting conditions. Six measurements were made per eye. Each measurement recorded was an average of sixteen measurements taken by the Lenstar.



Figure 2: Noncontact ocular biometer (Lenstar LS 900: Haag-Streit AG, Konig, Switzerland

Ocular component measurements included were: anterior chamber thickness, (measured from the anterior corneal surface to the front of the lens), lens thickness, vitreous chamber depth, retinal thickness, choroidal thickness, scleral thickness, and corneal curvature. Eye length was obtained as the distance from the anterior corneal surface to the posterior scleral surface. The Lenstar produced a reading that was the average of 16 measures that was represented by several peaks; anterior corneal surface, posterior cornea, anterior lens, posterior lens, anterior and posterior retina, posterior choroid and posterior scleral surface, respectively. Six measures were taken for each eye.

The curvature of the cornea was measured using images taken by the Lenstar LS 900, showing in-focus mires (ring of lights) positioned directly in the center of the pupil. Corneal images that contained misaligned or out of focus mires were rejected from analysis. Six images per eye were analyzed with Image J, by drawing a rectangular outline around the mires. Values of the rectangular dimensions gave the x and y components of the image. These values were then converted to corneal curvature measures along the horizontal and vertical meridians through calibration of ball bearings of known diameters. Using the refractive index of 1.33749, corneal curvatures were converted to corneal powers to use for analysis (Howland and Sayles, 1985). Corneal powers were analyzed as J0 as proposed by Thibos et al. (1997).

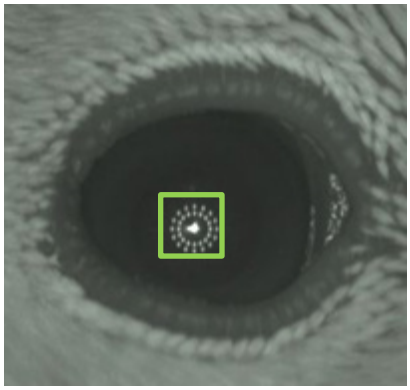


Figure 3: Measurement of corneal curvature using Lenstar images.

Refractive errors were measured with a Hartinger refractometer (Model 110: ausJENA, Jena, East Germany) (Wallman & Adams, 1987) while chicks were anesthetized with 1.5% isofluorane in oxygen with a 2L/min oxygen flow rate. Three measurements were taken in each meridian. Use of animals in this study was in compliance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the NECO Institutional Animal Care and Use Committee.



Figure 4: Hartinger refractometer (Model 110: ausJENA, Jena, East Germany)

Experimental Procedure

Chicks were 4-5 days old at the start of the experiment. During the experimental period, randomly selected chicks were placed in 32 x 20 inch diameter wire cages with free range access with both eyes exposed to a lighting condition for three days from the hours of 9AM to 5 PM (2 PM to 5 PM on day 1), with illumination by the modulated light sources placed above the cages. Chicks from the same batch were randomly assigned an illumination condition. A designated area was allotted for running the experiments; each condition was screened from the others by custom-made black-out curtains, and a double door system to separate the area from office area.

During non-experimental time, chicks were placed in a sound-and-light-proof chamber overnight with access to food and water at all times. No lenses were worn by the chicks before or during the experimental period. The number of chicks enrolled in each condition is shown in Table 1. Twice as many eyes were used, with the data from the two eyes averaged together.

	Temporal Frequency (Hz)					
Illumination Condition	**0 Hz**	**0.2 Hz**	**1 Hz**	**2 Hz**	**5 Hz**	**10 Hz**
With Blue light	**15**	**13**	**13**	**17**	**13**	**11**
Without Blue light	**15**	**13**	**14**	**12**	**15**	**14**

Table 1: Number of Chicks in Each Illumination and Temporal Frequency Condition

The experimental design contained two lighting conditions. The first lighting condition was in-phase modulated light containing red and green light components, the second lighting condition was in-phase modulated white light containing blue, red and green light components. Lights were modulated at 0.2, 1, 2, 5, or 10 Hz. Chicks were exposed to one lighting condition at one temporal frequency.

The illuminants were set to produce 80% contrast with sinusoidal modulation of the red (615 nm, half-bandwidth 20 nm), green (515 nm, half-bandwidth 35 nm) and blue (460nm, half-bandwidth 35nm) LEDs. Mean illuminations were measured with a photometer (Newport Model

818-SL serial number: 6915; Newport, Franklin, MA, USA) and set to 680 lux for all conditions. Intensity was digitally controlled, with fine adjustments made with neutral density filters.

The illuminants were Lamina Titans RGB LEDs driven by an eight channel, 12-bit Access I/O, USB-DA12-8A digital to analog converter with waveform generator functionality connected to BuckPucks (LuxDrive: 3021 D-E-500) that provided a linear current output over a range of 1.6 - 4.3 V. Light output was calibrated and a sinusoidal output was produced digitally using lookup tables, and confirmed by recording illuminance output (Newport Model 818-SL serial number: 6915).

Analysis

The effects of the illumination conditions on each of the ocular components (corneal astigmatism, anterior chamber depth, vitreous, choroidal thickness, eye length) and refraction were calculated as the change between pre- and post- measurements in both eyes of all chicks. Measurements for right and left eyes were averaged (Armstrong, 2013). The regression between the two eyes for all chicks was highly significant (R^2=44.2%; $p<0.001$). The results between the two eyes for with (R^2=48.8%; $p<0.001$), and without (R^2=45.1%; $p<0.0001$) blue chicks was highly significant. There were no outliers, due to strict criteria. 5% of subjects did not fall into the peak criteria. The average initial values for each of the ocular components can be found in Table 2.

Hz Frequency	Illumination	Ant. Chamber Mean (mm)	Lens Mean (mm)	Vitreous Mean (mm)	Eye Length Mean (mm)	Refraction Mean (D)
0	RGB	1.149	1.731	4.408	7.804	0.60
0.2	RGB	1.194	1.814	4.366	7.909	0.17
1	RGB	1.189	1.778	4.369	7.851	0.32
2	RGB	1.142	1.794	4.474	7.913	0.17
5	RGB	1.204	1.826	4.458	7.996	0.09
10	RGB	1.196	1.770	4.456	7.964	-0.10
0	RG	1.167	1.792	4.401	7.842	0.77
0.2	RG	1.161	1.803	4.406	7.852	0.93
1	RG	1.153	1.771	4.416	7.875	0.56
2	RG	1.172	1.815	4.492	8.022	0.49
5	RG	1.142	1.821	4.344	7.875	-0.32
10	RG	1.17	1.786	4.417	7.883	0.26

Table 2: Mean initial values (mm) in ocular components and refraction (D).

Changes in refraction and ocular dimensions were compared with regard to frequency (0, 0.2, 1, 5, 10 Hz) and illumination condition (with and without blue light) as well as, the interaction between frequency and illumination using two-way ANOVA. Unpaired, two-tailed comparisons were made between birds in different conditions if the F value was significant using Scheffé post hoc *t*-tests. Student's t-test was used for simple comparison at 0 Hz.

Results

Effects on Refractive Error

Changes in refractive error are shown in Figure 5. When changes in refractive error from both illumination conditions were compared, an interaction between frequency and illumination condition was shown (ANOVA: P=0.012). Initially, chicks presented with hyperopia (with blue: 0.23 ± 0.11 D; without blue: 0.37 ± 0.15 D). After exposure to the without blue illumination condition, there was a hyperopic shift at 5Hz (1.1 ± 0.44 D) and at 10 Hz (1.0 ± 0.45 D). This was in contrast to a myopic shift at 0.2 Hz (-0.93 ± 0.37 D; $P < 0.05$) and at 1 Hz (-0.67 ± 0.44 D; $P < 0.05$). No significant refractive change was found at 2 Hz (-0.59 ± 0.39 D; $p = 0.11$). The change in refractive error between groups at 5 and 10 Hz were not significant. Thus, temporal changes in frequency in without blue light produced refractive changes of approximately 2D.

Conversely, when chicks were exposed to with blue light condition, there was almost no change in refraction over the temporal frequency range tested. There was a mean change in refractive error of only -0.24 ± 0.30 D across the frequencies between 0 and 10 Hz.

In steady light the color of the illuminant had no effect on refractive changes. The comparison of steady light (0 Hz) showed no difference with (-0.18 ± 0.23 D) or without (-0.28 ± 0.31 D) blue light (P=0.8; Student's *t*-test). Thus, indicating that temporal contrast change is necessary for illuminant related changes in refraction, regardless of whether the illuminant contains blue light.

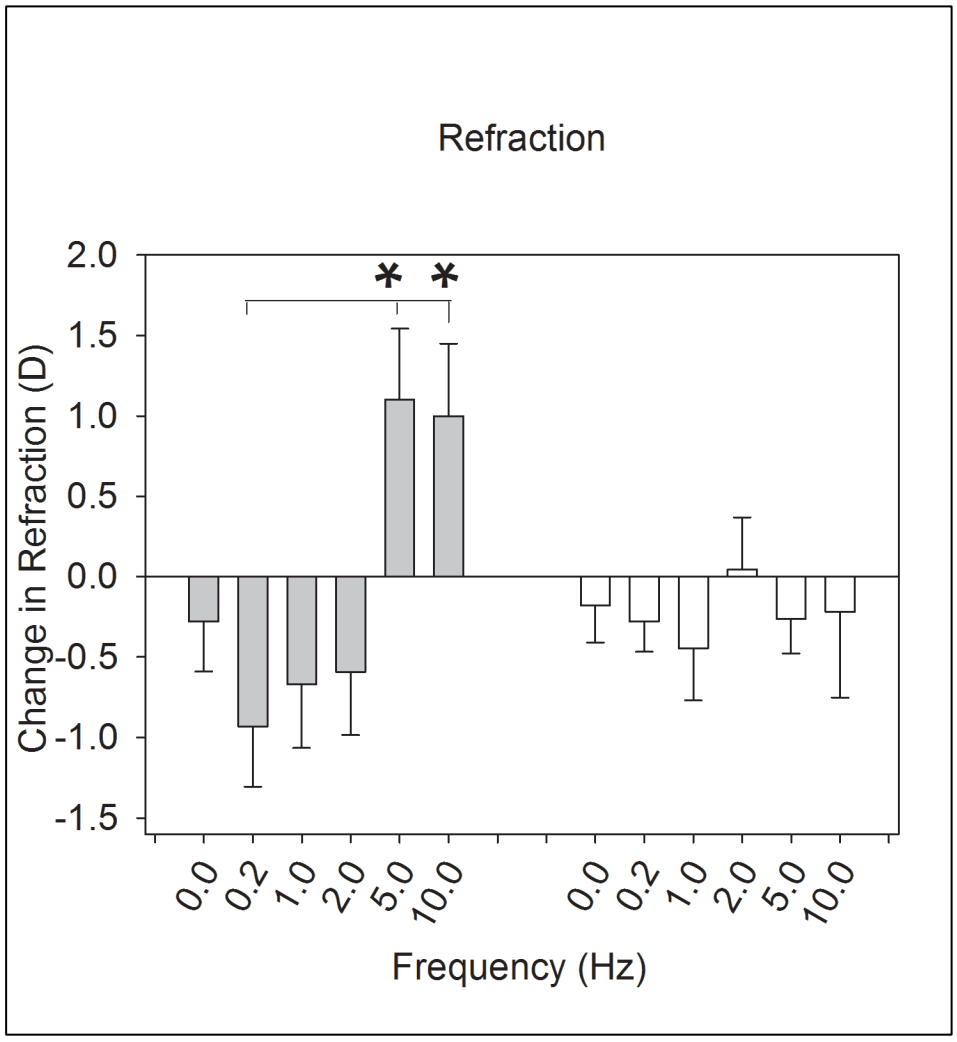


Figure 5: The change in refraction that occurs with exposure to different temporal frequencies without (grey bars) or with (white bars) a blue light component. In the absence of blue light, higher temporal frequencies produce a more hyperopic refraction compared to low temporal frequencies. Statistical difference is represented by * for *P* values less than 0.05. *Bars* indicate comparisons of refraction of higher temporal frequency 5 and 10 Hz with low temporal frequency 0.2Hz. Standard error bars are shown.

Effects on Eye Length

Changes in eye length are shown in Figure 6A. When data for changes in eye length for both illumination conditions were combined, it was shown that eye length changes were affected by temporal frequency (ANOVA: P=0.0001; R^2 of 15%). At 0 and 0.2 Hz, eye growth was greater (302 ± 28 μm and 311 ± 22 μm, respectively), than at 5 and 10 Hz (217 ± 0.15 μm and 201 ± 17 μm, respectively). Eye growth at 0 and 0.2 Hz was significantly greater than at 5 and 10 Hz (P< 0.01 both; Tukey HSD). The biggest effect of eye length on refraction was at 10 Hz (R^2=25%). Higher frequencies produced a reduction in eye growth, which corresponded to the hyperopic change in refractive error.

When eye length from the illumination conditions were compared, an interaction between lighting condition and temporal frequency of the stimulus was seen (Fig. 6B: ANOVA: P=0.034). When chicks were exposed to the with blue illumination condition, there was a much smaller change in eye growth, with only 77 μm more eye growth at 0.2 Hz than at 10 Hz (P = 0.44).. Conversely, when chicks were exposed to the without blue illumination condition, there was 145 μm more eye growth at 0.2 Hz than at 10 Hz (P<0.01; Tukey HSD), and 138 μm more growth at 0 Hz than at 10 Hz (P < 0.01; Tukey HSD). The lighting condition interacts with the frequency condition to produce differences in eye length. This was most pronounced when the high frequency with blue condition was compared with the 0 and 0.2Hz without blue condition (0Hz:p=0.016; 0.2Hz: p=0.019). Therefore, the presence of blue light was protective against temporal frequency sensitive changes in eye growth.

With steady light (0 Hz) in the two illumination conditions, there was no difference in eye growth with (269 ± 16 μm) or without (336 ± 31 μm) blue light (P = 0.06; Student's *t*-test). However, eyes were on average 67 μm shorter in the illumination condition containing blue

light. These results are in agreement with the refraction data, where the color of the illuminant did not significantly affect eye length in steady light.

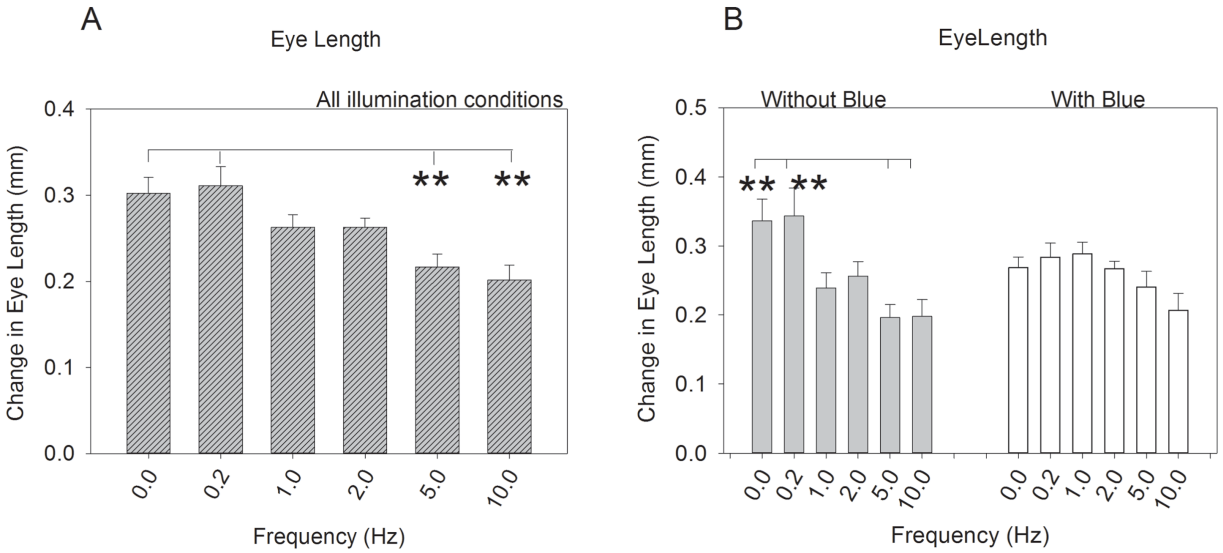


Figure 6: (**A**) Change in eye length in all conditions over a range of temporal frequencies. Eye length is dependent on temporal frequency of the lighting illuminant. (**B**) A comparison of the change in eye length between the two lighting conditions across a range of temporal frequencies. Without blue light, eyes show a change in eye length dependent on temporal frequency. P values less than 0.05 and 0.01 are shown as * and **, respectively.

Effects on Choroidal Thickness

Changes in choroidal thickness are shown in Figure 7. When data for changes in choroidal thickness were combined for both illumination conditions, changes in choroidal thickness with frequency was seen (ANOVA: P=0.03). At higher temporal frequencies, there was more choroidal thinning in comparison to lower frequencies. Specifically, at 10 Hz, there was 60 ± 9 µm of thinning, compared with 28 ± 6 µm at 2 Hz (10 vs. 2 Hz: P<0.05; Scheffé). At 0 Hz there was only 40 ± 6 µm of thinning (p <0.05). At higher temporal frequencies, the associated reduction in eye length is compensated for by thinner choroids.

When data for the changes in choroidal thickness with exposure to the illumination conditions were compared, an interaction between frequency and illumination condition was present (ANOVA: P=0.007). Choroids thinned the most at high temporal frequencies, but when chicks were exposed to the without blue condition, the choroid thinning increased from low to intermediate frequencies: 5Hz (73 ± 6 µm, P=0.02), 2 Hz (32 ± 7 µm, P=0.01) and 1 Hz (56 ± 8 µm, P=0.053) (Student's *t*-test). On the other hand, in the with blue condition the choroid thickened from low to intermediate temporal frequencies. Without blue light, choroidal thinning at lower and intermediate frequencies (0 to 2 Hz) was associated with increased eye growth (Figure 8 A) and myopic shifts in refraction, otherwise choroidal thickness changes maintained a constant vitreous chamber depth (Figure 8 B).

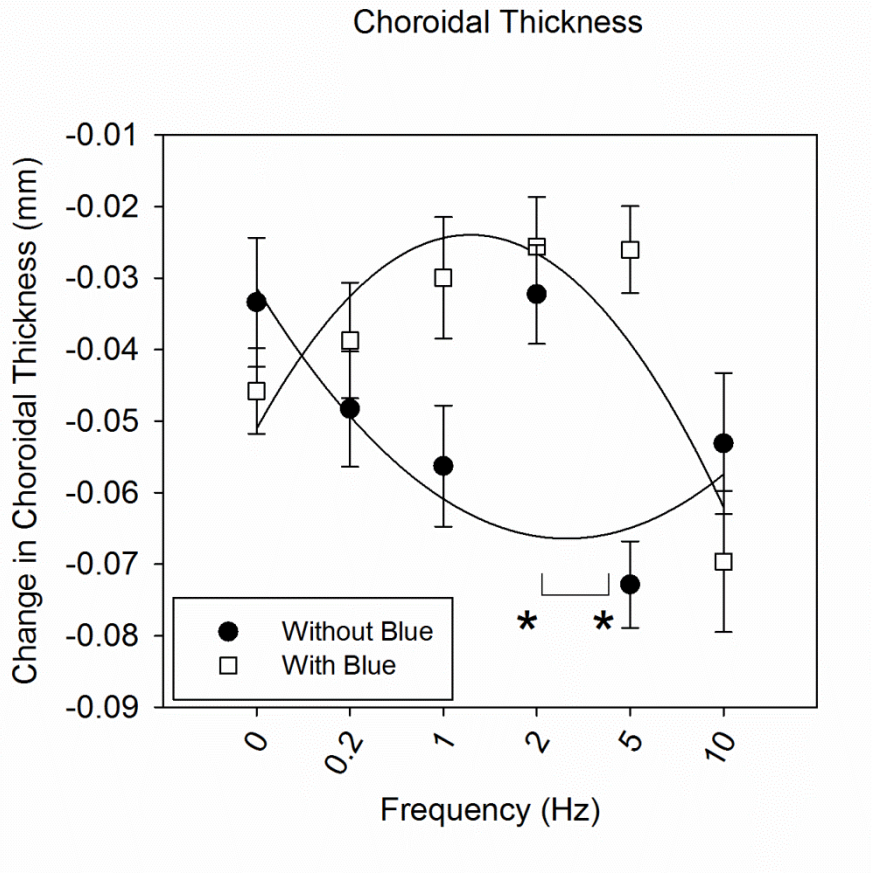


Figure 7: Change in choroidal thickness of chicks exposed under illumination conditions with (*white squares)* and without (*black circles)* blue light across a range of temporal frequencies. There was a significant difference in choroidal thickness change at 5 Hz in the without blue light condition, compared to choroidal thickness changes at 5 and 2 Hz in the with blue light condition. *Bars* and * show *P* values less than 0.05. Standard error bars are shown.

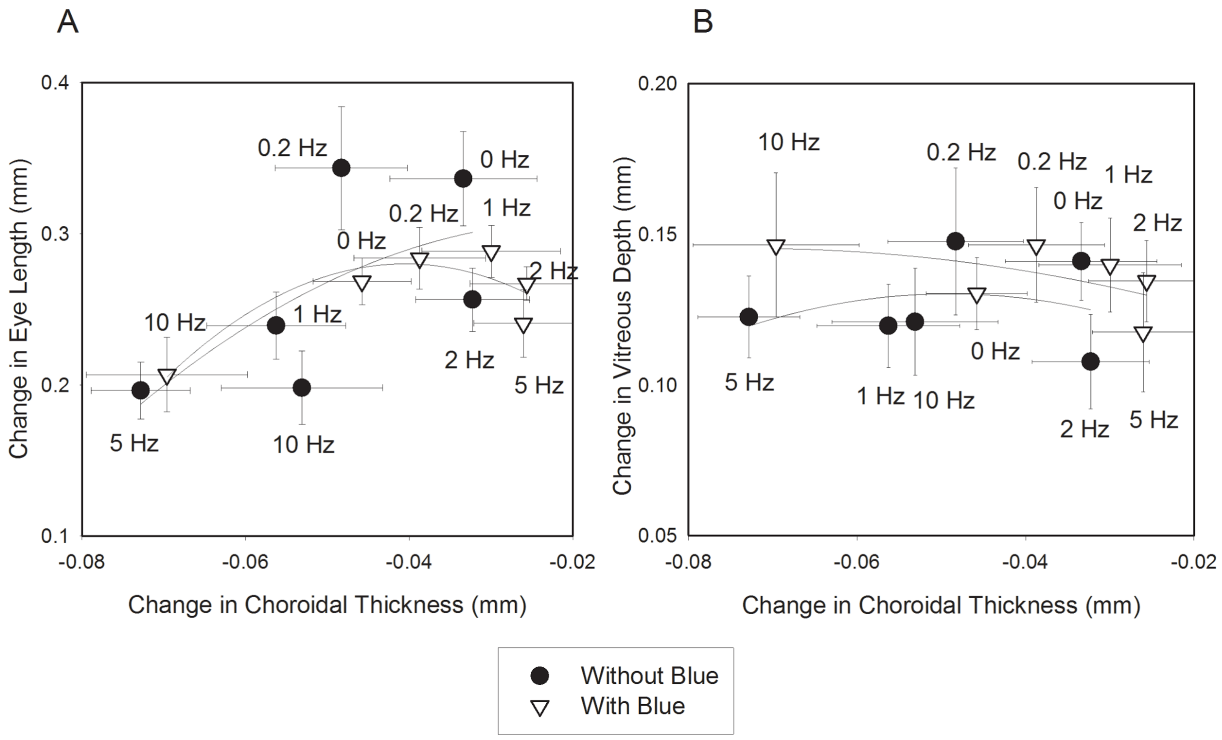


Figure 8: Changes in eye length **(A)** and vitreous **(B)** against changes in choroid under illumination conditions with (*white triangles)* and without (*black circles)* blue light. Each temporal frequency is separately plotted on the graph. Data for each illumination condition was fit with a polynomial function as shown by the *solid lines.*

Effects on anterior chamber depth

Changes in anterior segment were found to be sensitive to blue light exposure (ANOVA: p=0.006). Exposure to blue light produced shallower anterior chamber depths (with blue: 35 ± 6 µm; without blue: 46 ± 13 µm). Anterior chamber changes also showed a dependency on temporal frequency (ANOVA: P=0.003). Anterior chamber depths were found to be significantly shallower at 10 Hz in comparison to all other temporal frequencies (24-30 µm; P=< 0.05). Therefore, particularly at higher temporal frequencies, blue light reduced anterior chamber depths creating an overall increase in eye power, thus causing a myopic shift.

Effects on lens thickness

When data for changes in lens thickness was examined, an effect of temporal frequency was seen (ANOVA: p=0.001), specifically at lower frequencies. Combination of the two illumination conditions, indicated lens thickness increased by 137 μm at 0 Hz, but only by 115 μm at 10 Hz, and 108 μm at 5 Hz. The reduction in lens thickness at 5 Hz was significant compared to 0 Hz (P = 0.003), and may have contributed to a relative hyperopic shift found at higher temporal frequencies.

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

Table 3: Mean changes (in mm) in ocular components and refraction (in D) During exposure to flickering light with (RGB) and without (RG) blue Light at each Temporal Frequency

Effects on corneal power and astigmatism

Corneal astigmatism is reduced during emmetropization in the presence of blue light (Figure 9), but the effect did not show a temporal frequency response. With blue light, J_0 was reduced by -1.51± 0.09 D. This reduction in neonatal astigmatism was also seen in steady light. In the absence of blue light, only small changes were noted in J_0 (-0.39± 0.17 D) indicating that neonatal astigmatism was maintained. There was a significant change in J_0 between the illumination conditions (ANOVA: P<0.001). However, there was no effect with temporal frequency (ANOVA: P=0.436). The spherical equivalent corneal power (M) component was

similar in both illumination conditions, but, decreased slightly more with blue light (-5.27 ± 0.45 D), than without blue light (-4.53 ± 0.48 D).

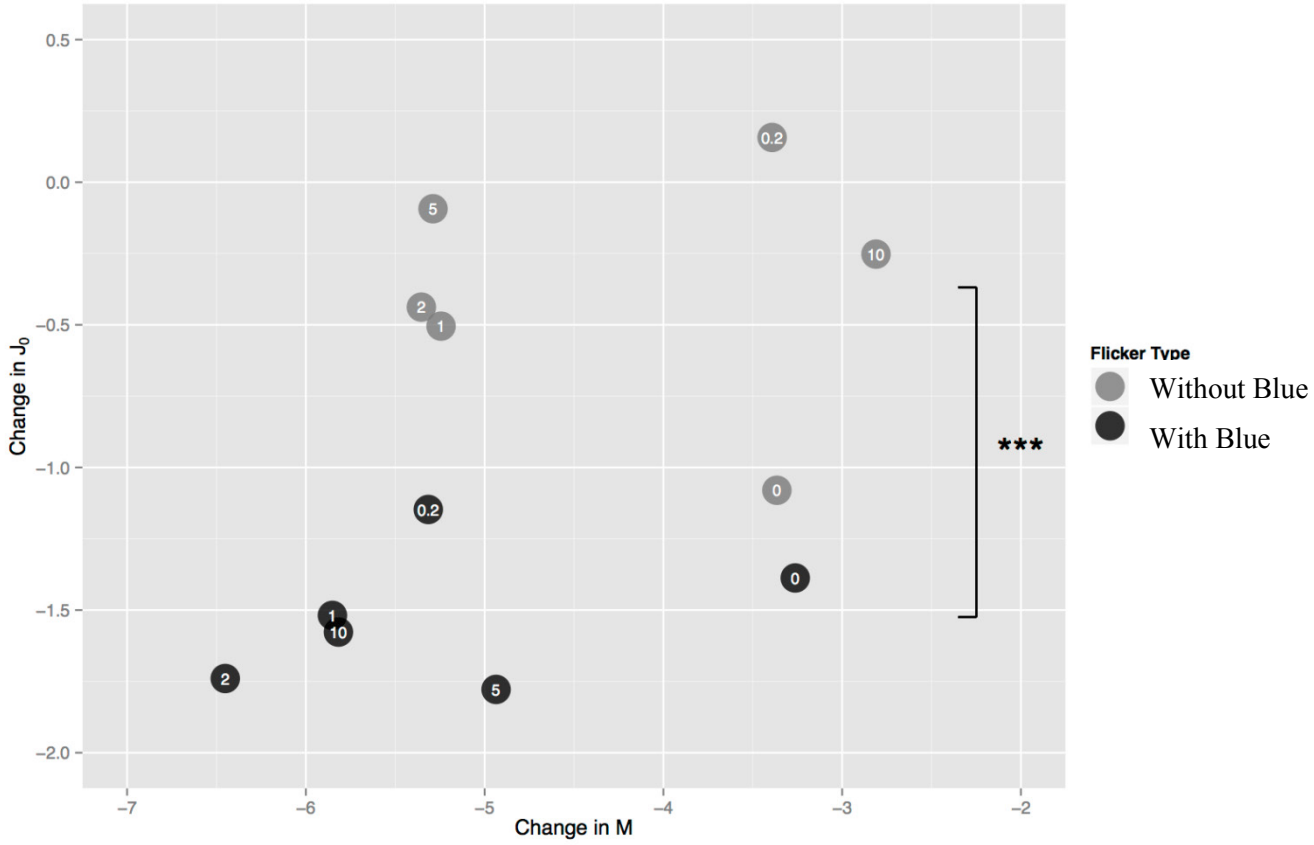


Figure 9: Change in corneal power (not spectacle correction) along J0 after exposure under illumination conditions with blue (*black circles)* and without blue (*gray circles). Bars* and *** indicate a change in corneal power along J0, with and without blue lighting conditions were significantly different ($p < 0.001$).

Discussion

In this present study, we examined how temporal frequency affects emmetropization in the chick model under two illumination conditions: with and without blue light. The results of this study support the hypothesis that emmetropization shows a temporal frequency response and blue light is an important factor for protection against myopic progression with exposure to low temporal frequencies. High temporal frequencies, which favor the luminance sensitive pathway, protected the eye against myopia. There was a reduction in eye length and a hyperopic shift in refraction. Conversely, low temporal frequencies, which favor the color sensitive pathway, created an increase in eye length and a myopic shift in the absence of blue light. Neonatal astigmatism also showed dependency on the presence of blue light by showing a reduction of astigmatism in the developing eye when blue light was present, but failed to do so when blue light was absent. This study supports the importance of a blue light environment during childhood development to prevent the development of myopia with exposure to natural sunlight (without blue light reducing filters).

How could blue light have an effect?

Blue light or UV light suppressive effects against myopia have been reported in recent studies (Foulds et al., 2013; Torii et al., 2017). However, the protective mechanism of outdoor light which is rich in blue wavelengths is not fully understood. Previous research on blue light proposed that the more myopic defocus of blue light provides a stimulus for a reduction in eye growth (Kroger et al., 1996; Seidemann et al., 2002). We suggest that at low temporal frequencies, the visual system has time to recognize and process the myopically defocused signal of blue light. Thus, when blue light is present the eyes respond by reducing growth, and respond with more growth when blue light is absent.

At higher temporal frequencies, the discrepancy in eye length was not as apparent between the two illuminant conditions but there was still a difference in refraction. Under the "with blue" illumination condition, the less hyperopic refractions could be a result of the visual system's inability to correctly detect the myopically defocused blue light, or from the introduction of the color signal due to signals from the S-cones progressing slower than signals from L- and M- cone through the visual system (Stromeyer et al., 1991; McKeefry et al., 2003, Smithson & Mollon, 2004; Rabin et al., 1994; Robson & Kulikowski, 1998; Lee et al., 2009; Lee & Sun, 2004; Stockman et al., 1991).

Comparison with previous studies-Posterior Segment:

Various studies have shown consistent results that a reduction in eye length and hyperopic shifts in refraction could be induced by exposure to high temporal frequencies (Gottlieb & Wallman, 1987; Schwahn & Schaeffel, 1997; Rucker et al., 2012; Rucker et al., 2017). Studies by Schwahn & Schaeffel (1997) exposed lens-wearing chicks to 150 W xenon light pulses (rich in blue light), with different duty cycles, at 6 or 12 Hz, and elicited a hyperopic shift and a reduction in eye length in both conditions. This present study extended temporal frequency parameters from earlier experiments in our lab (2 Hz; 100% modulation), for a sinusoidal waveform, by measuring over a range of temporal frequencies, 0, 0.2, 2, 5 and 10 Hz. Hyperopic refractive responses were found with exposure to 5 and 10 Hz with 80% modulation but not 2 Hz. This is most likely due to the reduction in modulation in the current experiment

Studies using various animal models: chicks (Crewther et al., 2006), mice (Yu et al., 2011), guinea pigs (Zhi et al., 2013) and cats (Cremieux et al., 1989), have found myopic refractive changes at low temporal frequencies. Crewther et al., exposed chicks to luminance

flicker in a slow on/fast off profile. Myopic refractions at 1 and 2 Hz were present in chicks wearing plano lenses, similar to the results of the present study. Chicks without lenses experienced no refractive changes. The study setup was designed with a halogen light source, which contains low illuminance levels (1.5 -180 lux) and limited illumination at the blue end of the spectrum. The reduction in blue light levels likely simulated a condition more comparable to the "without blue" condition in the current study, and support the study's similar results.

Comparison to previous studies- Corneal Astigmatism

Upon hatching, chicks present with against-the-rule astigmatism (Schmid & Wildsoet, 1997). This study's findings verify this and show a decrease in the amount of against-the-rule with development, similar to that found by Schmid & Wildsoet (1997), but only in chicks exposed to light containing a blue component. This finding signifies that the corneal emmetropization process was disrupted without blue light.

Howland et al., (1978) suggested that astigmatism may play a positive role during the emmetropization process. This study's results would support this hypothesis and suggest that blue light is necessary for the corneal emmetropization process to function normally. These findings can supplement earlier studies that show corneal flattening and shallowing of the anterior chamber occurs under continuous lighting conditions (Lauber & Oishi, 1987; Li et al., 1995; Stone et al., 1995), as well as with an increase in the intensity of ambient light (Cohen et al., 2008). Flattening of the cornea has the same effect as placing a negative lens in front of the eye, where the eye responds with vitreous elongation. In previous studies, when chicks were exposed to constant light and then were placed back in normal lighting conditions, the cornea was able to regain its normal curvature. Padmanabhan et al., (2007) and Wahl et al., (2015) both

presented evidence of recovery of emmetropia in refraction with the adjustment of corneal curvature and axial length when chicks were exposed to normal light after constant light conditions. However, restoration of normal ocular morphology was limited if original exposure to constant light exceeded 11 weeks. Earlier work by Troilo & Wallman (1991), reported similar findings of recovery of large eyes after dark induced hyperopia, where emmetropia was restored by increasing the size of an already enlarged eye.

This brings up an interesting area of research considering there is a debate as to whether the emmetropization mechanism is dependent upon a compensatory relationship between anterior and posterior segments or if they are independent mechanisms. Research presented by Lauber & Oishi (1987) suggest that they are independent processes contrary to what is suggested by Liu et al. (2004) who suggested that the changes found in anterior and posterior segment share a compensatory relationship. The present study's results support previous research that they share a compensatory relationship, with sensitivity to temporal frequency changes, as well as the importance of blue light exposure for proper emmetropization.

Clinical Relevance

With the introduction of digital devices, people's lifestyles and levels of light exposure have changed. People are spending more time indoors than they were before. For adults, this can be contributing to the increase in sleep disorders due to the reduction in sunlight and blue light exposure (Change et al., 2015; Czeisler, 2013). For children, a reduction in natural light can influence the emmetropization process. Several studies have shown support for spending time outdoors, where blue illumination is strongest, as a protective feature in delaying the onset of

myopia (French et al., 2013, Guggenheim et al., 2012, Jones-Jordan et al., 2012, Rose et al., 2008, Dirani et al., 2009).

In clinical practice many researchers are developing myopia control strategies to slow down progression. Clinical options include prescribing spectacles, multifocal contact lenses, orthokeratology, pharmaceutical agents, and lifestyle modifications. Many of these treatments are implemented once at least -0.50D of myopia has been identified. However, myopic progression may be detected earlier if a difference in refraction has exceeded the average 0.50D reduction in hyperopia over the course of one year. This may occur up to two years before the onset of myopia (Xiang et al., 2012). It is now well recognized that there is a genetic and environmental component to myopia. Genetic cases are characterized by high levels of myopia, very early onset, and identifiable chromosomal locations (Morgan & Rose, 2005). Of course, environmental conditions influence gene expressions, and upon expression, they can influence biochemical pathways of eye growth (Feldkämper & Schaeffel, 2003). Environmental factors have been shown numerous times in the chick model to influence axial length and refractive errors, through studies with lenses and various lighting conditions. There is strong evidence that the current increased incidence of myopia is due to the adaptation of environmental induced change (Wojciechowski, 2011). Indeed, Pan et al., (2015) showed that when the same environmental conditions were imposed on various ethnic groups, there was a mutual level of myopia despite significant genetic differences.

Many clinicians would find it hard to justify initiating treatment options before myopia has been diagnosed. This, as well as, the understanding of the influence of environmental aspects in myopia contributes to the importance of lifestyle changes in these patients. Lifestyle changes can be put in place at the start of the emmetropization process to promote proper

emmetropization. Prolonging the onset of myopia until later in childhood can reduce the likelihood that a patient becomes highly myopic. Holden et al., (2015) has identified high myopia as >6.00 D. At this diopter level and higher, a patient is more at risk for ocular disease such as cataracts, retinal detachment, glaucoma, maculopathy (Tano, 2002). A patient's quality of life in completing daily visual tasks with increasing myopia, as well as, candidacy for refractive surgery should also be considered. The results of this study as well as that of Rucker et al. (2017), would suggest that an important lifestyle modification would be exposure to natural sunlight (without any use of blue light reducing filters), or artificial lighting containing sufficient blue light as a protective measure against myopia.

Summary and Conclusions

In the absence of blue light, the mechanism of emmetropization in chick's eye is responding to temporal frequency of the lighting, becoming more myopic at lower frequencies and more hyperopic at higher frequencies. Blue light is, therefore, protective for normal emmetropization performance and against abnormal myopic (or hyperopic) changes at low (or high) temporal frequency of lighting conditions. These results are important for the clinical application of myopia control strategies, as well as understanding the possible effects the environment poses on visual development.

References

Armstrong, R.A. (2013). Statistical guidelines for the analysis of data obtained from one or both eyes. Ophthalmic Physiological Optics, 33:7-14.

Bedford, R.E. & Wyszecki, G. (1957). Axial chromatic aberration of the human eye. Journal of Optical Society of America, 47, 564-565.

Chang, A.M, Aeschbach, D., Duffy, J.F, Czeisler, C.A. (2015). Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next morning alertness. Proceedings of the National Academy of Sciences U.S.A. 2015; 112:1232-1237.

Charman, W.N, Tucker, J. (1978). Accommodation as a function of object form. American Journal of Optometry and Physiological Optics, 55(2):84-92.

Cohen, Y., Belkin, M., Yehezkel, O., Avni, I., Polat, U. (2008). Light intensity modulates corneal power and refraction in the chick eye exposed to continuous light. Vision Research 48, 2329-2335.

Cremieux, J., Orban, G.A., Duysens, J., Amblard, B., & Kennedy, H. (1989). Experimental myopia in cats reared in stroboscopic illumination. Vision Research, 29(8), 1033-1036.

Crewther, S.G., Barutchu, A., Murphy, M.J., & Crewther, D.P. (2006). Low frequency temporal modulation of light promotes a myopic shift in refractive compensation to all spectacle lenses. Experimental Eye Research, 83(2), 322-328.

Czeisler, C.A. (2013). Perspective: casting light on sleep deficiency. Nature, 497;p.S13.

Dacey, D.M., Crook, J.D., Packer, O.S. (2013). Distinct synaptic mechanisms create parallel S-ON and S-OFF color opponent pathways in the primate retina. Visual Neuroscience, 1-13.

Dacey, D.M. (1996). Circuitry for color coding in the primate retina. Proceedings of the National Academy of Science U S A, 93:582-588.

Dacey, D.M., Lee, B.B. (1994). The 'blue-on' opponent pathway in primate retina originates from a distinct bistratified ganglion cell type, Nature, 367:731-735.

Dacey, D.M., Peterson, B.B., Robinson, F.R., Gamlin, P.D. (2003). Fireworks in the primate retina: in vitro photodynamics reveals diverse LGN-projecting ganglion cell types. Neuron, 37:15-27.

DeMello, L.R., Foster, T.M., & Temple, W. (1992). Discriminative performance of the domestic hen in a visual acuity task. Journal of the Experimental Analysis of Behavior, 58, 147-157.

De Monasterio, F.M., Gouras, P. (1975). Functional properties of ganglion cells of the rhesus monkey retina. Journal of Physiology, 251:167-195.

Derrington, A.M., Krauskopf, J., Lennie, P. (1984). Chromatic mechanisms in lateral geniculate nucleus of macaque. Journal of Physiology, 357:241-265.

Derrington, A.M., Lennie, P. (1984). Spatial and temporal contrast sensitivities of neurons in lateral geniculate nucleus of macaque. Journal of Physiology, 357:219-240.

DeValois, R.L., Abramov, I., Jacobs, G.H. (1996). Analysis of response patterns of LGN cells. Journal of the Optical Society of America, 56, 966-977.

Di Y., Liu R.,Chu, R.Y., Zhou, X.T., Zhou, X.D. (2013). Myopia induced by flickering light in guinea pigs: a detailed assessment of susceptibility of different frequencies. International Journal of Ophthalmology, 6(2): 115-9.

Diether, S. & Schaeffel, F. (1997). Local changes in eye growth induced by imposed local refractive error despite active accommodation. Vision Research, 37, 659-668.

Dirani, M., Tong, L., Gazzard, G., Zhang, X., Chia, A., Young, T.L., Rose, K.A., Mitchell, P., Saw, S.M. (2009). Outdoor activity and myopia in Singapore teenage children. British Journal of Ophthalmology; 93(8): p. 997-1000.

Dreher, B., Fukada, Y., Rodieck, R.W. (1976). Identification, classification and anatomical segregation of cells with X-like and Y-like properties in the lateral geniculate nucleus of old-world primates. Journal of Physiology, 258:433-452.

Feldkämper, M., Schaeffel, S. (2003). Interactions of genes and environment in myopia. Developmental Ophthalmology, 37:34-49.

Fincham, E.F. (1951). The accommodation reflex and its stimulus. British Journal of Ophthalmology, 35(7), 381-393.

Flitcroft, D.I. (1990). A neural and computational model for the chromatic control of accommodation. Visual Neuroscience, 5(6), 547-555.

Flitcroft, D.I. (2012). The complex interactions of retinal, optical and environmental factors in myopia etiology. Progress in Retinal and Eye Research, 31(6): p. 622-60.

Foulds, W.S., Barathi, V.A., Luu, C.D. (2013). Progressive myopia or hyperopia can be induced in chicks and reversed by manipulation of the chromaticity of ambient light. Investigative Ophthalmology & Vision Science; 54:8004-8012.

French, A.N, Ashby, R.S., Morgan, I.G., Rose, K.A. (2013). Time outdoors and the prevention of myopia. Experimental Eye Research, 114: p. 58-68.

Gottlieb, M.D., & Wallman, J. (1987). Retinal activity modulates eye growth: Evidence from rearing in stroboscopic illumination. Society of Neuroscience Abstracts, 1297

Guggenheim, J.A, Northstone, K., McMahon, G., Ness, A.R., Deere, K., Mattocks, C., Pourcain, B.S., Williams, C. (2012). Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. Investigative Ophthalmology & Visual Science, 53(6): p. 2856-65.

Gwiazda J., Grice, K., Held, R., McLellan, J., Thorn, F. (2000). Astigmatism and the development of myopia in children. Vision Research, 40, 1019-1026.

Hodos, W., Erichsen, J.T. (1990). Lower-field myopia in birds: An adaptation that keeps the ground in focus. Vision Research, 30, 653-657.

Holden, B.A., Jong, M., Davis, S., Wilson, D., Fricke, T., Resnikoff, S. (2015). Nearly 1 billion myopes at risk of myopia-related sight-threatening conditions by 2050-time to act now. Clinical Experimental Optometry, 98(6);491-493

Howarth, P.A., & Bradley, A. (1986). The longitudinal chromatic aberration of the human eye and its correction. Vision Research, 26, 361-366.

Howland, H.C., Atkinson, J.,Braddick, O. and French, J. (1978). Infant astigmatism measured by photo-refraction. Science 202, 331-333

Howland, H.C., & Sayles, N. (1985). Photokeratometric and photorefractive measurements of astigmatism in infants and young children. Vision Research 25, 73-81

Hung, L.F., Wallman, J., Smith, E.L., 3rd. (2000). Vision-dependent changes in the choroidal thickness of macaque monkeys. Investigative Ophthalmology & Visual Science, 41:1259-69.

Jarvis, J.R., Taylor, N.B., Prescott, N.B., Meeks, I., & Wathes, C.M. (2002). Measuring and modeling the photopic flicker sensitivity of the chicken (Gallus gallus domesticus). Vision Research, 42, 99-106.

Jarvis, J.R., Prescott, N.B., & Wathes, C.M. (2003). A mechanistic inter-species comparison of flicker sensitivity. Vision Research, 43, 1723-1734.

Jarvis, J.R., Abeyesinghe, S.M., McMahon, C.E., & Wathes, C.M. (2009). Measuring and modeling the spatial contrast sensitivity of the chicken (Gallus gallus domesticus). Vision Research. Article in press.

Jones-Jordan, L.A., Sinnott, L.T., Cotter, S.A., Kleinstein, R.N., Manny, R.E., Mutti, D.O., Twelker, J.D., Zadnik, K. (2012).Time outdoors, visual activity, and myopia progression in juvenile-onset myopes. Investigative Ophthalmology & Visual Science, 53(11): p. 7169-75.

Kaiser, P.K., Lee, B.B., Martin, P.R., Valberg, A. (1990). The physiological basis of the minimally distinct border demonstrated in the ganglion cells of the macaque retina. The Journal of Physiology, 422, 153-183.

Kaplan, E., Shapley, R.M. (1986). The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. Proceedings of the National Academy of Sciences U S A., 83:2755-2757.

Kee, C.S., Marzani, D., Wallman, J. (1997). Differences in time course and visual requirements of ocular responses to lenses and diffusers. Investigative Ophthalmology & Visual Science, 42:575-583.

Kelly, D.H., & van Norren, D. (1997). Two-band model of heterochromatic flicker. Journal of the Optical Society of America, 67(8), 1081-1091

Klug, K., Herr, S., Ngo, I.T., Sterling, P., Schein, S. (2003). Macaque retina contains an S-cone OFF midget pathway. Journal of Neuroscience, 23:9881–9887.

Klug, K., Tsukamoto, Y., Sterling, P., Shein, S.J. (1993). Blue cone Off-midget ganglion cells in macaque. Investigative Ophthalmology & Vision Science, 3; 34(supp):986.

Kouyama, N., Marshak, D.W. (1992). Bipolar cells specific for blue cones in the macaque retina. Journal of Neuroscience, 12:1233-1252.

Kremers, J., Lee, B.B., Kaiser, P.K. (1992). Sensitivity of macaque retinal ganglion cells and human observers to combined luminance and chromatic temporal modulation. Journal of Optical Society of America, 9, 1477-1485.

Kroger, R.H., Wagner, H.J. (1996). The eye of the blue acara (Aequidens pulcher, Cichlidae) grows to compensate for defocus due to chromatic aberration. Journal of Comparative Physiology, 179:837–842.

Lauber, J.K., Boyd, J.E., Boyd, T.A. (1970). Intraocular pressure and aqueous outflow facility in light-induced avain buphthalmos. Experimental Eye Research, 9,181-187.

Lauber, J.K. (1991).Three avian eye enlargement protocols as myopia models: Effects of pharmacological intervention. Journal of Ocular Pharmacology, 7:65-75.

Lauber, J.K. & Oishi, T. (1987). Lid suture myopia in chicks. Investigative Ophthalmology & Visual Science, 28(11), 1851-1858.

Lee, B.B, Martin, P.R., Valberg, A. (1988). The physiological basis of heterchromatic flicker photometry demonstrated in the ganglion cells of the macaque retina. The Journal of Physiology, 404, 323-347.

Lee, B.B., Martin, P.R., Valberg, A. (1989). Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. The Journal of Physiology, 414, 223-243.

Lee, B.B., Pokorny, J., Smith, V.C., Martin, P.R., Valberg, A. (1990). Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. Journal of the Optical Society of America, 7:2223-2236.

Lee, R.J., Mollen, J.D., Zaidi, Q., Smithson, H.E. (2009). Latency characteristics of the short-wavelength-sensitive cones and their associated pathways. Journal of Visualized Experiments, 9(12):5.

Lee, B.B., Sun, H. (2004). Chromatic input to cells of the magnocellular pathway: mean chromaticity and the relative phase of modulated lights. Visual Neuroscience, 21:309-314.

Li, T., Troilo, D., Glasser, A., & Howland, H.C. (1995). Constant light produces severe corneal flattening and hyperopia in chickens. Vision Research, 35(9), 1203-1209.

Li, T., & Howland, H.C. (2003). The effects of Constant and Diurnal Illumination of the Pineal Gland and the Eyes on Ocular Growth in Chicks. Investigative Ophthalmology & Vision Science, 44; 3692-3697.

Liu, J., Pendrak, K., Capehart, C., Sugimoto, R., Schmid, G.F., & Stone, R.A. (2004). Emmetropisation under continuous but non-constant light in chicks. Experimental Eye Research, 79(5), 719-728.

Liu, R., Hu, M., He, J.C., Zhou, XT., Dai, J., Qu, X., Liu, H., Chu, R. (2014). The effects of monochromatic illumination on early eye development in rhesus monkeys. Investigative Ophthalmology & Visual Science; 55:1901-1909.

Mandelman T & Sivak J.G. (1983). Longitudinal chromatic aberration of the vertebrate eye. Vision Research; 23:1555-1559.

Marcos S, Burns S.A., Moreno-Barriusop E & Navarro R. (1999). A new approach to the study of ocular chromatic aberrations. Vision Research; 39:4309-4323.

Marshak, D.W. (2009). Retinal Ganglion Cells: Anatomy. Encyclopedia of Neuroscience, 211-218.

McBrien, N.A., Moghaddam, H.O., Cottriall, C.L, Leech, E.M., Cornell, L.M. (1995). The effects of blockade of retinal cell action potentials on ocular growth, emmetropization and form deprivation myopia in young chicks. Vision Research, 35, 1141-1152.

McKeefry, D.J., Parry, N.R., Murray, I.J. (2003). Simple reaction times in colour space: the influence of chromaticity, contrast, and cone opponency. Investigative Ophthalmology and Visual Science, 44:2267-2276.

Miles, F.A., Wallman, J. (1990). Local ocular compensation for imposed local refractive error. Vision Research, 30, 339-349.

Morgan, I., Rose, K. (2005). How genetic is school myopia? Progress in Retinal and Eye Research, 24:1-38.

Mutti, D.O., Mitchell, G.L., Jones, L.A., Friedman, N.E., Frane, S.L., Lin, W.K., Moeschberger, M.L., Zadnik, K. (2005). Axial growth and changes in lenticular and corneal power during emmetropization in infants. Investigative Ophthalmology & Vision Science, 46(9); 3074-80.

Nickla, D.L., Wildsoet, C., & Wallman, J. (1997) Compensation for spectacle lenses involves changes in proteoglycan synthesis in both the sclera and choroid. Current Eye Research, 16(4), 320-326.

Norton T. Experimental myopia in tree shrews. Bock G., Widdows K. (1990). (Eds), Myopia and the control of eye growth (Ciba Foundation Symposium 155), Wiley, Chichester , pp. 178-199.

Nuboer, J.F.W., Coemans, M.A.J.M., & Vos, J.J. (1992). Artificial lighting in poultry houses: do hens perceive the modulation of fluorescent lamps as flicker. British Poultry Science, 33, 123-133.

Osorio, D., Vorobyev, M., Jones, C.D. (1999). Color vision of domestic chicks. Journal of Experimental Biology, 202(pt 21):2951-2959.

Over, R., & Moore, D. (1981). Spatial acuity of the chicken. Brain Research, 221:424-426

Padmanabhan, V., Shih, J., Wildsoet, C.F. (2007). Constant light rearing disrupts compensation to imposed-but not induced- hyperopia and facilitates compensation to imposed myopia in chicks. Vision Research, 47(14)1855-1868.

Pan, C.W., Chen, Q., Sheng, X., Li, J., Niu, Z., Zhou, H., Wei, T., Yuan, Y., Zhong, H. (2015) Ethnic variations in myopia and ocular biometry among adults in a rural community in China: the Yunnan minority eye studies. Investigative Ophthalmology & Vision Science, 56:3235-3241.

Park, T.W., Winawer, J., Wallman, J. (2003). Further evidence that chick eyes use the sign of blur in spectacle lens compensation. Vision Research, 43(14):1519-1531.

Phillips, S. & Stark, L. (1977). Blur: A sufficient accommodative stimulus. Doc Ophthalmology, 43: 65.

Qiao-Grider, Y., Hung, L., Kee, C., Ramamirtham, R., Smith, E.L. (2007). Normal ocular development in young rhesus monkeys (Macaca mulatta). Vision Research, 47(,11); 1424-1444.

Rabin, J., Switkes, E., Crognale, M., Schneck, M.E., Adams, A.J. (1994). Visual evoked potentials in three-dimentsional color space: correlates of spatio-chromatic processing. Vision Research, 34:2657-2671.

Robson, A.G., Kulikowski, J.J. (1998). Objective specification of tritanopic confusion lines using visual evoked potentials. Vision Research, 38:3499-3503.

Rogers, L. (1996). Behavioral, structural and neurochemical asymmetries in the avian brain: a model system for studying visual development and processing. Neuroscience and Biobehavioral Reviews, 20:487-503.

Rose, K.A., Morgan, I.G., Ip, J., Kifley, A., Huynh, S., Smith, W., Mitchell, P. (2008). Outdoor activity reduces the prevalence of myopia in children. Ophthalmology; 115(8): p. 1279-1285.

Rucker, F., Henriksen, M., Yanase, T., Taylor, C. (2017). The role of temporal contrast and blue light in emmetropization. Vision Research. http://dx.doi.org/10.1016/j.visres.2017.07.003

Rucker, F.J., Wallman, J. (2012). Chicks use changes in luminance and chromatic contrast as indicators of the sign of defocus. Journal of Vision, 12(6):23.

Rucker, F.J., & Kruger, P.B. (2001). Isolate short-wavelength sensitive cones can mediate a reflex accommodation response. Vision Research; 41:911-922.

Rucker, F.J., & Kruger, P.B. (2004). The role of short-wavelength sensitive cones and chromatic aberration in the response to stationary and step accommodation stimuli. Vision Research, 44:197-208.

Rucker, F.J., & Wallman, J. (2008). Cone signals for spectacle-lens compensation: differential responses to short and long wavelengths. Vision Research; 48:1980-1991.

Saw, S.M., Wu, H.M., Hong, C.Y., Chua, W.H., Chia, K.S., Tan, D. (2001). Myopia and night lighting in children in Singapore. British Journal of Ophthalmology, 85, 527-528.

Saw, S.M., Zhang, M.Z., Hong, R.Z., Fu, Z.F., Pang, M.H., Tan, D.T. (2002). Near-work activity, night-lights, and myopia in the Singapore-China study. Archives of Ophthalmology, 120,620-627.

Schaeffel, F., Glasser, A., Howland, H.C. (1988). Accommodation, refractive error and eye growth in chickens. Vision Research, 28(5):639-57.

Schaeffel, F., Diether, S. (1999). The growing eye: an autofocus system that works on very poor images. Vision Research, 29:1585-1589.

Schiller, P.H., Logothetis, N.K., Charles, E.R. (1990). Role of the color opponent and broad-band channels in vision. Visual Neuroscience, 5:321-346.

Schmid, K.L., & Wildsoet, C.F. (1997). Natural and imposed astigmatism and their relation to emmetropization in the chick. Experimental Eye Research. 64, 837-847.

Schwahn, H.N., & Schaeffel, F. (1997). Flicker parameters are different for suppression of myopia and hyperopia. Vision Research, 37(19), 2661-2673.

Seidemann, A. & Schaeffel, F. (2002). Effects of longitudinal chromatic aberration on accommodation and emmetropization. Vision Research, 42:2409–2417.

Shapley, R.M., Perry, V.H. (1986). Cat and monkey retinal ganglion cells and their visual functional roles. Trends in Neuroscience, 9:229-235.

Shih, Y.F, Chiang, T.H, Lin, L.L. (2009). Lens thickness changes among schoolchildren in Taiwan. Investigative Ophthalmology and Vision Science, 50(6); 2637-44.

Smith, E.L. (1998). Environmentally induced refractive errors in animals. In Myopia and Nearwork (pp 57-90). Oxford: Butterworth-Heinemann.

Smithson, H.E, Mollon, J.D. (2004). Is the S-opponent chromatic subsystem sluggish? Vision Research, 44:2919-2929.

Stockman, A., MacLeod, D.I, DePriest, D.D. (1991). The temporal properties of the human short-wave photoreceptors and their associated pathways. Vision Research, 31:189-208.

Stone, R.A., Lin, T., Desai, D., & Capehart, C. (1995). Photoperiod, early post-natal eye growth, and visual deprivation. Vision Research, 35(9), 1195-1202.

Stromeyer, C.F., 3rd, Eskew, R.T. Jr, Kronauer, R.E., Spillmann, L. (1991). Temporal phase response of the short-wave cones singal for color and luminance. Vision Research, 31:787-803.

Tano, Y. (2002). Pathologic myopia: where are we now? American Journal of Ophthalmology, 134:645-660.

Thibos, L.N., Wheeler, W., Horner, D. (1997). Power vectors: an application of Fourier analysis to the description and statistical analysis of refractive error. Optometry & Vision Science 74:367–375.

Torii, H., Kurihara, T., Seko, Y., Negishi, K., Ohnuma, K., Inaba, T., Kawashima, M., Jiang, X., Kondo, S., Miyauchi, M., Miwa, Y., Katada, Y., Mori, K., Kato, K., Tsubota, K., Goto, J., Oda, M., Hatori, M., Tsubota, K. (2017). Violet light exposure can be a preventative strategy against myopia progression. EBioMedicine, 15; 210-219.

Troilo, D., Gottleib, M.D., Wallman, J. (1987). Visual deprivation causes myopia in chicks with optic nerve section. Current Eye Research, 6:993-999.

Troilo, D., Wallman, J. (1991). The regulation of eye growth and refractive state: An experimental study of emmetropization. Vision Research, 31: 1237-50.

Troelstra, A., Zuber, B.L., Miller, D., Stark, L. (1964). Accommodative tracking: a trial-and-error function. Vision Research, 4(11):585-94.

Wahl, C., Li, T., Howland, H. (2015). Plasticity in the growth of the chick eye: Emmetropization achieved by alternate morphologies. Vision Research; 110; 15-22.

Wald, G. & Griffin, D.R. (1947). The change in refractive power of the human eye in dim and bright light. Journal of Optical Society of America, 37, 321-336.

Wallman, J. and Adams, J.I. (1987). Developmental aspects of experimental myopia in chicks: Susceptibility, recovery and relation to emmetropization. Vision Research 27,1139-1163

Wallman, J., Wildsoet, C., Xu, A., Gottlieb, M.D., Nickla, D.L., Marran, L., Krebs, W., Christensen, A.M. (1995). Moving the retina: choroidal modulation of refractive state. Vision Research, 35(1):37-50.

Wallman, J. & Winawer, J. (2004). Homeostasis of eye growth and the question of myopia. Neuron, 43(4), 447-468.

Wildsoet, C.F. (2003). Neural pathways subserving negative lens-induced emmetropization in chicks-insights from selective lesions of the optic nerve and ciliary nerve. Current Eye Research, 27, 371-385.

Wildsoet, C.F. & Pettigrew, J.D. (1988). Experimental myopia and anomalous eye growth patterns unaffected by optic nerve section in chickens: evidence for local control of eye growth. Clinical Vision Sciences, 3, 99-107.

Wildsoet, C. & Wallman, J. (1995). Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. Vision Research, 35(9):1175-1194.

Wildsoet, C. (1998). Structural correlates of myopia. In M.Rosenfield & B. Gilmartin (Eds.), Myopia and Nearwork (pp.31-56), Oxford: Butterowrth-Heinemann.

Wojciechowski, R. (2011). Nature and Nurture: the complex genetics of myopia and refractive error. Clinical Genetics, 79:301-320.

Xiang, F., He, M., Morgan, I.G. (2012). Annual changes in Refractive Errors and Ocular Components before and after the onset of myopia in Chinese Children. Ophthalmology, 119:1478-1484.

Yu, Y., Chen, H., Tuo, J., & Zhu, Y. (2011). Effects of flickering light on refraction and changes in eye axial length of C57BL/6 mice. Ophthalmic Research, 46:80-87.

Zadnik, K., Jones, L.A., Irvin, B., Kleinstein, R.N., Manny, R.E., Shin, J.A., Mutti, D.O. (2000). Myopia and ambient night-time lighting. Nature, 404,143-144

Zhi, Z., Pan, M., Xie, R., Xiong, S., Zhou, X., Qu, J. (2013). The effect of temporal and spatial stimuli on the refractive status of guinea pigs following natural emmetropization. Investigative Ophthalmology & Vision Science, 54:890-897.

Zhu, X., Park, T.W., Winawer, J., Wallman, J. (2005). In a matter of minutes, the eye can know which way to grow. Investigative Ophthalmology and Visual Science, 46(7)2238-41.