AXIAL LENGTH IS ASSOCIATED WITH INDIVIDUAL DIFFERENCES IN ON- AND OFF- PATTERN DETECTION

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

William Hogue

May, 2022

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This manuscript has been read and accepted by the
Thesis Committee in satisfaction of the
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Abstract:

AXIAL LENGTH IS ASSOCIATED WITH INDIVIDUAL DIFFERENCES IN ON- AND OFF-PATTERN DETECTION

William Hogue
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Purpose:

This study aims to examine the post receptoral processing alterations of the ON/OFF visual pathways in axial myopia. Additionally, we sought to replicate evidence of choroidal thickening and thinning in response to ON/OFF pathway stimulation respectively. Finally, we examine the possibility diurnal choroidal thickness fluctuations can be altered by stimulation of the ON/OFF pathway.

Methods:

We measured the association between axial length and individual differences in psychophysical performance for positive (ON pathway stimuli) and negative (OFF pathway stimuli) contrast for 39 subjects (mean age 25.0 ± 3.73) with a wide range of axial lengths (20.9 to 27.9 mm). Subjects' contrast thresholds were measured with a 10 alternative forced choice letter identification task, and a two alternative forced choice difference of Gaussian (DoG) detection task. In the same subject pool, we measured changes in choroidal thickness after the completion of the positive and negative psychophysical sessions.

In a group of 9 subjects (mean age 24.2 ± 1.39), we measured choroidal thickness changes to positive and negative contrast in the morning and evening after completion of psychophysical testing in order to determine the effect on diurnal choroidal thickness fluctuations. Only the 10 alternative forced choice letter identification task was used for this experiment.

Stimuli were presented on a Vpixx 12-bit monitor. A Topcon KR-1W wavefront analyzer determined the patient's refractive error, and a Haag-Streit Lenstar 900 measured axial length and choroidal thickness. A mixture of Gaussian model applied to A-scans measured choroidal thickness.

Results:

There was a positive correlation between contrast threshold and axial length for positive ($t = 2.0$, p-value = 0.049, $r = 0.32$) and negative ($t = 2.9$, p-value = 0.006, $r = 0.44$) contrast DoG targets and positive ($t = 2.8$, p-value = 0.008, $r = 0.42$) and negative ($t = 2.5$, p-value = 0.02, $r = 0.38$) letter targets. There was a steeper correlation for the negative compared to positive contrast DoG targets ($t = 2.04$, p-value = 0.01, $r = 0.38$), but there was no detectable difference in the rate of increase in positive/negative contrast targets for the letter targets ($t = 0.03$, p-value = 0.98, $r = 0.005$).

Choroidal thickening (mean 5.4 ± 19 μm) was observed in response to positive contrast stimuli ($t = 1.8$, p-value = 0.04), but there was no detectable thinning effect in response to negative contrast stimuli ($t = -0.25$, p-value = 0.40). An effect on diurnal choroidal thickness fluctuations was not observed ($F = 2.3$ p-value = 0.13). Contrast thresholds did not differ between time of day or contrast polarity ($F = 2.3$, p-value = 0.13).

Conclusion:

Although the effect observed in asymmetries between ON and OFF thresholds for DoG targets was small, there seem to be underlying deficits in the OFF pathway more than the ON pathway of axial myopes. We hypothesize reduced sensitivity or increased internal noise within the OFF pathway leads to a dysregulation of the homeostatic set point and leads to stop signals for eye growth to be ignored. Choroidal thickness changes were inconclusive.

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1. General Introduction

1.1. Myopia epidemic

The prevalence of myopia is increasing worldwide, creating a burden on the healthcare system (Dolgin, 2015; Foster and Jiang, 2014; Hrynchak et. al, 2013). Over a 60 year period, the prevalence of myopia in China increased from 10 to 90 percent in children and young adults (Dolgin, 2015). The prevalence in the United States and Europe doubled over the last 50 years, with half of the young adults now being myopic (Dolgin, 2015). The detriments of myopia extend beyond the inconvenience of optical correction. An increase in myopia is linearly associated with an increased incidence of sight threatening conditions such as retinal detachments, glaucoma, early development of cataracts, and myopic maculopathy (Dolgin, 2015; Saw et al. 2005). Sight threatening conditions associated with myopia are predominantly due to long axial length (Jonas and Xu, 2014).

Individuals with an axial length greater than approximately 24.5 mm represent a failure of emmetropization (González Blanco et al. 2008; Meng et al. 2011; Wallman and Winawer, 2004). Humans are typically born hyperopic with a homeostatic set point of emmetropia (Varughese et al. 2005). Emmetropization requires visual input for guidance (Hung et al. 1995; McFadden 2004; Schaeffel et al. 1988; Varughese et al. 2005, Wallman et al. 1978). In monkeys, guinea pigs, and chickens, hyperopic defocus leads to compensatory axial elongation, while myopic defocus leads to slower axial growth (Howlett and McFadden, 2006; Hung et al. 1995; McFadden 2004, Schaeffel et al. 1988). The effect of lens blur on humans is less robust with mixed results showing minimal to no effect, however long term studies of lens induced axial changes are impossible to ethically recreate in humans (Chung et al. 2002; Gwiazda et al. 2003; Schaeffel and Feldkaemper, 2015). Read et al. (2010) did find axial length changed in humans in

response to defocus after only 30 minutes of exposed defocus, but the effect was small (mean 8 ± 14 μm).

Methods to control axial myopia progression include orthokeratology, soft multifocal contact lenses, and antimuscarinic agents (Walline, 2016). The long-term efficacy of myopia control treatments is questionable as the effect weakens after a year of treatment, and the result is limited to an approximate 0.5 mm reduction in axial length (Brennan et al. 2021). Time spent outdoors provides a protective effect against the development of myopia, but the mechanism is unknown (Dirani et al. 2009; Rose et al. 2008; Xiong et al. 2017). Given the shortcomings of myopia control, work addressing the underlying failures in emmetropization of human myopic eyes is necessary. Two areas that may provide clues as to what causes emmetropization to fail are differences in visual processing as myopia increases and the alterations in the anatomical makeup of myopic eyes beyond longer axial length.

1.2 Decreased visual performance and electrophysiological responses in myopes

1.2.1 Visual performance

Research examining the visual performance of myopes is mixed. High myopia is associated with increased contrast thresholds at high spatial frequencies and decreased spatial summation sensitivities (Ang, 2020; Collins and Carney, 1990; Jaworski et al. 2006; Liou and Chiu, 2001; Risse et al. 1996). Liou and Chiu (2001) found contrast thresholds were increased at high spatial frequencies for high (defined as -6.25 to -12 D) and severe (defined as less than -12 D) myopes but not for low to medium myopes (defined as -1D to -6D). Risse et al. (1996) found high myopes (defined as less than -8D) had increased contrast threshold at high spatial frequency but not low and medium spatial frequencies. Deficits in high (defined as -6.25 to -12 D) but not

severe (defined as less than -12 D) myopes were negated if contact lenses were used instead of spectacle correction (Liou et al. 2001).

Stoimenova (2007) measured contrast thresholds to positive and negative contrast in 60 myopes and 20 emmetropes and found the more myopic the subject was the higher their contrast threshold was to positive (lighter text on a darker background) and negative (darker text on a lighter background) contrast. For myopes, the negative contrast threshold was more reduced than the positive contrast threshold when compared to their emmetropic counterparts (Stoimenova, 2007). The asymmetry was more pronounced in the subjects with higher levels of myopia (Stoimenova, 2007). Radhakrishnan et al. (2004) found myopes showed greater contrast threshold increase when positive blur was introduced compared to negative blur, while emmetropes showed equal loss between the two.

Some deficits may relate to higher order aberrations in spectacle lenses (Collins and Carney 1990; Liou et al. 2001). Spectacle lenses induce aberrations such as oblique astigmatism, curvature of field, and chromatic aberrations (Collins and Carney, 1990; Liou et al. 2001). Higher power lenses used to correct higher refractive errors possess more aberrations, which could result in higher contrast thresholds the higher power the lens (Collins and Carney, 1990; Liou et al. 2001). Spectacle aberrations do not explain asymmetries in sensitivities to contrasts of opposite polarities, asymmetries in effect of positive or negative blur on contrast thresholds, or increased contrast thresholds in healthy severe myopes wearing contact lenses (Radhakrishnan et al. 2004; Risse et al. 1996; Stoimenova, 2007).

Another proposed explanation is myopic eyes have increased higher order aberrations (Zernike polynomials 3rd order and above). However, myopic and emmetropic eyes do not show

a substantial difference in higher-order aberrations when measured via adaptive optics (Atchison et al. 2006; Carkeet et al. 2002; Rossi et al. 2007).

Two remaining theories are local anatomical changes or interrupted post receptoral neural processing. Supporting post receptoral changes is preserved contrast thresholds at medium to low spatial frequencies in myopic observers (Jaworski et al. 2006). If an overall loss of photoreceptors were seen, contrast threshold would be increased more at high spatial frequencies due to increased photoreceptor spacing, but it would also be decreased across all spatial frequencies. Additionally, the asymmetric decrease in contrast thresholds to positive and negative contrast noted by Stoimenova (2007) points to involvement in alterations of the ON/OFF visual pathway. Physiologically, the ON and OFF pathways begin with the bipolar and ganglion cells in the retina, and the two remain segregated until the input layer IV alpha of the primary visual cortex (Chichilnisky and Wandell, 1996; Schwartz, 2021). Receptive fields of center-surround ON cells have a circular ON center and OFF surround configuration. Light presented to the ON center increases their biopotential, but light presented to the surround decreases their biopotential. The reverse is true for OFF cells (Chichilnisky and Wandell, 1996). OFF retinal ganglion cells are twice as numerous and half the size of ON retinal ganglion cells in the fovea (Chichilnisky and Wandell, 1996).

1.2.2 Electrophysiological response

In addition to changes in visual performance, electroretinogram (ERG) studies reveal alterations in the visual system of myopes. Both multifocal and full-field electroretinogram (mfERG, ffERG) studies find a decrease in amplitudes of the a- and b- waves and an increase in latencies in myopes compared to emmetropes (Kader, 2012; Perlman et al. 1984; Sachidanandam et al. 2017, Westall et al. 2001; Wolsley et al. 2008).

Full-field electroretinograms contain no spatial information, thus, peripheral aberrations altering the spatial image content are unlikely to be the sole factor in the altered ERG studies in myopes (Kader, 2012; Perlman et al. 1984; Sachidanandam et al. 2017, Westall et al. 2001). Full-field electroretinogram deficits were not present in myopes younger than 12 years old, suggesting deficits appear after emmetropization fails (Chia et al. 2013).

1.3 Anatomical differences in myopes

Ruling out anatomical differences requires an overview of what is known about the differences between myopic and emmetropic eyes beyond longer axial length. Myopic eyes have thinner subfoveal choroidal thickness (Fujiwara et al. 2009; Read et al. 2013). Choroidal changes are important in the discussion of myopia in light of their association with emmetropization. Choroidal thickening and thinning in chicks and marmosets is a biological precursor to decrements or increments in axial growth respectively (Troilo et al. 2000; Wallman et al. 1995). With respect to humans, orthokeratology for myopia control results in choroidal thickening followed by slowed axial length growth (Chen et al. 2016).

Choroidal thickness may change in response to visual stimuli (Aleman et al. 2018). Aleman et al. (2018) found exposure to negative contrast text leads to choroidal thinning (mean -16.15 ± 4.54 μm), and positive contrast text leads to choroidal thickening (mean 9.96 ± 6.51μm).

The effect of choroidal thickness on visual performance is unclear. Some studies have found thinner subfoveal choroidal thickness (not retinal thickness) is the greatest predictor of decreased visual acuity and retinal sensitivity (Nishida et al. 2012; Parravano et al. 2014; Zaben et al. 2015), while others find this correlation does not exist (Gupta et al. 2014; Gupta et al.

2016). Although choroidal thickness may affect visual performance, it is unlikely to be the sole cause given the inconsistent results.

An array of other anatomical alterations exist in myopic eyes. Myopic eyes have thinner scleras in the area posterior to the ora serrata (Jonas et al. 2019). Photoreceptor density is decreased in myopes if measured linearly (cells/mm^2) but increases if measured using angular density (cells/deg^2) (Wang et al. 2019). Retinal thickness and RPE density decrease in the equatorial region for myopes (Jonas et al. 2019). Wolsely et al. (2008) measured the area between the outer boundary of the OPL and the boundary of the RNFL to be thinner in myopic individuals, however, Jonas et al. (2019) reported retinal thickness within the posterior pole was no thinner in myopes than non-myopes. Macular basement membrane thickness, retinal thickness, and RPE density do not change in myopes (Jonas et al. 2019). None of these anatomical findings are likely to contribute to changes in visual performance, especially the asymmetric differences in positive and negative contrast thresholds.

1.4 Classification and quantification of myopia

In analyzing post receptoral changes it is important to clearly define the type of myopia being examined. Myopia from excessive axial length is the most common type of myopia, however, it is not the only etiology. Qualitative and quantitative classifications of myopia vary depending on the research group. Working with the International Myopia Institute (IMI) Flitcroft et al. (2019) proposed standardizing the terminology within myopia research. They suggest the qualitative categories of axial, refractive, and secondary myopia (Flitcroft et al. 2019). They suggest the quantitative categories of low myopia (less than or equal to -0.50 D but greater than -6.00 D), high myopia (less than or equal to -6.00 D), and pre-myopia (less than or equal to +0.75 D and greater than -0.50 D in children) (Flitcroft et al. 2019).

Individuals with secondary myopia ought to be excluded from myopia research studies due to etiologies not being generalizable to mechanisms related to failed emmetropization. Secondary myopia includes corneal conditions such as keratoconus, pharmacological induced myopia from drugs such as topiramate, and conditions of the crystalline lens such as cataracts or diabetic changes (de Jong, 2018; Cho et al. 2013; Craig et al. 2004; Flitcroft, 2019).

Refractive myopia (i.e., myopia driven by corneal curvature or atypical power of the crystalline lens) is less common than axial myopia (Flitcroft et al. 2019). Studies should separate axial and refractive myopia to improve consistency and application of findings.

Imperative in the classification of myopia is the quantitative measures used to define it. Defining a sample population via refractive error has the historical disadvantage of varying measurement techniques, contamination of spherical equivalents with astigmatism, and inability to parse out the types of myopia. Autorefraction without cycloplegia is convenient but is subject to instrument error and variance with accommodation (Choong et al. 2006). Autorefraction or retinoscopy with cycloplegia using cyclopentolate is accurate but has the disadvantage of inconvenience to the patient as the pupillary dilation lasts approximately 24 hours and the cycloplegia lasts approximately 6-24 hours (Bartlett and Jaanus 2007; Hashemi et al. 2016; McCullough et al. 2017; Morgan, 2015; Sun et al. 2018). Additionally, retinoscopy requires a skilled clinician. Other researchers choose subjective refraction, which varies based on clinical judgment and the subject's personality (Lauriola, 1997).

Although screening for ocular diseases limits the introduction of individuals with secondary myopia, subjects are not always aware of underlying conditions. Incidence of conditions such as keratoconus is more prevalent than previous estimates, and many individuals are undiagnosed (Godefrooij et al. 2017). In the case of cataracts, myopic shift precedes cataract

formation (Brown and Hill, 1987). One-third of the diabetic population is estimated to be undiagnosed, so lenticular changes from elevated blood sugar are easily missed (Gregg, 2004). Using axial length instead of refractive error circumvents the introduction of refractive myopia and most secondary myopia.

Axial length measures with ocular biometers are accurate, objective, noninvasive, and variance with accommodative status is largely avoided (Atchison and Smith, 2004; Chang et al. 2017; Mallen et al. 2006). The presence of axial length changes with accommodative status is debated, and the highest recorded changes are only around 0.05mm (Alderson et al. 2011; Atchison and Smith, 2004; Chang et al. 2017; Mallen et al. 2006). Assuming the primary driving factors behind myopia progression and degenerative conditions associated with it are caused by a dysregulation of the homeostatic process of eye growth, this thesis focuses on axial myopia (Dolgin, 2015; Flitcroft, 2019; Mutti 2005).

1.5 Motivation and aims

Given the evidence above, post receptoral processing alterations in axial myopia may explain decreased visual performance and altered electrophysiological responses. We believe changes in ON/OFF visual pathways are involved with post receptoral changes. ON/OFF retinal bipolar responses are involved in mfERG responses, and the ON/OFF visual pathways are responsible for contrast sensitivity to positive and negative contrast (Hood et al. 2002; Stoimenova, 2007).

Perhaps, the inconsistent results of visual performance and anatomy in myopes relate to the inconsistencies and lack of clarity in the classification of myopia (Flitcroft et al. 2019). We aim to analyze the ON/OFF visual pathway in axial myopes by measuring the association between axial length and individual differences in psychophysical performance for positive and

negative contrasts. If the difference between axial myopes and emmetropes is optical or anatomical in nature, we expect the detriments to remain uniform between positive and negative contrast. If instead asymmetries in positive and negative contrast thresholds exist in myopes, we can infer neural processing deficits exist in myopes. We hypothesize reduced psychophysical performance of axial myopic observers arises from the remnants of dysregulated emmetropization in either or both of the ON or OFF pathways.

Additionally, we measured changes in choroidal thickness after exposure to positive or negative contrast before and after performing two psychophysical tasks. Measuring choroidal thickness changes will serve to guide future research connecting anatomical changes with alterations in visual performance in axial myopia. We aim for our choroidal thickness study to serve as a replication and extension of previous work (Aleman et al. 2018).

Finally, we examine the possibility diurnal choroidal thickness fluctuations can be altered by exposure to positive or negative contrast. The effect on diurnal fluctuations could support the clinical recommendation that young observers, whose eyes remain plastic, use dark modes during night hours.

2. ON/OFF pattern detection

2.1 Introduction

Negative contrast (e.g., black text on a lighter background) predominantly stimulates the OFF pathway, while positive contrast (e.g., white text on a darker background) stimulates the ON pathway.

The distinction between the ON and OFF pathways in the visual system is supported by evidence of separate neural responses and psychophysical sensitivities. Spatial tuning and cortical connectivity between the ON and OFF pathways differ (Lu et al. 2012; Mazade et al. 2019; Tyler et al. 1992). Peak cortical responses are measured in response to small fast stimuli in the OFF pathway and to slower more global stimuli in the ON pathway (Mazade et al. 2019). When negative contrast stimuli are constructed with equal contrast energy to positive stimuli, positive contrast produces a larger visual response across 11 stimuli of different spatial and temporal properties (Lu and Sperling, 2012). Contrast thresholds extended to smaller letters with positive Raleigh contrast targets (Alexander et al. 1993). Taken together altered responses to ON/OFF stimuli support unique differences in the function of the ON and OFF system.

2.2 Purpose

We set out to collect evidence of asymmetries in psychophysical performance of positive and negative contrast in axial myopes. Uncovering asymmetries will assist in developing mechanistic hypotheses on the origin of failed emmetropization.

2.3 Methods

2.3.1 Observers

We recruited 39 observers from the New England College of Optometry. Subject ages ranged from 18 to 45 (mean age 25.0 ± 3.73). Observers were screened to exclude patients with a history of ocular disease, ortho-k lens wear, or ocular surgery. Spherical equivalent refractive errors ranged from -12 to +6.50 D (mean -3.42 ± 3.57 D). Axial length ranged from 20.9 to 27.9 mm (mean 24.8 ± 1.68 mm). The self identified gender makeup was 25 female and 14 male participants. For detailed demographic information see *table 1*.

2.3.2 Apparatus

A Topcon KR-1W wavefront analyzer (Topcon, Tokyo, Japan) was used to determine the patient's refractive error, and a Haag-Streit Lenstar 900 (Haag-Streit, Bern, Switzerland) was used to measure axial length. Testing was completed on a Vpixx 12-bit monitor (ViewPixx, Montreal, Canada) with an average luminance of 65 cd/m^2. Subjects were seated 114 cm from the monitor with their head stabilized using a chin-forehead rest.



Figure 1: Haag-Streit Lenstar 900 (Haag-Streit, Bern, Switzerland) (top left), Topcon KR-1W wavefront analyzer (Topcon, Tokyo, Japan) (top right), Vpixx 12-bit monitor (ViewPixx, Montreal, Canada) (bottom).

2.3.3 Stimuli and Procedure

Ocular biometry measurements were captured before and after each session using the Lenstar. The axial length measurement used in analysis was taken from the first measurement in the first session. The subject's refractive error was measured using the wavefront analyzer at the initial visit.

Stimuli were computed and displayed via MatLab and the video and psychophysics toolboxes (Brainard, 1997; Kleiner 2007). Two psychophysical tasks were used; a 10 alternative forced choice letter identification task and a two alternative forced choice Difference of Gaussian (DoG) detection task.

For the letter identification task, the observer identified a single Sloan font letter on each trial. The letter set included C, D, H, K, N, O, R, S, V, and Z (*figure 2)*. The letter size was 0.37° of visual angle (6.75 cycles per degree). The identity of the letter was randomly selected on each trial. Each letter stimulus was presented for 240 ms with a simultaneous audible tone to reduce temporal presentation uncertainty. After the letter was presented a selection screen appeared with all possible letters. The subject used a "Nintendo-style" gamepad to initiate each trial and record their response.

The selection screen contained all 10 letters (*figure 2)*. The selected letter was shown at maximum contrast, while the other letters were shown with slightly decreased contrast. The subject responded with buttons on the gamepad. At the beginning of each selection process, the letter initially highlighted was randomly selected to help maintain the subject's vigilance. A high pitched tone was played when the response was correct and a low pitched tone when the response was incorrect. Response time was recorded, but not analyzed. After the selection of the letter, the observers pressed a button to continue to the next trial.

For the next task, a DoG target was used with the equation in one dimension (Tyler, 1992):

$$DoG(x) = 2.56e^{-2.56x^2/2\alpha^2} - e^{-x^2/2\alpha^2}$$

DoG targets are circularly symmetric and mimic the anatomical arrangement of ON and OFF cells. The one dimensional equation extended to two dimensions produces the stimuli (*figure 3)*.

The center Gaussian of the DoG target extended 0.18° of visual angle (2.77 cycles per degree), while the surround was 0.27°.

The DoG detection task was a two interval forced choice procedure. The two intervals were each presented for 240 ms. A tone accompanied each interval with a blank 240 ms interstimulus interval. Each interval contained two horizontal lines with an empty space between them to minimize spatial uncertainty. In the space between the two horizontal lines, the DoG target was presented in the first or second interval. The subject used a gamepad to indicate the interval containing the stimuli by pressing the left arrow indicating the first presentation or the right arrow for the second. A high pitched tone was played when the response was correct, and a low pitched tone when the response was incorrect.

For our tasks, we opted to use RMS contrast. RMS contrast is defined as:

$$C_{RMS} = \sqrt{\frac{1}{MN} \sum_{i}^{M} \sum_{j}^{N} \left(L_{ij} - \bar{L} \right)^2}$$

where C_{RMS} is RMS contrast, M and N are the image size, L_{ij} is the luminance at a single pixel, and $\bar{L}$ is the mean luminance (Tiippana et al. 1994). RMS contrast values were randomly selected and presented using the method of constant stimuli. RMS contrast was logarithmically spaced from 1 to 10% contrast, and the sign of contrast was negative for OFF stimuli or positive for ON stimuli.



Figure 2: High contrast depictions of ON stimulus letter targets (top) and OFF stimulus letter targets (bottom).

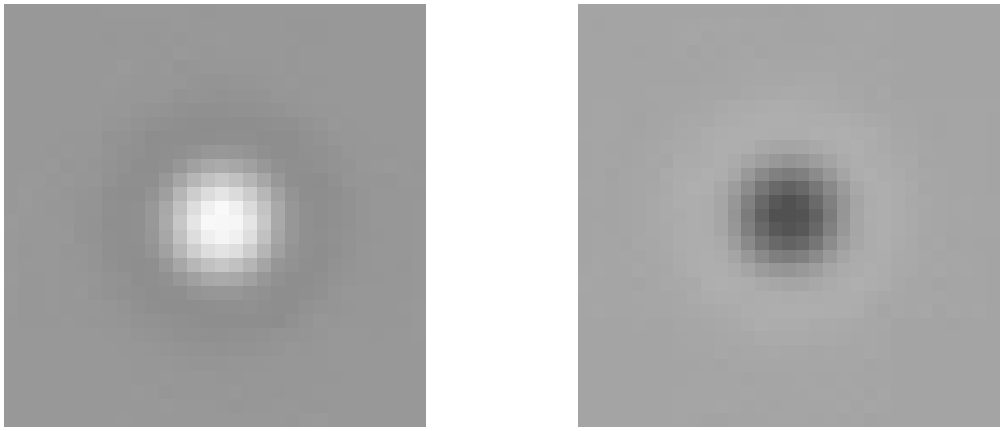


Figure 3**:** High contrast depictions of the ON (left) and OFF (right) Difference of Gaussian targets

Observers completed 10 practice trials of the DoG and letter tasks at a contrast level high enough so the target was easily identifiable. Successful completion of the practice trials indicated the subject could accurately perform the task. Observers underwent four sessions. Two sessions contained positive contrast stimuli, and two contained negative contrast stimuli. The order of positive and negative sessions was block randomized. During a session, both the letter identification and two interval forced choice tasks were completed in four blocks. Each block consisted of 150 letter identification and 150 two interval forced choice trials. The task each

session began with was randomized. The session was self paced and lasted 1-1.5 hours. Observers were able to take a break at any point. Upon completion of the first two sessions, observers were asked to return for a third and fourth session.

2.3.4 Analysis

At each of the seven levels of contrast d' values were calculated. The relative sensitivity index d' is the difference between the means of the distribution of internal noise and internal noise plus signal. One subject's result from one session is shown in *figure 4*. The contrast threshold was defined as a d' value of 0.75. Data analysis was completed using signal detection measures with R and the psyph R package (R Core Team, 2021; Revelle, 2021). Standardizing to a d' value allows comparison of the letter identification and DoG tasks. If a subject failed to achieve d' values of at least 0.75 at the maximum contrast level, they were excluded from the analysis. Of the 39 subjects, 2 subjects failed to meet the criteria. Leaving 37 subjects for threshold analysis.

We used Pearson product-moment correlation to measure the correlation between axial length and refractive error, axial length between eyes, log threshold and axial length, and log threshold and refractive error. The traditional cut off value of alpha is less than 0.05 was used for significance. Although we focus on axial length, the correlation between log threshold and refractive error was calculated to allow comparison to previous studies.

To compare OFF to ON thresholds we defined a relative sensitivity index as:

$$OFF/ONindex = \left(C_{off} - C_{on} \right) / \left(C_{off} + C_{on} \right)$$

C_{off} is the log threshold for the OFF target, and C_{on} is the log threshold for the ON target. The OFF/ON index was calculated for each subject. The greater the difference in OFF and ON

thresholds, the greater is the OFF/ON index. No difference in contrast thresholds will result in an OFF/ON index of zero, negative values indicate higher threshold for ON targets, and positive values indicate higher thresholds for OFF targets.

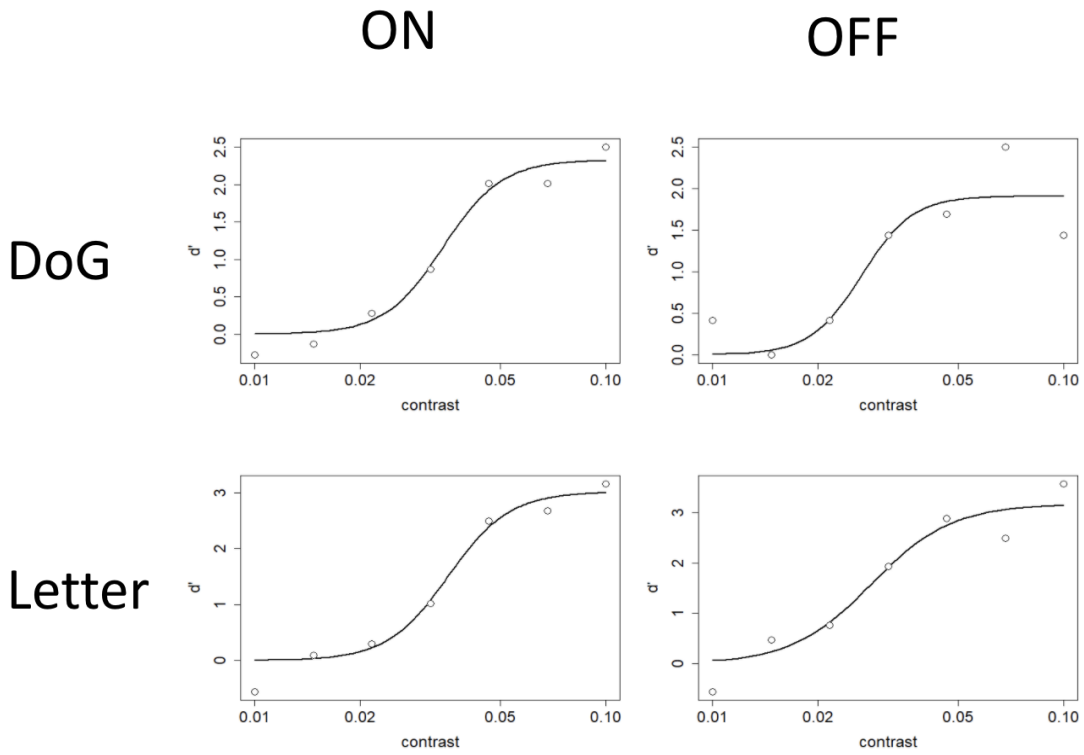


Figure 4: d' values are plotted as a function of contrast for ON DoG targets (top left), OFF DoG targets (top right), ON letter targets (bottom left), and OFF letter targets (bottom right) for one subject during one session.

2.4 Results

To ensure axial length and refractive error correlated in our subject pool, we plotted refractive error as a function of axial length for the right eye, left eye, and an average of the two eyes (*figure 5)*. We found the two correlated well for the right (r = 0.867) and left (r = 0.865) eye. As expected, there was a strong negative correlation between axial length and spherical

equivalent refractive error. Additionally, we analyzed the correlation of axial length between both eyes and found the two correlated well (r = 0.990).

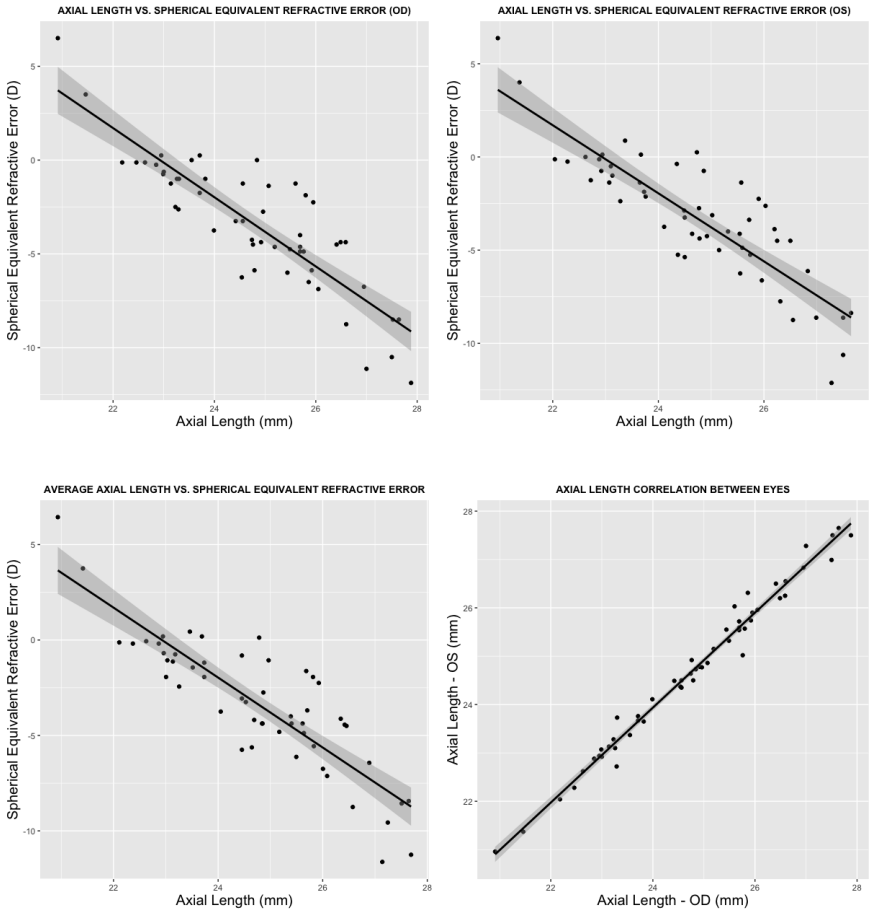


***Figure 5*:** Spherical equivalent refractive error as a function of axial length for the right (top left) and left eyes (top right), average between the two eyes (bottom left), and correlation of axial length between eyes (bottom right).

There was a positive correlation between log threshold and axial length for ON (t = 2.0284, df = 36, p-value = 0.04997, r = 0.32026) and OFF (t = 2.933, df = 36, p-value = 0.00580, r = 0.43917) DoG targets (*figure 6)*. There was a positive correlation between the DoG OFF/ON index and axial length (t = 2.04, df = 36, p-value = 0.012, r = 0.38).

For letter targets, there was also a positive correlation between log threshold and axial length for ON (t = 2.8165, df = 36, p-value = 0.00783, r = 0.42492) and OFF (t = 2.5027, df = 36, p-value = 0.017, r = 0.38497) targets (*figure 6).* There was not a positive or negative correlation between the OFF/ON index and axial length (t = 0.03063, df = 36, p-value = 0.9757, r = 0.00511).

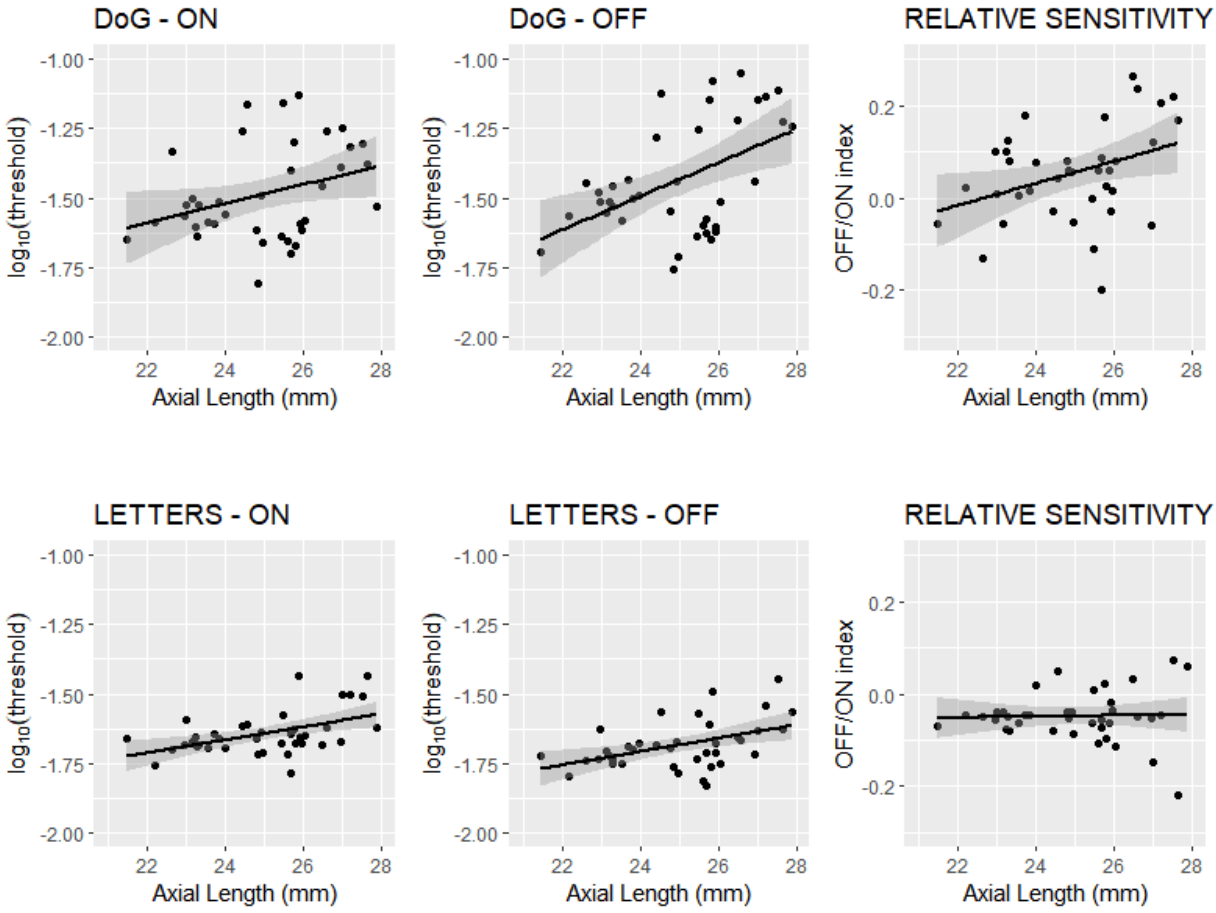
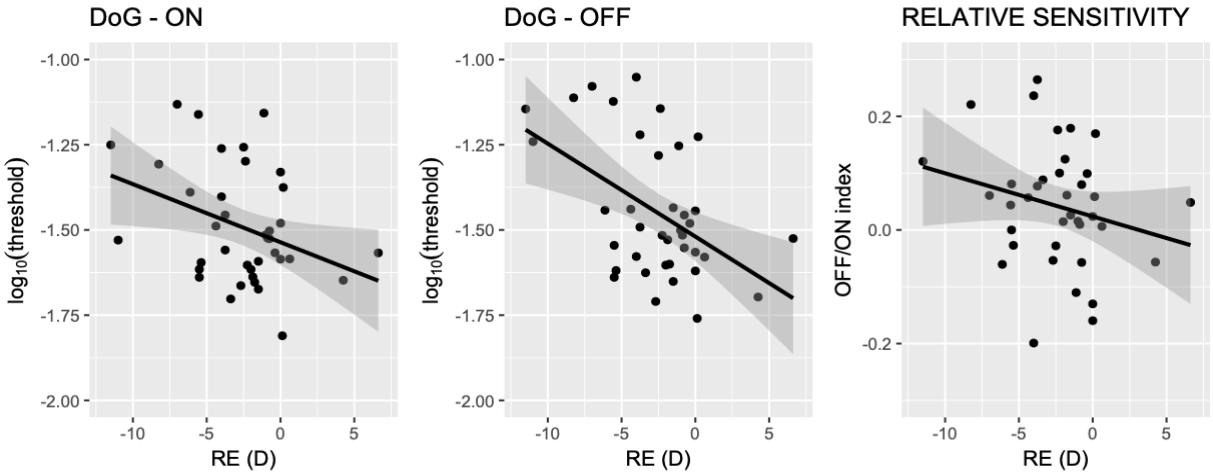


Figure 6*:* Log threshold for the ON DoG target is plotted as a function of axial length (top left). Log threshold for the OFF DoG target is plotted as a function of axial length (top middle). The OFF/ON DoG index is plotted as a function of axial length (top right). Log threshold for the ON letter target is plotted as a function of axial length (bottom left). Log threshold for the OFF letter

target is plotted as a function of axial length (bottom middle). The OFF/ON letter index is plotted as a function of axial length (bottom right).

In order to compare with previous literature, we calculated the previous findings using spherical equivalent refractive error instead of axial length. The results remained the same (*figure 7).* There was a negative correlation between log threshold and spherical equivalent refractive error for ON (t = -2.2881, df = 36, p-value = 0.02811, r = -0.35632) and OFF (t = -3.4367, df = 36, p-value = 0.001501, r = -0.49703) DoG targets. There was a negative correlation between the OFF/ON index and spherical equivalent refractive error (t = -2.2758, df = 36, p-value = 0.02891, r = -0.35464).

There was a negative correlation between log threshold and spherical equivalent refractive error for ON (t = -3.0141, df = 36, p-value = 0.0047, r = -0.44889) and OFF (t = -2.3688, df = 36, p-value = 0.02333, r = -0.36722) letter targets, but again, there was no positive or negative correlation between the OFF/ON index and refractive error (t = -1.2553, df = 36, p-value = 0.2175, r = -0.20478).



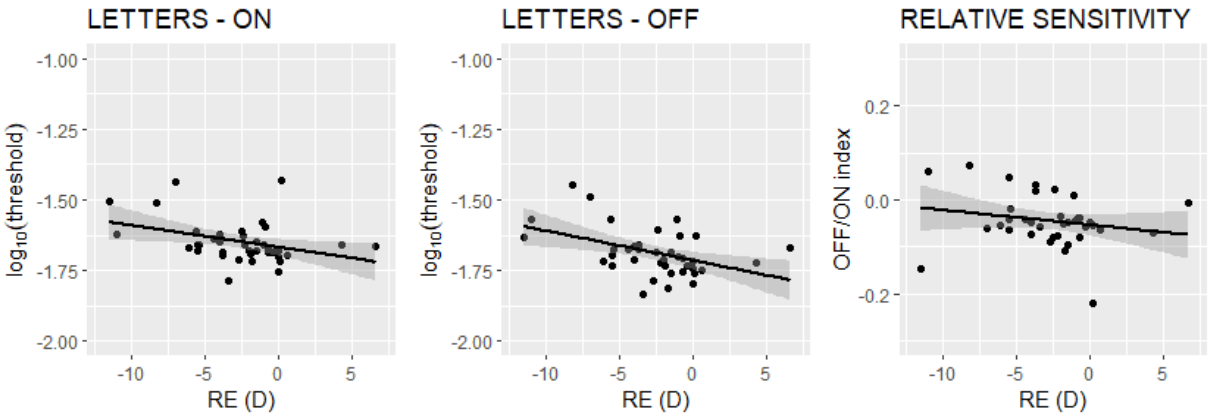


Figure 7: Log threshold for the ON DoG target is plotted as a function of spherical equivalent refractive error (top left). Log threshold for the OFF DoG target is plotted as a function of spherical equivalent refractive error (top middle). The OFF/ON DoG index is plotted as a function of spherical equivalent refractive error (top right). Log threshold for the ON letter target is plotted as a function of spherical equivalent refractive error (bottom left). Log threshold for the OFF letter target is plotted as a function of spherical equivalent refractive error (bottom middle). The OFF/ON letter index is plotted as a function of spherical equivalent refractive error (bottom right).

2.5 Discussion

In summary, we found contrast thresholds were increased for both ON and OFF targets in axial myopes. We found the longer the axial length was for a given subject the greater the difference in the threshold between the OFF and ON DoG target was. Axial myopes had higher thresholds to OFF compared to ON targets, whereas emmetropes had approximately similar thresholds. The asymmetries in thresholds of the ON and OFF targets were not observed for letter targets. Although the effect observed in asymmetries between ON and OFF thresholds for

DoG targets was small, there seem to be underlying deficits in the OFF more than the ON pathway of axial myopes.

Understanding the underlying mechanism leading to deficiencies in the OFF visual pathway in axial myopia will lead to more effective means of mitigating or altogether preventing the onset of myopia. For example, if a lack of exposure to OFF stimuli were the culprit, a rich diet of OFF stimuli could provide a therapeutic effect. We hypothesize reduced sensitivity or increased internal noise within the OFF pathway leads to a dysregulation of the homeostatic set-point and leads to stop signals for eye growth to be ignored.

Exposure to a rich visual diet is an important component of emmetropization (Bodnarenko and Chalupa, 1993; Wong et. al; 2000). A rich visual diet includes exposure to ON and OFF stimuli. Natural scenes contain a wide array of spatial variation in local contrast (Frazor and Geisler, 2006). Outdoor scenes providing a diverse visual experience may improve contrast sensitivity and visually guided emmetropization. Using software analysis, we found signals generated by the OFF pathway are more robust for naturalistic rather than artificial stimuli. Robust OFF stimulation in natural images provides a link between deficits in the OFF pathway and the preventative effect of time spent outdoors.

The mechanism of increased time outdoors preventing the onset of myopia is uncertain (Dirani et al. 2009; Rose et al. 2008; Xiong et al. 2017). Hypotheses include the magnitude of outdoor light intensity, the rich short-wavelength spectral composition of outdoor light, circadian rhythm regulation via the stimulation of the intrinsically photosensitive retinal ganglion cells (ipRGCs), stimulation of the peripheral retina, pupillary constriction resulting in an increased depth of focus, and variance in accommodative stimuli (French et al. 2013; Xiong et al. 2017). Given our findings showing the OFF pathway is affected in myopes more than the ON pathway

is affected, diverse spatial variation in local contrast of natural scenes, and computational analysis revealing increased OFF stimulation in outdoor environments, we hypothesize the lack of exposure to outdoor scenes in children who become myopic leads to faulty development of OFF pathway and subsequent axial elongation. More work is needed to support this theory and determine the underlying reasoning why faulty development of the OFF pathway may lead to failure in emmetropization.

Decreased visual performance may leave myopes at a disadvantage in sports activities. A feedback loop of poor sports performance reducing time spent outdoors may further promote myopia progression (Dirani et al. 2009; Rose et al. 2008; Xiong et al. 2017). This is a lofty assertion requiring more studies.

Another theory linking OFF and ON cells to emmetropization is their function as blur detectors. In the mouse model, Mani and Schwartz (2017) uncovered unique ON cells they called ON delayed cells. ON delayed cells integrate information over longer periods of time and larger spatial scales (Mani and Schwartz, 2017). Sensitivity to contrast present across the full visual field makes ON delayed cells prime candidates for full field blur detectors (Mani and Schwartz, 2017). The existence of OFF delayed cells is unknown, but their discovery would bolster our theory. Blur detection plays a key role in the process of emmetropization, but the mechanism for blur detection is yet to be uncovered (Benavente-Perez et al. 2012; Diether and Wildsoet, 2005; Schmucker and Schaeffel, 2004). Taking our results with the findings of Mani and Schwartz (2017), The ON/OFF system may play an important role in blur detection. Disruption to the ON/OFF blur detection system may lead to failed emmetropization.

Similar to our DoG targets, but unlike our letter identification task, Stoimenova et al. (2007) found that for myopes the negative contrast threshold was more reduced than the positive

contrast threshold when compared to their emmetropic counterparts. There are several possibilities for why we were unable to reproduce asymmetric contrast sensitivities using letter targets. The Stoimenova (2007) experiment was monocular, while ours was binocular. There are well-documented differences in monocular versus binocular contrast thresholds as binocular summation is especially beneficial for low contrast letters (Schneck, 2010). Stoimenova (2007) used Cyrillic letters, while we used standard Sloan letters. Stoimenova (2007) failed to provide the font of Cyrillic letters used. It is impossible to assess their similarities to Sloan letters. Sloan letters are carefully constructed to produce reliable, reproducible measures, while there are no rigorous criteria for Cyrillic fonts (Plainis et al. 2013).

In the future, it would be valuable to include a range of spatial frequencies for the letter and DoG target. It may be that at certain spatial frequencies there would be asymmetries in the letter target as well. A potential shortcoming of our experiment was the use of two targets with spatial frequencies at different points on the contrast sensitivity curve. Additional shortcomings include the use of both spectacle and contact lenses by subjects. Future studies should only use one or the other to avoid the introduction of peripheral aberrations in some but not all subjects.

Our study adds to the body of evidence showing myopic visual performance is impaired. Without data of children undergoing emmetropization, it is impossible to determine whether deficits in the ON/OFF system lead to a dysregulation of the homeostatic set point during emmetropization as we hypothesize, or if instead, the deficiencies are a result of axial myopia. Future research is needed to evaluate contrast thresholds to positive and negative stimuli in children.

3. Choroidal changes following ON/OFF stimulation

3.1 Introduction

Exposure to negative and positive contrast stimuli may lead to choroidal thinning and thickening respectively. In a small sample size of seven subjects, Aleman et al. (2018) found negative contrast text produces a thinning effect on the choroid (mean -16.15 ± 4.54 μm), and positive contrast produces a thickening effect (mean 9.96 ± 6.51μm). Aleman et al. (2018) interpreted opposing choroidal thickness changes to positive and negative contrast in the context of the ON and OFF pathways in the visual system.

Hoseini-Yazdi et al. (2021) found the thinning effect of OFF pathway stimulation was enhanced when the subject accommodated to a near target. Increased thinning to OFF targets while accommodating provides a link to the association between increased time spent reading and myopia (Huang et al. 2016). Other near tasks, such as time spent on the computer, are not associated with an increase in myopia (Huang et al. 2016). Text contained within a book consists of predominantly negative contrast text whereas the visual landscape of a computer screen is varied (Aleman et al. 2018).

Wang et al. (2019) found passive exposure to ON and OFF pattern stimuli also produced choroidal thickening and thinning after three hours. However, changes seen with pattern stimuli were small (around five microns), and their sample size was only 11 subjects (Wang et al. 2019). The same study found three hours of ON stimuli a day for seven days increased retinal dopamine levels in chicken eyes (Wang et al. 2019). Dopamine levels influence the process of emmetropization (Feldkaemper and Schaeffel, 2013). Animals with lens induced and form deprivation myopia have lower levels of dopamine, and injected dopamine decreases the amount of myopia in lens induced and form deprivation myopia (Feldkaemper and Schaeffel 2013).

Choroidal changes are important in the discussion of myopia in light of their association with emmetropization. Choroidal thickening and thinning in chicks and marmosets is a biological precursor to decrements or increments in axial growth respectively (Nickla et al. 2005; Troilo et al. 2000; Wallman et al. 1995; Wildsoet 1997). Troilo et al. (2000) documented 20 μm of choroidal thinning in marmoset eyes with negative lens induced myopia and 12 μm of thickening in eyes with positive lens induced hyperopia. The mechanism underlying the process of choroidal changes leading to axial length changes is unknown. One hypothesis is that the choroid acts as a mechanical buffer or a diffusion barrier for the sclera resulting in altered growth (Troilo et al. 2000).

The plasticity of the human choroid is an important link to animal models. Read et al. (2013) found among a group of 39 myopic individuals subfoveal choroidal thickness was 50 μm thinner than nonmyopic age matched children (Read et al. 2013). Chen et al. (2016) found orthokeratology used for myopia control resulted in choroidal thickening followed by less axial length growth. Fontaine et al. (2017) found thinner choroids in children predicted future myopic shifts.

Animal studies reveal mixed results regarding the direction of axial length change with ON or OFF stimuli. Inactivation of the ON pathway with D,L-2-amino-4-phosphonobutryic acid in kittens resulted in less axial length growth (Smith et al. 1991). Crewther and Crewther (2003) found the same results of ON pathway inactivation in chickens. With genetic inactivation of the ON channel in mice, however, Chakraborty et al. (2015) and Pardue et al. (2008) found an increase in axial length growth. Although animal study results vary, it is likely that ON and OFF visual pathways play a role in emmetropization. It is possible the effect of stimulating the ON or

OFF pathway in humans is comparable to pharmacological inhibition as adaptation to an ON or OFF stimulus leads to a dampening of the signal strength (Aleman et al. 2018).

3.2 Purpose

We aim to replicate the choroidal thickness changes found by Aleman et al. (2018) with a larger sample size. We anticipate retinal choroidal thickness will increase in response to ON pathway stimulation and decrease in response to OFF pathway stimulation. Understanding anatomical changes in myopic observers and linking these findings to psychophysical studies will serve to elucidate the process underlying the ON/OFF pathway's involvement in emmetropization.

3.3 Methods

3.3.1 Observers

We recruited 39 observers from the New England College of Optometry. These subjects were the same participants from the first experiment. Ocular biometry measurements were taken in conjunction with psychophysical testing. Subject ages ranged from 18 to 45 (mean 25.0 ± 3.73). Observers were screened to exclude patients with a history of ocular disease, ortho-k lens wear, or ocular surgery. Spherical equivalent refractive errors ranged from -12 to +6.50 D (mean -3.42 ± 3.57 D). Axial length ranged from 20.9 to 27.9 mm (mean 24.8 ± 1.68 mm). The self identified gender makeup was 25 female and 14 male participants. For detailed demographic information see *table 1*.

3.3.2 Apparatus

A-scan measurements were captured with a Haag-Streit Lenstar 900 (Haag-Streit, Bern, Switzerland) (*figure 1*). The Lenstar uses optical low coherence reflectometry to produce an A-scan measurement (Read et al. 2011). A Vpixx 12-bit monitor (ViewPixx, Montreal, Canada) displayed the stimuli with an average luminance of 65 cd/m^2. The subject was seated 114 cm from the monitor with their head stabilized with a chin-forehead rest (*figure 1*).

3.3.3 Stimuli and Procedure

A-scan measurements were captured before and after psychophysical tasks. Tasks contained either positive or negative contrast. Two sessions contained positive contrast stimuli, and two sessions contained negative contrast stimuli. Stimuli were the DoG and letter targets from the previous experiment seen in *figures 2 and 3*. Subjects were exposed to both letters and DoG targets during the experiment. Paired ON and OFF sessions were done at approximately the same time of day in order to account for diurnal fluctuations in choroidal thickness (Chakraborty et al. 2011; Lee, et al. 2014; Tan et al. 2012). Haag-Streit Lenstar 900 A-Scan results were analyzed to determine choroidal thickness. Measurements of choroidal thickness from A-scans captured on the Lenstar correlate well with those from ocular coherence tomography (OCT) (Read et al. 2011). We pre-registered our hypothesis using Open Science Framework (OSF) prior to data analysis. Pre-registration provides robust accountability for the intended direction of our research.

3.3.4 Analysis

We used a mixture of Gaussian model to determine choroidal thickness developed in R (R Core Team 2021) (*figure 8)*. To implement the mixture of Gaussian models we isolated peaks

on the A-scan representing the RPE and the choroid/sclera border using Gaussian fits. The RPE produces a narrow spectral density while the choroid scleral border produces a wider spectral density. Using the mixture of Gaussian model, these two peaks can be repeatedly isolated. The distance between the peaks of the two Gaussian functions was selected as the biomarker of choroidal thickness. The distance was initially recorded in arbitrary units and converted to microns. Manual segmentation of OCT scans is the popular method for analysis of choroidal thickness but has the disadvantage of reliance on subjective measures as well as being a tedious process. Automated segmentation of choroidal thickness on OCT is becoming increasingly prevalent but requires more complex algorithms that are not yet widely available (Zhang et al. 2020)

We used Pearson's product-moment correlation to determine if choroidal thickness measurements were in agreement between the two eyes of a subject if choroidal thickness changes varied with axial length, and if axial length correlated with choroidal thickness. Following our pre-registered methods, we used directional one-sided t-tests to analyze choroidal thickness changes after exposure to ON or OFF stimuli. The traditional cut off value of alpha is less than 0.05 was used for significance.

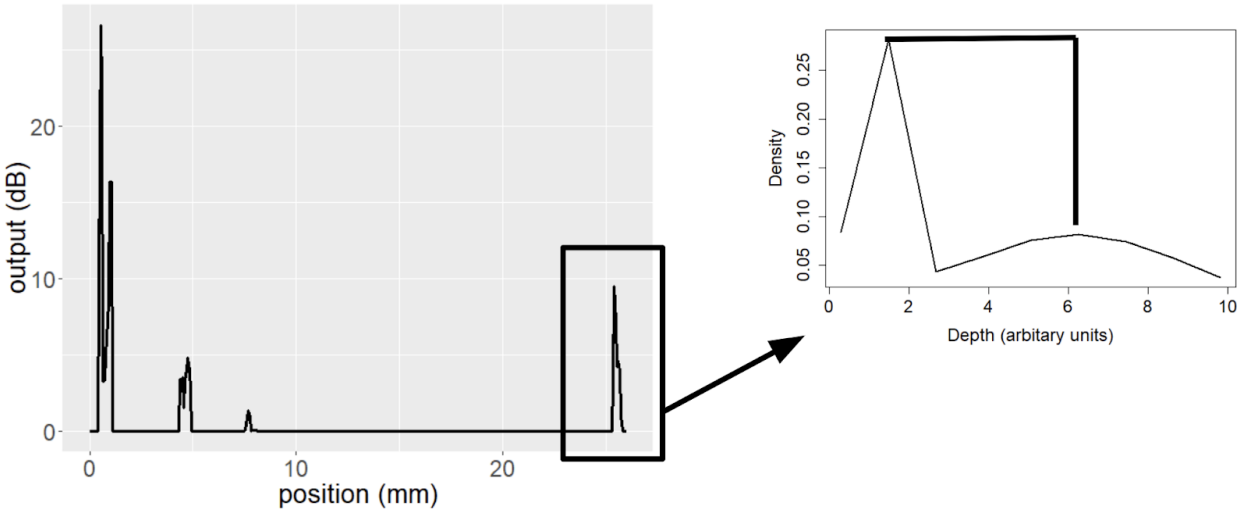


Figure 8: A-scan of an eye (left). The first double peak represents the anterior and posterior cornea. The next peak is the anterior lens, followed by the posterior lens. The final peak is the retina. If zoomed in, the final peak has spikes at the RPE and choroid sclera border. The right graph shows the Gaussian fits of these peaks with the distance between the peaks quantifying the choroidal thickness in arbitrary units. A final choroidal thickness measurement was calculated after arbitrary units were converted to microns.

3.4 Results

Mean choroidal thickness was 352 ± 81 μm in the right eye and 366 ± 80 μm in the left eye. There was a negative correlation between choroidal thickness and axial length before ($t = -3.1519$, $df = 76$, p-value = 0.00232, $r = -0.34000$) and after ($t = -3.4352$, $df = 76$, p-value = 0.00096, $r = -0.36661$) psychophysical testing (*figure 9)*.

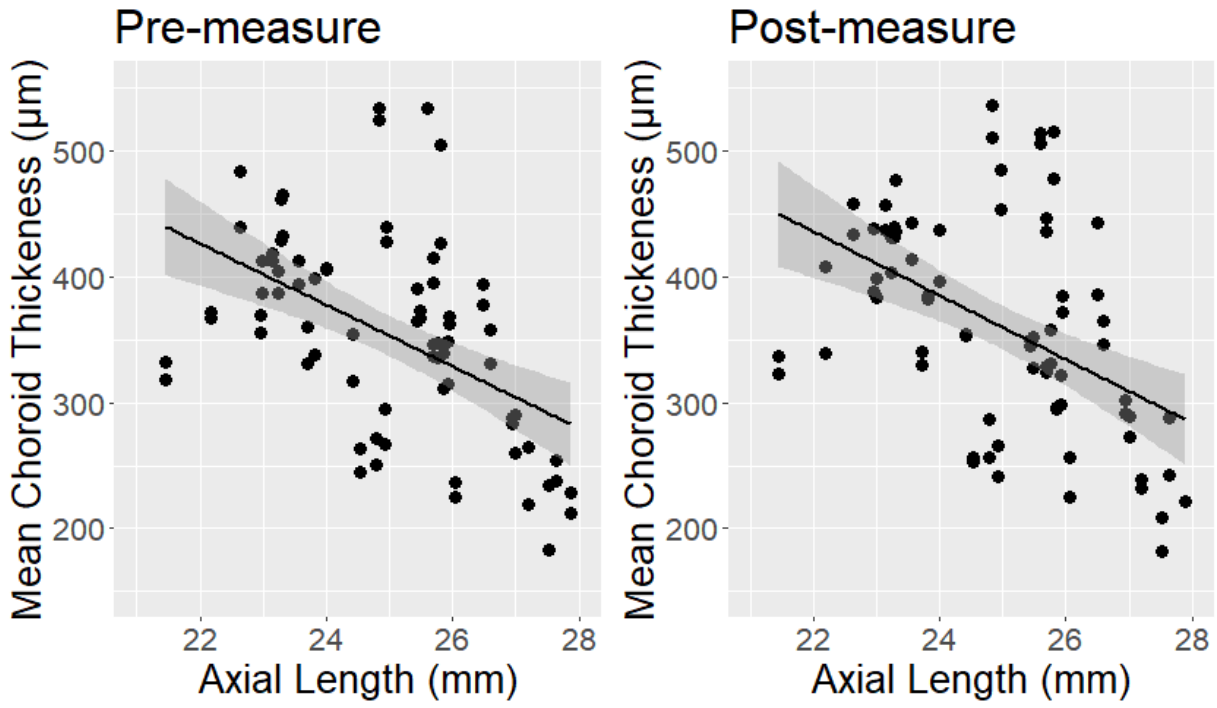


Figure 9: Mean choroidal thickness plotted as a function of axial length before (left) and after (right) psychophysical testing

Choroidal thickness measurements between eyes correlated closely before psychophysical testing for ON (t = 9.3855, df = 37, p-value = 2.511e-11, r = 0.83917) and OFF (t = 8.4327, df = 37, p-value = 3.85e-10 r = 0.88534) sessions and after psychophysical testing for ON (t = 11.582, df = 37, p-value = 7.226e-14, r = 0.88534) and OFF (t = 11.733, df = 37, p-value = 4.954e-14, r = 0.88778) sessions.

We found that ON stimuli lead to choroidal thickening (mean 5.4 ± 19 µm) (t = 1.7506, df = 38, p-value = 0.04404), however OFF stimuli did not produce detectable choroidal thinning (t = -0.25147, df = 38, p-value = 0.4014). Choroidal thickness changes did not correlate with

axial length for ON (t = -1.4216, df = 37, p-value = 0.1635, r = -0.22758) or OFF (t = -1.4343, df = 37, p-value = 0.1599, r = -0.22950) stimuli (*figure 10).*

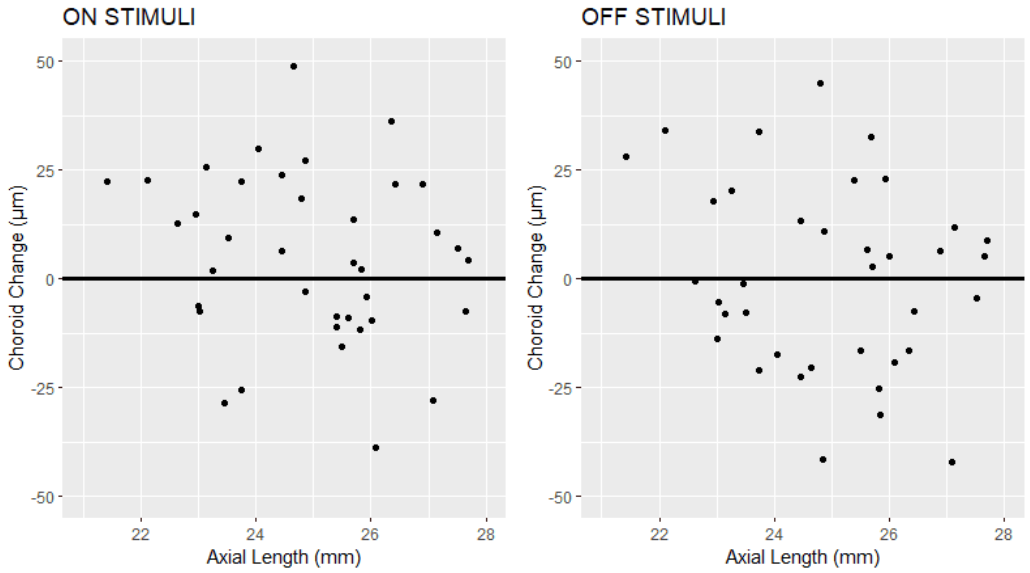


Figure 10: Choroidal thickness changes plotted as a function of axial length for ON (right) and OFF (left) stimuli. ON stimuli lead to choroidal thickening and OFF stimuli lead towards a trend of choroidal thinning.

3.5 Discussion

Our choroidal thickness measurements are in agreement with previously described average choroidal thickness in healthy subjects (Entezari et al. 2018). Entezari et al. (2018) reported a mean thickness of 371 ± 78 µm in 208 eyes using enhanced depth imaging OCT. Our measures showed a mean thickness of 352 ± 81 µm in the right eye and 366 ± 80 µm in the left eye. Our thinner measurements are expected due to our skew toward a myopic cohort as myopes are known to have thinner choroids (Read et al. 2013). Our mean refractive error was -3 D while Entezari et al. (2018) excluded subjects with more than -1 D. Thinner choroids in myopic

observers were confirmed in our study as there was the expected negative correlation between chorodial thickness and spherical equivalent refractive error (*figure 9*).

Another way of confirming our methods is to check the correlation of choroidal thickness between two eyes of one subject. As discussed in *figure 5,* axial lengths between the two eyes of the subjects correlated closely, we expected choroidal thickness measurements would also correlate, which they did.

Given our choroidal thickness measurements are similar to previously reported measures, correlate with axial length, and correlate between two eyes, we propose A-scans captured on a Haag-Streit Lenstar 900 and analyzed with a mixture of Gaussian model is a simple yet effective means of measuring choroidal thickness. We predicted we would find choroidal thickening after exposure to ON stimuli and choroidal thinning after exposure to OFF stimuli. We did not replicate the thinning effect after exposure to OFF stimuli. We did find thickening after exposure to ON stimuli, but the effect size was small to moderate (Cohen's d = 0.56).

There are a few possible explanations for why we were unable to reproduce the OFF thinning effect. Previous studies found changes in choroidal thickness using letter targets only, while we incorporated a DoG target. It may be that our DoG stimuli were adequate for stimulation of ON but not OFF retinal cells. Additionally, their subject pool was very small with only 7 subjects (Aleman et al. 2018). Their subjects were evenly distributed between emmetropic and myopic, whereas our subjects skewed towards myopic refractive errors. As previously mentioned the choroid scleral border Gaussian fit had a wide spectral density. This may have led to a relative insensitivity to choroidal thinning.

One important difference between the Aleman et al. (2018) study and our study was the size of the stimulus. Our letter targets were significantly larger than the target Aleman et al.

(2018) used (22.2 minutes of arc versus 11.8 minutes of arc). OFF retinal ganglion cells are twice as numerous and half the size of ON retinal ganglion cells in the fovea (Chichilnisky and Wandell, 1996). It may be that the size of our letters was adequate for stimulating the ON pathway but too large to isolate the OFF pathway. Dependence of ON/OFF stimulation on letter size is supported by the fact Hoseini-Yazdi et al. (2021) used a small target (11.8 minutes of arc) and found a thinning effect of OFF stimulation, but no thickening effect on ON stimulation. For choroidal thickening or thinning to occur, the ON/OFF cells may need to be optimally stimulated.

In addition to stimulus size, because we incorporated a psychophysical task into the experiment, our stimulus presentation was inherently different. Aleman et al. (2018) used a free reading task where the subjects were exposed to a page full of letters of a given contrast. We presented all the letters in between stimulus presentations, but the screen was only filled with letters in the center.

With our null finding, we cannot make any reliable connections to our psychophysical data showing an asymmetric increase in contrast thresholds for positive and negative stimuli in myopes. Perhaps future studies evaluating contrast polarity's effect on choroidal thickness should focus on conditions more similar to those used by Aleman et al. (2018).

4. Diurnal choroidal thickness changes following ON/OFF stimulation

4.1 Introduction

Choroidal thickness undergoes diurnal fluctuations (Chakraborty et al. 2011; Lee, et al. 2014; Tan et al. 2012). Choroidal thickness reaches a maximum at around 9:00 AM (averaging 372.2 μm) and a minimum at around 5:00 PM (averaging 340.6 μm) (Tan et al. 2012). Measurements of choroidal thickness at two hour intervals reveal a linear decline throughout the day (Tan et al. 2012). Axial length also undergoes diurnal fluctuation with the longest measurements occurring around 12:00 PM and the shortest around 12:00 AM (Chakraborty et al. 2011).

In animal models, choroidal thickness diurnal fluctuations are affected by visual input (Nickla et al. 1998; Stone et al. 2020). Form deprivation and lens induced myopia lead to dysregulations in the normal diurnal fluctuations of the choroid. As mentioned in our previous experiment, visual input may induce changes in choroidal thickness as the choroid may thin in response to the negative contrast and thicken in response to positive contrast (Aleman et al. 2018). Whether positive and negative contrast affect the diurnal fluctuation of human choroidal thickness is unknown.

Some studies have found thinner subfoveal choroidal thickness predicts decreased visual acuity and retinal sensitivity (Nishida et al. 2012; Parravano et al. 2014; Zaben et al. 2015), while other studies find this correlation does not exist (Gupta et al. 2014; Gupta et al. 2016). If choroidal thickness does affect visual performance, contrast thresholds may be increased at night when the choroid is thinner (Nishida et al. 2012; Parravano et al. 2014; Zaben et al. 2015). There is little research evaluating diurnal fluctuations in contrast thresholds. Two factors shown to affect visual performance throughout the day are contact lens wear and dry eye syndrome. Both

dry eye syndrome and contact lens wear lead to decreased visual performance in the evening due to poor tear film quality (Belda-Salmeron et al. 2013; Walker et al. 2010).

4.2 Purpose

With this follow up experiment, we aim to examine the impact of positive and negative contrast on choroidal thickness at peak and trough times of diurnal choroidal thickness fluctuations. In general terms, we will be able to assess the impact of night mode versus normal mode device settings on the diurnal fluctuations of the choroid. Normal mode contains predominantly negative contrast, while night mode contains positive contrast. As the choroid is thinnest at night, normal mode may lead to increased thinning, while positive mode could mitigate some of the thinning.

On average, children eight to 10 years old spend an astonishing eight hours per day looking at screens, and older children increase this further to over 11 hours (Rideout et al. 2010). Understanding how manipulations to screen polarity affect emmetropization presents a major opportunity for myopia control. Ultimately, these measurements could support the clinical recommendation that young observers, whose eyes remain plastic, use dark modes during night hours when the choroid is at its thinnest.

4.3 Methods

4.3.1 Observers

We recruited nine observers from the New England College of Optometry. Subject ages were between 22 and 26 (mean age 24.2 ± 1.39). Observers were screened to exclude patients with a history of ocular disease, ortho-k lens wear, or ocular surgery. Spherical equivalent

refractive errors ranged from -8 to 0 D (mean -4.11 ± 2.67 D). Axial length ranged from 20.9 to 27.9 mm (mean 25.4 ± 1.32 mm). The self identified gender makeup was six female and three male participants.

4.3.2 Apparatus

Similar to our first experiment, a Topcon KR-1W wavefront analyzer (Topcon, Tokyo, Japan) was used to determine the patient's refractive error, and a Haag-Streit Lenstar 900 (Haag-Streit, Bern, Switzerland) was used to measure ocular biometry, including choroidal thickness. Testing was completed on a Vpixx 12-bit monitor (ViewPixx, Montreal, Canada) with an average luminance of 65cd/m^2. The subject was seated 114 cm from the monitor with their head stabilized with a chin-forehead rest.

4.3.3 Stimuli and Procedure

Given we were unable to replicate the findings of Aleman et al. (2018) in our previous study, we chose to exclude the DoG and only use the letter stimuli similar to the ones they used. Subjects completed a 10 alternative forced choice letter identification task. The task had seven contrast levels presented randomly via the method of constant stimuli. The letters were in Sloan font and only the letters C, D, H, K, N, O, R, S, V, and Z were used as shown in *figure 2*. Each letter was presented for 240 ms with a concomitant tone. After a letter was presented, a selection screen appeared with all possible letters. The time spent reading the letters on the selection screen was meant to simulate the free reading that subjects performed in the Aleman et al. (2018) study. The subjects used a gamepad to indicate their responses. A high pitched tone was played when the response was correct and a low pitched tone when the response was incorrect. The

experiment was self paced as the subjects press a button on the gamepad when they are ready for the next presentation.

Ocular biometry was performed before and after completing psychophysical testing to measure change in choroidal thickness. Refractive error was measured via autorefraction with the wavefront analyzer at the first visit. Before testing, observers completed 10 practice trials at high contrast to ensure they understood and could perform the task. Subjects underwent six days of morning and evening sessions. Days were broken down into two days of positive stimuli testing, two days of negative stimuli testing, and two days of baseline measurements. On baseline days, subjects were asked to go about their normal routine for an hour in between measurements. Morning sessions were between approximately 8:00 AM and 10:00 AM. Evening sessions were between approximately 4:00 PM and 6:00 PM. The order of positive and negative contrast tasks was block randomized. Each session of psychophysical testing lasted for approximately one hour and consisted of 600 letter identification tasks. Subjects were allowed breaks at any point during testing.

4.3.4 Analysis

As described in the previous study, we used a mixture of Gaussian model applied to the A-scan measurements to calculate choroidal thickness. We isolated peaks on the A-scan representing the RPE and the choroid/sclera border using Gaussian fits and measured the distance between these peaks using arbitrary units. The arbitrary units were then converted into microns. We calculated repeated measures ANOVA for choroidal thickness and psychophysics data. The traditional cut off value of alpha is less than 0.05 was used for significance.

4.4. Result

Change in choroidal thickness did not vary with stimulus condition (F = 0.231, p-value = 0.796) or time of day (F = 1.558, p-value = 0.252). Given stimulus condition, there was a trend towards stimulus conditions having an effect on choroidal thickness change, but the value was not significant (F = 2.343 p-value = 0.133). There was no detectable effect of time of day (F = 0.009, p-value 0.928) or stimulus polarity (F = 0.200, p-value = 0.666) on contrast threshold . Given time of day, stimulus polarity did not have a detectable effect on contrast threshold (F = 0.004, p-value = 0.952).

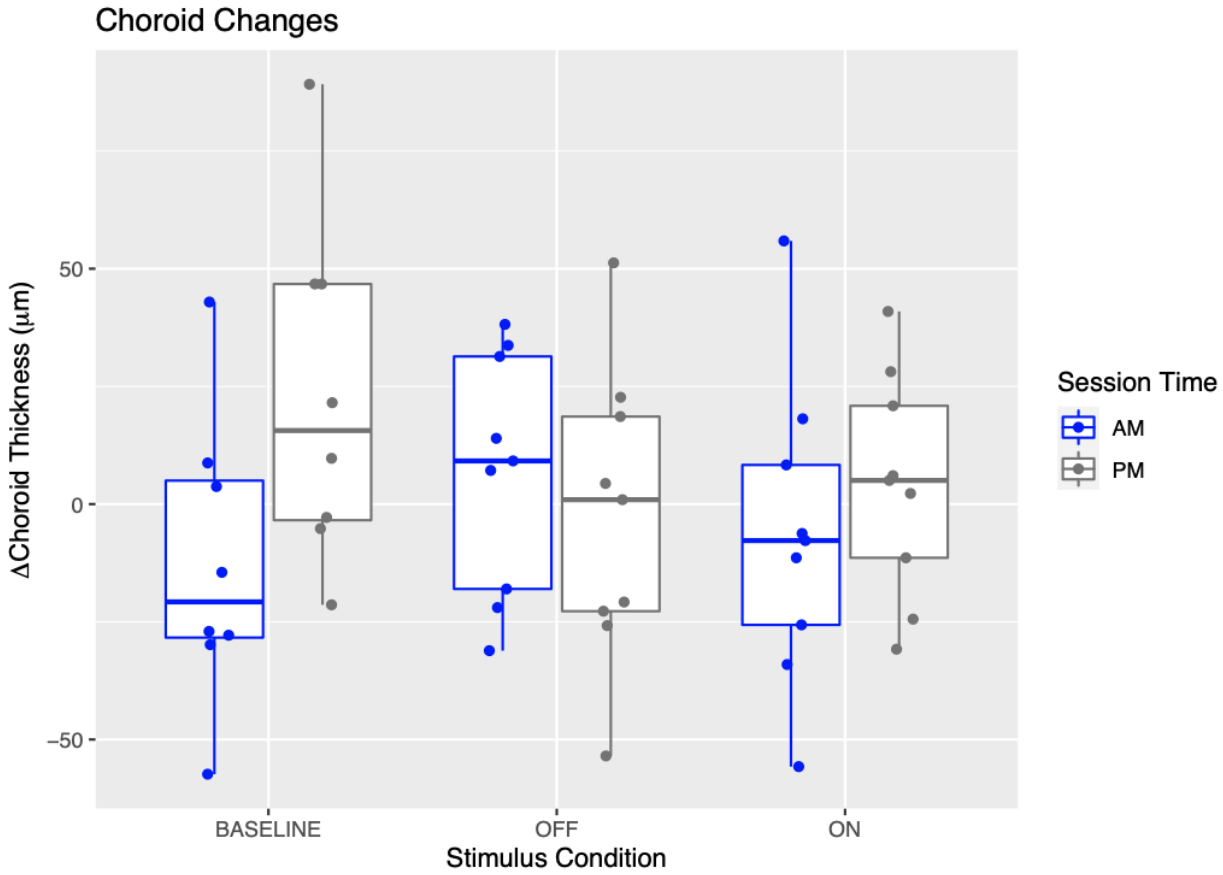


Figure 11: Change in choroid thickness is plotted as a function of stimulus conditions for the morning (blue) and evening (gray) sessions.

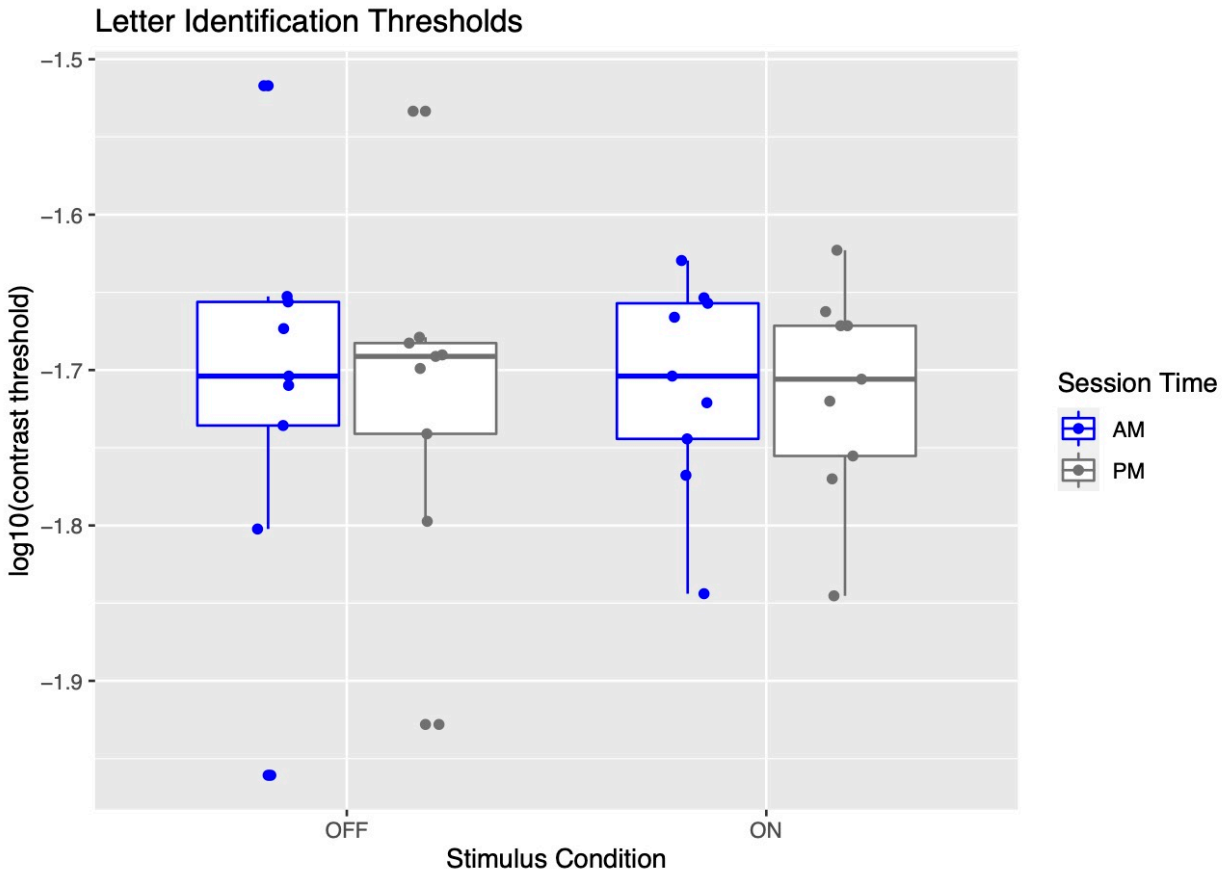


Figure 12: Contrast threshold to letter targets is plotted as a function of ON and OFF stimulus conditions for morning (blue) and evening (gray) sessions.

4.5 Discussion

In summary, we found time of day had no detectable effect on changes in choroidal thickness after exposure to positive and negative contrast. We also found no detectable difference in contrast thresholds between ON and OFF stimuli in the morning and evening. Shortcomings of this experiment include a small subject pool of only nine observers.

Qualitatively there was a trend towards thickening for evening baseline measures and thinning for morning baseline measures. It may be that we are picking up the thinning taking place after reaching peak thickness in the morning and thickening after reaching the thinnest

measurement in the evening. This trend was not observed with exposure to positive or negative stimuli that lead to choroidal thickness closer in value for morning and evening.

One hour long sessions may be too short to observe a change in thickness. If we stretched our sessions out to a period longer than an hour, we may have seen a change in the choroidal thickness secondary to expected diurnal fluctuations. Previous research measuring diurnal choroidal thickness every two hours found a change within this time interval (Tan et al. 2012).

We used the letter target to more closely replicate the Aleman et al. (2018), but it is possible, given our findings of greater asymmetry in contrast thresholds to positive and negative contrast, the DoG target would have been more appropriate for the contrast threshold portion of this experiment.

Overall, we are unable to make any recommendations for the contrast polarity of screens in the morning and evening. In order to more closely replicate the Aleman et al. (2018) study, it would be beneficial to repeat this experiment using free reading with the page being filled with letters of a certain polarity.

5. General Conclusion

There was a positive correlation between axial length and threshold for the letter and DoG targets for both positive and negative contrast stimuli. Results remained true if refractive error was used instead of axial length. The rate of increase in threshold is greater for OFF than ON DoG stimuli. The asymmetry in the rate of contrast dependent threshold increase with axial length and refractive error was not observed for our letter targets.

Asymmetries in DoG target thresholds in axial myopes adds to growing evidence that post-receptoral processing of myopes is faulty. In our study, one of the pathways affected appears to be the ON/OFF visual pathway. Understanding the mechanism leading to changes in the ON/OFF post receptoral processing will help uncover the deficits leading to failed emmetropization. We hypothesize reduced sensitivity or increased internal noise within the OFF pathway leads to a dysregulation of the homeostatic set-point and leads to stop signals for eye growth to be ignored.

Associations of faulty visual processing with changes in choroidal thickness are unclear. Choroidal thickness increased when ON stimuli were used (although the effect was small), but no detectable changes occurred to OFF stimuli. The observed lack of choroidal thinning following OFF stimulation is inconsistent with previous research (Aleman et al. 2018; Hoseini-Yazdi et al. 2021; Wang et al. 2019).

As children spend nearly a third of their day on screens, understanding the role of contrast polarity in emmetropization opens massive opportunities for intervention (Rideout et al. 2010). Although we did not find substantial anatomical changes, future research investigating the asymmetries in contrast threshold to positive and negative contrast of young subjects may reveal effective suggestions for alterations to the visual diet as to provide a protective effect against the

development of myopia. For example, if displayed content could be tuned to better match the contrast characteristics present in an outdoor environment, the same protective effect against myopia development could be observed.

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Supplemental Material

Demographics ON/OFF pattern detection and Choroidal changes following ON/OFF Stimulation

Subject	Sex	Age	Myopia Age	Axial OD	Sphere OD	Cyl OD	Axis OD	SE OD	Axial OS	Sphere OS	Cyl OS	Axis OS	SE OS	Session 1 and 2	Session 3 and 4
1	M	23	8	26.05	-6.75	-0.25	114	-6.875	25.96	-6.50	-0.25	157	-6.625	afternoon	afternoon
2	M	42	16	23.99	-3.75	0.00	0	-3.750	24.11	-3.75	0.00	0	-3.750	afternoon	afternoon
3	M	24	12	24.96	-2.75	0.00	0	-2.750	24.77	-2.50	-0.50	84	-2.750	afternoon	morning
4	F	36	14	23.29	-2.50	-0.25	57	-2.625	22.72	-1.25	0.00	0	-1.250	afternoon	afternoon
5	F	23	12	23.30	0.00	-2.00	177	-1.000	23.73	-1.00	-1.75	1	-1.875	morning	morning
6	F	23	16	22.99	-0.50	-0.50	96	-0.750	23.07	-1.25	-0.25	67	-1.375	morning	morning
7	F	24	NA	22.95	0.50	-0.50	166	0.250	22.94	0.25	-0.25	6	0.125	morning	afternoon
8	F	23	12	25.69	-3.50	-1.00	172	-4.000	25.72	-3.25	-0.25	38	-3.375	morning	morning
9	F	23	5	20.91	6.75	-0.50	117	6.500	20.96	6.50	-0.25	73	6.375	afternoon	afternoon
10	F	24	16	23.14	-0.75	-1.00	118	-1.250	23.13	-0.75	-0.50	95	-1.000	afternoon	afternoon
11	M	24	7	25.49	-1.25	-7.00	3	-4.750	25.32	-1.00	-6.00	177	-4.000	afternoon	morning
12	M	24	23	26.95	-6.50	-0.50	9	-6.750	26.83	-5.75	-0.75	162	-6.125	afternoon	afternoon
13	F	24	NA	22.63	0.00	-0.25	94	-0.125	22.62	0.00	0.00	0	0.000	afternoon	afternoon
14	M	28	12	24.42	-3.00	-0.50	5	-3.250	24.49	-2.75	-0.25	180	-2.875	morning	afternoon
15	F	25	11	21.46	4.00	-1.00	14	3.500	21.37	4.50	-1.00	178	4.000	afternoon	afternoon
16	F	26	12	25.69	-4.25	-0.75	73	-4.625	25.54	-3.75	-0.75	93	-4.125	afternoon	afternoon
17	M	23	7	25.86	-6.50	0.00	0	-6.500	26.31	-7.50	-0.50	94	-7.750	morning	morning
18	F	26	NA	22.18	0.00	-0.25	159	-0.125	22.04	0.00	-0.25	1	-0.125	morning	afternoon
19	F	23	6	27.64	-7.75	-1.50	37	-8.500	27.65	-7.25	-2.25	37	-8.375	morning	afternoon
20	M	22	12	26.59	-4.00	-0.75	16	-4.375	26.25	-4.00	-1.00	173	-4.500	afternoon	afternoon
21	F	27	5	27.00	-11.00	-0.25	18	-11.125	27.28	-12.00	-0.25	137	-12.125	afternoon	afternoon
22	F	25	11	24.92	-4.25	-0.25	92	-4.375	24.78	-4.25	-0.25	72	-4.375	afternoon	afternoon
23	M	25	7	25.44	-5.75	-0.50	14	-6.000	25.55	-6.00	-0.50	155	-6.250	afternoon	morning
24	M	23	15	25.80	-1.75	-0.25	117	-1.875	25.57	-1.25	-0.25	48	-1.375	afternoon	morning
25	F	23	21	24.79	-5.75	-0.25	129	-5.875	24.50	-5.25	-0.25	78	-5.375	morning	afternoon
26	F	24	10	25.95	-2.00	-0.50	161	-2.250	25.90	-2.00	-0.50	167	-2.250	morning	morning
27	F	23	NA	24.84	0.00	0.00	0	0.000	24.73	0.25	0.00	0	0.250	morning	afternoon
28	F	23	8	26.49	-4.25	-0.25	25	-4.375	26.20	-3.25	-1.25	160	-3.875	morning	morning

29	M	30	10	23.71	-1.25	-1.00	87	-1.750	23.76	-1.75	-0.75	99	-2.125	afternoon	afternoon
30	F	24	18	23.82	-0.75	-0.50	96	-1.000	23.65	-1.25	-0.25	25	-1.375	morning	afternoon
31	F	24	12	24.54	-5.75	-1.00	162	-6.250	24.37	-4.50	-1.50	5	-5.250	afternoon	afternoon
32	M	23	8	27.52	-8.25	-0.50	11	-8.500	27.50	-8.25	-0.75	9	-8.625	afternoon	morning
33	F	23	10	25.92	-5.75	-0.25	141	-5.875	25.74	-4.75	-1.00	174	-5.250	afternoon	afternoon
34	F	23	10	23.23	-2.25	-0.50	177	-2.500	23.28	-2.25	-0.25	26	-2.375	afternoon	afternoon
36	M	23	11	25.60	-1.00	-0.50	123	-1.250	26.03	-2.50	-0.25	167	-2.625	afternoon	afternoon
37	M	25	12	25.76	-4.75	-0.25	22	-4.875	25.02	-3.00	-0.25	169	-3.125	afternoon	afternoon
38	F	25	6	27.88	-11.50	-0.75	152	-11.875	27.50	-10.50	-0.25	53	-10.625	afternoon	morning
39	F	26	13	25.19	-4.50	-0.25	15	-4.625	25.15	-4.50	-1.00	172	-5.000	morning	morning

Demographics for Diurnal choroidal thickness changes following ON/OFF stimulation

Subject	Sex	Age	Axial OD	Sphere OD	Cyl OD	Axis OD	SE OD	Axial OS	Sphere OS	Cyl OS	Axis OS	SE OS
1	M	25	26.05	-6.75	-0.25	114	-6.875	25.96	-6.50	-0.25	157	-6.625
2	F	24	25.50	-4.50	-0.50	4	-4.750	25.36	-3.00	-0.50	19	-3.250
3	F	24	24.70	-3.50	-1.75	94	-4.375	24.89	-3.75	-1.50	91	-4.500
4	M	26	25.86	-5.25	-0.50	7	-5.500	25.09	-2.75	-0.75	158	-3.125
5	M	22	24.60	-0.25	-0.50	78	-0.500	24.48	-0.25	-0.50	96	-0.500
6	F	23	24.84	-3.50	-0.25	150	-3.625	24.83	-3.25	-0.75	17	-3.625
7	F	26	28.49	-8.00	-1.25	7	-8.625	28.27	-8.25	-1.75	2	-9.125
8	F	25	23.50	0.00	-0.25	76	-0.125	23.41	-0.25	0.00	0	-0.250
9	F	23	25.88	-4.25	-0.75	166	-4.625	25.52	-3.50	-1.00	12	-4.000