

Electroretinograms Following Short-Term Chromatic Light Adaptation in High Myopes and Non-Myopes

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Purpose: Worldwide, myopia cases are on the rise and the need for finding a definitive mechanism by which myopia develops has become more imperative than ever before. Axial elongation in myopic eyes is linked to short wavelength (λ) light-dependent increases in retinal dopamine (DA). DA is associated with enhanced electroretinogram (ERG) amplitudes. This thesis seeks to examine short-term adaptation to short and long λ light at moderate and strong levels. I hypothesize that short λ light exposure differentially affects full-field ERGs (ffERGs) and central ERGs in myopic eyes compared to controls.

Methods: In a multi-visit cross-over design, we compared ERGs before and after 20 minutes of full-field adaptation to long (red LED peak λ (627 nm) or short (blue LED peak λ 448 nm) λ light (Espion ColorDome™, Diagnosys LLC). Human participants (ages 18-30) were healthy high myopes (≤ -5 diopters (D)) and emmetropes (+1 D to -0.25 D). Monocular, light-adapted (LA) ffERGs were recorded using skin electrodes and a handheld system that adjusted for pupil diameter in real time (RETeval™). ERG stimuli were LA standard white flash and flicker (85 Td.s), presented at 2 and 28.3 Hz, respectively. The a-wave, b-wave and flicker ERG amplitudes, and implicit times were compared as a function of pre/post adaption (time), adapting stimulus (λ), and refractive error (RE) group using a mixed model ANOVA. Monocular multi-focal ERGs (mfERGs; 61 hexagons) and pattern ERGs (PERGs; 15° field, 15' reversing checks) were recorded with natural pupils in keeping with ISCEV standards using DTL electrodes. The Espion™ console and amplifier (Diagnosys LLC) was used for both ERG tests. The primary outcome measures analyzed were the amplitudes and implicit times of the mfERG central wavelet, mfERG average of the surrounding rings and the PERG P50 peak and were compared as a function of pre/post adaption (time), adapting stimulus (λ), and RE group using a mixed model ANOVA.

Results: There were no significant differences between controls and myopes prior to adaptation (all $p \geq 0.05$) for ffERG tests. The luminance of the light had an effect such that changes in b-wave implicit time decreased with adaptation to 300 cd/m² light in controls (blue 30: $-0.451 \pm 1.28\%$; blue 300: $-2.28 \pm 2.56\%$; $p \leq 0.001$, $\eta^2 G = 0.14$) but less so in the high myope group (blue 30: $+0.05 \pm 1.3\%$; blue 300: $-1.26 \pm 2.43\%$; $p = 0.01$, $\eta^2 G = 0.05$). Changes in flicker implicit time decreased with the stronger luminance level but were not different between refractive error groups (i.e. controls: blue 30: $0.15 \pm 1.0\%$; blue 300: $-1.05 \pm 1.61\%$, myopes: blue 30: $+0.44 \pm 2.74\%$; blue 300: $-1.1 \pm 1.50\%$, $p \leq 0.001$, $\eta^2 G = 0.13$). Stronger light conditions caused b-wave amplitudes to become less negative (smaller) (i.e. controls: blue 30: $-5.38 \pm 15.6\%$; blue 300: $-3.50 \pm 17.7\%$, myopes: blue 30: $-10.9 \pm 15.4\%$; blue 300: $+0.85 \pm 10.9\%$, $p = 0.02$, $\eta^2 G = 0.05$). Similarly, with

flicker amplitudes, they became less negative (smaller) with stronger luminance adaptation (i.e. controls: blue 30: $-4.1 \pm 13.4\%$; blue 300: $-0.62 \pm 17.1\%$, myopes: blue 30: $-9.9 \pm 18.3\%$; blue 300: $+4.7 \pm 12.0\%$, $p=0.00$, $\eta^2G=0.07$). When comparing between controls and high myopes or between short and long wavelengths, changes in b-wave amplitudes did not differ. There was a significant difference between RE groups for the PERG P50 amplitude prior to adaptation ($p=0.03$; Cohen's $d=1.06$) such that they were significantly smaller in myopes ($2.63 \pm 1.0 \mu\text{V}$) than controls ($3.95 \pm 1.5 \mu\text{V}$). The N95 amplitudes were also smaller (i.e. less negative) in myopes ($-4.39 \pm 1.82 \mu\text{V}$) than controls ($-6.58 \pm 1.55 \mu\text{V}$; $p=0.01$; Cohen's $d=-1.3$). The mfERGs showed no significant pre-adaptation RE differences (all $p \geq 0.05$; all $\eta^2G \leq 0.07$). For the PERG, in both controls and myopes, the change in N95 amplitudes decreased more with long λ light (controls: $-16.6 \pm 23.4\%$; myopes: $-24.7 \pm 43.4\%$) than short λ light (controls: $+10.4 \pm 23.5\%$; myopes: $+1.88 \pm 26.7\%$; $p \leq 0.001$; $\eta^2G=0.541$). There were no effects of adaptation on the P50 amplitude or implicit time. For mfERG, the change in N1P1 magnitude was not different between controls and myopes and was not different when comparing λ s for any of the five rings ($p \geq 0.05$).

Conclusions: There is no evidence that chromatic adaptation has a differential effect on post-adaptation fERGs in high myopes. Long λ adaptation, especially with stronger luminance, prolongs ERG implicit times, probably reflecting relatively reduced input from the faster long cone system. There is evidence to suggest smaller central retinal responses with the PERG P50 but not with the central wavelet of the mfERG, indicating altered retinal ganglion cell function but not altered central inner retinal function in young adults with myopia. Further studies should focus on confirming whether altered central retinal function persists in adult myopes and whether longer adaptation times would yield a greater RE difference.

Acknowledgements

Praise the LORD

"In all your ways acknowledge Him and He shall direct your paths"

- Proverbs 3:6

First and foremost, I must begin by thanking the LORD. As one of my favourite hymns say: "I will not boast in anything. No gifts, no power, no wisdom. But I will boast in Jesus Christ, His death and resurrection."

To my supervisors Dr. Vivian Choh and Dr. Daphne McCulloch, your unwavering support, guidance, and mentorship have been invaluable. Thank you for the opportunity to explore such an ever-evolving and *beautiful* field of study. The last two years have only confirmed and furthered my passion for vision science. I have gleaned so much wisdom from both of you, thank you for always challenging me to never settle for less than the best. A heartfelt thank you to my committee members Dr. Vivan Dayeh and Dr. Jennifer Hunter for your valued knowledge and timely advice. I know my thesis is better because of it.

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Last but certainly not least, I would like to thank my fellow colleagues and graduates in vision science for making the past two years so enjoyable. I have made lasting friendships and I hope to stay in touch and continue to celebrate the wonderful things we will accomplish in the years ahead!

Dedication

I lovingly dedicate this thesis to my parents. Mom and Dad, I cannot thank you enough for the many sacrifices you have made over the years to support my dreams. You not only taught me how to fly, you let me soar. I know that no matter where life takes me, your daily prayers, instructions, and constant encouragement will forever echo in my ears, guiding my every step.

May this and all that follows be a testament to your labour, the fruit it has borne and will continue to bear. I love you.

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List of Abbreviations

AWA a-wave amplitude

AWT a-wave implicit time

AXL axial length

BWA b-wave amplitude

BWT b-wave implicit time

DA retinal dopamine

DACs dopaminergic amacrine cells

DTL Dawson, Trick and Litzkow electrodes

ERGs electroretinograms

ETDRS early treatment diabetic retinopathy study

FDM form deprivation myopia

ffERG full-field electroretinogram

GABA gamma-aminobutyric acid

GCL ganglion cell layer

ILM inner limiting membrane

INL inner nuclear layer

IPL inner plexiform layer

ipRGCs intrinsically photosensitive ganglion cells

ISCEV The International Society for Clinical Electrophysiology of Vision

LASIK laser-assisted in situ keratomileusis

LCA longitudinal chromatic aberration

LIM lens-induced myopia

LogMAR logarithm of the minimum angle of resolution

mfERG multifocal electroretinogram

NFL nerve fiber layer

ONL outer nuclear layer

OPL outer plexiform layer

PERG pattern electroretinogram

PRK photorefractive keratectomy

RGCs retinal ganglion cells

RPE retinal pigmented epithelium

SER spherical equivalent refraction

Td trolands

Td.s troland seconds

WA flicker waveform amplitude

WIT flicker waveform implicit time

List of Symbols

η^2 Eta squared

λ Wavelength

Chapter 1

Overall Objectives

Myopia, or short-sightedness, occurs most commonly due to an elongation of the axial length—the distance from the apex of the cornea to the retina. This elongation causes light from distant objects to focus in front of the retina rather than directly on it, resulting in blurred vision. Additionally, a longer axial length can lead to retinal thinning, increasing the risk of retinal tears, macular degeneration, and other retinal diseases (Morgan et al., 2012).

With global rates of myopia rising rapidly, understanding the environmental and biological factors driving its progression is more critical than ever (Holden et al., 2016). Although the exact cause of this surge remains unclear, risk factors can be broadly categorized into two groups. The first involves the visual environment, where increased educational demands and reduced time spent outdoors are strongly associated with higher myopia risk (Pan et al., 2012). The second is genetic predisposition—particularly when both parents are highly myopic, the likelihood of their child developing myopia significantly increases (Morgan et al., 2021a). However, these factors often confound one another and a definitive mechanism behind myopia progression has yet to be identified.

Despite a growing body of research linking outdoor light exposure to reduced eye growth—particularly through the dopaminergic-melanopsin pathway—the molecular mechanisms remain poorly understood. My study explores the potential of outdoor activity as a treatment for myopia since outdoor light is dominated by short-wavelength blue light (480 nm) which may influence retinal function through intrinsically photosensitive retinal ganglion cells (ipRGCs) and dopamine pathways (Zhang et al., 2008a). Previous experiments have suggested a link between dopamine and myopia (Zhou et al., 2017a), with evidence showing that electroretinograms (ERGs) in myopic individuals tend to be smaller and less responsive to short-wavelength stimuli (Westall et al., 2001; Kader, 2012). While the direct impact of dopamine on ERG signals is not fully established, some findings in-

dicating that dopamine may enhance ERG responses. For example, Li et al. found that 6-hydroxy dopamine in chickens was able to stimulate dopaminergic pathways enough to block development of myopia (Li et al., 1992). It has also been suggested that dopamine has an excitatory effect on ON bipolar cells, potentially enhancing the photopic b-wave response (Popova and Kuppenova, 2013). Another study also suggested that short-term blue light exposure on the optic nerve could play a role (Amorim-de Sousa et al., 2021), however, questions remain about how blue light directed solely at the optic nerve could affect ipRGCs, and whether blue light has a broader impact on the peripheral retina.

To address these uncertainties, this study was designed to determine whether blue light adaptation enhances ERGs and if myopes respond differently to blue light compared to controls. It also examined whether the retinal response varies depending on the location within the retina, aiming to clarify whether myopic individuals generally exhibit distinct and localized reactions to blue light. Following a comprehensive review of relevant literature, my work is presented through three interconnected manuscripts. The first examines changes in full-field ERG (ffERG) in high myopes after short-term chromatic light exposure at standard luminance. The second builds on this by testing ffERG responses under higher luminance levels, more representative of outdoor light. The third investigates the effects of light adaptation on central retinal ERG tests, including pattern ERG (PERG) and multifocal ERG (mfERG). Together, these studies offer a layered perspective on how the outdoor light environment may affect retinal physiology and contribute to myopia development.

Chapter 2

Literature Review

2.1 The Human Eye

Vision is widely regarded as one of the most fundamental senses, allowing us to appreciate and engage with the world we live in, to gain knowledge and to communicate through visual expression (Kolb, 2020). Light enters the eye through a circular aperture called the pupil. The coloured part of our eyes, known as the iris, has sphincter muscles that reduce the size of the pupil and dilator muscles that increase the size of the pupil. The transparent corneal layer that covers the iris and pupil plays an important role in focusing light. The convex crystalline lens changes its shape becoming more round in order to bring near objects into focus (Oyster, 1999). The crystalline lens shape is controlled by surrounding suspensory ligaments that are also attached to the ciliary muscles (Kolb, 2020). Contraction of the ciliary muscles results in relaxation of the suspensory ligaments that normally hold the lens taut.

The eye contains chambers filled with two types of fluids that work to maintain the interocular pressure through a process of circulation and reabsorption. The first fluid is called the aqueous humour that fills both the area from the cornea to the front of the iris (the anterior chamber) and the area between the iris and the lens (the posterior chamber). In addition to helping the eye to maintain its shape, the aqueous fluid contains nutrients vital to the health of the anterior structures. The second fluid type is a gel-like substance known as the vitreous humour and the longest distance of the visual pathway from the cornea to the retina is through the vitreous (Oyster, 1999).

The retina is a thin, non-regenerative layer at the back of the eye that captures photons, converting them to electrical signals that get sent to the visual cortex of the brain (Kolb, 2020). The outermost layer of the retina is called the retinal pigmented epithelium (RPE) while the rest of the cells of the retina comprise the neural retina. The human retina

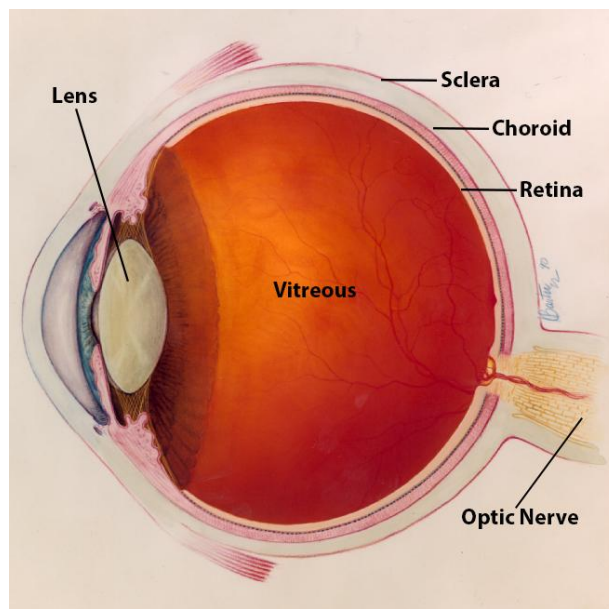


Figure 2.1: Graphic showing the basic ocular structures. Used with permission © National Eye Institute.

is inverted, where light must travel through the entirety of the neural retina in order to reach the light-sensitive cells. These cells, the photoreceptors (i.e. rods and cones), sit adjacent to the RPE. The RPE is responsible for producing and recycling the chromophore retinal, a vitamin A derivative, which allows for the initiation of phototransduction (i.e. the capturing and transduction of light into an electrical signal) (Oyster, 1999). This signal makes its way to the front of the retina and out of the eye to the brain for further processing. The RPE is directly adjacent to the choroid layer, which helps to form a blood-retinal barrier and to regulate nutrient exchange between the highly vascularized choroid and the layers of the neural retina (Oyster, 1999; Vanderah and Gould, 2020) (Figure 2.1). The choroid (along with the RPE) is highly pigmented due to the presence of melanin which aids in absorbing stray light, reducing aberration and light scatter, thus helping to improve the clarity of the image on the retina (Vanderah and Gould, 2020). Lastly, the sclera is the opaque, white connective tissue that comprises the supporting wall of the eyeball, and serves as the attachment site for extra-ocular muscles that enables movements of our eyes (Oyster, 1999).

2.2 Retinal Organization & Physiology

The retina, developed from the embryonic forebrain, is considered a part of the brain. Although it is only half a millimeter thick, there are 10 distinct layers of the retina that

each play a critical role in image formation (Kolb, 2003; Vanderah and Gould, 2020). With respect to the nomenclature of these layers, "nuclear" layers contain cell-bodies, "plexiform" layers indicate synaptic regions and "inner" and "outer" refer to the distance from the optic nerve and brain where outer is farthest from the front of the eye (Vanderah and Gould, 2020).

Light must travel through all 10 layers in order to reach the light-sensitive cells (i.e. rods and cones) where phototransduction can actually occur. Beginning with the outermost layer, the RPE contains melanin pigment which aids greatly in absorbing reflected light to ensure a crisp image (Oyster, 1999; Vanderah and Gould, 2020).

Moving anteriorly, the outer nuclear layer (ONL) contains the cell bodies of the rods and cones. The rods are slow-responding and most sensitive to dim light conditions, found with greatest frequency in the peripheral retina. They have long, thin inner and outer segments that project into the sub-retinal space. The inner segments are filled with mitochondria and the outer segments contain neatly stacked membranous discs that are free-floating and not directly attached to the plasma membrane (Oyster, 1999). Each disc has multiple trans-membrane opsin proteins known as rhodopsin that houses retinal, a molecule that plays an important role in the phototransduction cascade. The cone photoreceptors differ from rods in several ways. There are three types of cones each maximally sensitive to light at either short (419 nm), medium (531 nm) or long (559 nm) λ s whereas there is only one kind of rod (Kolb, 2003). Each type of cone has a slightly altered version of the opsin protein with relatively identical structures but substituted amino acids. These opsins also house retinal. Cones exist in greatest concentration at the fovea and there are 20 times less cones than rods in the human retina. Cones are generally shorter in size and although their inner segments are also filled with mitochondria, their outer segments are conical with a wider base and top that tapers off, much like their name suggests. The membranous discs within are connected to the plasma membrane unlike their rod counterparts (Brown, 1965).

The region where the synapses between horizontal cells and the synaptic spherules of the photoreceptor cells takes place is the outer plexiform layer (OPL) (Brown, 1965). Horizontal cells work to adjust the signals before they get sent to bipolar cells by responding oppositely (i.e. inhibitory or excitatory depending on the photoreceptor signal), boosting our ability to detect contrast in luminance. (Oyster, 1999).

The next layer, the inner nuclear layer (INL), contains the cell bodies of various types of bipolar, amacrine and horizontal cells. The bipolars directly synapse with the retinal ganglion cells (RGCs). The amacrine cells receive impulses from the bipolars, passing this information onto the RGCs as well as other bipolar and amacrine cells (Oyster, 1999). The inner plexiform layer (IPL) is the synaptic layer where RGCs connect with the bipolar and

amacrine cells. Most amacrine cells send inhibitory signals such as gamma-aminobutyric acid (GABA) or glycine when synapsing with bipolar cells which helps to merge, modulate and add information about timing to the the visual signals. Although there are many types of amacrine cells that have been discovered (Freed et al., 1996), dopaminergic amacrine cells (DACs) play an important role in adjusting for different lighting conditions and supporting high acuity light-adapted vision. DACs are the sole source of retinal dopamine (DA) in the eye and follow a circadian rhythm of production (high levels of production during the day, low at night). Additionally, the dendrites of DACs spread into the RGC layer and have been shown to interact with different types of RGCs in the dopaminergic pathway (Gonon, 1988; Dacey, 1990; Zhang et al., 2008b). There are displaced amacrine cells (up to 25% in the primate (non-human and human) retina (Martin-Martinelli et al., 1994)) that are found in the ganglion cell layer (GCL), some of which may be DACs. RGCs and ipRGCs may also be displaced into the INL (Bordt et al., 2022). These displaced cells along with those in the normal synaptic pathway all contribute to the details of the final image created by the brain.

The next two layers, GCL and nerve fiber layer (NFL) contain the cell bodies and axons of the RGCs, respectively. There are many different types of RGCs but they all carry electrical impulses to the brain via the optic nerve (Kolb, 2003). One of these types are called intrinsically photosensitive ganglion cells (ipRGCs) which are a subtype of RGCs that are not involved in the image forming process but are light sensitive due to the melanopsin pigment they contain (Provencio et al., 1998; Hatori and Panda, 2010). The ipRGCs are coupled to amacrine cells and help to control light-induced circadian rhythms via the retina-hypothalamic pathway that then signals to the suprachiasmatic nucleus in the brain, ultimately controlling release of melatonin (Pickard and Sollars, 2012).

The most anterior layer is the inner limiting membrane (ILM). Made of extracellular matrix, it serves as a thin barrier between the vitreous humour and ends of the Müller cells, which help maintain retinal homeostasis and structural support to the rest of the retina (Brown, 1965).

While the entire retina is important for image formation and perception, the central area called the macula is where images are focused with the highest resolution. The central retina is cone-dominated (Kolb, 2003). Here, the cones are thinner than even rods and tightly packed. There are also more cone bipolar cells, cone-related horizontal cells and amacrine cells as well as more ganglion cells and synaptic connections, leading to thicker retinal layers as you move centrally. The centre-most point of the macula is the fovea, a small depression in the centre of the retina and the point of highest visual acuity where there are only cones present. All other retinal layers are pushed aside in a unique formation known as the bundle of Henle (Brown, 1965; Kolb, 2003). The absence of any blood vessels

and displacement of all other layers allows for a relatively high quality image, additionally making the central fovea an important area for pattern vision. Potentially due to the lack of nerve layers for protection, there is a yellow macular pigment that covers the central area of the fovea which protects the cones against too much exposure to harmful short wavelength light radiation (Brown, 1965).

Eye & Visual Pathway

In an emmetropic, unaccommodated eye, light from infinity refracted at the cornea, enters through the pupil in order for light rays from a distant point to focus at a single point directly on the retina. The retina makes use of an intracellular phototransduction pathway enabling it to preform the first stages of image processing. Light must travel through the entirety of the eye in order to reach the outer segments of the photoreceptors where the photo-pigment molecules are located. The photoreceptors need to be close to the RPE which produces retinal (chromophore and derivative of vitamin A). When exposed to light, the 11-cis retinal changes to an all trans conformation (Kolb, 2003) and is then shuttled back into the RPE.

The now activated opsin proteins then trigger a downstream cascade of events that eventually shuts cation channels, resulting in hyperpolarization of the photoreceptors. Phototransduction prevents the release of glutamate, an amino acid important for communicating with ON bipolar cells. The ON bipolar cells depolarize in response to the decrease in glutamate from the photoreceptors which then triggers the ON ganglion cells. These signals are modified by horizontal cells which provide input from the surrounding retina. The signals are also fine-tuned by amacrine cells, which incorporate information about borders and timing of complex stimuli. The electrical signals generated are then transmitted to the ganglion cells, transferred through the optic nerve fibers and ultimately to the brain for further processing (Brown, 1965).

In the dark, the opposite happens where the opsin are inactivated and therefore the cation channels stays open and allow cations such as sodium and calcium to enter the photoreceptor cytoplasm and the resulting depolarization leads to the ultimate release of glutamate to OFF bipolar cells. The release of glutamate inactivates the OFF bipolar cells (Kolb, 2003). The resulting polarization caused by light stimuli not only sends information about the image to the brain but light also induces an electrical change in the cornea relative to the back of the eye. This spread of electrical current can be quantitatively measured via electroretinograms (ERGs) (Brown, 1968).

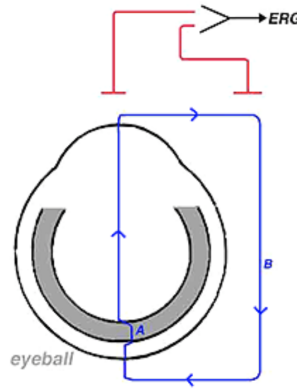


Figure 2.2: There are two pathways that the light-induced current travels. Pathway A stays within the retina layers and pathway B spreads to the front of the eye and is measured by the ERG. From The Electoretinogram: ERG by Ido Perlman (Permission from Webvision <http://webvision.med.utah.edu/>).

2.2.1 Electroretinograms

Today, ERGs are used regularly in clinical settings but the discovery of light-induced voltage potentials dates back to 1865, when Holmgren discovered that a light stimulus can change electrical potential in an amphibian eye. In 1903, Gotch (Gotch, 1903) separated the electrical potential into a positive and negative wave, paving the way for Einthoven and Jolly's work in 1908 (Einthoven and Jolly, 1908) where they confirmed the generation of three waves: a negative wave (a-wave) and positive wave (b-wave). In 1911, Piper contributed the idea that the above ERG components last as long as the light flash. In 1933, Granit recorded ERGs in cats and was able to isolate wave responses by increasing the anesthesia and observing the disappearance of the waves (Granit, 1933; Riggs, 1965; Perlman, 2018; Bhatt et al., 2023).

ERG recordings are possible due to the dark and light currents. As was mentioned earlier, in the dark, there is an influx of Na^+ which leads to a depolarization of the photoreceptors. In light environments, there is a hyperpolarization of the photoreceptors. The parallel and radial arrangement of photoreceptors sum together to form an extracellular current (the current generated from lateral cells such as amacrine and horizontal cells cancels out). These radial electrical currents flow through two different pathways. The first pathway (A) stays within the retina and the second one (B) travels through the retina, vitreous, anterior tissues and then returns to RPE via sclera. The light induced current through pathway B is what is measured by the external ERG electrodes (Figure 2.2). Through basic physics and Ohms law we can deduce that if the active electrode is placed

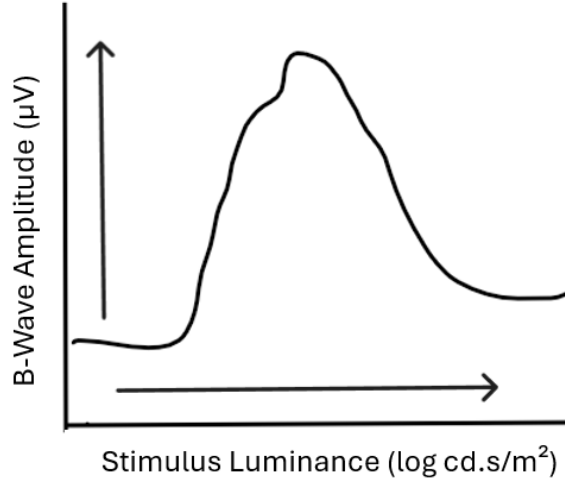


Figure 2.3: The photopic hill phenomenon refers to the drop in b-wave amplitude as the stimulus luminance increases. This decrease and subsequent plateau originates from the ON and OFF bipolar cells. Adapted from (Hamilton et al., 2007).

as close to the corneal surface as possible and the reference electrode is placed at the outer canthus or another temporal and neutral area, the electrical voltage at the reference can be subtracted from the voltage at the active and is what generates the ERG waveform.

The origins of each of the three main ERG components are as follows. The a-wave which represents the functionality of the photoreceptors in dark adapted conditions. In light adapted conditions, the a-wave reflects cone function and bipolar function. This difference in cell origin can be attributed to the fact that dark adaptation of 10-20 min will suppress the cone system. Although the dark-adapted ERG a-wave incorporates input from both rods and cones, it predominantly emphasizes the rod system. Consequently, this response is slower but exhibits a markedly larger amplitude. In contrast, the light-adapted ERG is characterized by a rapid time to peak amplitude and a smaller overall amplitude, thereby representing cone system activity (Brown and Wiesel, 1961; Brown, 1968; Hood and Finkelstein, 1986).

Though still the subject of debate, the b-wave in both the dark adapted and light adapted ERG primarily represents activity from ON bipolar cells. However, it is also modulated by contributions from OFF bipolar cells, amacrine cells, ganglion cells and potentially Müller cells (Miller and Dowling, 1970). In clinical ERG recordings, the b-wave reflects the combined electrical signals from all these cell types. Nevertheless, certain pharmacological agents and analytical techniques can help isolate the specific contributions of individual cells (Miller, 1973; Sieving et al., 1994).

An important concept of the light adapted b-wave is the photopic hill which states that

as light intensity increases, the b-wave amplitude also increases until it reaches a maximum saturation. After this point, light at greater luminance levels will fail to increase the b-wave amplitude and it instead decreases until it finally reaches a plateau (Wali and Leguire, 1992) (Figure 2.3). The origin of this unique shape is found in the ON and OFF bipolar cells in that the ON-response amplitude reduces and the positive peak of the OFF-response becomes delayed (Ueno et al., 2004; Hamilton et al., 2007).

Types of Electroretinograms

The International Society for Clinical Electrophysiology of Vision (ISCEV) has established standards for administering ERG tests in clinical settings enabling ERGs to be utilized as a non-invasive measure of retinal function. The three main types of ERGs (Bhatt et al., 2023) are the full-field electroretinogram (ffERG), pattern electroretinogram (PERG) and multifocal electroretinogram (mfERG) (Figure 2.4). The ffERG evaluates overall retinal function by applying brief light flashes. Under light-adapted conditions, the standard stimulus consists of a $3 \text{ cd}\cdot\text{s}/\text{m}^2$ flash and a 30 Hz flicker, while the dark-adapted test uses flashes ranging from 0.01 to $10 \text{ cd}\cdot\text{s}/\text{m}^2$ (Robson et al., 2022). As mentioned earlier, in the light-adapted test, the a-wave primarily represents cone and bipolar cell responses, whereas the b-wave is largely attributed to the activity of ON bipolar cells (Figure 2.4). In contrast, the pattern electroretinogram (PERG) is a more region-specific test targeting the macula, particularly retinal ganglion cells (RGCs), using a contrast-reversing black-and-white checkerboard stimulus (Thompson et al., 2024). Both ffERG and PERG are limited in their ability to assess electrical responses from multiple retinal areas simultaneously.

To overcome this limitation, the multifocal electroretinogram (mfERG) employs an array of either 61 or 103 hexagons that flicker between black and white in a pseudorandomized sequence known as an m-sequence (Hoffmann et al., 2021). Although the m-sequence is the same for each hexagon, the timing is slightly different for each one. This allows the system to separate their individual signals by automatically comparing the recorded electrical activity with the on/off timing of each hexagon to ultimately figure out which area of the retina the response originates from. These responses are not captured directly from a specific location; instead, they are pulled out from the continuous signal based on the timing embedded in the m-sequence (Hoffmann et al., 2021). Waveforms from each hexagon can be analyzed individually or averaged in rings for broader interpretation (refer to Figure 2.4 for the ring layout) such that the innermost ring, ring 1, captures electrical activity from the central retinal region. Overall, the mfERG provides a valuable assessment of cone and bipolar cell function across multiple retinal sites.

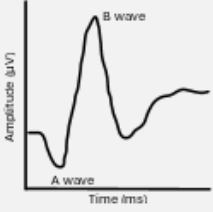
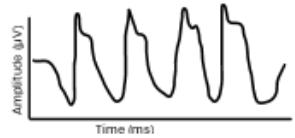
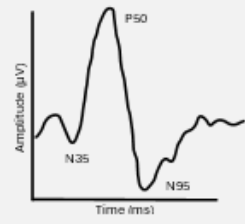
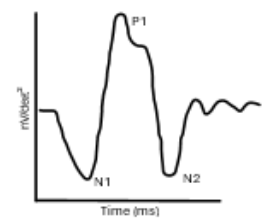
ERG Type	Retinal Layer Origin	Stimulus Parameters	Waveform Components
Full field	Flash A wave: Cone + OFF bipolar B wave: ON/OFF bipolar	White flashes at 3 cd.s/m ² at rate of 30 Hz Background luminance of 30 cd/m ²	
	Flicker Cone response	Brief white flashes (< 5 ms) at 3 cd.s/m ² at rate of 30 Hz Background luminance of 30 cd/m ²	
Pattern	N35: Baseline P50: Macular cone, RGC, bipolar N95: RGC close to optic nerve	Contrast reversing checkerboard pattern at rate of 4 rps Luminance of >80 cd/m ² 15° field 0.8° check width	
Multifocal	Ring 1: Central cone system and bipolar Rings 2-5: Peripheral cone system and bipolar	Pseudorandomized 61 hexagon pattern Luminance of 100 cd/m ² 40°-50° field	

Figure 2.4: Summary of the three main types of ERG tests: ffERFG, PERG and mfERG, their retinal origins, stimulus parameters and patterns and the representative normal waveforms. Note: rps stands for reversals per second, cd stands for candelas.

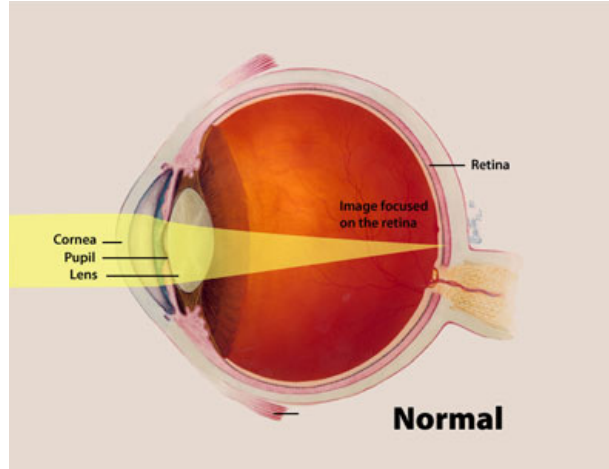


Figure 2.5: Light refraction in a normal, emmetropic eye. Light from infinity focuses at a point directly on the retina. Used with permission © National Eye Institute.

2.3 Myopia

Myopia, also known as short-sightedness, occurs when there is a mismatch between the optics of the eye and its length causing light from infinity to focus at a point in front of the photoreceptors of the retina (Figures 2.5, 2.6) (Oyster, 1999; Morgan et al., 2012). The most common way that myopia manifests is via an abnormal elongation of the vitreous chamber resulting in an increase in the axial length (AXL) of the eyeball (Vera-Diaz, 2010; Morgan et al., 2012). Alternatively, myopia can also occur when the optical power at the front of the eye is too large, commonly a result of the cornea being too steep (Oyster, 1999; Morgan et al., 2012). In contrast, hyperopia, also known as farsightedness, occurs when the eye is too short for its optics; light from objects at all distances theoretically focuses at a point behind the retina (Figure 2.7). After birth, a child’s eyes are typically hyperopic (Cook and Glasscock, 1951); however, through a process called emmetropization, the eyeball actively reshapes, decreasing the range of hyperopia as the child ages (Ingram and Barr, 1979; Mayer et al., 2001; Mutti and Marks, 2011). Usually, ocular growth and corneal curvature develop so that distant objects are focused correctly on the retina but the axial length of the eye can still continue to grow. Since an increase in axial length is closely linked to the development of myopia, managing axial length during growth is a key focus for prevention (Oyster, 1999; Benjamin et al., 1957). When refractive error is not equivalent along the minimum and maximum meridians of the eye (i.e. astigmatism), the spherical equivalent refraction (SER) is the average of the refractive powers. Myopia can be quantified as ≤ -0.5 D and high myopia is variably defined as ≤ -5.0 to -6.0 D (Morgan et al., 2012).

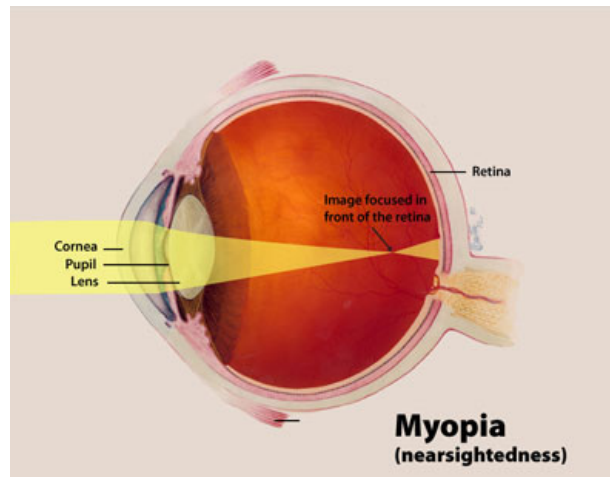


Figure 2.6: Light refraction in a myopic eye. Light from infinity focuses at a point in front of the retina causing distant objects to appear blurry. Used with permission © National Eye Institute.

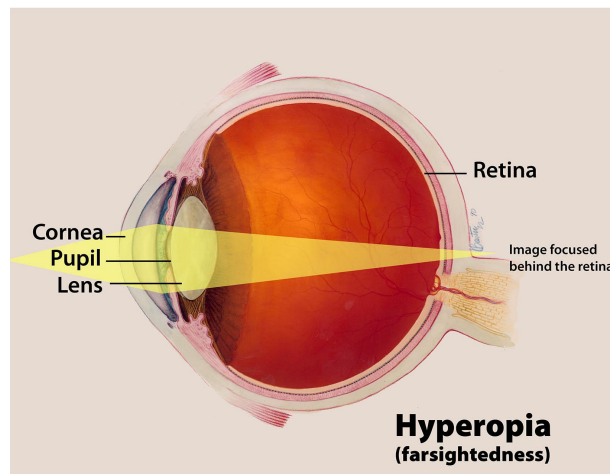


Figure 2.7: Light refraction in a hyperopic eye. Light from infinity focuses at a point behind the retina causing objects to appear blurry. Used with permission © National Eye Institute.

Clinically, refractive error is managed with spectacles and contact lenses that provide optical correction and refractive surgery procedures such as laser-assisted in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) that flatten the cornea. These solutions can be an economic burden to the consumer. Additionally, the cost of managing myopia with specialized treatment options such as scleral or orthokeratology lenses (that target the sclera and cornea, respectively), pharmaceutical products (i.e. low dose atropine) presents an even larger economic burden reaching \$250 billion/year (USD) (Chua and Foster, 2020; Foo et al., 2021). Moreover, vision loss due to conditions like myopia can severely impair visually demanding tasks, hindering educational and work performance (Cieza et al., 2017). If left uncorrected, distance viewing problems has been estimated to result in a loss in productivity equal to \$202 billion (USD) annually (Smith et al., 2009).

None of the current remedies for myopia affect the axial length of the eye and thus, does not ameliorate the risk of developing the sight-threatening conditions often associated with high myopia. Those with high myopia are at a greater risk of developing sight-threatening conditions such as macular degeneration, retinal detachments and glaucoma; as the axial length increases, retinal layers, especially the NFL, can thin, increasing susceptibility to retinal layer separation and optic nerve damage (Saw et al., 2005; Resnikoff et al., 2008; Smith and Walline, 2015; Williams and Hammond, 2019).

Worldwide, the prevalence of myopia is steadily increasing (Dolgin, 2015) reaching levels of over 90% in young adults in parts of East Asia including China, Taiwan, Hong Kong, Japan and Korea (Lim et al., 2009; Morgan et al., 2012; Holden et al., 2016). Over the last two decades, myopia cases have almost doubled in North America from 28% to 42% (Holden et al., 2016) and in Canada, about 30% of the population is affected by myopia (Vitale et al., 2009; Yang et al., 2018). It is predicted that by 2050, over half of the entire world’s population will be myopic (Holden et al., 2016).

2.3.1 Risk Factors

There are many factors that can increase the probability of developing myopia including genetic background, environmental factors or both (Au Eong et al., 1993; Wong et al., 2000; Pan et al., 2011; Morgan et al., 2021b). Mutti et al. showed that an individual’s risk for developing myopia was dependent on the number of myopic parents where the risk was greater with two myopic parents compared to one or no myopic parents (Mutti et al., 2002; Jones et al., 2007). Also, the prevalence of myopia is higher in identical twins than fraternal twins (Dirani et al., 2006; Ding et al., 2013; Kim et al., 2013). Some of the genes that might explain the inheritability of refractive error development have been identified and depending on the amount and types that are present in an individual, the likelihood

of developing myopia could increase tenfold (Verhoeven et al., 2013). These genes are involved in diverse functions such as ion transport, extracellular matrix remodeling, and overall eye development. Thus, the risk of myopia is likely influenced by multiple genetic factors rather than a single underlying cause.

While a genetic predisposition may increase the likelihood of developing myopia, it does not necessarily lead to its onset. Myopia may arise through genetic susceptibility triggered by the environment. One of the findings that sparked a shift in the understanding of the role of environmental cues was the study of Iñupiat (Eskimo) tribes in Barrow, Alaska (Young et al., 1969). This research revealed that children whose lifestyles now included schooling developed myopia while the parents and grand-parents showed no signs of myopia. This striking shift within a single generation challenged the long-held belief that genetics alone dictated myopia prevalence.

Further support for visual environmental cues came from animal studies using diffusers and lenses to alter the visual environment of the animal. In response to these cues, the eye attempts to emmetropize (Schaeffel et al., 1988; Schippert et al., 2008). Emmetropization occurs when the unaccommodated eyeball grows till the eye becomes emmetropic to ensure that light refracts at a single spot on the retina. The two main kinds of visual cues used in animal models are form deprivation (loss of high spatial frequency, for example, from translucent but not transparent diffusers) and lenses that alter where the image focuses in the eye (positive or negative lenses). These processes do not require that the image reach the brain; ocular growth can still occur with optic nerve lesion, indicating that the signals for emmetropization are controlled within the retina itself (Troilo et al., 1987; Wallman, 1993; Norton, 1999). There is the thought that form deprivation myopia (FDM) and lens-induced myopia (LIM) triggers the retinal response through different types of blur, while others believe there is no difference between the two mechanisms (Morgan et al., 2013)

It has been suggested that increased near work tasks elevates the time spent focusing at short distances and introduces peripheral defocus from the surrounding objects. This defocus can induce hyperopic blur and result in increased axial length (Smith et al., 2005, 2009). This mechanism could explain with successive generations developed myopia and were noted to have spent significantly more time on near-focused activities (Young et al., 1969).

It has become clear that the prevalence of myopia seems to grow with the number of years of education such that adults who have degrees in higher education tend to be more myopic (Mirshahi et al., 2014; Mountjoy et al., 2018). In areas where children are less educated, myopia cases are more rare (Morgan et al., 2018; Peckham et al., 1977). Furthermore, the countries that have the highest amount of myopia (i.e. Singapore, Taiwan, China) are also the countries that score high in educational outcomes and tend to put more

of an emphasis on educational training and homework from as early as preschool (Saw et al., 2007; Morgan and Rose, 2013). Although there is not enough evidence to suggest causality, increased time spent in the education system is a clear risk factor for developing myopia.

The onset of the digital age and the long hours spent in front of screens has only exacerbated the myopia epidemic (Morgan et al., 2021b). Additionally, when comparing myopic refraction during 2020 and the previous 5 years, at home confinement during the COVID-19 pandemic in China was associated with a shift towards myopia progression in children from 6-8 years of age (Wang et al., 2021). While peripheral defocus may explain what is causing the eye to grow, this theory is confounded by the likelihood that the increased screen time and near work are associated with a reduction of time spent outdoors (Rose et al., 2008; Flitcroft, 2012; He et al., 2015; Jin et al., 2015; Wu et al., 2018). A minimum of 2 hours per day outdoors has been advocated for all children to reduce the risk of myopia (Rose et al., 2008). In fact, Rose et al. found that children spending high amounts of time outdoors might be protective against myopia development even if these children engage in high amounts of near work (Rose et al., 2008; Flitcroft, 2012; He et al., 2015; Repka, 2015).

2.3.2 Mechanisms for the Outdoor Light Theory

While the underlying mechanism for how outdoor light might be protective against myopia development is not well understood, there are many theories that have arisen to help highlight the possible cause. For instance, increased sunlight exposure often increases circulating levels of vitamin D in the body (Mutti and Marks, 2011; YAM et al., 2017) which may be associated with a shortening of axial length by either interfering with the retinoic acid induced retinal-scleral growth signaling pathway or by decreasing choroidal thickness (Tavera-Mendoza et al., 2006; Bailey et al., 2008).

Another interesting concept is the dynamic nature of outdoor scenes that cannot be replicated indoors. When outside, our eyes responds to the varying shapes, contrasts, movement of light and these kinds of visual stimulation may activate the spatiotemporal image-response mechanisms in the retina to slow the progression of myopia (Rucker, 2013; Flitcroft et al., 2020).

Furthermore, during the process of emmetropization, where the eye adjusts its growth to reduce refractive error, longitudinal chromatic aberration (LCA) has been suggested as a possible cue to detect the direction of blur (Zhu et al., 2005; Rucker and Wallman, 2009; Zhang and Zhu, 2022). Essentially the two directions can be summarized as either myopic defocus where light focuses in front of the retina, causing the eye to grow inwards or hyperopic defocus such that light is focused behind the retina, resulting in longer eyes (Carr

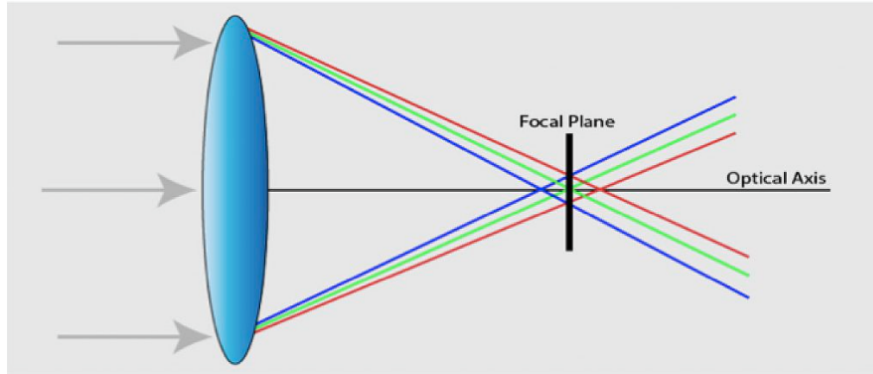


Figure 2.8: Longitudinal chromatic aberration occurs when different sized wavelengths are refracted through a lens. Shorter wavelengths are refracted in front of the focal plane, medium wavelengths close to the centre of the focal plane and long wavelengths behind the focal plane. From *The Science Behind Myopia* by Brittany J. Carr and William K. Stell (Permission from Webvision <http://webvision.med.utah.edu/>).

and Stell, 1995). LCA occurs due to differences in wavelength and how they refract through a lens (Figure 2.8). As a result, short-wavelengths (blue light, 430–470 nm) focus at a point in front of the retina, medium-wavelengths (yellow/green light, 490–570 nm) approximately at the focal plane and long-wavelengths (red light, 600–760 nm) focus behind the retina (Carr and Stell, 1995).

In the human eye, the fovea contains no cones sensitive to short wavelengths and instead contains cones sensitive to medium and long wavelengths (Swiatczak and Schaeffel, 2022). One hypothesis suggests that the eye grows to a size optimized for focusing light in the middle of the visible spectrum—specifically yellow-green wavelengths. If this idea holds true, then an eye that is hyperopic would have shorter wavelengths in focus, while a myopic eye would have longer wavelengths in focus. Thus, since outdoor light contains more short-wavelength light, exposure to it for extended periods of time may trigger a decrease in the growth response in a myopic eye (Carr and Stell, 1995).

This theory was tested across species (Figure 2.9). In fish (Timucin et al., 2016), guinea pigs (Long et al., 2009; Liu et al., 2011) and chickens (Rucker and Wallman, 2009) short-wavelength light (UV and blue) acts as an inhibitor of myopia and supports the idea that adaptation to short-wavelengths may cause a hyperopic shift in the eye and long-wavelengths results in a myopic shift and increase in axial length. The same was true in human studies (Lou and Ostrin, 2020; Thakur et al., 2021). The overall accumulation of evidence suggests that mono-chromatic short-wavelength light may be protective against myopia and outweighs the long-wavelength light evidence (Figure 2.9).

Wavelength	Species	Author	Shift	Effect on Axial Length
Violet (360-400 nm)	Chicken	Torii, 2017	Hyperopic	Decreased
	Mice	Strickland, 2021 Jiang, 2021		
	Human	Torii, 2022		
Blue (430-470 nm)	Fish	Timucin, 2016	Hyperopic	Decreased
	Guinea pig	Liu, 2011		
		Jiang, 2014		
		Zou, 2013		
		Yu, 2021		
		Qian, 2013		
	Chicken	Seidman, 2002 Foulds, 2014 Wang, 2018	Myopic	Increased
	Human	Lou, 2020 Thakur, 2021		
Red (600-760 nm)	Tree Shrew	Gawne, 2017	Myopic	Increased
	Guinea pig	Long, 2009		
		Jiang, 2018		
	Chicken	Foulds, 2017 Seidman, 2002 Wang, 2018	Hyperopic	Decreased
	Human	Lou, 2020 Thakur, 2021		
	Rhesus Monkey	Gawne, 2018 Ward, 2018 Hung, 2018 Smith, 2015		

Figure 2.9: Summary of the recent studies that have examined the effects of different wavelengths of monochromatic light (violet: 360-400 nm, blue: 430-470 nm and red: 600-760 nm) on axial length and whether adaptation to these wavelengths results in a myopic or hyperopic shift in various species (e.g. chicks, mice, human, guinea pig, tree shrew etc.). Modified from (Thakur et al., 2021).

In contrast, in rhesus monkeys (Smith et al., 2015; Hung et al., 2018) and tree shrews (Gawne et al., 2017; Ward et al., 2018) long-wavelength light leads to a decreased axial length instead of an increase. Numerous studies over the past few years that have reported positive results using low-level red-light therapy for myopia control. A general consensus exists on the protocol used in many of these human studies: participants are exposed to 650 nm red light from a laser at a luminance level of approximately 1600 lux for 3 minutes, twice a day, separated by a minimum of 4 hours, for 5 to 6 days a week. This treatment has been associated with a significant reduction in axial elongation (by up to 69%) and a slowing of myopic refraction (by up to 77%) (Chung et al., 2012; Xiong et al., 2021; Jiang et al., 2022; Zhou et al., 2022).

The proposed mechanism for this effect is photobiomodulation, which is based on the Arndt-Schultz law. This principle suggests that a specific balance of power density and exposure time is necessary to stimulate cellular activity. If the power density or exposure time is excessive, it can lead to cellular inhibition (de Freitas and Hamblin, 2016). While the exact biological mechanism remains unknown, it is hypothesized that long-wavelength red light impacts mitochondrial function by increasing ATP production through the cytochrome-c oxidase cascade (Hamblin, 2018). In the eye, this could potentially enhance mitochondrial function in the sclera and stimulate collagen production and fibroblast health (Hamblin, 2018). The benefits of red light therapy depend on continual use in order to sustain cellular changes and discontinuing therapy can have rebound effects (Xiong et al., 2022).

However, some concerns have been raised regarding the safety and true mechanism of action. A study by Ostrin and colleagues compared the safety of three commonly used red-light therapy lasers and found that two of the three exceeded the maximum permissible exposure for a three-minute viewing duration (Ostrin and Schill, 2024). Furthermore, it is plausible that the observed decrease in axial length could be an artifact of increased choroidal thickness due to inflammation, rather than a genuine slowing of myopic progression.

The use of blue light in research and clinical applications can also be controversial due to its biological effects. Blue light refers to short-wavelength visible light, typically in the range of 400–500 nm. Shorter wavelengths carry more energy and excessive exposure can lead to damage of ocular tissues. For example, intense blue or ultraviolet (UV) light can harm proteins in the cornea (as seen in photokeratitis or "welder's flash"), contribute to cataract formation, and accelerate retinal degeneration, potentially increasing the risk of age-related macular degeneration (Zuclich, 1989). While sunlight contains damaging wavelengths, including UV, the concern arises primarily with prolonged or intense exposure.

The more commonly cited concern with blue light from screens and artificial sources

relates to its impact on the circadian system. Short-wavelength light strongly stimulates intrinsically photosensitive retinal ganglion cells (ipRGCs), which play a key role in regulating circadian rhythms (Ospri et al., 2017). Evening exposure to blue light can suppress melatonin production and disrupt sleep patterns, even at moderate intensities but this effect is regulatory rather than damaging (Hysing et al., 2015; Chang et al., 2015; Tordjman et al., 2017). If blue light exposure is controlled, non-intensive, and administered outside of evening hours, there is no significant risks related to blue light that would be anticipated.

Blue light can regulate circadian rhythms since the melanopsin contained within ipRGCs have a peak sensitivity to light around 480 nm and are involved in dopaminergic mechanisms. The ipRGCs project their axons onto DACs (Repka, 2015; Flitcroft et al., 2019) which are a main source of dopamine in the eye (Zhang et al., 2008b; Sankaran et al., 2018).

Many animal models suggest increased dopamine levels to be associated with a reduced axial elongation (Feldkaemper and Schaeffel, 2013; Zhou et al., 2017b). In chicks, retinal dopamine is down-regulated in lens-induced myopia and dopamine agonists inhibits myopia progression (Stone et al., 1989; Guo et al., 1995). When apomorphine, an agonist of dopamine, was injected into chicks via intravitreal injections just posterior to the superior limbus, myopic shift and axial length elongation was inhibited. However, it is proposed that apomorphine acts via a different pathway than atropine, a pharmaceutical drug often associated with decreased myopia, evident by the fact that the combined effect of apomorphine and atropine was no more than just atropine alone (Schmid and Wildsoet, 2004). Also, following release, dopamine acts on multiple targets in the retina including the RPE, potentially regulating the growth cascade from the choroid to sclera (Rymer and Wildsoet, 2005; Feldkaemper and Schaeffel, 2013; Stone et al., 2013). Studies have shown that exposing chicks, monkeys, and mice to high-intensity light can prevent the onset of form-deprivation myopia (Ashby et al., 2009). However, when a dopamine (D2) receptor antagonist is introduced, it blocks the recovery from form-deprivation myopia and the protective benefits of bright light exposure (Pendrak et al., 1997; McCarthy et al., 2007; Nickla and Totonelly, 2011). Therefore, dopamine is clearly linked to the mechanism underlying outdoor light and decreased myopia progression.

Chapter 3

General Materials & Methods

The following section describes the general protocols and analytical procedures employed across all three studies included in this thesis. Study-specific protocols and adaptations are detailed separately within each respective study chapter.

Sample Size Calculation: Sample sizes were calculated using the effect size F value of 0.25, two-tailed alpha error of 0.05, correlation among repeated measures of 0.5 and non-sphericity correction of 1, resulting in total required sample size of 24 for a power of 80%. All power and sample size calculations were made using G*Power 3.1.9.4.

Participants: Healthy trichromatic myopes (spherical equivalent of ≤ -5 D; $n=24$) and controls (spherical equivalent between $+1$ D and -0.25 D; $n=24$), aged 18-30 years, were recruited. I excluded any participant with a best-corrected visual acuity (BCVA) poorer than 0.1 logarithm of the minimum angle of resolution (LogMAR), anyone with ocular or neurological disorders, and anyone using neuroactive medications or having a significant concussion within the past 5 years. This study was performed at the University of Waterloo School of Optometry and Vision science in Ontario, Canada. Ethical clearance for this study was granted by a University of Waterloo Research Ethics Board (REB 43930), and informed consent was obtained from all participants.

Protocols for Ocular Examinations: The BCVA was measured using the early treatment diabetic retinopathy study (ETDRS) visual acuity chart scored in LogMAR units. Corrective lens prescriptions were confirmed using a manual lensometer (Reichert, Inc. ML1 Lensometer®), Depew, NY, USA). The axial length of the eye was measured using an IOLMaster 700 (Carl Zeiss Meditec, Inc., Dublin, CA, USA).

ERG Measurements: The most myopic eye in the high myope group and the most emmetropic eye in the control group were selected for monocular ERG testing. ERGs were recorded using the handheld RETeval® system (LKC Technologies, Gaithersburg, MD, USA). The skin beneath the lower eyelid and temple were cleaned with NuPrep® abrasive

gel (D.O. Weaver & Co., Colorado, USA). SensorStrip™ skin electrode contact regions were centered below the pupil as close as possible to the margin of the lower eyelid and outer canthus. The electrode was then connected to the RETeval® device.

Data Analysis: Statistical analysis was carried out using Jamovi (version 2.6, The jamovi project: <https://www.jamovi.org>) and R (version 4.4.3, [r-project.org](https://www.r-project.org)). Prior to analysis the percent change for each component was calculated (post minus pre divided by pre). The data were tested for normalcy and variances were tested for homogeneity (Shapiro-Wilk test, Levene’s test, respectively). Repeated measures mixed model ANOVAs (CRAN: Package rstatix) were used to analyze the a-wave, b-wave and flicker ERG amplitudes and implicit times as a function of pre- and post-adapting stimulus wavelength (within factor), refractive error group (between factor) and interaction effects of refractive error on adaptation. Greenhouse-Geisser corrections were used if necessary. Alpha values were set to $p \leq 0.05$. Bonferroni post-hoc tests were used when indicated. For all effect size calculations, eta-squared (η^2G) was used to estimate the clinical significance of any differences between control and high myopes. The η^2G is calculated by using the sum of squares for that effect plus the sum of squares for the error associated with it ($\eta^2G = SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$). The η^2G is grouped as follows: small ($\eta^2G \geq 0.01$), medium ($\eta^2G \geq 0.06$) and large ($\eta^2G \geq 0.14$) effects.

The relationship between refractive error and ERG output was investigated. Axial length can be an accurate parameter due to it being unaffected by corneal curvature, in this study cohort, refractive error and axial length were negatively correlated ($R^2 = 0.77$); all high myopes in this group had long eye globes compared to controls ($p \leq 0.001$, Appendix C for graphical representation), I therefore used refractive error to compare the ERG in high myopes and non-myopes. All means were reported with confidence intervals (95% CI) unless otherwise noted.

Chapter 4

Effects of Standard Light Adaptation on the Full-Field Electroretinogram (ffERG)

4.1 Introduction

Given that dopamine levels cannot be directly measured in humans, there is a need for efficient biomarkers that may aid in the indirect quantification of retinal dopamine levels and help to identify the signals and pathways that influence myopia development. One way to objectively measure retinal function is by using electroretinograms (ERG). ERGs use different visual stimuli such as brief flashes and flickers to elicit a response from the retinal cells. The subsequent electrical activity recorded forms ERG waveforms and can be used to differentiate myopes. High myopes are known to have reduced full-field ERG amplitudes (Westall et al., 2001; Kader, 2012; Gupta et al., 2022). For instance, Perlman et al. found reduced light-adapted b-wave amplitudes with increased degree of myopic refractive error and axial length as well as delayed implicit times also proportional to increased axial length (Perlman et al., 1984). Malik et al. found reduced a-waves and b-waves as the degree of myopia increased especially in eyes with degenerative retinæ (Malik et al., 1969). Koh et al. found reduced photopic a-waves, b-waves and flicker amplitudes with higher myopes (Koh et al., 2014). The decreased functionality of the retina may or may not be related to retinal thinning and may or may not be related to the larger eye globe as a result of increased axial length (Perlman et al., 1984). Two recent publications indicated that the b-waves of the ERG in response to short-wavelength light shone onto the optic nerve heads were greater in myopic than in non-myopic eyes (Amorim-de Sousa et al., 2021; Schilling et al., 2023) possibly explained by the fact that myopic retinæ, lacking DA (Stone et al.,

1989; Guo et al., 1995), were more responsive to DA release.

However, stimulating the entire retina with short-wavelength light may be a more effective way to trigger the ipRGC-melanopsin pathway (Lou and Ostrin, 2020). There is also evidence to suggest that non-myopic young adults have increased pupil constriction under blue light. The pupillary light response is known to be mediated by the ipRGC-melanopsin system (Lucas et al., 2003; Gamlin et al., 2007; Ostrin, 2018). The pupil will constrict to reduce the amount of light reaching the eye. Overall, normal pupillary response times are about one second for initial constriction. Increased levels of retinal dopamine results in activation of the cyclic-AMP pathway (Sodhi and Hartwick, 2014) within ipRGCs in rat models. In the event that a similar process occurs in humans, it might explain why Mutti et al. saw less pupillary constriction in myopes; either light stimulation failed to evoke the same rise in retinal dopamine levels or the ipRGCs themselves are less responsive to dopamine in the myopic group (Mutti et al., 2020a). It remains unclear if short-term light adaptation of the entire retina could affect the ERG waveforms

In the present study, I seek to compare the impact of short-term, full-field adaptation to long (red) and short (blue) wavelength light on the amplitude and implicit times of light-adapted ERGs. My main objective is to examine whether DA release mechanisms in high myopes are different than in non-myopes following stimulation of the short-wavelength cone and ipRGCs pathways in an effort to help clarify the affect of outdoor light on the ERGs of myopic individuals.

4.2 Protocol

I used the methods outlined in the general materials and methods (Chapter 3). The ERG and adaptation protocol specific to this study are outlined below.

ERG Measurements: ISCEV standard (Robson et al., 2022) flash and flicker ERGs were elicited by white flashes of 85 troland seconds (Td.s) presented at 2 and 28.3 Hz, respectively with a white background at 850 trolands (Td). The RETeval® system adjusts for pupil diameter using the troland protocol in real-time which avoids the need for mydriasis. The flash ERG a-wave was measured from the baseline to the minimum negative trough in a time window between 10 and 20 ms after flash onset, and the b-wave was measured from the trough of the a-wave to the b-wave maximum between 20 and 35 ms after flash onset (Figure 4.1). The flicker ERG waveform was measured from peak to trough (Figure 4.1) and flicker timing was measured from onset to the first positive peak.

Adaptation Protocol: A two-visit cross-over design was used with each visit at least 24 h apart. Participants were randomly assigned a full-field adaptation stimulus of either short

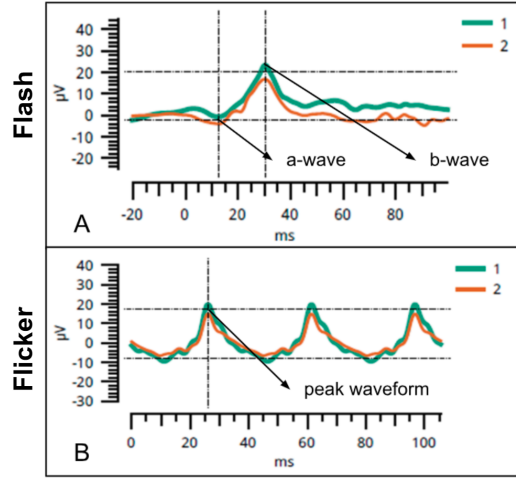


Figure 4.1: Graphs showing the pre-adaptation LA ERG recording measured in an emmetropic participant. The green and orange coloured lines represent two replicate measurements. Panel A reflects the flash ERG a-wave (measured from the baseline to minimum negative trough in a time window between 10 and 20ms after flash onset) and b-wave (measured from the trough of the a-wave to the b-wave maximum between 20 and 35ms after flash onset). Panel B represents the reconstructed flicker ERG waveform (measured from trough to maximum peaks of each harmonic, beginning at 20-30ms after flicker onset). The ERGs were recorded using SensorStrip™ skin electrodes connected to the RETeval® device.

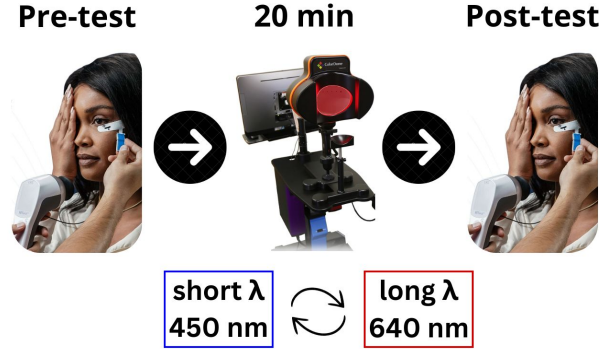


Figure 4.2: A pre-ERG test for each participant was taken using the RETeval® followed by adaptation to coloured light for 20 minutes using the Espion Colour Dome™ (Diagnosys LLC). Each participant was randomly assigned adaptation to either short (450 nm) or long (640 nm) wavelength light at 30 cd/m² at an initial visit and the other wavelength at the second visit. The 20 minute adaptation period was followed by a post-ERG test. Copyright permissions obtained for use of images.

or long wavelength light for 20 minutes using the Espion ColorDome™ (Diagnosys LLC). The luminance level was set at 30 cd/m² and the peak wavelengths of the narrow-band LEDs were 448 nm for short wavelength adaptation and 627 nm for the long wavelength adaptation. A second set of ERG recordings were collected following coloured light adaptation. At their second visit, participants were adapted to the other wavelength (Figure 4.2)

4.3 Results

Pre-adaptation ERG amplitudes and implicit times were highly variable across visits as is clear from the large degree of scatter on the graphs below (Figure 4.3). Between the two visits, the baseline value ranges for controls are as follows: AWT values ranged from 10.20 to 12.51 ms for blue light adaptation and 10.17 to 13.22 ms for red light adaptation. AWA ranges were -7.79 to -2.35 μ V for blue and -9.66 to -1.66 μ V for red light adaptation. BWT values ranged from 26.14 to 30.21 ms for blue and 26.21 to 30.32 ms for red. BWA ranged from 12.33 to 49.85 μ V for blue and 7.11 to 55.18 μ V for red. WIT values ranged from 23.48 to 26.05 ms for blue and 23.57 to 25.96 ms for red. WA ranged from 10.55 to 40.30 μ V for blue and 10.65 to 44.43 μ V for red. The baseline value ranges across the

Table 4.1: Characteristics of the control and high myope groups included in the final analysis.

	n	Number of Females	Median Age [min, max]	Median SER [min, max]	Median AXL [min, max]
Controls	23	14	23 [18, 28]	0.00 [-0.50, 1.25]	23.60 [22.41, 25.35]
Myopes	24	17	24.5 [19, 27]	-6.86 [-9.75, -5.25]	26.22 [24.63, 28.00]

two visits for myopes are as follows: AWT values ranged from 8.93 to 13.62 ms for blue light adaptation and 9.65 to 13.79 ms for red light adaptation. AWA ranged from -11.05 to -0.77 μV for blue and -11.55 to -1.56 μV for red light adaptation. BWT values ranged from 26.10 to 28.98 ms for blue and 26.21 to 29.11 ms for red. BWA ranged from 6.83 to 52.28 μV for blue and 8.68 to 51.87 μV for red. WIT values ranged from 23.68 to 25.61 ms for blue and 23.81 to 25.93 ms for red. WA amplitudes showed the greatest spread, from 5.11 to 52.38 μV for blue and 9.21 to 56.14 μV for red.

It should be noted that the above control baseline values reflect the ranges excluding one control participant. This participant was removed due to inconsistency in their data results between visits as is especially evident for the a-wave implicit time (AWT) and b-wave implicit time (BWT) components (i.e. AWT V1: 10.86 ms; V2: 32.13 ms and BWT V1: 26.77 ms; V2: 50.46 ms) (Appendix A for further notes on outlier removal decision). Thus, the final analysis was carried out on a sample size $N=47$ and the descriptive statistics for these participants are in Table 4.1.

There were no significant differences between controls and myopes prior to adaptation (all $p \geq 0.05$, Appendix B) however the discrepancy between visits confounds these results. To circumvent this inconsistency, the relative change of each component was used. None of the data for the ERG components were normally distributed. However, the variances for each component were homogeneous. Repeated measures ANOVAs for all components yielded similar results as the non-parametric testing (Appendix B).

Adaptation to either short (blue) or long (red) wavelength light did not change the ERG amplitudes of any of the waveform components significantly (all $p \geq 0.05$) (4.4). No distinct patterns or significant differences between controls and myopes could be identified (Table 4.2).

Adaptation to either short (blue) or long (red) wavelength light did not change the ERG amplitudes of any of the waveform components significantly (all $p \geq 0.05$) (4.4). No distinct patterns or significant differences between controls and myopes could be identified

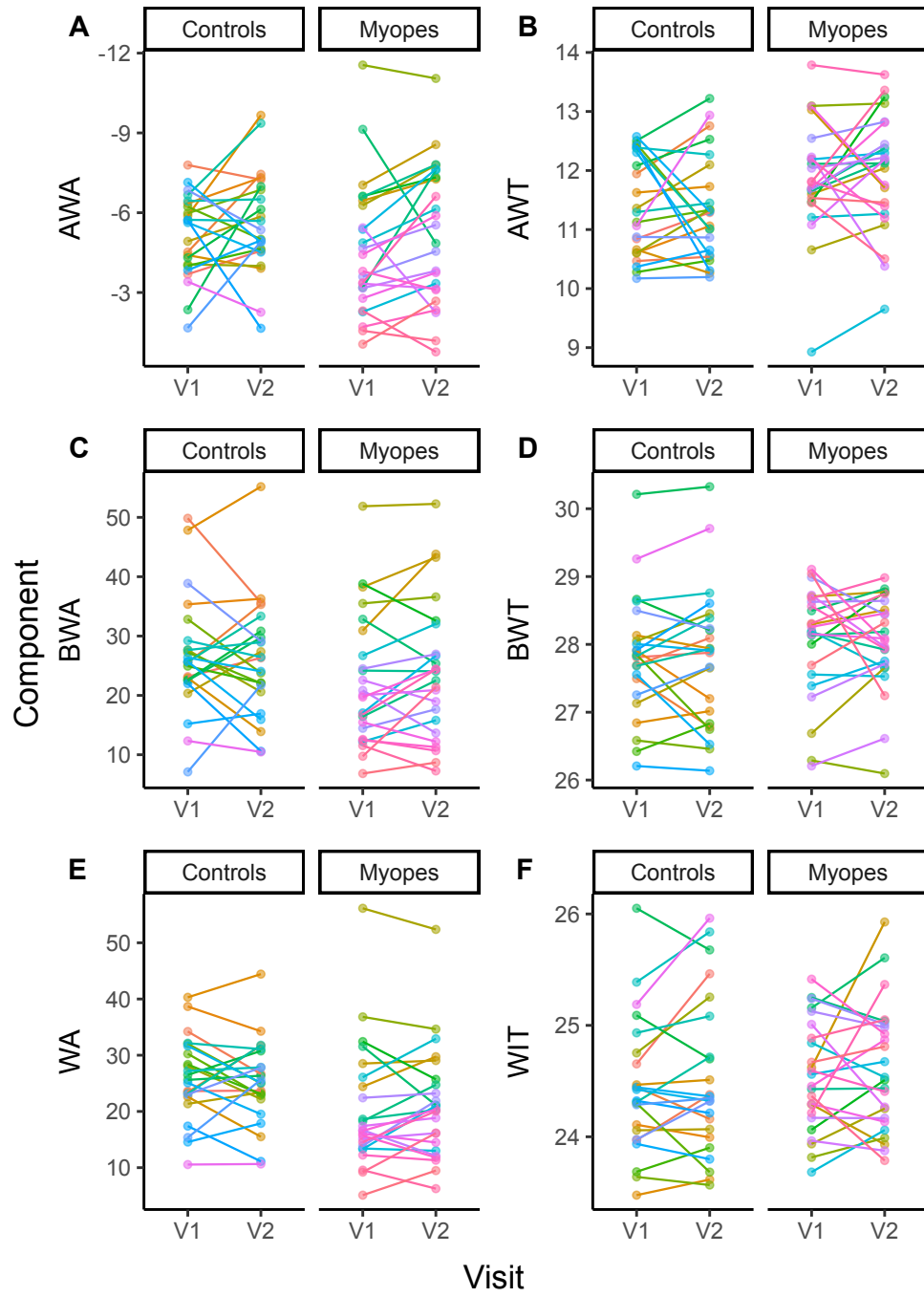


Figure 4.3: Pre-adaptation data for all 48 participants, controls ($n=24$) and high myopes ($n=24$), at visit 1 (V1) and visit 2 (V2) for each component AWA, AWT, BWA, BWT, WA and WIT in panels A-F, respectively. The relative change was used for analysis due to the inconsistency between visits. In panel B and D, the outlier in the control group (as indicated by the salmon coloured line extending to the top of the graph) was removed from the final analysis.

Table 4.2: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for AWA are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	0.05	0.83	0.00	0.05
2	Colour	1.00	45.00	0.00	0.97	0.00	0.05
3	RE:Colour	1.00	45.00	0.02	0.88	0.00	0.05

(Table 4.2).

For both the controls and high myopes, adaptation to long wavelengths resulted in a significant prolonged implicit time in ERG b-waves ($+1.9 \pm 2.1\%$ for controls; $+0.09 \pm 1.5\%$ for high myopes; $p=0.01$; $\eta^2G=0.08$) when compared to changes following short wavelength light adaptation. Similarly, flicker ERG implicit times had a significant positive percent change for both refractive error groups following adaptation to long wavelength where the mean values were $+1.1 \pm 1.0\%$ for controls, $+1.4 \pm 1.5\%$ for high myopes ($p \leq 0.001$, $\eta^2G=0.28$). Adaptation to short wavelength light did not significantly prolong implicit times for either refractive error group. Adaptation to either short or long wavelengths had no other effects on the ERG parameters measured and there were no differences between the high myope group and control group (Table 4.2).

Table 4.3: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for AWT are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	0.20	0.66	0.00	0.05
2	Colour	1.00	45.00	2.54	0.12	0.02	0.48
3	RE:Colour	1.00	45.00	2.03	0.16	0.02	0.48

Table 4.4: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for BWA are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	1.60	0.21	0.02	0.48
2	Colour	1.00	45.00	0.00	1.00	0.00	0.05
3	RE:Colour	1.00	45.00	0.23	0.63	0.00	0.05

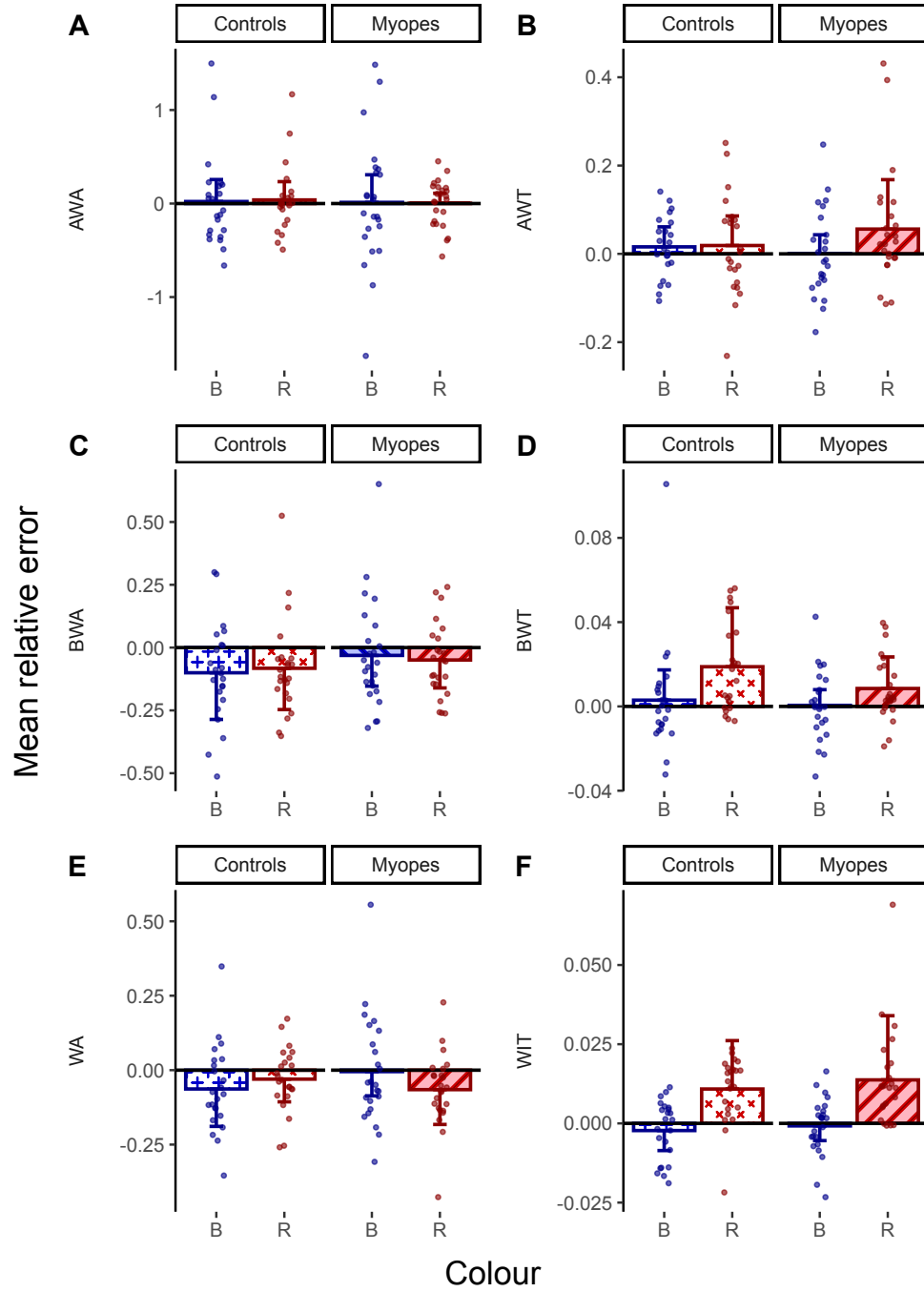


Figure 4.4: The mean relative change of each component (AWA, AWT, BWA, BWT, WA and WIT in panels A-F, respectively) following adaptation to blue and red light for controls and myopes. The error bars reflect the 95% confidence intervals of the mean.

Table 4.5: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for BWT are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	2.37	0.13	0.03	0.66
2	Colour	1.00	45.00	8.17	0.01*	0.08	0.98
3	RE:Colour	1.00	45.00	0.88	0.35	0.01	0.27

Table 4.6: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for WA are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	0.18	0.67	0.00	0.05
2	Colour	1.00	45.00	0.19	0.66	0.00	0.05
3	RE:Colour	1.00	45.00	2.24	0.14	0.03	0.66

4.4 Discussion

Unlike other studies examining ERG differences between myopes and controls (Westall et al., 2001; Kader, 2012; Koh et al., 2014), I did not find pre-adaptation ERG amplitudes to be smaller in myopes, This was similar to what Stapley and colleagues found; they looked at non-pathological myopia and attributed the lack of difference between groups to the wide range of refractive error amongst their participants (Stapley et al., 2025). Although the current study is well powered (92%) and focused on high myopes (≤ -5.00 D) it could be that larger refractive errors would help detect differences. For instance, the participants in Westall et al.’s study where a significant difference between myopes and controls for a- and b-waves was found, had larger refractive errors (between -6.00 D and -14.50 D, compared to the current study’s range of -5.00 D to -8.50 D) (Westall et al., 2001).

For the one control participant that was removed from analysis, (section 4.3) the inconsistent ERG measurements were likely influenced by blink artifacts and excessive noise due to movement; this participant was notably uncomfortable with the flashes of light and had difficulty not blinking during the duration of the tests. Furthermore, the inconsistency between visits can confound the results (Figure 4.3). Stapley et al. is the only study aside from the present that has looked at using the RETeval handheld device to conduct light-adapted full-field ERGs using SensorStrips™ in high myopes (Stapley et al., 2025). They also found no significant differences between controls and myopes for light-adapted (LA) flash and flicker amplitudes or implicit times. They pointed to the fact that typically, amplitude measurements are known to be less reproducible (Asakawa et al., 2017). Indeed,

Table 4.7: The p-values, effect sizes and power for effects based on refractive error (RE) and colour (λ) for WIT are listed (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	45.00	0.72	0.40	0.01	0.27
2	Colour	1.00	45.00	43.34	0.00*	0.28	1.00
3	RE:Colour	1.00	45.00	0.11	0.74	0.00	0.05

Hobby et al. found that variations in electrode placement, especially with RETeval SensorStrips™ affects the amplitudes. If SensorStrips™ are incorrectly placed too far from the lid margin, the response amplitudes are significantly reduced but the implicit times are unaffected (Hobby et al., 2018). Although care was taken to ensure consistent positioning, even small changes in placement could have exacerbated the existing variability. RETeval has recently updated the software to help with electrode positioning so that when the device is held up to the participant’s eye, the area where the electrode should be placed is highlighted on the screen and the test will not proceed until it detects correct placement. This software update was not available at the time of data collection but this update may help minimize test, re-test variability in the future.

With regards to changes post-adaptation to either wavelength, there were no significant differences between controls and myopes when comparing the changes in b-wave and flicker amplitudes (Figure 4.4.) Though not significant, adaptation seemed to result in a negative percent change in all cases. While it is expected that adaptation of any kind would decrease cellular responsitivity, (Govardovskii et al., 2000; Wong et al., 2005) this result is contrary to the original hypothesis since short-term adaptation to short wavelength light did not differentially up-regulate the b-wave amplitude. One possibility is that the adaptation duration was not long enough for the short wavelength light to produce sufficient dopamine to affect the ERG amplitudes perhaps a longer than 20 minutes of adaptation time is necessary for ipRGCs integration time when adapting the entire retina. Additionally, perhaps an immediate post measurement does not allow for adequate ipRGC recovery time since as Wong et al. found, light-adapting backgrounds significantly reduces the sensitivity of ipRGCs, perhaps interfering with downstream dopaminergic mechanisms (Wong et al., 2005). A longer wait time before taking the ERG measurement may allow ipRGC-mediated dopamine release to take place. Moreover, 30 cd/m² may not be a good representation of light outdoors but rather, is closer to the luminance of a well-lit indoor room. Perhaps a strong light that is more representative of outdoor experience would better stimulate the ipRGCs and elicit an increase in ERG amplitude (Stone et al., 1989; Rose et al., 2008).

Lastly, long wavelength adaptation prolonged ERG b-wave and flicker implicit times.

Flicker ERGs, being cone-isolating, reflect long (L-) and medium (M-) cone inputs. At high frequencies (≥ 20 Hz), responses from the L-cone system exhibit shorter latencies compared to M-cones (Kommanapalli et al., 2014). Given that the implicit time delay was only seen following long wavelength adaptation, this may reflect suppressed input from the faster, long wavelength cone system since the slower medium wavelength cone system is left intact to respond to the stimulus after adaptation (Kommanapalli et al., 2014). Overall, I find no evidence that short-term, chromatic adaptation has differential effects associated with high myopia; however, as part of my next step, I aim to examine whether short term adaptation to chromatic light at stronger light levels may result in a differential retinal response in myopic eyes.

Chapter 5

Effects of Strong Light Adaptation on the Full-Field Electroretinogram (ffERG)

5.1 Introduction

High intensity light (up to 100 000 lux outdoors) has been shown to be protective against myopia progression in children (Lanca et al., 2019). He et al. found that adding 40 minutes of outdoor activity everyday for 6-year-olds at school in China, reduced the 3-year cumulative incidence rate of myopia with a 10% difference of incidence between the intervention and control group (He et al., 2015). In a slightly older population of children (10-15 years), Read et al. examined ambient light exposure using a wearable light sensor wrist band over a two-week period and concluded that longitudinal changes in axial length were greatest in the group that experienced the lowest level of average daily light exposure. These authors suggest that exposure to light ≥ 3000 lux seems to have the greatest impact on slowing axial length growth (Read et al., 2015). These results are further supported by Rose et al. who found that being outdoors is protective against myopia development even in children who engage in high levels of near work. They also suggest that physical activity is not a critical factor in controlling eye growth (Rose et al., 2008). This theory concurs with what Read et al. found when they observed that the amount of physical activity was not significantly different between refractive error groups but light exposure levels were. Although, there is less established literature on how outdoor light exposure impacts myopia progression in young adults, wearable light sensor data showed that established myopes have less exposure to light in the UV spectrum (Alvarez and Wildsoet, 2013; Schmid et al., 2013). These aspects of outdoor light, including high luminance and distribution

of short-wavelength light has been shown to trigger melanopsin-mediated pathways. The subsequent release of dopamine has been clearly identified as playing an important role in the regulation of eye growth potentially via signals to the sclera (Swiatczak and Schaeffel, 2022; Amorim-de Sousa et al., 2024).

Full-field ERGs allow for the indirect measurement of retinal function. Past studies have found significantly reduced amplitudes in myopes (Perlman et al., 1984; Koh et al., 2014; Stapley et al., 2025). Dopamine is upregulated when ON bipolar circuits are stimulated and ERG a- and b-waves are affected by decreased DAC production of retinal dopamine (Dumitrescu et al., 2009). In the mouse retina, b-wave amplitudes are enhanced after exposure to high-intensity light. Also, a mutation in the dopamine receptor reduces the age-related increase in b-wave amplitudes (Tian et al., 2015). Stimulating the optic nerve with short-term flickering short-wavelength light using a virtual-reality headset at a luminance of 15 cd/m² may have been enough to up-regulate the b-wave (Amorim-de Sousa et al., 2021), but as was stated in the previous chapter, I did not find a significant difference in ERG amplitudes between controls and myopes following short-term adaptation to short-wavelength light at a standard luminance of 30 cd/m².

Thus, there is a gap in the understanding of effects of short-term high-intensity light adaptation of the entire retina and how this impacts ERGs in the young adult population. The aim of this study is to examine whether short-term adaptation to high-intensity short-wavelength light (300 cd/m²) has a differential effect on the ERG in controls compared to those of high myopes and compared to the effects of standard luminance (30 cd/m²) adaptation.

5.2 Protocol

I used the methods outlined in the general materials and methods (Chapter 3). The ERG protocol is the same as what has been outlined in paper one (Chapter 4.2). The adaptation protocol specific to this study is outlined below.

Adaptation Protocol: I used a four-visit cross over design, with each visit at least 24 h apart. Following a pre-ERG test, participants were randomly assigned a full-field adaptation stimulus of either short or long wavelength light for 20 minutes using the Espion ColorDome™ (Diagnosys LLC) at a luminance level of either 30 cd/m² or 300 cd/m² (Figure 5.1). The peak wavelengths of the narrow-band LEDs were 448 nm for short wavelength adaptation and 627 nm for the long wavelength adaptation. A second set of ERG recordings were collected following coloured light adaptation. Note that, for the participants that participated in study 1, their data was re-used in study 2 (30 cd/m²).

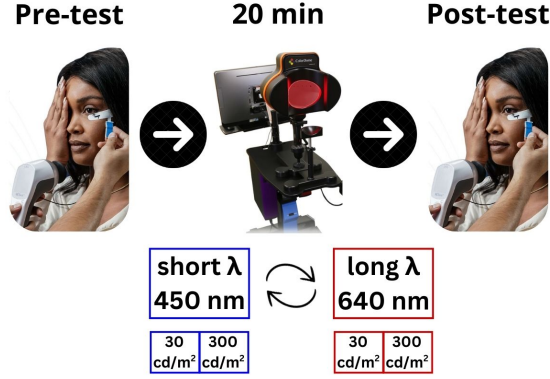


Figure 5.1: The protocol for study 2 to take a pre-ERG test for each participant was taken using the RETeval[®] followed by adaptation to coloured light for 20 minutes using the Espion Colour Dome[™] (Diagnosys LLC). Each participant was randomly assigned adaptation to either short (450 nm) or long (640 nm) wavelength light at either 30 cd/m² or 300 cd/m² for a total of four visits. The 20 minute adaptation period was followed by a post-ERG test. Copyright permissions obtained for use of images.

5.3 Results

Pre-adaptation ERG amplitudes and implicit times were highly variable across visits as is clear from the large degree of scatter on the graphs below (Figure 5.2). Across the four visits the baseline value ranges for controls are as follows: AWT values for blue light adaptation ranged from 10.20 to 12.45 ms at 30 cd/m² and 9.54 to 12.92 ms at 300 cd/m², while red light adaptation ranged from 9.81 to 12.94 ms at 30 cd/m² and 10.24 to 12.58 ms at 300 cd/m². AWA for blue light adaptation ranged from -6.67 to -3.14 μ V at 30 cd/m² and -11.19 to -2.72 μ V at 300 cd/m², and red light adaptation from -9.66 to -1.67 μ V at 30 cd/m² and -9.22 to -1.37 μ V at 300 cd/m². BWT values spanned 26.04 to 29.71 ms across all conditions. BWA values ranged from 7.11 to 56.78 μ V. WIT values remained clustered between 23.45 and 25.96 ms across all conditions. Lastly, WA values ranged from 7.53 to 49.38 μ V. The baseline value ranges across all four visits for myopes are as follows: AWT values ranged from 8.93 to 13.62 ms under blue 30 cd/m² stimulation, 9.63 to 13.17 ms under blue 300 cd/m², 9.65 to 13.79 ms under red 30 cd/m², and 10.45 to 13.39 ms under red 300 cd/m². AWA values ranged from -11.05 to -1.06 μ V under blue 30 cd/m², -10.00 to -2.07 μ V under blue 300 cd/m², -11.90 to -2.68 μ V under red 30 cd/m², and -9.05 to -2.20 μ V under red 300 cd/m². BWT values ranged from 25.91 to 29.57 ms across

Table 5.1: Characteristics of the control and high myope groups

	n	Number of Females	Median Age [min, max]	Median SER [min, max]	Median AXL [min, max]
Controls	17	9	24 [18,28]	0.00 [-0.50, 1.25]	23.62 [23.24, 25.21]
Myopes	14	11	24 [19, 27]	-7.50 [-9.75, -5.38]	26.22 [24.63, 27.62]

all conditions. BWA values ranged from 6.83 to 52.28 μV under blue 30 cd/m^2 and 14.07 to 43.40 μV under red 300 cd/m^2 . WIT values ranged from 23.68 to 26.31 ms across all conditions. WA values ranged from 5.11 to 55.90 μV under blue 30 cd/m^2 and 12.82 to 44.58 μV under red 300 cd/m^2 .

There were no significant differences between controls and myopes prior to adaptation (all $p \geq 0.05$) however the discrepancy between visits confounds these results. To circumvent this inconsistency, the relative change (Chapter 3) of each component was used. The characteristics for the sample size of the 31 participants are in Table 5.1. None of the data for the ERG components were normally distributed. However, the variances for each component were homogeneous. Repeated measures ANOVAs for 5 of the 6 components yielded similar results as the non-parametric testing (Appendix B) with only AWA results not matching.

Table 5.2: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance($\lambda^*\text{BR}$), refractive error for AWA (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	$\eta^2\text{G}$	Power
1	RE	1.00	29.00	0.00	1.00	0.00	0.05
2	Colour	1.00	29.00	10.15	0.00*	0.04	0.59
3	Brightness	1.00	29.00	2.39	0.13	0.03	0.47
4	RE:Colour	1.00	29.00	2.09	0.16	0.01	0.19
5	RE:Brightness	1.00	29.00	0.97	0.33	0.01	0.19
6	Colour:Brightness	1.00	29.00	4.36	0.05*	0.02	0.34
7	RE:Colour:Brightness	1.00	29.00	1.57	0.22	0.01	0.19

There was an overall effect of luminance such that changes in b-wave implicit time decreased with adaptation to 300 cd/m^2 light in controls (blue 30: $-0.451 \pm 1.28\%$; blue 300: $-2.28 \pm 2.56\%$; $p \leq 0.001$, $\eta^2\text{G}=0.14$) and less so in the high myope group (blue 30:

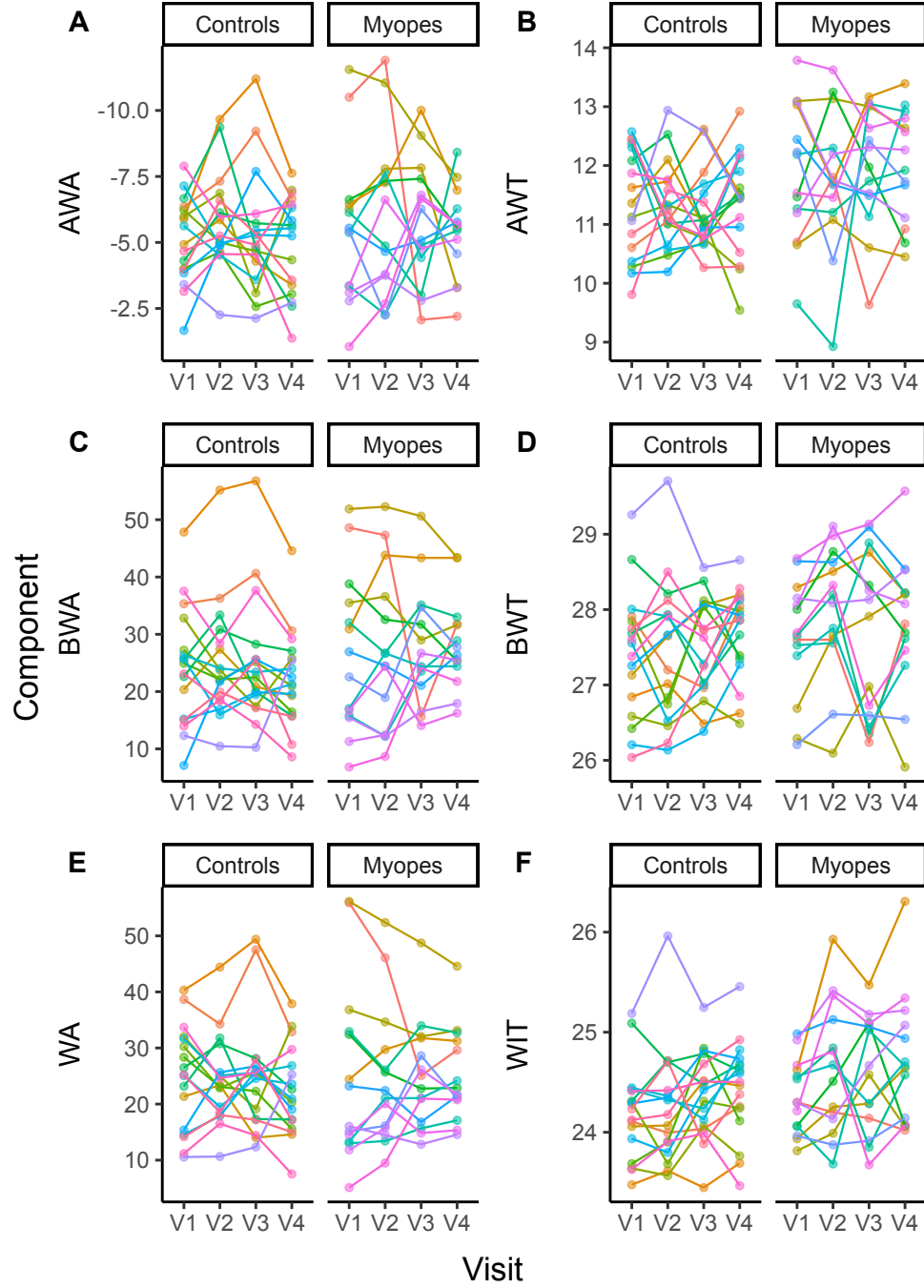


Figure 5.2: The pre-adaptation data for all 31 participants, controls (n=17) and high myopes (n=14), at visit 1 (V1), visit 2 (V2), visit 3 (V3) and visit 4 (V4) for each component AWA, AWT, BWA, BWT, WA and WIT in panels A-F, respectively. Percent error was used for analysis due to the inconsistency between visits.

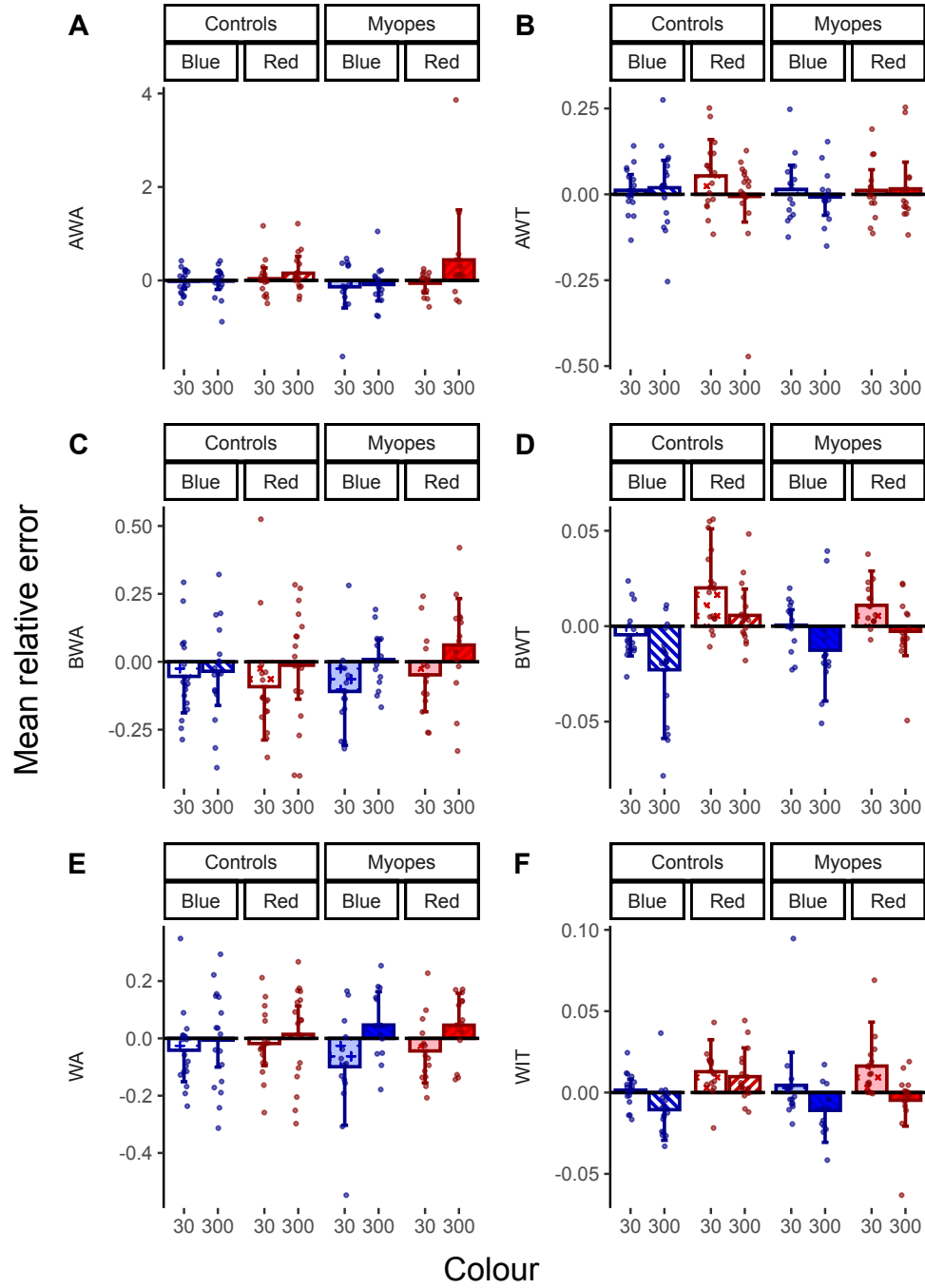


Figure 5.3: The mean relative change of each component (AWA, AWT, BWA, BWT, WA and WIT in panels A-F, respectively) following adaptation to blue (short λ) and red (long λ) light at standard and strong luminance levels for both controls and myopes. The blue and red bars reflect the mean and the dots show individual data. The error bars reflect the 95% confidence intervals of the mean.

Table 5.3: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance(λ *BR), refractive error for AWT (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	29.00	0.35	0.56	0.00	0.05
2	Colour	1.00	29.00	0.26	0.61	0.00	0.05
3	Brightness	1.00	29.00	0.78	0.38	0.01	0.19
4	RE:Colour	1.00	29.00	0.00	0.95	0.00	0.05
5	RE:Brightness	1.00	29.00	0.19	0.67	0.00	0.05
6	Colour:Brightness	1.00	29.00	0.43	0.52	0.00	0.05
7	RE:Colour:Brightness	1.00	29.00	2.40	0.13	0.01	0.19

Table 5.4: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance(λ *BR), refractive error for BWA (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	29.00	0.49	0.49	0.01	0.19
2	Colour	1.00	29.00	0.90	0.35	0.00	0.05
3	Brightness	1.00	29.00	6.27	0.02*	0.05	0.69
4	RE:Colour	1.00	29.00	1.57	0.22	0.01	0.19
5	RE:Brightness	1.00	29.00	1.00	0.33	0.01	0.19
6	Colour:Brightness	1.00	29.00	0.21	0.65	0.00	0.05
7	RE:Colour:Brightness	1.00	29.00	0.36	0.55	0.00	0.05

+0.05 $\pm$ 1.3%; blue 300: -1.26 $\pm$ 2.43%; p=0.01, η^2G =0.05) (Figure 5.3). Similarly, changes in flicker implicit time decreased with the stronger luminance level but were not different between refractive error groups (i.e. controls: blue 30: 0.15 $\pm$ 1.0%; blue 300: -1.05 $\pm$ 1.61%, myopes: blue 30: +0.44 $\pm$ 2.74%; blue 300: -1.10 $\pm$ 1.50%, p $\leq$ 0.001, η^2G =0.13). This effect of brightness with b-wave implicit time and flicker implicit time was significantly different between short and long wavelength. Specifically, changes in implicit time showed a greater decrease with long-wavelength light (p $\leq$ 0.001, η^2G =0.20 (BWT), η^2G =0.12 (WIT)). For both control and myopes, adaptation had no effect on a-wave implicit time (Figure 5.2).

Post-adaptation changes in b-wave amplitudes became less negative (smaller) under stronger light conditions (i.e. controls: blue 30: -5.38 $\pm$ 15.6%; blue 300: -3.50 $\pm$ 17.7%, myopes: blue 30: -10.9 $\pm$ 15.4%; blue 300: +0.85 $\pm$ 10.9%, p=0.02, η^2G =0.05). Again,

Table 5.5: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance(λ *BR), refractive error for BWT (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	29.00	0.02	0.89	0.00	0.05
2	Colour	1.00	29.00	35.88	0.00*	0.20	1.00
3	Brightness	1.00	29.00	22.98	0.00*	0.14	0.99
4	RE:Colour	1.00	29.00	7.14	0.01*	0.05	0.69
5	RE:Brightness	1.00	29.00	0.23	0.64	0.00	0.05
6	Colour:Brightness	1.00	29.00	0.06	0.81	0.00	0.05
7	RE:Colour:Brightness	1.00	29.00	0.10	0.76	0.00	0.05

Table 5.6: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance(λ *BR), refractive error for WA (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	29.00	0.00	0.99	0.00	0.05
2	Colour	1.00	29.00	0.79	0.38	0.01	0.19
3	Brightness	1.00	29.00	8.27	0.01*	0.07	0.84
4	RE:Colour	1.00	29.00	0.01	0.92	0.00	0.05
5	RE:Brightness	1.00	29.00	2.56	0.12	0.02	0.34
6	Colour:Brightness	1.00	29.00	0.48	0.49	0.00	0.05
7	RE:Colour:Brightness	1.00	29.00	0.39	0.54	0.00	0.05

a similar result was found with flicker amplitudes (i.e. controls: blue 30: $-4.10 \pm 13.4\%$; blue 300: $-0.62 \pm 17.1\%$, myopes: blue 30: $-9.90 \pm 18.3\%$; blue 300: $+4.70 \pm 12.0\%$, $p=0.00$, $\eta^2G=0.07$). When comparing between controls and high myopes or between short and long wavelengths, changes in b-wave amplitudes did not differ.

Additionally, a modest but statistically significant increase in the change in a-wave amplitude was found following adaptation to long-wavelength light ($p=0.00$, $\eta^2G=0.04$) at 300 cd/m^2 ($p=0.05$, $\eta^2G=0.02$; controls: red 30: $+3.66 \pm 37.8\%$; red 300: $+15.4 \pm 40.1\%$, myopes: red 30: $-6.13 \pm 25.1\%$; red 300: $+44.1 \pm 109\%$). This result was not significantly different between controls and myopes (Figure 5.2).

Table 5.7: The p-values, effect sizes and power for effects based on colour (λ), adapting luminance (BR), refractive error (RE) and interactions between colour, luminance(λ *BR), refractive error for WIT (*denotes a significant p-value).

	Effect	DFn	DFd	F	p	η^2G	Power
1	RE	1.00	29.00	0.36	0.55	0.00	0.05
2	Colour	1.00	29.00	23.09	0.00*	0.12	0.98
3	Brightness	1.00	29.00	16.23	0.00*	0.13	0.99
4	RE:Colour	1.00	29.00	1.72	0.20	0.01	0.19
5	RE:Brightness	1.00	29.00	2.81	0.10	0.02	0.34
6	Colour:Brightness	1.00	29.00	0.07	0.79	0.00	0.05
7	RE:Colour:Brightness	1.00	29.00	1.40	0.25	0.01	0.19

5.4 Discussion

Consistent with my previous study, no significant baseline differences were observed between the refractive groups prior to adaptation. This lack of differentiation may be attributed to the same factors discussed in study 1 (Chapter 4) although this study was also well powered at 91%. However, adaptation to long-wavelength light at 300 cd/m² resulted in a notable decrease in b-wave and flicker implicit times, suggesting a shorter response time for ON/OFF bipolar cells under high-intensity red light (Dumitrescu et al., 2009). Although both refractive groups exhibited this trend, the reduction in implicit time was more pronounced in emmetropic controls than in myopes. This observation may reflect the presence of prolonged implicit times in myopic retinæ at baseline, which may or may not be related to structural abnormalities and reduced retinal functionality (Perlman et al., 1984).

Amplitude changes in b-wave and flicker waveforms were attenuated following stronger adaptation conditions across both wavelength types. Specifically, the reductions observed following 30 cd/m² were diminished when adaptation luminance was increased to 300 cd/m² (i.e. increase in the relative change in amplitude). Although not statistically significant, there was a trend toward increased amplitude with higher luminance, independent of wavelength. This outcome diverges from my initial hypothesis which suggested that short-wavelength light differentially up-regulates the b-wave amplitude in myopes versus controls. According to Read et al.’s measures of outdoor light, luminance levels of greater than 3000 lux has the most impact on myopia progression. In the present study, 300 cd/m² was the highest luminance level that the Espion ColorDome™ could achieve. This is approximately 942 lux (calculated by multiplying value in lux by 180 degrees for an extended surface such as the Ganzfeld), which is equivalent to a typical overcast day. Due to instru-

mental limitations it is difficult to replicate outdoor light levels indoors. Perhaps, future studies could examine affect of 20 minutes of outdoor time using wearable light sensors and distance trackers (Read et al., 2015), measuring the effects on ERG amplitudes.

Interestingly, a-wave amplitudes significantly increased following adaptation to long-wavelength light at 300 cd/m² but no such increase was seen with b-wave amplitudes. The absence of change at 30 cd/m² suggests that the higher luminance level may be sufficient to selectively stimulate the OFF-response pathway but not ON and OFF systems together (Brown, 1968). However, the results for a-wave amplitude should be interpreted with caution since this was the only component where the non-parametric ANOVA differs from the repeated measured ANOVA. Also, the effect size and power ($\eta^2G=0.02$, power=0.34) for the colour and brightness interaction is one of the lowest among the AWA component factors where the effect size and power of the brightness factor are greater yet not significant (Appendix B). Thus it is unclear which statistical test reflects the true effect of adaptation on a-wave amplitudes given that the non-parametric testing uses linear mixed modeling in its calculations.

Overall, aside from a modest reduction in responsiveness of retinal cells as measured by b-wave implicit time among the myopic cohort, the present study does not provide evidence of a differential retinal response between myopes and controls. However, increased adapting luminance appears to influence ERG outcomes, as adaptation to short-wavelength light at 300 cd/m² was sufficient to reduce cellular response time. Stronger luminance adaptation also demonstrated a greater propensity to modulate ERG amplitudes, although these effects were likely dampened by noise introduced through skin electrode recordings, although implicit time variability remained minimal. Future investigations will incorporate DTL electrodes to enhance measurement reliability and reduce variability given that prior research has demonstrated that contact electrodes, such as Dawson, Trick and Litzkow electrodes (DTL) or Burian-Allen corneal electrodes, offer improved signal consistency and yield larger amplitudes (Bradshaw et al., 2004; Mortlock et al., 2010; Tang et al., 2018). Future work will also focus on central ERG assessments since it is possible that full-field ERG tests masks any localized ERG changes in the retina.

Chapter 6

Effects of Light Adaptation on the Pattern (PERG) and Multi-Focal (mfERG) Electroretinogram

6.1 Introduction

The PERG uses a checkerboard stimulus to evoke responses primarily from retinal ganglion cells, with additional input from bipolar cells and cones (Thompson et al., 2024). The mfERG records responses from multiple retinal regions (Hoffmann et al., 2021). Both PERG and mfERG are considered central retinal tests, as their pattern stimuli target the central retinal region and are predominantly detected by central foveal cones (Riggs, 1965). Observations of lower P50 amplitudes (first positive peak of the PERG waveform) in myopes have been found using PERGs (Oner et al., 2009). Other researchers point to a significant negative correlation between axial length and P50 amplitudes (Hidajat et al., 2003). Similarly, low amplitudes in myopes were observed with mfERGs (Chan and Mohidin, 2003; Kader, 2012; Koh et al., 2014). Interestingly, the central component of mfERGs were smaller in the emmetropic children who later became myopic (Li et al., 2017) suggesting that ERGs may have predictive value in identifying individuals at risk for myopia.

It is well established that short-wavelength light can stimulate the release of dopamine in the eye (Zhou et al., 2017b). The effects of dopamine on PERGs and mfERGs are less understood. Studies done on individuals with Parkinson’s disease, a condition characterized by a dopamine deficiency (Kouli et al., 2018) have highlighted diminished PERG amplitudes and others detected a delayed peak times (Langheinrich et al., 2000; Ikeda et al., 1994). Similarly, with mfERGs, amplitudes were significant smaller in patients with

Parkinson's and response time was longer in patients compared to controls (Palmowski-Wolfe et al., 2006).

Recent evidence suggests that short-wavelength light may selectively modulate retinal activity in myopes. The up-regulation of PERG activity was reported in myopes and reflects a difference between controls and myopes 20 minutes after stimulating the blind spot with short-wavelength light for 1 min (Amorim-de Sousa et al., 2021). In the previous two studies, I did not detect a post short-wavelength adaptation difference between controls and myopes with full-field ERGs but it is possible that more central, pattern-based retinal tests may reveal refractive error-related differences. Therefore, this study aims to investigate the effects of short-term, full-field, short and long wavelength light adaptation on PERG and mfERG responses in myopic and non-myopic individuals.

6.2 Protocol

I used the methods outlined in the general materials and methods (Chapter 3). The ERG protocol specific to this study is outlined below. A two-visit cross over adaptation design was used (Figure 6.2, Chapter 5).

ERG Measurements: The most myopic eye in the high myope group and the most emmetropic eye in the control group were selected for monocular ERG testing without mydriasis. Anesthetic drops (1-2 drops of Tetracaine 0.5% Minims) were administered to improve comfort and participant compliance. The skin at the outer canthus and temple were cleaned with NuPrep® abrasive gel (Weaver & Company, Colorado, USA). The string of the DTL™ was draped along the lower eyelid making contact with the conjunctiva and corneal surface (Figure 6.1).

Ten20® electrode paste (Weaver & Company, Colorado, USA) and electrode tape were used to help improve contact. 3M™ Red Dot™ Monitoring Electrodes were used as ground electrodes and were placed on the forearm. The reference silver chloride electrode was placed on the outer canthus of the eye being tested. The Espion™ E2 Diagnosys LLC system was used to measure the ISCEV standard PERG (15° field, 0.8° reversing checks) in ordinary ambient room lighting. Automated N35, P50 and N95 cursors were moved such that N35 reflected the baseline 0 μ V, P50 is highest point between 45 ms and 55 ms and N95, defined as the distance from the peak of the P50 to the middle of the negative, broad trough, around 95 ms from onset (Figure 6.3). The N95 component does not always have a sharp, well-defined peak. Instead, the trough is often wide or flattened, making it difficult to pinpoint the exact moment of its peak. Therefore, the N95 implicit time is not often reported as a reliable measure of retinal function and will not be

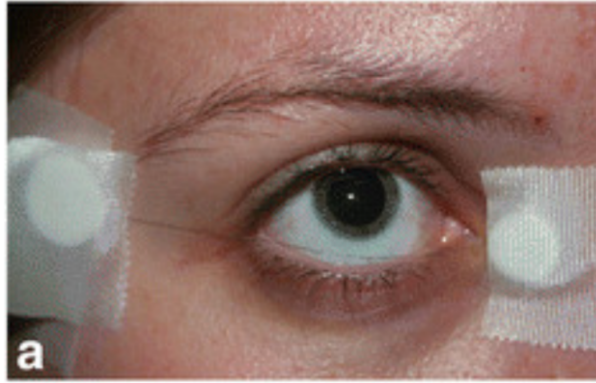


Figure 6.1: The thread of the DTL™ was draped along the lower lid such that the white adhesive pads were secured at the inner canthus of the eye and tip of the eyebrow. Since this is the active electrode, it was then connected to the amplifier of the Espion™ recording console. Used with permission. Copyright © 2016, Anastasia Lapkovska et al.

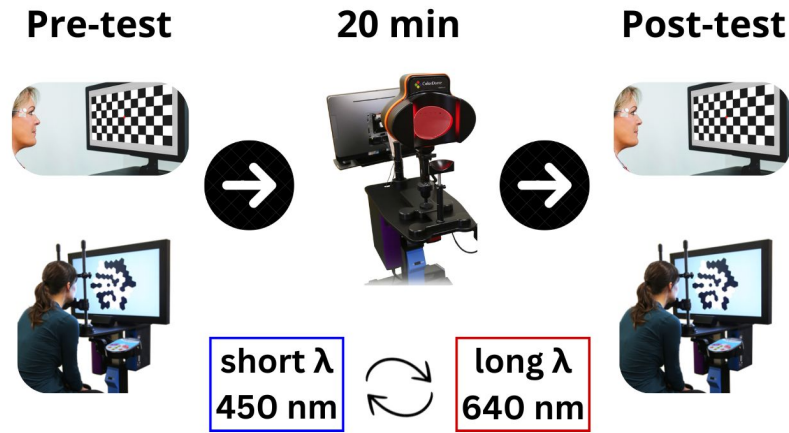


Figure 6.2: The protocol followed was to take a preliminary PERG and mfERG test for each participant was taken using the Espion™ followed by adaptation to coloured light for 20 minutes using the Espion Colour Dome™ (Diagnosys LLC). Each participant was randomly assigned adaptation to either short (450 nm) or long (640 nm) wavelength light at 300 cd/m² for a total of two visits. The 20 minute adaptation period was followed by a post PERG and mfERG test. Copyright permissions obtained for use of images.

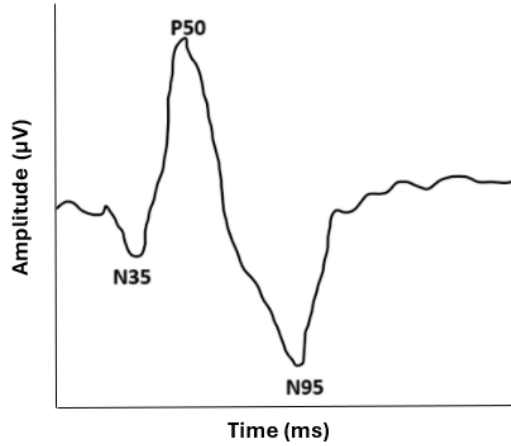


Figure 6.3: An example of an original pre-adaptation PERG recording measured in an emmetropic participant. N35 marks the trough before the P50. The P50 is highest point between 45 ms and 55 ms and N95 with a peak time in the region of 95 ms. The PERG was recorded using DTL™ skin electrodes connected to the Espion™ device.

reported here (Thompson et al., 2024). The device automatically averages the waveforms and rejects data contaminated by eye movements or blinks or 60 Hz noise with a max amplitude of 100 μV . On average, less than 10% of total sweeps were removed from the average waveform as outliers. Next, the Espion E2 Diagnosys system™ was used to measure the ISCEV standard mfERG (61 hexagons, 30 second sequences). The N1P1 magnitude (nV/deg^2) and respective implicit times for each of the 5 rings were analyzed (Types of ERGs in Chapter 2.3.2 and Figure 6.4). The ring averages are a biphasic wave with an initial negative trough followed by a positive peak and another negative trough, termed N1, P1, and N2, respectively.

6.3 Results

Pre-adaptation amplitudes and implicit times were variable across visits for the PERG and mfERG as is clear from the degree of scatter on the graphs below (Figures 6.5,6.6). Between the two visits, the baseline value ranges are as follows: P50 amplitude: controls 0.84 to 6.24 μV ; myopes 0.75 to 5.00 μV , P50 time: controls 40.5 to 47.0 ms; myopes 37.5 to 55.5 ms, N95 amplitude: controls -12.5 to -3.51 μV ; myopes -9.19 to -1.02 μV , N1P1 ring 1: controls 127.0 to 923.0 nV/deg^2 ; myopes 97.0 to 2129.0 nV/deg^2 , and N1P1 ring 2: controls 12.8 to 1039.3 nV/deg^2 ; myopes 95.8 to 1736.7 nV/deg^2 , N1P1 ring 3: controls 103.8 to 700.7 nV/deg^2 ; myopes 75.9 to 1799.6 nV/deg^2 , N1P1 ring 4: controls

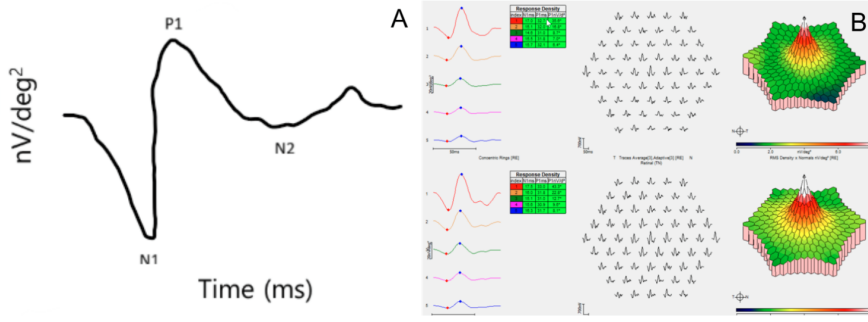


Figure 6.4: An original pre-adaptation mfERG recording measured in an emmetropic participant. Panel A depicts a sample wavelet with the peaks and troughs labeled. Panel B depicts the ring traces from each hexagon. The five ring averages are on the left side, each with a negative trough followed by a positive peak and another negative trough titled N1, P1 and N2 respectively. Typically the N1P1 magnitude is used for analysis. The mfERG was recorded using DTL™ skin electrodes connected to the Espion™ device.

97.5 to 855.1 nV/deg²; myopes 80.3 to 1078.4 nV/deg², N1P1 ring 5: controls 109.4 to 970.7 nV/deg²; myopes 86.6 to 1079.2 nV/deg².

It should be noted that the values for mfERG exclude one control participant since scheduling conflicts prevented them from returning for a final mfERG measurement and was therefore unable to be included in the mfERG analysis. The characteristics of the sample size used for final analysis is in Table 6.1 (minus one control participant for the mfERG).

There was a significant difference between refractive error groups for the PERG P50 amplitude prior to adaptation ($p=0.03$; Cohen's $d=1.06$) such that they were significantly smaller in myopes ($2.63 \pm 1.00 \mu V$) than controls ($3.95 \pm 1.50 \mu V$) (Figure 6.7). The N95 amplitudes were also smaller (i.e. less negative) in myopes ($-4.39 \pm 1.82 \mu V$) than controls ($-6.58 \pm 1.55 \mu V$; $p=0.01$; Cohen's $d=-1.3$). The mfERGs showed no significant pre-adaptation refractive error differences (all $p \geq 0.05$; all Cohen's $d \leq 0.07$) (Figure 6.8). However, the discrepancy between visits confounds these results and to circumvent this inconsistency, the relative change of each component was used. The data for the PERG were normal however the mfERG data did not follow a normal distribution. However, the variances for each component were homogeneous. Repeated measures ANOVAs for all components yielded similar results as the non-parametric testing (Appendix B).

For the PERG, in both controls and myopes, the proportional change in N95 amplitudes was greater with long-wavelength light (controls: $-16.6 \pm 23.4\%$; myopes: $-24.7 \pm 43.4\%$) than short-wavelength light (controls: $+10.4 \pm 23.5\%$; myopes: $+1.88 \pm 26.7\%$; $p \leq 0.001$;

Table 6.1: Characteristics of the control and high myope groups. Note: abbreviations SER = spherical equivalent refraction and AXL = axial length.

	n	Number of Females	Median Age [min, max]	Median SER [min, max]	Median AXL [min, max]
Controls	9	5	23 [19, 29]	0.00 [-0.50, 0.25]	23.435 [22.64, 25.21]
Myopes	11	6	24 [21, 29]	-7.625 [-11.38, -5.00]	26.97 [25.34, 28.05]

Table 6.2: PERG p-values and effect sizes for effects based on colour adaptation (λ) and based on refractive error (RE) group are listed (* denotes a significant p-value).

Component	Effect	P-value	Effect Size (η^2G)	Power
P50 AMP	RE:	0.896	0.001	0.06
	λ :	0.619	0.005	0.17
P50 IT	RE:	0.333	0.029	0.51
	λ :	0.787	0.002	0.08
N95 AMP	RE:	0.521	0.019	0.26
	λ :	$\leq 0.001^*$	0.170	1.00

$\eta^2G=0.170$). There were no effects of adaptation on the P50 amplitude or implicit time (Figures 6.9, 6.2). For mfERG, the change in N1P1 magnitude was not different between controls and myopes and was not different when comparing wavelengths for any of the five rings ($p \geq 0.05$; Figures 6.10, 6.3).

6.4 Discussion

Our findings of a significantly smaller P50 and N95 amplitude in myopes at baseline agrees with what has been reported in literature in that the more myopic the individual, the lower the P50 and N95 amplitude (Hidajat et al., 2003; Oner et al., 2009). The participant profile of my study is comparable to Oner et al. and Hidajat et al. and although they had greater sample sizes, the same difference in P50 and N95 amplitudes was detected with my sample size of 11 high myopes. These results are suggestive of an altered ganglion cell response in high myopes (Thompson et al., 2024) and may have clinical relevance especially if a smaller P50 or N95 can be detected in early myopes.

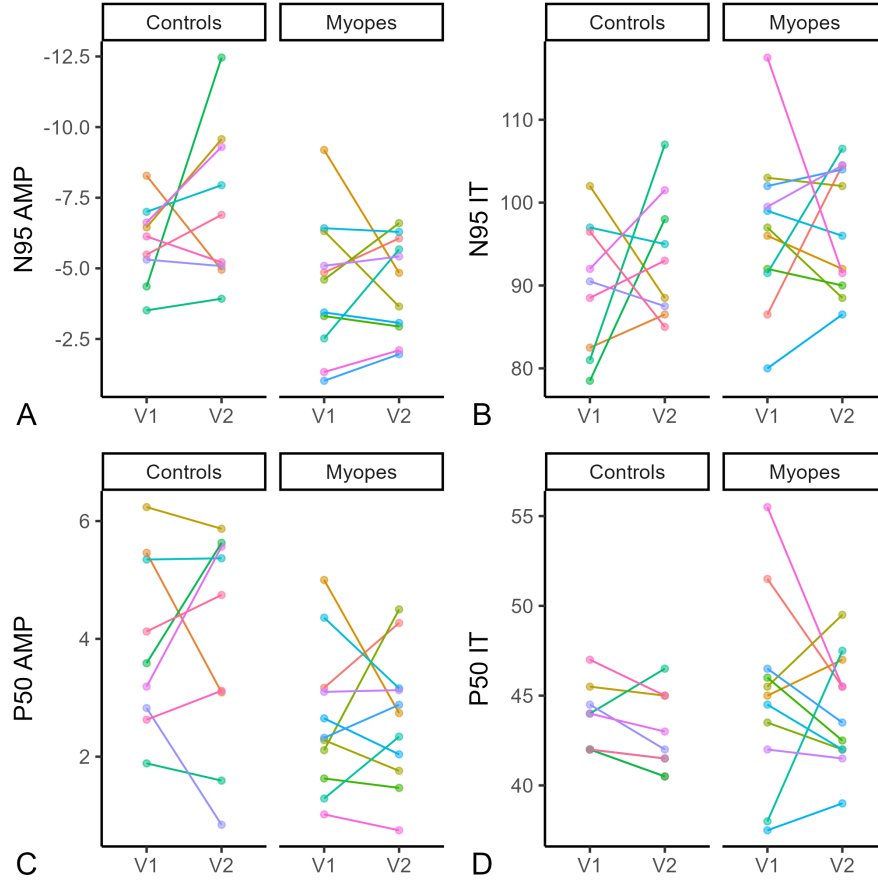


Figure 6.5: The pre-adaptation PERG data for all 20 participants at visit 1 (V1) and visit 2 (V2) for each component (N95 amplitude and implicit time and P50 amplitude and implicit time, A-D, respectively).

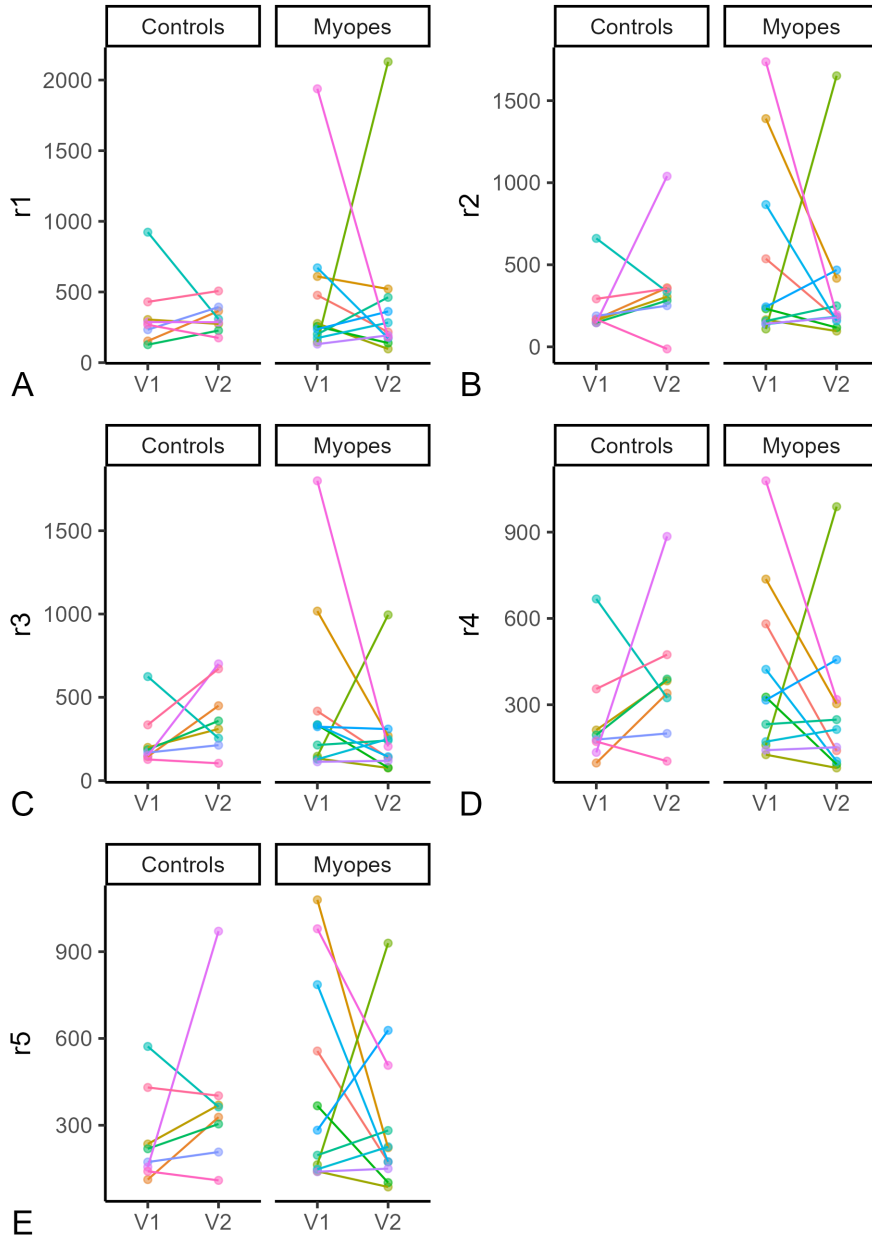


Figure 6.6: The pre-adaptation mfERG data for all 19 participants at visit 1 (V1) and visit 2 (V2) for the N1P1 magnitude for all 5 rings (A-E, respectively).

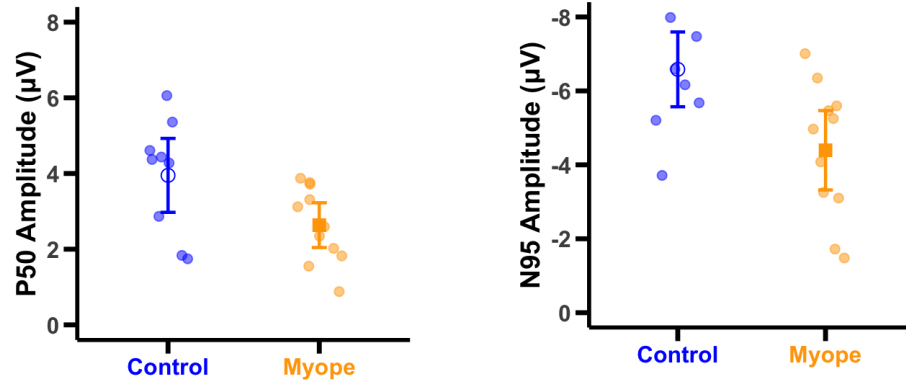


Figure 6.7: The pre-adaptation average P50 and N95 amplitudes in controls (in blue) and myopes (in orange). The large symbols represent the mean. The error bars represent the 95% confidence interval of the mean. Note that the negative axis of the N95 amplitude graph has been flipped for ease of interpretation.

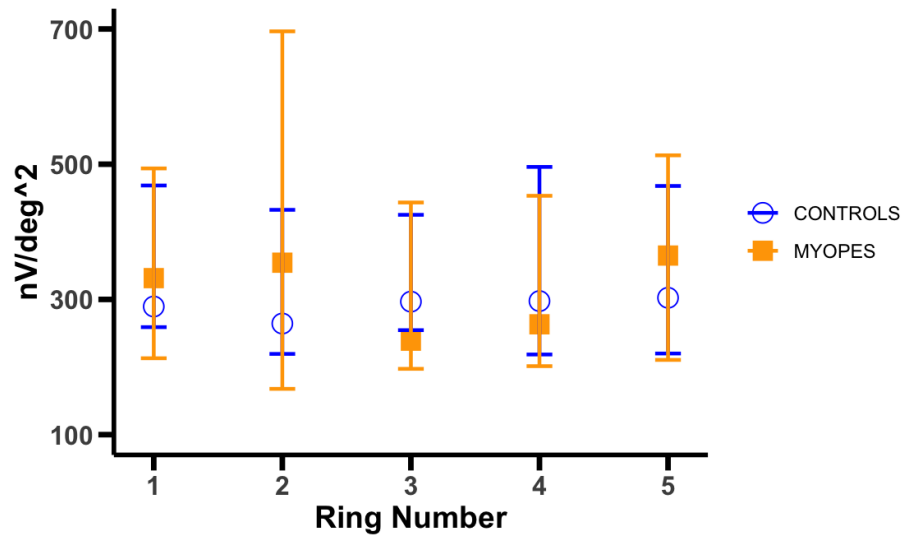


Figure 6.8: The median N1P1 magnitude in controls (in blue) and myopes (in orange). The error bars represent the inter-quartile range of the data.

Table 6.3: mfERG N1P1 magnitude p-values and effect sizes for effects based on colour (λ) and based on refractive error (RE) group are listed (** denotes a significant p-value).

Component	Effect	P-value	Effect Size (η^2G)	Power
Ring 1	RE:	0.943	0.00	0.05
	λ :	0.723	0.006	0.12
Ring 2	RE:	0.268	0.032	0.65
	λ :	0.423	0.022	0.39
Ring 3	RE:	0.521	0.007	0.27
	λ :	0.448	0.024	0.36
Ring 4	RE:	0.649	0.005	0.15
	λ :	0.964	0.00	0.05
Ring 5	RE:	0.189	0.034	0.80
	λ :	0.839	0.002	0.08

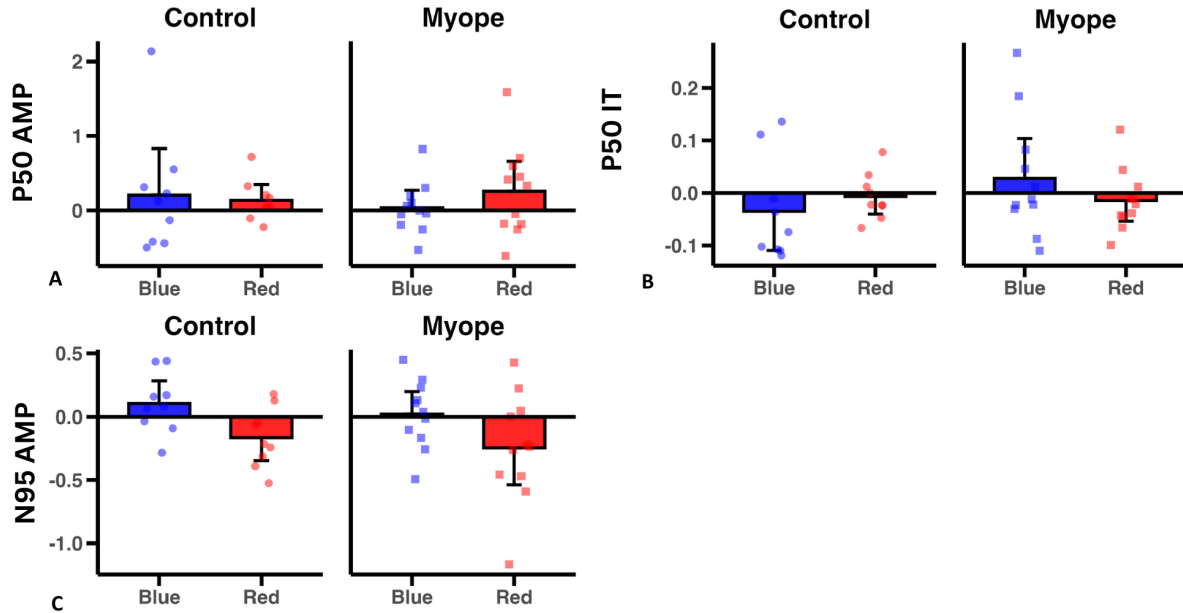


Figure 6.9: The average relative change for the PERG P50 amplitude, P50 implicit time and N95 amplitude for the control and myope group (A-C, respectively). The error bars represent the 95% confidence interval of mean.

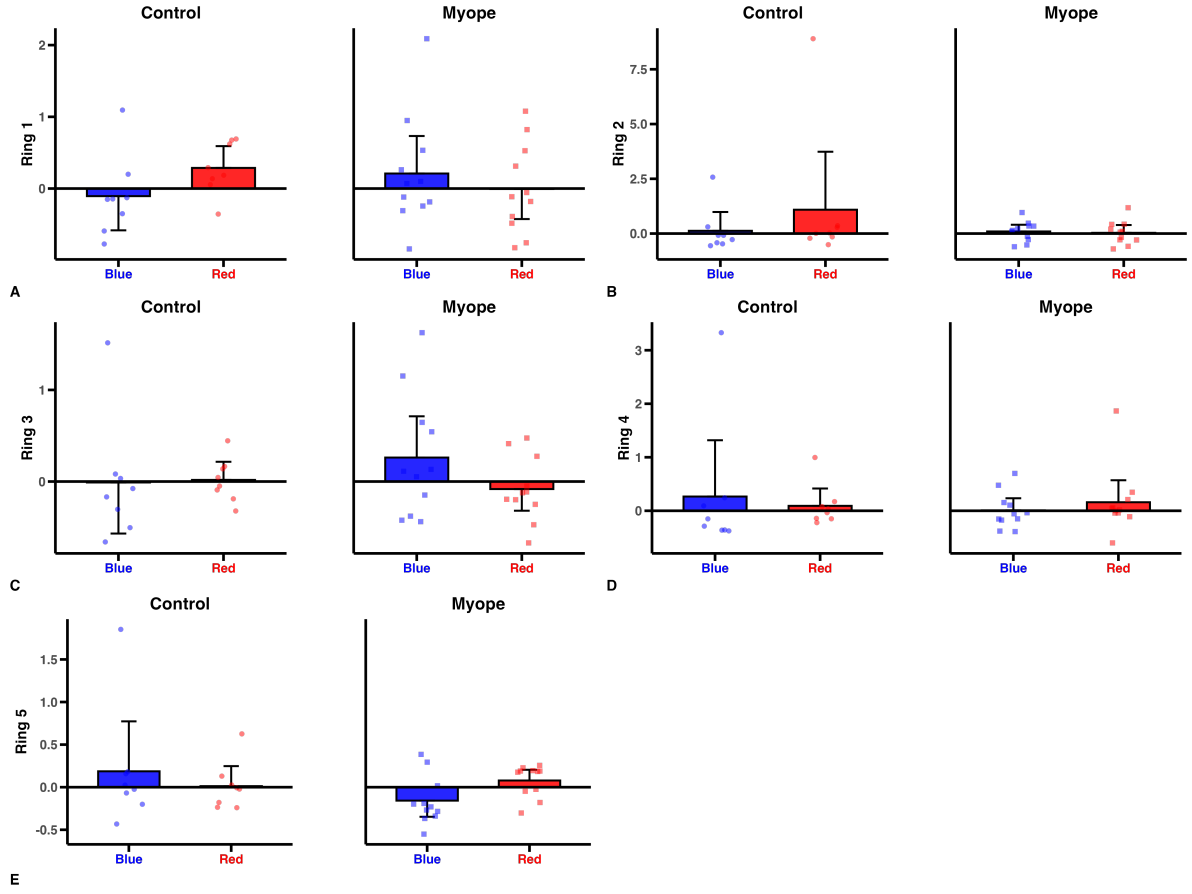


Figure 6.10: The average relative change for the mfERG N1P1 magnitude for each of the five rings (A-E, respectively). The error bars represent the 95% confidence interval of the mean.

However, the failure to detect a refractive error difference with mfERG does not line up with what others have found (Chan and Mohidin, 2003; Kader, 2012; Koh et al., 2014). This lack of difference is unexpected given that the degree of myopia in the present study was again comparable to previous investigations by Kader, Koh et al., and Chan et al., all of which defined high myopia as a refractive error of -6.00 D or greater (Chan and Mohidin, 2003; Kader, 2012; Koh et al., 2014). Although my study used a slightly more inclusive threshold of -5.00 D to define high myopia, the majority of participants exhibited a refractive error of -8.00 D, representing 36.4% of the myopic sample. Notably, Kader's study included 20 high myopes and Koh et al. and Chan et al. each recruited over 30 subjects in their respective high myopia groups. These differences in sample size may have contributed to the lack of statistical significance in my findings although my study was powered to detect these differences (power= 56%). Future research should incorporate larger cohorts to improve statistical power and detect subtle refractive error-related effects in mfERGs.

Following short-wavelength light adaptation, the significant reduction in N95 amplitude in myopic participants suggests reduced responsiveness of retinal ganglion cells, potentially reflecting structural or metabolic alterations associated with axial elongation (Perlman et al., 1984). Prior studies have reported thinning of the retinal nerve fiber layer and diminished contrast sensitivity in myopic eyes, which may underlie the observed electrophysiological attenuation (Saw et al., 2005; Resnikoff et al., 2008).

The multifocal electroretinogram (mfERG) revealed no significant differences between control and myopic groups following adaptation to either wavelength. To my knowledge, this study is the first to assess mfERG responses following 20 minutes of chromatic light adaptation in a myopic population. Refractive error-related differences were not observed even though this study is powered to detect differences as was seen with the PERG results (power=56%). It remains possible that a more diverse and larger sample size is required to detect subtle changes in mfERG parameters regardless of whether measurements are taken pre- or post-adaptation.

Chapter 7

Pilot: S-Cone Electroretinogram and High Myopia

7.1 Introduction

There has been much debate regarding S-cone (short-wavelength light sensitive cones) functionality in highly myopic individuals. Literature reveals a significant reduction in S-cone sensitivity in high myopes using visually evoked cortical potentials (Kawabata et al., 1997) and via selective chromatic adaptation (Koike and Tokoro, 1986). Yamamoto et al. measured S-cone ERGs and found reduced S-cone amplitudes as well as reduced fERGs with increased myopia progression (Yamamoto et al., 1997). It is unclear in the literature whether myopia progression selectively damages S-cones or if this decrease in responsitivity needs to be taken in proportion to the general loss of sensitivity in L-(long-wavelength light sensitive) and M-cones (medium-wavelength light sensitive).

The previous two studies used the RETeval® to perform light-adapted full-field ERG (LA fERG) in this thesis but the LA fERG is dominated by responses from the L- and M-cones and does not include responses from the S-cones since there are far fewer and their summed responses are of smaller amplitudes and slower peak times (Gouras and MacKay, 1990). The S-cone ERG is a method of measuring the S-cone retinal pathway by using a background that suppresses the rods and minimizes contributions from the L- and M-cones (Perlman et al., 2020).

I hypothesize that S-cone responsitivity will be reduced in high myopes compared to controls. To test this hypothesis I performed a baseline S-cone ERG test to determine if a refractive error difference can be detected using the S-cone protocol and DTL electrodes.

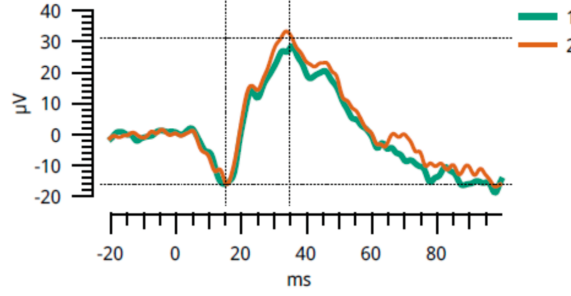


Figure 7.1: A sample S-cone measurement for an emmetropic participant taken using DTL electrodes connected to the RETeval®. The green and orange coloured lines represent two replicate measurements. The S-cone a-wave was measured from baseline to the minimum negative trough (between 10 and 20 ms). The b-wave is measured from the trough of the negative a-wave to its respective maximum peak (between 30-50 ms).

7.2 Protocol

I used the methods outlined in the general materials and methods (Chapter 3). The ERG protocol specific to this study are outlined below.

ERG Measurements: A baseline S-cone ERG was recorded using the RETeval® system (LKC Technologies, Gaithersburg, MD, USA). The most myopic eyes in the high myope group and the most emmetropic eyes in the control group were selected for monocular ERG testing. The skin at the outer canthus and temple were cleaned with NuPrep® abrasive gel (Weaver & Company, Colorado, USA). The string of the Dawson, Trick, and Litzkow (DTL) electrodes™ was draped along the lower eyelid making slight contact with the conjunctiva and corneal surface. Ten20® electrode paste (Weaver & Company, Colorado, USA) and electrode tape were used to help improve contact. 3M™ Red Dot™ Monitoring Electrodes were used as ground electrodes were placed on the forearm and the reference electrode was placed on the outer canthus of the eye being tested. The electrode was then connected to the RETeval® device a custom troland protocol was used according to ISCEV guidelines ((Perlman et al., 2020)). The blue flash of the S-cone ERG had a rate of 28 td.s at 2 Hz with a red background of 8500 Td. The a-wave of the S-cone ERG was measured from baseline to the minimum negative trough between 10 and 20 ms after flash onset. The b-wave of the S-cone is measured from the trough of the negative a-wave to the maximum peak in the region of 30-50 ms after flash onset.

Table 7.1: Characteristics of the control and high myope groups. Note: abbreviations SER = spherical equivalent refraction and AXL = axial length.

	n	Number of Females	Median Age [min, max]	Median SER [min, max]	Median AXL [min, max]
Controls	11	9	25 [19, 29]	0.00 [-0.50, 0.50]	23.26 [22.64, 24.69]
Myopes	10	6	24 [21, 29]	-7.60 [-11.40, -5.00]	26.97 [25.34, 28.05]

Table 7.2: The non-parametric p-values and effect sizes for each S-cone component are listed (*denotes a significant p-value).

Component	P-value	Effect Size (η^2G)	Power
AWA	0.29	0.06	0.19
AWT	0.06	0.17	0.48
BWA	0.01*	0.27	0.73
BWT	0.86	0.00	0.05

7.3 Results

The data did follow a normal distribution and variances were homogeneous. The descriptive statistics for these participants are in Table 7.1. The high myope group had significantly lower b-wave amplitudes (32.4 ± 10.1 μV) compared to controls (48.1 ± 16.3 μV) ($p=0.01$, $\eta^2G=0.27$) (Figure 7.2). Both refractive error groups showed no differences in their a-wave amplitudes ($p=0.29$), a-wave implicit times ($p=0.06$) or b-wave implicit times ($p=0.86$) (Table 7.2).

7.4 Discussion

Compared to controls, high myopes exhibited smaller b-wave amplitudes, reflecting a dampened response from ON/OFF bipolar cells along the S-cone pathway. These results are similar to that of Yamamoto et al. (1997) who also found decreased S-cone amplitudes but saw no differences with implicit time (Yamamoto et al., 1997). These results agree with previous reports of reduced functionality in the S-cones of myopes (Koike and Tokoro,

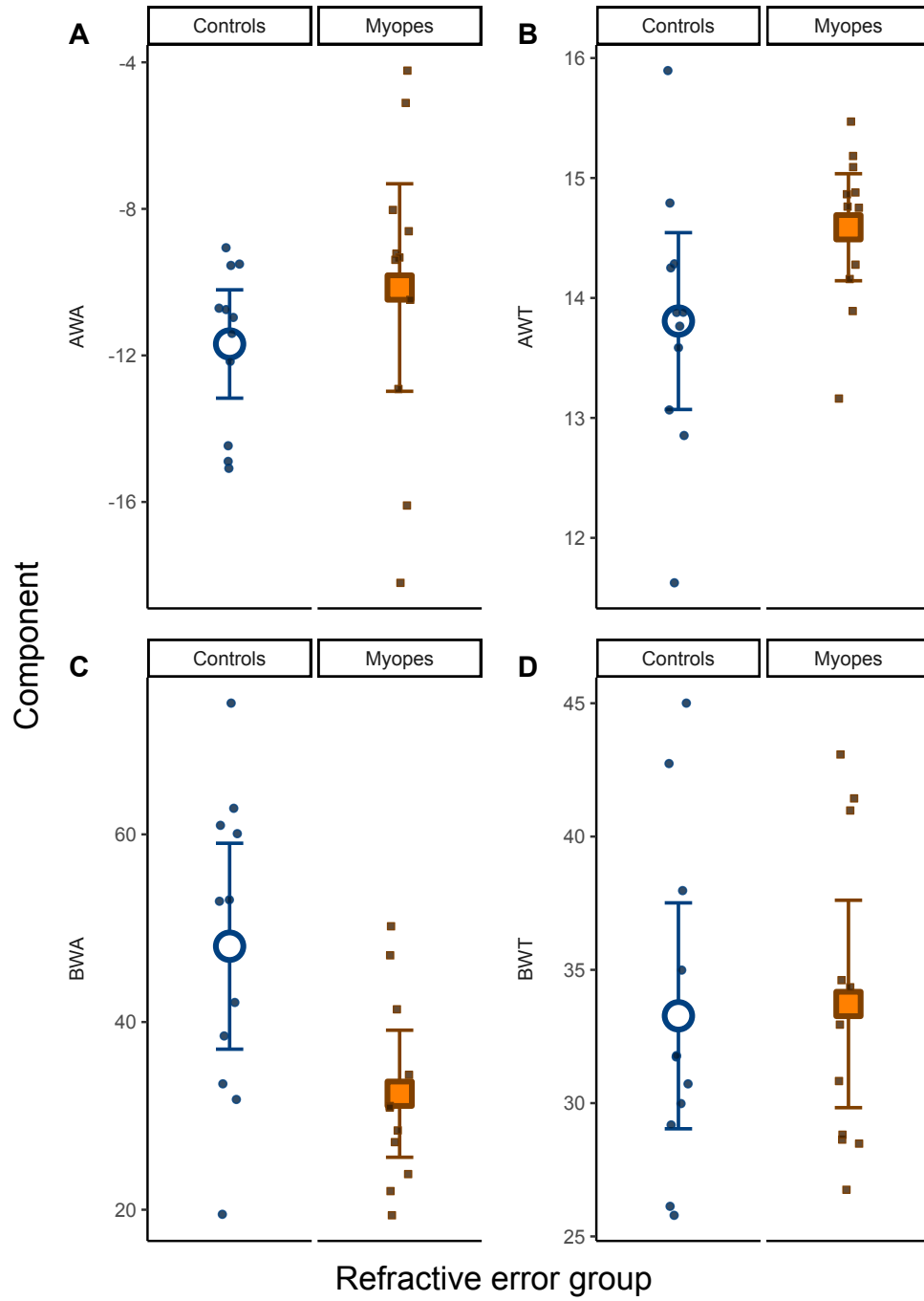


Figure 7.2: The average baseline S-cone ERG measurements for S-cone AWA, AWT, BWA and BWT (A-D, respectively) for controls (blue) and high myopes (orange). The error bars reflect the 95% confidence interval of the mean.

1986; Kawabata et al., 1995). On the other hand, the absence of a reduction in LA fERGs (Studies 1 & 2), does not agree with what Yamamoto et al. (1997) found since they saw an equal reduction in amplitude for S-, L- and M-cones (Yamamoto et al., 1997). They conclude that perhaps, in myopia, S-cones are affected as much as, or even less than, L- and M-cones. Yamamoto et al.'s study had a cohort with a more inclusive age group (13-40) whereas my sample contains individuals ages 18-30. Thus, their sample may represent an earlier stage in the progression toward pathological myopia (Yamamoto et al., 1997). It is not well-understood how age affects cone degradation but a longitudinal study, although more involved, may elucidate a temporal relation if S-cone ERGs and fERGs are taken in progressing myopes over the span of several years.

It is also plausible that the use of DTL thread electrodes contributed to the ability to detect the refractive error-related differences in S-cone ERGs only. Unlike sensor strips, which are known to attenuate signal amplitudes and introduce noise, DTL electrodes provided clearer recordings (Kriss, 1994; Tang et al., 2018). Although the *RETeval* proved effective in measuring S-cone responses, the misidentification of the peaks and troughs of the ERG waveform (Appendix A) demonstrates its susceptibility to measurement error, underscoring the importance of analyzing raw data directly rather than relying solely on machine-generated averages.

Given the selective reduction in S-cone ERGs, it is potentially of interest that Schmidt et al., found M-cone activity to be dependent on S-cone input. This work may suggest that early S-cone impairment could contribute to subsequent L- and M-cone deficits in advanced myopia (Schmidt et al., 2018). Furthermore, Taylor et al. demonstrated a negative correlation between S-cone stimulus thresholds and refractive error in myopic individuals. With the knowledge that longitudinal chromatic aberration has been proposed to help fine-tune emmetropization cues, if myopes are predisposed to reduced S-cone functionality, this impairment may interfere with normal emmetropization processes (Taylor et al., 2018).

While my findings may not definitively resolve the question of which cone type degenerates first in myopia, they do reveal the potential of selective reduction in S-cone sensitivity in high myopes. Also, the results of this study demonstrate that the *RETeval* can reliably detect differences in S-cone functionality even with a relatively small sample size (10 myopes, power of 56%), supporting its potential use as a biomarker or endpoint for monitoring myopia progression. Future research would include use of a larger sample size (20 to 30 myopes) with a higher degree of myopia (-8.00 to -15.00 D) and use of the DTL electrodes for the LA fERGs to reduce the noise introduced by skin electrodes.

Chapter 8

General Discussion & Conclusion

This thesis investigated the effects of short-term, short-wavelength light exposure on retinal function in individuals with high myopia compared to non-myopes, using electroretinogram (ERG) measurements to assess functional differences. The first experiment used standard luminance levels and full-field ERG tests, followed by trials with increased luminance to evaluate its impact. The focus then shifted to the central retina, where light adaptation effects were examined using pattern ERG (PERG) and multifocal ERG (mfERG). As part of a pilot study, baseline S-cone ERGs were also recorded in high myopes.

Prior to adaptation, no refractive error differences were observed following LA fERG to white light but baseline PERG and S-cone ERG were reduced in myopes. These specific ERGs may serve as clinically relevant biomarkers, particularly in pediatric populations, for detecting and potentially predicting the onset and progression of myopia. Reduced PERG and S-cone ERG responses could signal early retinal ganglion cell or S-cone dysfunction, possibly due to myopiagenic factors such as retinal thinning or detachment.

While there were post-adaptation refractive error differences with any of the ERG tests, the findings do suggest that higher luminance levels enhance stimulation of ON and OFF bipolar cells in both control and high myope groups, as evidenced by modest, yet significant increases in ERG amplitudes with stronger light. Long-wavelength light adaptation was associated with prolonged implicit times, whereas short-wavelength light did not significantly alter implicit time. Although it is not possible to confirm, this result may indicate that long-wavelength systems are more sensitive to short-term adaptation.

My findings diverge from those of Amorim-de-Sousa, one of the only other studies that looked at ERGs following short-term short-wavelength light adaptation in myopes. This group reported an up-regulation of b-wave and P50-N95 amplitudes following stimulation (Amorim-de Sousa et al., 2021). There are certain methodological differences that may account for the discrepancy in my results and theirs. First, their sample size had only five

myopic participants and the absence of a control group, while all the studies in the present thesis had more than double the number of myopes and controlled for both non-myopes and different wavelengths of light.

In addition, Amorim-de-Sousa employed a virtual reality headset to deliver a 1-minute of flickering retinal stimulation, whereas my protocol involved 20 minutes of chromatic light adaptation. It is not completely clear why this group would use flickering light since there is evidence to suggest a myopic shift with flickering blue light adaptation in tree shrews (Gawne et al., 2017). Also, flickering light has been recently shown to enhance electro-retinal responses and retinal blood flow (Rai et al., 2025), offering a potential alternative explanation for the increased b-wave amplitudes observed by this group following adaptation. I chose to use static full-field light in order to remove any possible confounds with flickering light.

The impact of adaptation duration remains unclear; some studies have shown that 10–20 minutes of exposure is sufficient to activate ipRGCs (Mutti et al., 2020b), while others have required up to 1 hour to observe changes in axial length and choroidal thickness, presumably mediated via the ipRGC pathway (Thakur et al., 2021; Lou and Ostrin, 2020). Furthermore, their measurements were taken 20 minutes post-stimulation, while ours were recorded immediately. Delayed measurement may be necessary to allow ipRGC-mediated dopamine release to manifest (Wong et al., 2005) however, given that controls and myopes followed the same patterns in all our results, it is more likely that adaptation time of 20 minutes was simply not enough to stimulate the ipRGCs to release a significant amount of dopamine to produce a difference measurable by the ERG.

It is possible that brief stimulation is adequate, but other variables may explain the divergence in results such as the targeting of the blind spot to isolate retinal ganglion cell axons more directly. Melanopsin is known to be found predominately in the cell bodies of the ipRGCs and not the axons (Spitschan, 2019) and so it is not exactly clear how short-term light stimulation of the blind spot enables the excitation of melanopsin. My protocol involved full-field retinal stimulation and although I did not directly stimulate the cell-bodies of the ipRGCs, it is established that ipRGCs are maximally sensitive to wavelengths around 450 nm and therefore it is likely that I was able to stimulate the ipRGC pathway. Although, like Amorim-de-Sousa, the exact pathway for how post-adaptation effects were induced remains unclear.

The results of this thesis underscore the importance of further investigating the relationship between refractive error and light-induced retinal responses using objective tools like ERG. However, several limitations to my study should also be acknowledged. First, the Espion Colourdome™ used for adaptation stimuli was limited in its maximum luminance output due to calibration issues and outdated hardware, reducing the ability to go above

300 cd/m², limiting real-world light exposure conditions. While the RETeval® system offers user-friendly, non-invasive ERG recording without dilation and meets ISCEV standards, its susceptibility to blink artifacts, noise from skin electrodes, and inaccuracies in auto-generated averages were difficult and time consuming to correct as it requires manual review of raw data. Additionally, caffeine intake was not controlled for. Studies do show an affect of caffeine on increasing choroidal thickness, which may have influenced axial length measurements and ERG amplitudes, given their inverse relationship (Altinkaynak et al., 2016). Although most testing occurred between 9 a.m. and 5 p.m., occasional scheduling outside these hours may have introduced variability due to ipRGC-mediated circadian influences.

Future research could build on these findings by including a larger and more diverse sample with varying degrees of myopia. Moreover, long-term exposure to short-wavelength light (1–2 hours) and real-world adaptation studies using wearable light sensors and distance vision trackers could provide deeper insights. It is also possible that the ipRGC-mediated dopamine release needed time to manifest and waiting 20 to 30 minutes after adaption may reveal differences in ERG amplitudes relative to short-wavelength light adaptation (Amorim-de Sousa et al., 2021). Also, integrating ocular biometry (i.e. axial length measurements and OCT imaging) alongside ERG data may help correlate structural and functional changes and align findings with previous studies on short-term light adaptation (Thakur et al., 2021; Lou and Ostrin, 2020).

In summary, this thesis found no conclusive evidence that short-term light exposure differentially affects retinal function in myopic individuals, as measured by ERG. Nonetheless, the results contribute to the growing body of research on the interplay between environmental light exposure and refractive error, and highlight the need for further investigation into the mechanisms driving myopia development.

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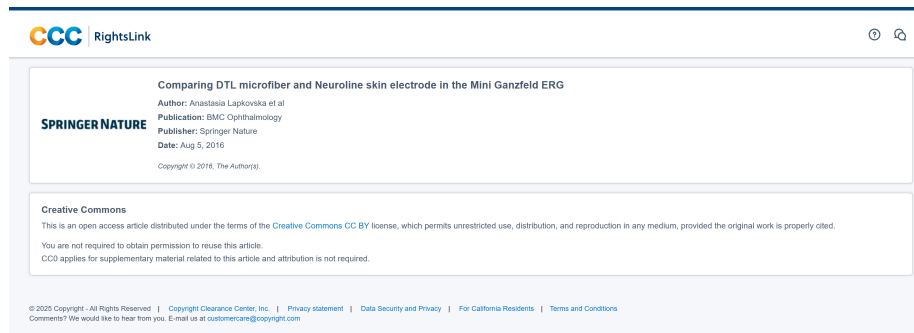


Figure 8.1: DTL image open access copyright permission

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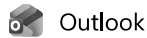
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Figure 8.2: LKC Technologies photo license agreement for RETeval Image



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Date Wed 8/20/2025 9:32 AM

To Ashita Bidarkar <abidarkar@uwaterloo.ca>

Cc Daphne McCulloch <daphne.mcculloch@uwaterloo.ca>; Vivian Choh <vivian.choh@uwaterloo.ca>

 2 attachments (674 KB)

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Hi Ashita

Thanks for checking. Yes you have our permission to use our photos in your thesis. You can reference the entire system as "E3 system (Diagnosys LLC, USA)"

We have some more patient setup photos at this link, including some pattern. Those we took about a year ago.
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Regards

Ryan

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Sent: Tuesday, August 19, 2025 2:11 PM

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Thank you,
Ashita

Figure 8.3: Email from Diagnosys representative, Ryan Farmer, expressing permission to use images from their webpage in my thesis.

References

- Altinkaynak, H., Ceylan, E., Kartal, B., Keleş, S., Ekinçi, M., and Olcaysu, O. O. (2016). Measurement of Choroidal Thickness Following Caffeine Intake in Healthy Subjects. *Current Eye Research*, 41(5):708–714.
- Alvarez, A. A. and Wildsoet, C. F. (2013). Quantifying light exposure patterns in young adult students. *Journal of Modern Optics*, 60(14):1200–1208.
- Amorim-de Sousa, A., Chakraborty, R., Collins, M. J., Fernandes, P., González-Méijome, J., Hannibal, J., Hoseini-Yazdi, H., Read, S. A., Ellrich, J., and Schilling, T. (2024). Blue light stimulation of the blind spot in human: from melanopsin to clinically relevant biomarkers of myopia. *Bioelectronic Medicine*, 10(1):26.
- Amorim-de Sousa, A., Schilling, T., Fernandes, P., Seshadri, Y., Bahmani, H., and González-Méijome, J. M. (2021). Blue light blind-spot stimulation upregulates b-wave and pattern ERG activity in myopes. *Scientific Reports*, 11(1):9273. Publisher: Nature Publishing Group.
- Asakawa, K., Amino, K., Iwase, M., Kusayanagi, Y., Nakamura, A., Suzuki, R., Yuuki, T., and Ishikawa, H. (2017). New Mydriasis-Free Electroretinogram Recorded with Skin Electrodes in Healthy Subjects. *BioMed Research International*, 2017:8539747.
- Ashby, R., Ohlendorf, A., and Schaeffel, F. (2009). The effect of ambient illuminance on the development of deprivation myopia in chicks. *Investigative Ophthalmology & Visual Science*, 50(11):5348–5354.
- Au Eong, K. G., Tay, T. H., and Lim, M. K. (1993). Race, culture and Myopia in 110,236 young Singaporean males. *Singapore Medical Journal*, 34(1):29–32.
- Bailey, M. D., Sinnott, L. T., and Mutti, D. O. (2008). Ciliary Body Thickness and Refractive Error in Children. *Investigative Ophthalmology & Visual Science*, 49(10):4353–4360.

- Benjamin, B., Davey, J. B., Sheridan, M., Sorsby, A., and Tanner, J. M. (1957). Emmetropia and its aberrations; a study in the correlation of the optical components of the eye. *Special Report Series (Medical Research Council (Great Britain))*, 11(293):1–69.
- Bhatt, Y., Hunt, D. M., and Carvalho, L. S. (2023). The origins of the full-field flash electroretinogram b-wave. *Frontiers in Molecular Neuroscience*, 16:1153934.
- Bordt, A. S., Patterson, S. S., Kuchenbecker, J. A., Mazzaferri, M. A., Yearick, J. N., Yang, E. R., Ogilvie, J. M., Neitz, J., and Marshak, D. W. (2022). Synaptic inputs to displaced intrinsically-photosensitive ganglion cells in macaque retina. *Scientific Reports*, 12(1):15160.
- Bradshaw, K., Hansen, R., and Fulton, A. (2004). Comparison of ERGs recorded with skin and corneal-contact electrodes in normal children and adults. *Documenta Ophthalmologica. Advances in Ophthalmology*, 109(1):43–55.
- Brown, J. L. (1965). *The Structure of the Visual System in Vision and Visual Perception*. John Wiley & Sons Inc.
- Brown, K. T. (1968). The electroretinogram: Its components and their origins. *Vision Research*, 8(6):633–IN6.
- Brown, K. T. and Wiesel, T. N. (1961). Localization of origins of electroretinogram components by intraretinal recording in the intact cat eye. *The Journal of Physiology*, 158(2):257–280.
- Carr, B. J. and Stell, W. K. (1995). The Science Behind Myopia. In Kolb, H., Fernandez, E., Jones, B., and Nelson, R., editors, *Webvision: The Organization of the Retina and Visual System*. University of Utah Health Sciences Center, Salt Lake City (UT).
- Chan, H. L. and Mohidin, N. (2003). Variation of multifocal electroretinogram with axial length. *Ophthalmic and Physiological Optics*, 23(2):133–140. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1475-1313.2003.00097.x>.
- Chang, A.-M., Aeschbach, D., Duffy, J. F., and 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 of the United States of America*, 112(4):1232–1237.
- Chua, S. Y. L. and Foster, P. J. (2020). The Economic and Societal Impact of Myopia and High Myopia. In Ang, M. and Wong, T. Y., editors, *Updates on Myopia: A Clinical Perspective*, pages 53–63. Springer, Singapore.

- Chung, H., Dai, T., Sharma, S. K., Huang, Y.-Y., Carroll, J. D., and Hamblin, M. R. (2012). The nuts and bolts of low-level laser (light) therapy. *Annals of Biomedical Engineering*, 40(2):516–533.
- Cieza, A., Kocur, I., Mariotti, S., and McCoy, M. (2017). The future of eye care in a changing world: call for papers. *Bulletin of the World Health Organization*, 95(10):667.
- Cook, R. C. and Glasscock, R. E. (1951). Refractive and ocular findings in the newborn. *American Journal of Ophthalmology*, 34(10):1407–1413.
- Dacey, D. M. (1990). The dopaminergic amacrine cell. *Journal of Comparative Neurology*, 301(3):461–489. [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/cne.903010310](https://onlinelibrary.wiley.com/doi/pdf/10.1002/cne.903010310).
- de Freitas, L. F. and Hamblin, M. R. (2016). Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE journal of selected topics in quantum electronics: a publication of the IEEE Lasers and Electro-optics Society*, 22(3):7000417.
- Ding, X., Wang, D., Huang, Q., Zhang, J., Chang, J., and He, M. (2013). Distribution and heritability of peripheral eye length in Chinese children and adolescents: the Guangzhou Twin Eye Study. *Investigative Ophthalmology & Visual Science*, 54(2):1048–1053.
- Dirani, M., Chamberlain, M., Garoufalis, P., Chen, C., Guymer, R. H., and Baird, P. N. (2006). Refractive errors in twin studies. *Twin Research and Human Genetics: The Official Journal of the International Society for Twin Studies*, 9(4):566–572.
- Dolgin, E. (2015). The myopia boom. *Nature*, 519(7543):276–278.
- Dumitrescu, O. N., Pucci, F. G., Wong, K. Y., and Berson, D. M. (2009). Ectopic retinal ON bipolar cell synapses in the OFF inner plexiform layer: Contacts with dopaminergic amacrine cells and melanopsin ganglion cells. *Journal of Comparative Neurology*, 517(2):226–244. [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/cne.22158](https://onlinelibrary.wiley.com/doi/pdf/10.1002/cne.22158).
- Einthoven, W. and Jolly, W. (1908). The form and magnitude of the electrical response of the eye to stimulation by light at various intensities. *Quarterly Journal of experimental physiology*.
- Feldkaemper, M. and Schaeffel, F. (2013). An updated view on the role of dopamine in myopia. *Experimental Eye Research*, 114:106–119.

- Flitcroft, D., He, M., Jonas, J., Jong, M., Naidoo, K., Ohno-Matsui, K., Rahi, J., Resnikoff, S., Vitale, S., and Yannuzzi, L. (2019). IMI – Defining and classifying myopia: A proposed set of standards for clinical and epidemiologic studies. *Investigative Ophthalmology and Visual Science*, 60(3):M20–M30.
- Flitcroft, D. I. (2012). The complex interactions of retinal, optical and environmental factors in myopia aetiology. *Progress in Retinal and Eye Research*, 31(6):622–660.
- Flitcroft, D. I., Harb, E. N., and Wildsoet, C. F. (2020). The Spatial Frequency Content of Urban and Indoor Environments as a Potential Risk Factor for Myopia Development. *Investigative Ophthalmology & Visual Science*, 61(11):42.
- Foo, L. L., Lanca, C., Wong, C. W., Ting, D., Lamoureux, E., Saw, S.-M., and Ang, M. (2021). Cost of Myopia Correction: A Systematic Review. *Frontiers in Medicine*, 8. Publisher: Frontiers.
- Freed, M. A., Pflug, R., Kolb, H., and Nelson, R. (1996). ON-OFF amacrine cells in cat retina. *The Journal of Comparative Neurology*, 364(3):556–566.
- Gamlin, P. D. R., McDougal, D. H., Pokorny, J., Smith, V. C., Yau, K.-W., and Dacey, D. M. (2007). Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Research*, 47(7):946–954.
- Gawne, T. J., Ward, A. H., and Norton, T. T. (2017). Long-wavelength (red) light produces hyperopia in juvenile and adolescent tree shrews. *Vision Research*, 140:55–65.
- Gonon, F. G. (1988). Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Neuroscience*, 24(1):19–28.
- Gotch, F. (1903). The time relations of the photo-electric changes in the eyeball of the frog. *The Journal of Physiology*, 29(4-5):388–410.
- Gouras, P. and MacKay, C. J. (1990). Electoretinographic responses of the short-wavelength-sensitive cones. *Investigative Ophthalmology & Visual Science*, 31(7):1203–1209.
- Govardovskii, V. I., Calvert, P. D., and Arshavsky, V. Y. (2000). Photoreceptor Light Adaptation. *The Journal of General Physiology*, 116(6):791–794.
- Granit, R. (1933). The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *The Journal of Physiology*, 77(3):207–239.

- Guo, S. S., Sivak, J. G., Callender, M. G., and Diehl-Jones, B. (1995). Retinal dopamine and lens-induced refractive errors in chicks. *Current Eye Research*, 14(5):385–389.
- Gupta, S. K., Chakraborty, R., and Verkicharla, P. K. (2022). Electroretinogram responses in myopia: a review. *Documenta Ophthalmologica. Advances in Ophthalmology*, 145(2):77–95.
- Hamblin, M. R. (2018). Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. *Photochemistry and photobiology*, 94(2):199–212.
- Hamilton, R., Bees, M. A., Chaplin, C. A., and McCulloch, D. L. (2007). The luminance-response function of the human photopic electroretinogram: a mathematical model. *Vision Research*, 47(23):2968–2972.
- Hatori, M. and Panda, S. (2010). The emerging roles of melanopsin in behavioral adaptation to light. *Trends in Molecular Medicine*, 16(10):435–446.
- He, M., Xiang, F., Zeng, Y., Mai, J., Chen, Q., Zhang, J., Smith, W., Rose, K., and Morgan, I. G. (2015). Effect of Time Spent Outdoors at School on the Development of Myopia Among Children in China: A Randomized Clinical Trial. *JAMA*, 314(11):1142–1148.
- Hidajat, R., Mclay, J., Burley, C., Elder, M., Morton, J., and Goode, D. (2003). Influence of axial length of normal eyes on PERG. *Documenta Ophthalmologica. Advances in Ophthalmology*, 107(2):195–200.
- Hobby, A. E., Kozareva, D., Yonova-Doing, E., Hossain, I. T., Katta, M., Huntjens, B., Hammond, C. J., Binns, A. M., and Mahroo, O. A. (2018). Effect of varying skin surface electrode position on electroretinogram responses recorded using a handheld stimulating and recording system. *Documenta Ophthalmologica*, 137(2):79–86.
- Hoffmann, M. B., Bach, M., Kondo, M., Li, S., Walker, S., Holopigian, K., Viswanathan, S., and Robson, A. G. (2021). ISCEV standard for clinical multifocal electroretinography (mfERG) (2021 update). *Documenta Ophthalmologica*, 142(1):5–16.
- Holden, B. A., Fricke, T. R., Wilson, D. A., Jong, M., Naidoo, K. S., Sankaridurg, P., Wong, T. Y., Naduvilath, T. J., and Resnikoff, S. (2016). Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. *Ophthalmology*, 123(5):1036–1042.
- Hood, D. and Finkelstein, M. (1986). *Sensitivity to light*. In: Boff, KR., Kaufman, L., Thomas, JP., editors. *Handbook of perception and human performance*. Vol 1: Sensory processes and perception. John Wiley and Sons.

- Hung, L.-F., Arumugam, B., She, Z., Ostrin, L., and Smith, E. L. (2018). Narrow-band, long-wavelength lighting promotes hyperopia and retards vision-induced myopia in infant rhesus monkeys. *Experimental Eye Research*, 176:147–160.
- Hysing, M., Pallesen, S., Stormark, K. M., Jakobsen, R., Lundervold, A. J., and Sivertsen, B. (2015). Sleep and use of electronic devices in adolescence: results from a large population-based study. *BMJ Open*, 5(1):e006748.
- Ikeda, H., Head, G. M., and Ellis, C. J. (1994). Electrophysiological signs of retinal dopamine deficiency in recently diagnosed Parkinson’s disease and a follow up study. *Vision Research*, 34(19):2629–2638.
- Ingram, R. M. and Barr, A. (1979). Changes in refraction between the ages of 1 and 3 1/2 years. *The British Journal of Ophthalmology*, 63(5):339–342.
- Jiang, Y., Zhu, Z., Tan, X., Kong, X., Zhong, H., Zhang, J., Xiong, R., Yuan, Y., Zeng, J., Morgan, I. G., and He, M. (2022). Effect of Repeated Low-Level Red-Light Therapy for Myopia Control in Children: A Multicenter Randomized Controlled Trial. *Ophthalmology*, 129(5):509–519.
- Jin, J.-X., Hua, W.-J., Jiang, X., Wu, X.-Y., Yang, J.-W., Gao, G.-P., Fang, Y., Pei, C.-L., Wang, S., Zhang, J.-Z., Tao, L.-M., and Tao, F.-B. (2015). Effect of outdoor activity on myopia onset and progression in school-aged children in northeast china: the sujiatun eye care study. *BMC Ophthalmology*, 15(1):73.
- Jones, L. A., Sinnott, L. T., Mutti, D. O., Mitchell, G. L., Moeschberger, M. L., and Zadnik, K. (2007). Parental history of myopia, sports and outdoor activities, and future myopia. *Investigative Ophthalmology & Visual Science*, 48(8):3524–3532.
- Kader, M. A. (2012). Electrophysiological study of myopia. *Saudi Journal of Ophthalmology*, 26(1):91–99.
- Kawabata, H., Fujimoto, N., and Adachi-Usami, E. (1997). [Sensitivity loss of short wavelength sensitive cones in myopic eyes by blue-on-yellow perimetry]. *Nippon Ganka Gakkai Zasshi*, 101(8):648–655.
- Kawabata, H., Murayama, K., Kakisu, Y., and Adachi-Usami, E. (1995). Blue cone spectral sensitivity in eyes with high myopia, as estimated by flash VECF. *Folia Ophthalmologica Japonica*, 46(5):535–539.
- Kim, M. H., Zhao, D., Kim, W., Lim, D.-H., Song, Y.-M., Guallar, E., Cho, J., Sung, J., Chung, E.-S., and Chung, T.-Y. (2013). Heritability of myopia and ocular biometrics

- in Koreans: the healthy twin study. *Investigative Ophthalmology & Visual Science*, 54(5):3644–3649.
- Koh, V., Tan, C., Nah, G., Zhao, P., Yang, A., Lin, S. T., Wong, T. Y., Saw, S. M., and Chia, A. (2014). Correlation of structural and electrophysiological changes in the retina of young high myopes. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 34(6):658–666.
- Koike, A. and Tokoro, T. (1986). Spectral sensitivities in high myopic eyes. *Journal of Japanese Ophthalmological Society*, 90(4):556–560.
- Kolb, H. (2003). How the retina works. *Sigma Xi The Scientific Society*.
- Kolb, H. (2020). *Gross Anatomy of the Eye*. Webvision.
- Kommanapalli, D., Murray, I. J., Kremers, J., Parry, N. R. A., and McKeefry, D. J. (2014). Temporal characteristics of L- and M-cone isolating steady-state electroretinograms. *Journal of the Optical Society of America. A, Optics, Image Science, and Vision*, 31(4):A113–120.
- Kouli, A., Torsney, K. M., and Kuan, W.-L. (2018). Parkinson’s Disease: Etiology, Neuropathology, and Pathogenesis. In Stoker, T. B. and Greenland, J. C., editors, *Parkinson’s Disease: Pathogenesis and Clinical Aspects*. Codon Publications, Brisbane (AU).
- Kriss, A. (1994). Skin ERGs: their effectiveness in paediatric visual assessment, confounding factors, and comparison with ERGs recorded using various types of corneal electrode. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 16(2-3):137–146.
- Lanca, C., Teo, A., Vivagandan, A., Htoon, H. M., Najjar, R. P., Spiegel, D. P., Pu, S.-H., and Saw, S.-M. (2019). The Effects of Different Outdoor Environments, Sunglasses and Hats on Light Levels: Implications for Myopia Prevention. *Translational Vision Science & Technology*, 8(4):7.
- Langheinrich, T., Tebartz van Elst, L., Lagrèze, W. A., Bach, M., Lücking, C. H., and Greenlee, M. W. (2000). Visual contrast response functions in Parkinson’s disease: evidence from electroretinograms, visually evoked potentials and psychophysics. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 111(1):66–74.
- Li, S. Z.-C., Yu, W.-Y., Choi, K.-Y., Lam, C. H.-I., Lakshmanan, Y., Wong, F. S.-Y., and Chan, H. H.-L. (2017). Subclinical Decrease in Central Inner Retinal Activity

- Is Associated With Myopia Development in Children. *Investigative Ophthalmology & Visual Science*, 58(10):4399–4406.
- Li, X.-X., Schaeffel, F., Kohler, K., and Zrenner, E. (1992). Dose-dependent effects of 6-hydroxy dopamine on deprivation myopia, electroretinograms, and dopaminergic amacrine cells in chickens. *Visual Neuroscience*, 9(5):483–492.
- Lim, M. C. C., Gazzard, G., Sim, E.-L., Tong, L., and Saw, S.-M. (2009). Direct costs of myopia in Singapore. *Eye (London, England)*, 23(5):1086–1089.
- Liu, R., Qian, Y.-F., He, J. C., Hu, M., Zhou, X.-T., Dai, J.-H., Qu, X.-M., and Chu, R.-Y. (2011). Effects of different monochromatic lights on refractive development and eye growth in guinea pigs. *Experimental Eye Research*, 92(6):447–453.
- Long, Q., Chen, D., and Chu, R. (2009). Illumination with monochromatic long-wavelength light promotes myopic shift and ocular elongation in newborn pigmented guinea pigs. *Cutaneous and Ocular Toxicology*, 28(4):176–180.
- Lou, L. and Ostrin, L. A. (2020). Effects of Narrowband Light on Choroidal Thickness and the Pupil. *Investigative Ophthalmology & Visual Science*, 61(10):40.
- Lucas, R. J., Hattar, S., Takao, M., Berson, D. M., Foster, R. G., and Yau, K.-W. (2003). Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science (New York, N.Y.)*, 299(5604):245–247.
- Malik, S. R. K., Gupta, A. K., Gupta, P. C., and Singh, G. (1969). E. R. G. in myopia. *Indian Journal of Ophthalmology*, 17(2):48.
- Martin-Martinelli, E., Savy, C., and Nguyen-Legros, J. (1994). Morphometry and distribution of displaced dopaminergic cells in rat retina. *Brain Research Bulletin*, 34(5):467–482.
- Mayer, D. L., Hansen, R. M., Moore, B. D., Kim, S., and Fulton, A. B. (2001). Cycloplegic refractions in healthy children aged 1 through 48 months. *Archives of Ophthalmology (Chicago, Ill.: 1960)*, 119(11):1625–1628.
- McCarthy, C. S., Megaw, P., Devadas, M., and Morgan, I. G. (2007). Dopaminergic agents affect the ability of brief periods of normal vision to prevent form-deprivation myopia. *Experimental Eye Research*, 84(1):100–107.
- Miller, R. F. (1973). Role of K⁺ in generation of b-wave of electroretinogram. *Journal of Neurophysiology*, 36(1):28–38.

- Miller, R. F. and Dowling, J. E. (1970). Intracellular responses of the Müller (glial) cells of mudpuppy retina: their relation to b-wave of the electroretinogram. *Journal of Neurophysiology*, 33(3):323–341.
- Mirshahi, A., Ponto, K. A., Hoehn, R., Zwiener, I., Zeller, T., Lackner, K., Beutel, M. E., and Pfeiffer, N. (2014). Myopia and level of education: results from the Gutenberg Health Study. *Ophthalmology*, 121(10):2047–2052.
- Morgan, I. G., Ashby, R. S., Nickla, D. L., and Guggenheim, J. A. (2013). Form deprivation and lens-induced myopia: are they different? *Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians (Optometrists)*, 33(3):355–361.
- Morgan, I. G., French, A. N., Ashby, R. S., Guo, X., Ding, X., He, M., and Rose, K. A. (2018). The epidemics of myopia: Aetiology and prevention. *Progress in Retinal and Eye Research*, 62:134–149.
- Morgan, I. G., Ohno-Matsui, K., and Saw, S.-M. (2012). Myopia. *The Lancet*, 379(9827):1739–1748.
- Morgan, I. G. and Rose, K. A. (2013). Myopia and international educational performance. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 33(3):329–338.
- Morgan, I. G., Wu, P.-C., Ostrin, L. A., Tideman, J. W. L., Yam, J. C., Lan, W., Baraas, R. C., He, X., Sankaridurg, P., Saw, S.-M., French, A. N., Rose, K. A., and Guggenheim, J. A. (2021a). IMI Risk Factors for Myopia. *Investigative Ophthalmology & Visual Science*, 62(5):3.
- Morgan, I. G., Wu, P.-C., Ostrin, L. A., Tideman, J. W. L., Yam, J. C., Lan, W., Baraas, R. C., He, X., Sankaridurg, P., Saw, S.-M., French, A. N., Rose, K. A., and Guggenheim, J. A. (2021b). IMI Risk Factors for Myopia. *Investigative Ophthalmology & Visual Science*, 62(5):3.
- Mortlock, K. E., Binns, A. M., Aldebasi, Y. H., and North, R. V. (2010). Inter-subject, inter-ocular and inter-session repeatability of the photopic negative response of the electroretinogram recorded using DTL and skin electrodes. *Documenta Ophthalmologica. Advances in Ophthalmology*, 121(2):123–134.
- Mountjoy, E., Davies, N. M., Plotnikov, D., Smith, G. D., Rodriguez, S., Williams, C. E., Guggenheim, J. A., and Atan, D. (2018). Education and myopia: assessing the direction of causality by mendelian randomisation. *BMJ (Clinical research ed.)*, 361:k2022.

- Mutti, D. O. and Marks, A. R. (2011). Blood Levels of Vitamin D in Teens and Young Adults with Myopia. *Optometry and Vision Science*, 88(3):377.
- Mutti, D. O., Mitchell, G. L., Moeschberger, M. L., Jones, L. A., and Zadnik, K. (2002). Parental myopia, near work, school achievement, and children’s refractive error. *Investigative Ophthalmology & Visual Science*, 43(12):3633–3640.
- Mutti, D. O., Mulvihill, S. P., Orr, D. J., Shorter, P. D., and Hartwick, A. T. E. (2020a). The Effect of Refractive Error on Melanopsin-Driven Pupillary Responses. *Investigative Ophthalmology & Visual Science*, 61(12):22.
- Mutti, D. O., Mulvihill, S. P., Orr, D. J., Shorter, P. D., and Hartwick, A. T. E. (2020b). The Effect of Refractive Error on Melanopsin-Driven Pupillary Responses. *Investigative Ophthalmology & Visual Science*, 61(12):22.
- Nickla, D. L. and Totonelly, K. (2011). Dopamine antagonists and brief vision distinguish lens-induced- and form-deprivation-induced myopia. *Experimental Eye Research*, 93(5):782–785.
- Norton, T. T. (1999). Animal Models of Myopia: Learning How Vision Controls the Size of the Eye. *ILAR journal*, 40(2):59–77.
- Oner, A., Gumus, K., Arda, H., Karakucuk, S., and Mirza, E. (2009). Pattern Electroretinographic Recordings in Eyes With Myopia. *Eye & Contact Lens*, 35(5):238.
- Osprey, L. L., Prusky, G., and Hattar, S. (2017). Mood, the Circadian System, and Melanopsin Retinal Ganglion Cells. *Annual review of neuroscience*, 40:539–556.
- Ostrin, L. A. (2018). The ipRGC-driven pupil response with light exposure and refractive error in children. *Ophthalmic and Physiological Optics*, 38(5):503–515. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/opo.12583>.
- Ostrin, L. A. and Schill, A. W. (2024). Red light instruments for myopia exceed safety limits. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 44(2):241–248.
- Oyster, C. W. (1999). *The Human Eye - Structure and Function*. Sinauer Associates, Inc.
- Palmowski-Wolfe, A. M., Perez, M. T., Behnke, S., Fuss, G., Martziniak, M., and Ruprecht, K. W. (2006). Influence of dopamine deficiency in early Parkinson’s disease on the slow stimulation multifocal-ERG. *Documenta Ophthalmologica. Advances in Ophthalmology*, 112(3):209–215.

- Pan, C.-W., Ramamurthy, D., and Saw, S.-M. (2012). Worldwide prevalence and risk factors for myopia. *Ophthalmic and Physiological Optics*, 32(1):3–16. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1475-1313.2011.00884.x>.
- Pan, C.-W., Wong, T.-Y., Lavanya, R., Wu, R.-Y., Zheng, Y.-F., Lin, X.-Y., Mitchell, P., Aung, T., and Saw, S.-M. (2011). Prevalence and risk factors for refractive errors in Indians: the Singapore Indian Eye Study (SINDI). *Investigative Ophthalmology & Visual Science*, 52(6):3166–3173.
- Peckham, C. S., Gardiner, P. A., and Goldstein, H. (1977). Acquired myopia in 11-year-old children. *British Medical Journal*, 1(6060):542–545.
- Pendrak, K., Nguyen, T., Lin, T., Capehart, C., Zhu, X., and Stone, R. A. (1997). Retinal dopamine in the recovery from experimental myopia. *Current Eye Research*, 16(2):152–157.
- Perlman, I. (2018). *The Electroretinogram: ERG*. Webvision.
- Perlman, I., Kondo, M., Chelva, E., Robson, A. G., and Holder, G. E. (2020). ISCEV extended protocol for the S-cone ERG. *Documenta Ophthalmologica. Advances in Ophthalmology*, 140(2):95–101.
- Perlman, I., Meyer, E., Haim, T., and Zonis, S. (1984). Retinal function in high refractive error assessed electroretinographically. *The British Journal of Ophthalmology*, 68(2):79–84.
- Pickard, G. E. and Sollars, P. J. (2012). Intrinsically photosensitive retinal ganglion cells. *Reviews of Physiology, Biochemistry and Pharmacology*, 162:59–90.
- Popova, E. and Kuppenova, P. (2013). Effects of dopamine receptor blockade on the intensity-response function of ERG b- and d-waves in dark adapted eyes. *Vision Research*, 88:22–29.
- Provencio, I., Jiang, G., De Grip, W. J., Hayes, W. P., and Rollag, M. D. (1998). Melanopsin: An opsin in melanophores, brain, and eye. *Proceedings of the National Academy of Sciences of the United States of America*, 95(1):340–345.
- Rai, M., Lakshmanan, Y., Choi, K. Y., and Chan, H. H.-L. (2025). Effects of flickering light stimulation on retinal blood flow and full-field electroretinogram in mice. *Documenta Ophthalmologica. Advances in Ophthalmology*.
- Read, S. A., Collins, M. J., and Vincent, S. J. (2015). Light Exposure and Eye Growth in Childhood. *Investigative Ophthalmology & Visual Science*, 56(11):6779–6787.

- Repka, M. X. (2015). Prevention of Myopia in Children. *JAMA*, 314(11):1137–1139.
- Resnikoff, S., Pascolini, D., Mariotti, S. P., and Pokharel, G. P. (2008). Global magnitude of visual impairment caused by uncorrected refractive errors in 2004. *Bulletin of the World Health Organization*, 86(1):63–70.
- Riggs, L. A. (1965). *Electrophysiology of Vision in Vision and Visual Perception*. John Wiley & Sons Inc.
- Robson, A. G., Frishman, L. J., Grigg, J., Hamilton, R., Jeffrey, B. G., Kondo, M., Li, S., and McCulloch, D. L. (2022). ISCEV Standard for full-field clinical electroretinography (2022 update). *Documenta Ophthalmologica*, 144(3):165–177.
- Rose, K. A., Morgan, I. G., Ip, J., Kifley, A., Huynh, S., Smith, W., and Mitchell, P. (2008). Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology*, 115(8):1279–1285.
- Rucker, F. J. (2013). The role of luminance and chromatic cues in emmetropisation. *Ophthalmic and Physiological Optics*, 33(3):196–214. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/opo.12050>.
- Rucker, F. J. and Wallman, J. (2009). Chick eyes compensate for chromatic simulations of hyperopic and myopic defocus: evidence that the eye uses longitudinal chromatic aberration to guide eye-growth. *Vision Research*, 49(14):1775–1783.
- Rymer, J. and Wildsoet, C. F. (2005). The role of the retinal pigment epithelium in eye growth regulation and myopia: a review. *Visual Neuroscience*, 22(3):251–261.
- Sankaran, M., Keeley, P. W., He, L., Iuvone, P. M., and Reese, B. E. (2018). Dopaminergic amacrine cell number, plexus density, and dopamine content in the mouse retina: Strain differences and effects of Bax gene disruption. *Experimental Eye Research*, 177:208–212.
- Saw, S.-M., Cheng, A., Fong, A., Gazzard, G., Tan, D. T. H., and Morgan, I. (2007). School grades and myopia. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 27(2):126–129.
- Saw, S.-M., Gazzard, G., Shih-Yen, E. C., and Chua, W.-H. (2005). Myopia and associated pathological complications. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 25(5):381–391.
- Schaeffel, F., Glasser, A., and Howland, H. C. (1988). Accommodation, refractive error and eye growth in chickens. *Vision Research*, 28(5):639–657.

- Schilling, T., Soltanlou, M., Nuerk, H.-C., and Bahmani, H. (2023). Blue-light stimulation of the blind-spot constricts the pupil and enhances contrast sensitivity. *PLOS ONE*, 18(5):e0286503. Publisher: Public Library of Science.
- Schippert, R., Schaeffel, F., and Feldkaemper, M. P. (2008). Microarray analysis of retinal gene expression in chicks during imposed myopic defocus. *Molecular Vision*, 14:1589–1599.
- Schmid, K. L., Leyden, K., Chiu, Y.-h., Lind, S.-R., Vos, D. J., Kimlin, M., and Wood, J. M. (2013). Assessment of daily light and ultraviolet exposure in young adults. *Optometry and Vision Science: Official Publication of the American Academy of Optometry*, 90(2):148–155.
- Schmid, K. L. and Wildsoet, C. F. (2004). Inhibitory Effects of Apomorphine and Atropine and Their Combination on Myopia in Chicks. *Optometry and Vision Science*, 81(2):137.
- Schmidt, B. P., Sabesan, R., Tuten, W. S., Neitz, J., and Roorda, A. (2018). Sensations from a single M-cone depend on the activity of surrounding S-cones. *Scientific Reports*, 8(1):8561. Publisher: Nature Publishing Group.
- Sieving, P. A., Murayama, K., and Naarendorp, F. (1994). Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. *Visual Neuroscience*, 11(3):519–532.
- Smith, E. L., Hung, L.-F., Arumugam, B., Holden, B. A., Neitz, M., and Neitz, J. (2015). Effects of Long-Wavelength Lighting on Refractive Development in Infant Rhesus Monkeys. *Investigative Ophthalmology & Visual Science*, 56(11):6490–6500.
- Smith, E. L., Kee, C.-S., Ramamirtham, R., Qiao-Grider, Y., and Hung, L.-F. (2005). Peripheral vision can influence eye growth and refractive development in infant monkeys. *Investigative Ophthalmology & Visual Science*, 46(11):3965–3972.
- Smith, M. J. and Walline, J. J. (2015). Controlling myopia progression in children and adolescents. *Adolescent Health, Medicine and Therapeutics*, 6:133–140. Publisher: Dove Medical Press _eprint: <https://www.tandfonline.com/doi/pdf/10.2147/AHMT.S55834>.
- Smith, T. S. T., Frick, K. D., Holden, B. A., Fricke, T. R., and Naidoo, K. S. (2009). Potential lost productivity resulting from the global burden of uncorrected refractive error. *Bulletin of the World Health Organization*, 87(6):431–437.
- Sodhi, P. and Hartwick, A. T. E. (2014). Adenosine modulates light responses of rat retinal ganglion cell photoreceptors through a cAMP-mediated pathway. *The Journal of Physiology*, 592(19):4201–4220.

- Spitschan, M. (2019). Melanopsin contributions to non-visual and visual function. *Current Opinion in Behavioral Sciences*, 30:67–72.
- Stapley, V., Anderson, R. S., Saunders, K., and Mulholland, P. J. (2025). Measuring differences in the ERG in myopia using the RETeval device with skin electrodes. *Ophthalmic and Physiological Optics*, 45(3):666–680. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/opo.13460>.
- Stone, R. A., Lin, T., Laties, A. M., and Iuvone, P. M. (1989). Retinal dopamine and form-deprivation myopia. *Proceedings of the National Academy of Sciences of the United States of America*, 86(2):704–706.
- Stone, R. A., Pardue, M. T., Iuvone, P. M., and Khurana, T. S. (2013). Pharmacology of myopia and potential role for intrinsic retinal circadian rhythms. *Experimental Eye Research*, 114:35–47.
- Swiatczak, B. and Schaeffel, F. (2022). Myopia: why the retina stops inhibiting eye growth. *Scientific Reports*, 12(1):21704. Publisher: Nature Publishing Group.
- Tang, J., Hui, F., Hadoux, X., Sarossy, M., van Wijngaarden, P., Coote, M., and Crowston, J. G. (2018). A Comparison of the RETeval Sensor Strip and DTL Electrode for Recording the Photopic Negative Response. *Translational Vision Science & Technology*, 7(6):27.
- Tavera-Mendoza, L., Wang, T., Lallemand, B., Zhang, R., Nagai, Y., Bourdeau, V., Ramirez-Calderon, M., Desbarats, J., Mader, S., and White, J. H. (2006). Convergence of vitamin D and retinoic acid signalling at a common hormone response element. *EMBO reports*, 7(2):180–185. Num Pages: 185 Publisher: John Wiley & Sons, Ltd.
- Taylor, C. P., Shepard, T. G., Rucker, F. J., and Eskew, R. T. (2018). Sensitivity to S-Cone Stimuli and the Development of Myopia. *Investigative Ophthalmology & Visual Science*, 59(11):4622–4630.
- Thakur, S., Dhakal, R., and Verkicharla, P. K. (2021). Short-Term Exposure to Blue Light Shows an Inhibitory Effect on Axial Elongation in Human Eyes Independent of Defocus. *Investigative Ophthalmology & Visual Science*, 62(15):22.
- Thompson, D. A., Bach, M., McAnany, J. J., Šuštar Habjan, M., Viswanathan, S., and Robson, A. G. (2024). ISCEV standard for clinical pattern electroretinography (2024 update). *Documenta Ophthalmologica*, 148(2):75–85.

- Tian, N., Xu, H.-p., and Wang, P. (2015). Dopamine D2 receptors preferentially regulate the development of light responses of the inner retina. *European Journal of Neuroscience*, 41(1):17–30. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/ejn.12783>.
- Timucin, O. B., Arabaci, M., Cuce, F., Karatas, B., Onalan, S., Yasar, M., Yildirim, S., and Karadag, M. F. (2016). The effects of light sources with different spectral structures on ocular axial length in rainbow trout (*Oncorhynchus mykiss*). *Experimental Eye Research*, 151:212–221.
- Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., and Fougrou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, 15(3):434–443.
- Troilo, D., Gottlieb, M. D., and Wallman, J. (1987). Visual deprivation causes myopia in chicks with optic nerve section. *Current Eye Research*, 6(8):993–999.
- Ueno, S., Kondo, M., Niwa, Y., Terasaki, H., and Miyake, Y. (2004). Luminance dependence of neural components that underlies the primate photopic electroretinogram. *Investigative Ophthalmology and Visual Science*, 45(3):1033–1040.
- Vanderah, T. W. and Gould, D. J. (2020). *Nolte’s The Human Brain*. Elsevier, Inc.
- Vera-Diaz, F. A. (2010). Myopia. In Dartt, D. A., editor, *Encyclopedia of the Eye*, pages 98–105. Academic Press, Oxford.
- Verhoeven, V. J. M., Hysi, P. G., Wojciechowski, R., Fan, Q., Guggenheim, J. A., Höhn, R., MacGregor, S., Hewitt, A. W., Nag, A., Cheng, C.-Y., Yonova-Doing, E., Zhou, X., and Ikram, M. K. e. a. (2013). Genome-wide meta-analyses of multi-ancestry cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nature Genetics*, 45(3):314–318.
- Vitale, S., Sperduto, R. D., and Ferris, F. L. (2009). Increased prevalence of myopia in the United States between 1971–1972 and 1999–2004. *Archives of Ophthalmology (Chicago, Ill.: 1960)*, 127(12):1632–1639.
- Wali, N. and Leguire, L. E. (1992). The photopic hill: a new phenomenon of the light adapted electroretinogram. *Documenta Ophthalmologica. Advances in Ophthalmology*, 80(4):335–345.
- Wallman, J. (1993). Chapter 6 Retinal control of eye growth and refraction. *Progress in Retinal Research*, 12:133–153.

- Wang, J., Li, Y., Musch, D. C., Wei, N., Qi, X., Ding, G., Li, X., Li, J., Song, L., Zhang, Y., Ning, Y., Zeng, X., Hua, N., Li, S., and Qian, X. (2021). Progression of Myopia in School-Aged Children After COVID-19 Home Confinement. *JAMA ophthalmology*, 139(3):293–300.
- Ward, A. H., Norton, T. T., Huisinigh, C. E., and Gawne, T. J. (2018). The hyperopic effect of narrow-band long-wavelength light in tree shrews increases non-linearly with duration. *Vision Research*, 146-147:9–17.
- Westall, C. A., Dhaliwal, H. S., Panton, C. M., Sigesmund, D., Levin, A. V., Nischal, K. K., and Héon, E. (2001). Values of electroretinogram responses according to axial length. *Documenta Ophthalmologica*, 102(2):115–130.
- Williams, K. and Hammond, C. (2019). High myopia and its risks. *Community Eye Health*, 32(105):5–6.
- Wong, K. Y., Dunn, F. A., and Berson, D. M. (2005). Photoreceptor Adaptation in Intrinsically Photosensitive Retinal Ganglion Cells. *Neuron*, 48(6):1001–1010. Publisher: Elsevier.
- Wong, T. Y., Foster, P. J., Hee, J., Ng, T. P., Tielsch, J. M., Chew, S. J., Johnson, G. J., and Seah, S. K. (2000). Prevalence and risk factors for refractive errors in adult Chinese in Singapore. *Investigative Ophthalmology & Visual Science*, 41(9):2486–2494.
- Wu, P.-C., Chen, C.-T., Lin, K.-K., Sun, C.-C., Kuo, C.-N., Huang, H.-M., Poon, Y.-C., Yang, M.-L., Chen, C.-Y., Huang, J.-C., Wu, P.-C., Yang, I.-H., Yu, H.-J., Fang, P.-C., Tsai, C.-L., Chiou, S.-T., and Yang, Y.-H. (2018). Myopia Prevention and Outdoor Light Intensity in a School-Based Cluster Randomized Trial. *Ophthalmology*, 125(8):1239–1250.
- Xiong, F., Mao, T., Liao, H., Hu, X., Shang, L., Yu, L., Lin, N., Huang, L., Yi, Y., Zhou, R., Zhou, X., and Yi, J. (2021). Orthokeratology and Low-Intensity Laser Therapy for Slowing the Progression of Myopia in Children. *BioMed Research International*, 2021(1):8915867. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1155/2021/8915867>.
- Xiong, R., Zhu, Z., Jiang, Y., Kong, X., Zhang, J., Wang, W., Kiburg, K., Yuan, Y., Chen, Y., Zhang, S., Xuan, M., Zeng, J., Morgan, I. G., and He, M. (2022). Sustained and rebound effect of repeated low-level red-light therapy on myopia control: A 2-year post-trial follow-up study. *Clinical & Experimental Ophthalmology*, 50(9):1013–1024.

- YAM, J. C., Tang, S., Lau, T., Rong, S. S., and Chen, L. J. (2017). Vitamin D and Myopia: A Systematic Review and Meta-analysis. *Investigative Ophthalmology & Visual Science*, 58(8):2379.
- Yamamoto, S., Nitta, K., and Kamiyama, M. (1997). Cone electroretinogram to chromatic stimuli in myopic eyes. *Vision Research*, 37(15):2157–2159.
- Yang, M., Luensmann, D., Fonn, D., Woods, J., Jones, D., Gordon, K., and Jones, L. (2018). Myopia prevalence in Canadian school children: a pilot study. *Eye (London, England)*, 32(6):1042–1047.
- Young, F. A., Leary, G. A., Baldwin, W. R., West, D. C., Box, R. A., Harris, E., and Johnson, C. (1969). THE TRANSMISSION OF REFRACTIVE ERRORS WITHIN ESKIMO FAMILIES*. *Optometry and Vision Science*, 46(9):676.
- Zhang, D.-Q., Wong, K. Y., Sollars, P. J., Berson, D. M., Pickard, G. E., and McMahon, D. G. (2008a). Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proceedings of the National Academy of Sciences*, 105(37):14181–14186. Publisher: Proceedings of the National Academy of Sciences.
- Zhang, D.-Q., Wong, K. Y., Sollars, P. J., Berson, D. M., Pickard, G. E., and McMahon, D. G. (2008b). Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proceedings of the National Academy of Sciences*, 105(37):14181–14186. Publisher: Proceedings of the National Academy of Sciences.
- Zhang, P. and Zhu, H. (2022). Light Signaling and Myopia Development: A Review. *Ophthalmology and Therapy*, 11(3):939–957.
- Zhou, L., Xing, C., Qiang, W., Hua, C., and Tong, L. (2022). Low-intensity, long-wavelength red light slows the progression of myopia in children: an Eastern China-based cohort. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, 42(2):335–344.
- Zhou, X., Pardue, M. T., Iuvone, P. M., and Qu, J. (2017a). Dopamine Signaling and Myopia Development: What Are the Key Challenges. *Progress in retinal and eye research*, 61:60–71.
- Zhou, X., Pardue, M. T., Iuvone, P. M., and Qu, J. (2017b). Dopamine Signaling and Myopia Development: What Are the Key Challenges. *Progress in retinal and eye research*, 61:60–71.

- Zhu, X., Park, T. W., Winawer, J., and Wallman, J. (2005). In a matter of minutes, the eye can know which way to grow. *Investigative Ophthalmology & Visual Science*, 46(7):2238–2241.
- Zuclich, J. A. (1989). Ultraviolet-induced photochemical damage in ocular tissues. *Health Physics*, 56(5):671–682.

Appendices

Appendix A

Outlier Identification & Calculations

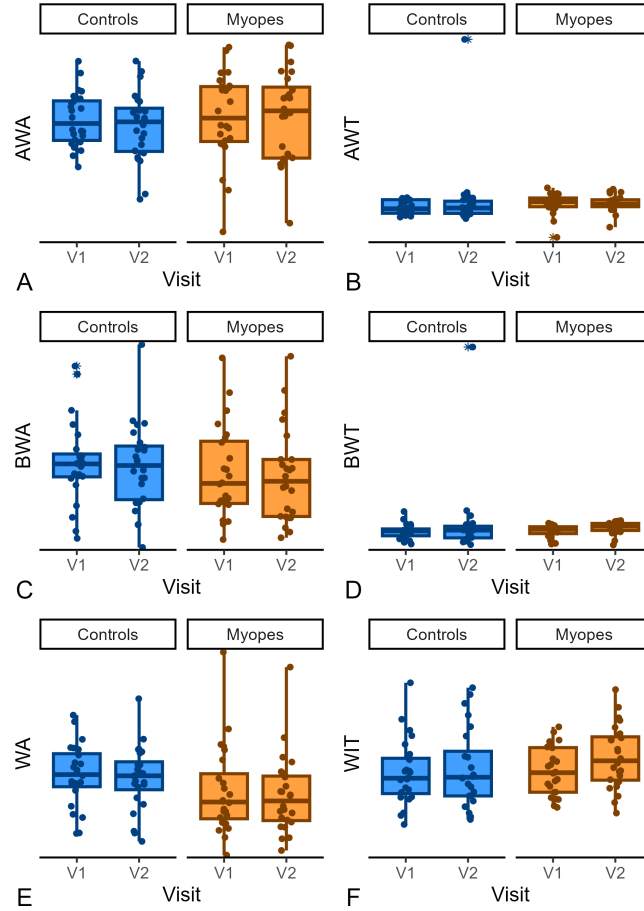


Figure A.1: Box plots of the pre-adaptation study 1 data for controls (blue) and myopes (orange) at visit 1 (V1) and visit 2 (V2) for each component a-wave amplitude (AWA), a-wave implicit time (AWT), b-wave amplitude (BWA), b-wave implicit time (BWT), flicker waveform amplitude (WA) and flicker waveform implicit time (WIT) in panels A-F, respectively. These graphs help to explain why one participant was removed as an outlier and no other outliers were removed. As is clear from panel B and D, the outlier in the control group (as indicated by the blue dot with asterisk at the top of the graph) was removed since their data was 3x what we accepted as the outlier threshold limit. When compared to the outliers in panel C, it is clear that they are not 3x more than the defined limit and thus were not removed from the final analysis.

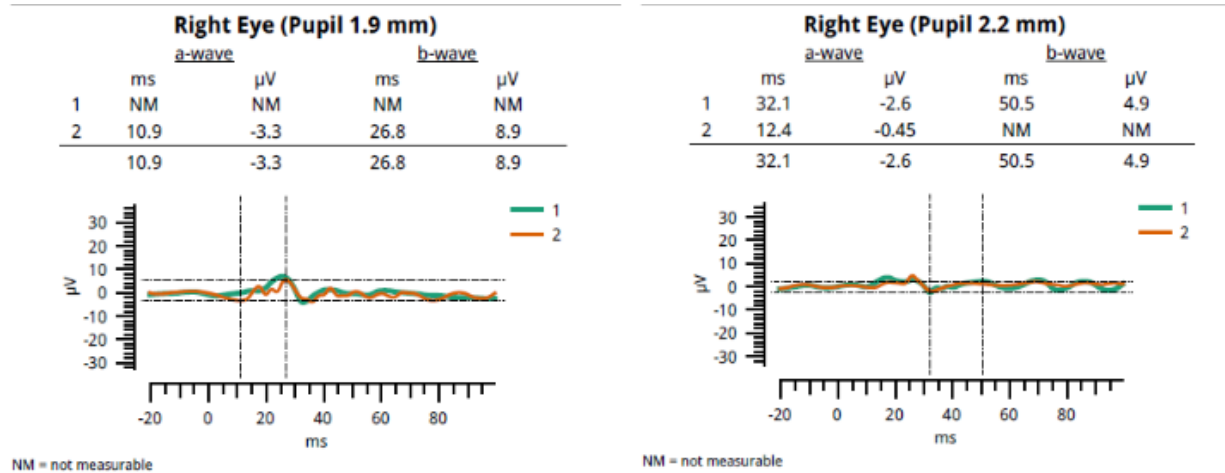


Figure A.2: Sample data showing the pre-adaptation RETeval® measurements (Study 1) for the outlier participant during their first visit (left) and second visit (right). The two lines in green and then orange reflect the replicate measurements. The NM stands for not measurable and it is clear that this individuals ERG waveform is very diminished most likely attributed to the participants inability to restrain from blinking and their discomfort due to bright lights of the flash ERG test. In the second visit, the default peak time and amplitude markers have been placed at incorrect locations, hence the large increase in data. It could also be attributed to electrode placement or physiological interference due to skin electrodes.

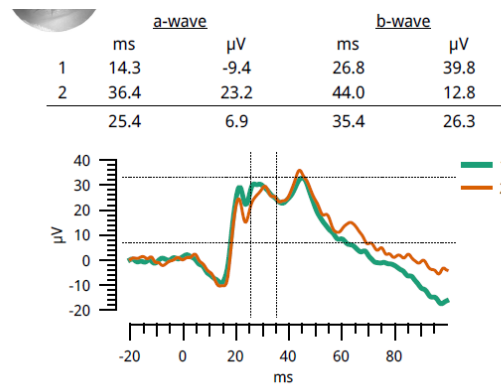


Figure A.3: Sample baseline RETeval® S-cone ERG (Study 4) for one of the myopic participants. The two lines in green and then orange reflect the replicate measurements. It is clear that this individuals ERG waveform is relatively consistent between replicates and was clearly measurable but the autotomized average selection of the RETeval® has selected the incorrect portion of the waveform.

Appendix B

Non-Parametric Tests for All Studies

Repeated Measures ANOVA - visits AWT

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_G	η^2	η^2_P
WVL	0.02425	1	0.02425	0.0415	0.840	0.000	0.000	0.001
WVL * RE	0.00767	1	0.00767	0.0131	0.909	0.000	0.000	0.000
Residual	26.31809	45	0.58485					

Note. Type 3 Sums of Squares
[3]

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_G	η^2	η^2_P
RE	5.51	1	5.51	3.71	0.061	0.056	0.056	0.076
Residual	66.94	45	1.49					

Note. Type 3 Sums of Squares

Assumptions

Homogeneity of Variances Test (Levene's)

	F	df1	df2	p
fst_AWT_bre_B	1.70	1	45	0.199
fst_AWT_bre_R	1.35	1	45	0.251

Figure B.1: Sample output from Jamovi for pre-adaptation a-wave implicit times (Study 1). There were no significant differences between controls and myopes ($p=0.061$) and no differences between blue and red light adaptation ($p=0.840$).

Table B.1: AWA Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.06	1	45	0.8011
Colour	Colour	0.17	1	45	0.6777
RE:Colour	RE:Colour	0.01	1	45	0.9158

Table B.2: AWT Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.00	1	45	0.9678
Colour	Colour	1.37	1	45	0.2482
RE:Colour	RE:Colour	1.83	1	45	0.1832

Table B.3: BWA Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	1.24	1	45	0.2722
Colour	Colour	0.00	1	45	0.9835
RE:Colour	RE:Colour	0.04	1	45	0.8501

Table B.4: BWT Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	1.00	1	45	0.3227
Colour	Colour	12.91	1	45	0.0008
RE:Colour	RE:Colour	1.25	1	45	0.2695

Table B.5: WA Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.03	1	45	0.8645
Colour	Colour	0.02	1	45	0.8819
RE:Colour	RE:Colour	2.72	1	45	0.1059

Table B.6: WIT Non Parametric ANOVA Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.01	1	45	0.9358
Colour	Colour	59.63	1	45	0.0000
RE:Colour	RE:Colour	0.18	1	45	0.6696

Table B.7: AWA Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.17	1	29	0.6793
Colour	Colour	3.59	1	87	0.0613
Brightness	Brightness	2.24	1	87	0.1385
RE:Colour	RE:Colour	1.16	1	87	0.2847
RE:Brightness	RE:Brightness	0.16	1	87	0.6908
Colour:Brightness	Colour:Brightness	1.61	1	87	0.2082
RE:Colour:Brightness	RE:Colour:Brightness	0.49	1	87	0.4844

Table B.8: AWT Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	2.88	1	29	0.1003
Colour	Colour	0.44	1	87	0.5073
Brightness	Brightness	0.61	1	87	0.4385
RE:Colour	RE:Colour	0.20	1	87	0.6556
RE:Brightness	RE:Brightness	0.03	1	87	0.8591
Colour:Brightness	Colour:Brightness	0.38	1	87	0.5383
RE:Colour:Brightness	RE:Colour:Brightness	0.52	1	87	0.4741

Table B.9: BWA Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.94	1	29	0.3398
Colour	Colour	0.68	1	87	0.4135
Brightness	Brightness	13.05	1	87	0.0005
RE:Colour	RE:Colour	1.76	1	87	0.1886
RE:Brightness	RE:Brightness	0.81	1	87	0.3697
Colour:Brightness	Colour:Brightness	1.03	1	87	0.3137
RE:Colour:Brightness	RE:Colour:Brightness	0.69	1	87	0.4087

Table B.10: BWT Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.02	1	29	0.8845
Colour	Colour	48.19	1	87	0.0000
Brightness	Brightness	27.57	1	87	0.0000
RE:Colour	RE:Colour	5.12	1	87	0.0261
RE:Brightness	RE:Brightness	0.00	1	87	0.9662
Colour:Brightness	Colour:Brightness	0.20	1	87	0.6565
RE:Colour:Brightness	RE:Colour:Brightness	0.37	1	87	0.5434

Table B.11: WA Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.00	1	29	0.9652
Colour	Colour	1.14	1	87	0.2882
Brightness	Brightness	8.53	1	87	0.0044
RE:Colour	RE:Colour	0.05	1	87	0.8263
RE:Brightness	RE:Brightness	1.99	1	87	0.1617
Colour:Brightness	Colour:Brightness	0.04	1	87	0.8444
RE:Colour:Brightness	RE:Colour:Brightness	0.06	1	87	0.7994

Table B.12: WIT Non-Parametric Output

	Term	F	Df	Df.res	Pr(>F)
RE	RE	1.12	1	29	0.2995
Colour	Colour	45.28	1	87	0.0000
Brightness	Brightness	25.95	1	87	0.0000
RE:Colour	RE:Colour	0.58	1	87	0.4483
RE:Brightness	RE:Brightness	0.36	1	87	0.5485
Colour:Brightness	Colour:Brightness	0.19	1	87	0.6675
RE:Colour:Brightness	RE:Colour:Brightness	2.43	1	87	0.1225

Table B.13: mfERG non-parametric ANOVA output Ring 1

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.14	1	17	0.7159
WL	WL	0.38	1	17	0.5483
RE:WL	RE:WL	2.64	1	17	0.1226

Table B.14: mfERG non-parametric ANOVA output Ring 2

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.12	1	17	0.7333
WL	WL	0.03	1	17	0.8579
RE:WL	RE:WL	0.87	1	17	0.3639

Table B.15: mfERG non-parametric ANOVA output Ring 3

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.37	1	17	0.5519
WL	WL	0.42	1	17	0.5241
RE:WL	RE:WL	2.53	1	17	0.1300

Table B.16: mfERG non-parametric ANOVA output Ring 4

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.06	1	17	0.8134
WL	WL	0.88	1	17	0.3620
RE:WL	RE:WL	0.00	1	17	0.9914

Table B.17: mfERG non-parametric ANOVA output Ring 5

	Term	F	Df	Df.res	Pr(>F)
RE	RE	0.49	1	17	0.4940
WL	WL	3.29	1	17	0.0875
RE:WL	RE:WL	3.66	1	17	0.0726

Appendix C

Refractive Error & Axial Length Graphs for All Studies

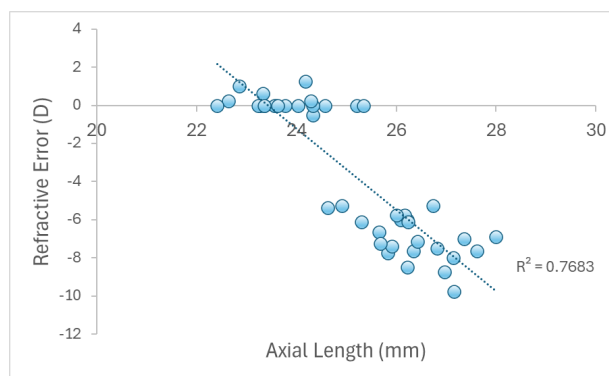


Figure C.1: Study 1: Scatter-plot showing the correlation between refractive error and axial length. There is a negative correlation ($R^2 = 0.7683$) such that those with higher refractive errors had longer eyes

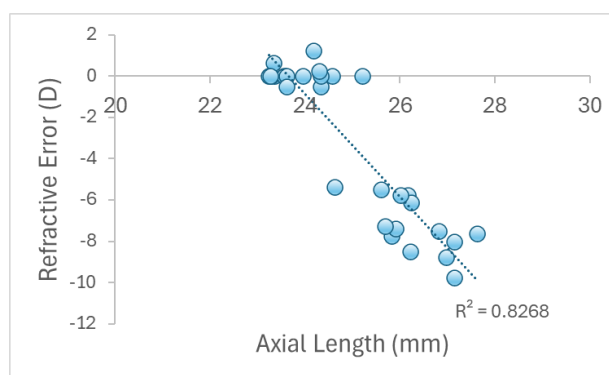


Figure C.2: Study 2: Scatter-plot showing the correlation between refractive error and axial length. There is a negative correlation ($R^2 = 0.8268$) such that those with higher refractive errors had longer eyes

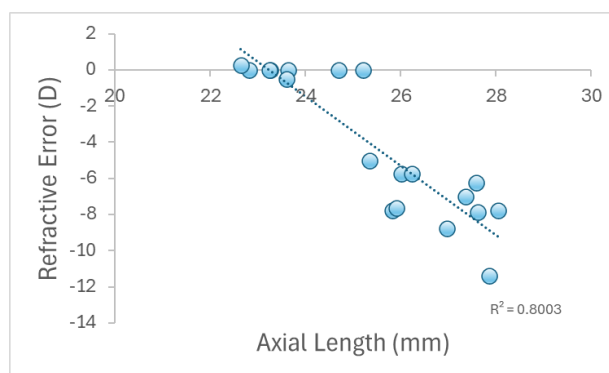


Figure C.3: Study 3: Scatter-plot showing the correlation between refractive error and axial length. There is a negative correlation ($R^2 = 0.8003$) such that those with higher refractive errors had longer eyes

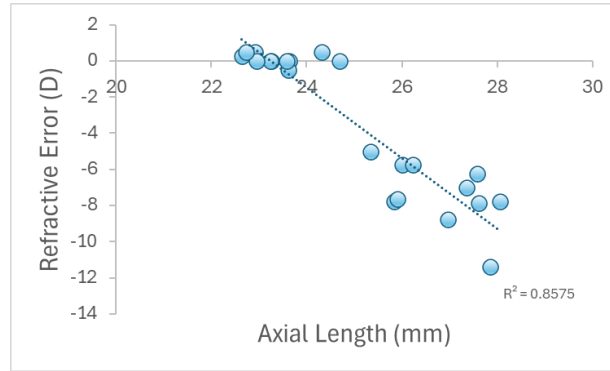


Figure C.4: Study 4: Scatter-plot showing the correlation between refractive error and axial length. There is a negative correlation ($R^2 = 0.8575$) such that those with higher refractive errors had longer eyes

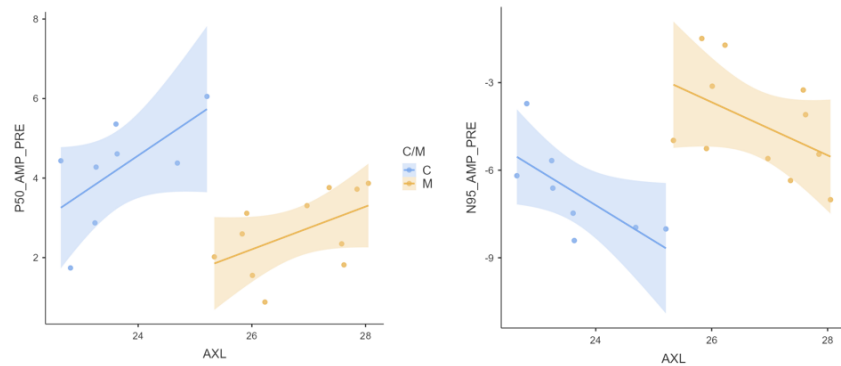


Figure C.5: Study 3: PERG pre-adaptation results plotted against axial length. This graph further supports the finding that high myopes have smaller ERGs.