Regional Differences in the Relationship Between Retinal Structure and ON-OFF Pathway Function in Myopic Patients

By

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Thesis

In partial satisfaction of the requirements for the degree of

Master of Science in Vision Science

State University of New York

College of Optometry

May, 2023

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Abstract

Purpose: The purpose of this study was to measure the effect of myopia on ON and OFF pathway asymmetries displayed between 5° to 30° of eccentricity and examine the structure-function relationship between retinal thickness and visibility of light and dark stimuli in eccentric quadrants of myopic eyes.

Methods: Eighteen eyes were randomly selected from human subjects and all myopic subjects underwent testing with habitual soft contact lens correction. Subjects underwent ON-OFF perimetric testing in the test eye. The complete procedure is referenced and discussed in the body of the manuscript. Stimuli were presented at various contrasts across 30-degrees of the visual field and stimuli increased in size as a function of eccentricity. Structural and functional testing, including ultra-wide field macular optical coherence tomography (OCT), 30-2 static automated perimetry (SAP) mean sensitivity, peripheral autorefactive (AR) measurements, and axial length (AL), were also measured. All testing, except axial length measurements, were taken with subjects fully corrected in soft contact lenses.

Results: There was a statistically significant positive correlation between AL and combined light and dark errors across the entire testing area of 5-30° (p=0.0019) as well as each eccentric range (5-10° p=0.0389; 11-20° p=0.0015; 21-30° p =0.0008). There was a statistically significant positive correlation between errors to light stimuli as a function of AL across the entire testing area of 5-30° (p=0.0251), 11-20° (p=0.0207) and 21-30° (p =0.0178). There was a statistically significant positive correlation between AL and dark stimuli errors across the entire testing area of 5-30° (p=0.0461), 11-20° (p=0.0424) and 21-30° (p =0.024). There was no statistically significant correlation when analyzing errors to light and dark stimuli separately at the 5-10° eccentricity. There was a statistically significant negative correlation between RE and combined light and dark errors across the entire testing area of 5-30° (p=0.0444) and a statistically significant negative correlation at the most peripheral eccentric range of 21-30° (p=0.0128). When analyzing errors to light and dark stimuli separately as a function of RE, there was no statistically significant correlation (entire field dark p=0.19, light p=0.1519; 5-10° dark p=0.429, light p=0.4421; 11-20° dark p=0.2449, light p=0.1529; 21-30° dark p=0.0823,
light p=0.087). Subjects displayed higher errors to light stimuli over the entire testing area 5-30° (p=0.0166) and 21-30° (p=0.0007), but not at 5-10° or 11-20° (5-10° p=0.7043; 11-20° p=0.2572). Total retinal thickness (TRT) ranged from 265.44 ± 18.85 microns in the interotemporal (IT) quadrant to 213.28 ± 11.34 microns in the superonasal (SN) quadrant (p = 3.81 x 10^-7, Wilcoxon test for IT vs SN comparison). The quadrant with the greatest average retinal thickness (IT) was associated with the lowest %errors (6.45 ± 6.56) and highest visual field mean sensitivity (VFMS, 30.9 ± 1.08 dB), whereas the quadrant with the least average retinal thickness (SN) was associated with the highest % errors (22.77 ± 15.93, p=8.91 x 10^-9 for IT vs SN comparison) and among the lowest MS (29.43 ± 1.34, p=0.0020 for IT vs SN comparison).

Conclusion: Higher levels of myopia are associated with greater response errors during ON-OFF perimetric testing, with higher error rates in response to light targets compared to dark targets. Both findings are most pronounced at the 21–30-degree eccentricity and have a stronger correlation with axial length compared to refractive error. Higher rates of error on ON-OFF perimetry correspond to thinner retinal thickness in the corresponding retinal quadrant. The highest average percent errors on ON-OFF perimetric testing were present in the superonasal visual field, which coincides with the thinnest total retinal thickness in the corresponding region of the retina (interotemporal). Better understanding of the structural and corresponding functioning relationship between ON-OFF perimetric testing and retinal thickness may further enhance our understanding of myopic refractive development.
INTRODUCTION:

Myopia (near-sightedness) has been a topic of scientific study for over four hundred years but has only recently been recognized as a significant public health risk (Flitcroft et al., 2019). The rise in global prevalence of myopia is so significant that the World Health Organization predicts that half of the global population will be myopic by the year 2050 (Morgan et al., 2012; Wang et al., 2021). In China and Seoul, 90-95% of teenagers and young adults are myopic (Zhou et al., 2017). Uncorrected refractive error was estimated to be the most common cause of distance vision impairment and the second most common cause of global blindness after cataracts (Bourne et al., 2013). This is especially relevant considering the recent surge in myopia since the start of the COVID-19 pandemic. Several proposals made to explain this increase include increased screen time, accommodative response with prolonged near work, reduced exposure to sunlight, more indoor ambient and digital screen lighting and longer time between eye exams (Rosenfield, 2022).

Although blurred vision secondary to myopia may be managed with corrective lenses or refractive surgery, high myopia substantially increases the risk of potentially blinding sequelae (Sherwin et al., 2012). Ocular health risks associated with high myopia may be related to excessive axial elongation, which may increase the risk for developing conditions such as glaucoma, retinal detachments, choroidal neovascularization, foveoschisis, and early onset cataracts (Flitcroft et al., 2019; Sherwin et al., 2012). Risk for myopia development in a pediatric population includes parental myopia, with a six-fold increase in risk if both parents are myopic, ethnicity, age of onset and time spent
outdoors, which highlights the risk imposed both by genetic predisposition as well as environmental exposure (Recko & Stahl, 2015).

The vertebrate retina consists of a large diversity of cells that form distinct circuitry that work in parallel to produce complex visual output (Hoon et al., 2014; Palczewski et al., 2000). It is a stratified, layered structure beginning with the rod and cone photoreceptors, which initiate the process of converting light energy into electrochemical signals to be interpreted by the visual centers in the brain. The photoreceptors, which synapse with each other as well as horizontal cells, relay information to bipolar cells and the signal continues directly or indirectly (via amacrine cells) to the ganglion cells (Hoon et al., 2014; Rice et al., 2001).

Reduced light exposure has been shown to be associated with myopia progression. Conversely, increased time spent outdoors may offer some level of protection against myopic progression (Pons et al., 2017; Smith et al., 2012), which may be related to increased retinal dopamine release (Rose et al., 2008; Zhang et al., 2007).

Dopamine is a necessary neurotransmitter that mediates various retinal functions including, visual signaling and refractive development. Although extensive studies do support the important role of dopamine in mediating myopia development, pharmacology studies are limited by the partial specificity in various species used in myopia research (Zhang et al., 2007; Zhou et al., 2017). Retinal dopamine synthesis has a graded response to increasing irradiances of white light and retinal dopamine levels have been shown to be reduced in form deprivation myopia (Brainard & Morgan, 1987; Rohrer et al., 1993). Stone et al. (1989) found that administration of agents known to interact with dopamine receptors in deprived chick eyes cause a reduction in axial growth and when dopamine
agonists are administered alone, it decreases axial growth in a dose-dependent manner (Stone et al., 1989). Dopamine also mediates adapting light/dark retinal circuits and serves as a regulator of the retinal circadian clock (Witkovsky, 2004).

There is much interest in examining the structural and refractive differences that exist in myopic eyes. There is an abundance of literature assessing choroidal thickness in myopes, as thinner choroids have been demonstrated to precede myopia onset and progression (Read et al., 2013). Several studies have reported regional changes in retinal thickness of the macula of myopes, however it is unclear if there is retinal thickening or thinning within the peri-foveal region in myopic eyes (Kim et al., 2020; Lam et al., 2007; Song et al., 2014; Wu et al., 2008). Analysis of the peripheral refractive changes in myopic eyes have also been investigated for myopia control methods, such as orthokeratology and soft contact lenses that induce peripheral hyperopic defocus. Studies have identified regional differences in peripheral refractive error in myopic eyes compared to emmetropic and hyperopic eyes (Atchison et al., 2006; Yelagondula et al., 2022). Understanding these changes that are seen in myopic eyes, particularly those with progressing myopia, are of great importance to better identify those at risk of developing high myopia to prevent potentially vision threatening changes.

Previous work (Chakraborty et al., 2015; Pons et al., 2017) has suggested neural pathways that respond to local luminance changes, the ON and OFF pathways, may influence refractive development. Animals that use vision to navigate through their environment depend on these two parallel visual pathways to detect luminance changes. The ON pathway responds to local luminance increments, or highlights, whereas the OFF pathway responds to local luminance decrements, or shadows, in visual space or within
an image (Jansen et al., 2019). These two pathways begin with retinal signaling in the eye
then converge in the visual cortex where they combine information for higher level
processing and interpretation (Nelson & Kolb, 2004). The only known dopamine
synthesizing neuronal cells in the retina are dopaminergic amacrine (DA) cells. Studies
have suggested that light can increase DA cell activity by stimulating rod and cone
photoreceptors and melanopsin-expressing intrinsically photosensitive retinal ganglion
cells (ipRGCs) (Qiao et al., 2016). DA cells and ipRGCs are ON pathway driven,
providing a potential link between myopia and ON pathway activity. If myopia
progression is associated with a deficit in retina dopamine, visual environments that that
lead to weaker ON responses may also lead to greater myopia progression (Pons et al.,
2017; Vuong et al., 2015).

The ON and OFF pathways begin at the level of the ON and OFF cone bipolar
cells which signal ON and OFF ganglion cells, respectively. A distinguishing
characteristic of these two bipolar cells is the type of glutamate receptors each expresses.
ON bipolar cells express metabotropic glutamate receptors (mGluR6) as well as
 glutamatergic ionotropic receptors while OFF bipolar cells express a variety of ionotropic
 glutamate receptors (Chalupa & Günhan, 2004). In a light adapted state at rest, cone
photoreceptors are continuously releasing glutamate, which stimulates the mGluR6
receptors of the ON bipolar cells which cause hyperpolarization. Light stimulation
hyperpolarizes cone photoreceptors, inhibiting glutamate release leading to
depolarization of the ON bipolar cells initiating ON pathway stimulation. The opposite is
ture for OFF bipolar cells; light decrements lead to increase glutamate release from cone
photoreceptors, further inhibiting the ON bipolar cells (hyperpolarization) but stimulating

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the OFF bipolar cells (depolarization) and the OFF pathway (Chalupa & Günhan, 2004; Snellman et al., 2008).

There are several important differences between the ON and OFF pathways. ON pathways have a peak response to a light stimulus, matching the ON center receptive field size, on a dark background, whereas OFF pathway displays peak response to dark stimuli on a light background (Chalupa & Günhan, 2004; Jansen et al., 2019; Nelson & Kolb, 2004). There is an apparent OFF dominance in both human and animal models, as the OFF pathway covers more cortical territory, makes stronger connections, and drives stronger cortical responses compared to the ON pathway (Pons et al., 2017). The ON and OFF pathways also terminate within separate strata of the retinal inner plexiform layer (IPL), with OFF ganglion cell dendrites being localized in the more distal region of the IPL and the ON localized in the more proximal region of the IPL (Chalupa & Günhan, 2004). Neuronal blur, explained by Pons et al in 2017, is the enlargement of light (ON pathway) stimuli in high contrast conditions. This is due to the earlier saturation of the luminance-response of the ON pathway in high contrast conditions and is exacerbated in conditions that are known to be associated with myopia progression (Kremkow et al., 2014; Pons et al., 2017). Pons et al suggested that, due to the ON pathway characteristics, the enlargement of light stimuli in conditions such as optical blur or dim lighting conditions could cause decreased ON pathway stimulation leading to decreased retinal dopamine levels and myopia progression.

Asymmetries between ON and OFF pathway functioning in myopia have been shown, primarily using electrodiagnostic testing such as electroretinograms (Brainard & Morgan, 1987; Chakraborty et al., 2015; Pardue et al., 2008; Zhang et al., 2007).
Neuronal blur, which is suggested to play a role in OFF cortical dominance, is exacerbated in conditions associated with myopia progression, i.e. optical blur and dim lighting conditions (Pons et al., 2017). Genetic links between ON pathway functioning and high myopia exist in nature, such as in congenital stationary night blindness type 1, as well as in experimental animal models (Chakraborty et al., 2015; Pardue et al., 2008; Zhang et al., 2007). The purpose of this study was to measure the effect of myopia on ON and OFF pathway asymmetries in an effort to further our understanding of myopic refractive error development. We asked observers to respond to light and dark targets on a binary noise background in a virtual reality environment. To assure accurate fixation and consequently, assurance of the retinal location of the presented stimuli, the observer’s fixation was restricted to the central 5° of vision. Structural and functional testing, including ultra-wide field macular optical coherence tomography (OCT), 30-2 static automated perimetry (SAP) mean sensitivity, peripheral autorefractive measurements, and axial length, were measured for further analysis.

MATERIALS & METHODS:

Subjects:

Eighteen human subjects (14 females/4 males) were recruited. The asymmetric distribution in males versus females in our study reflects the asymmetry of greater females enrolled in optometry school compared to males. Subjects’ axial length ranged from 23.04 mm to 28.75 mm (mean: 26.03 ± 1.53) and spherical equivalent (SE) refractive error (RE) ranged from emmetropia to -9.50 D (mean: -5.35D ± 2.79). One eye per subject was randomly chosen and tested. The inclusion criteria for this study required an absence of known eye diseases, Snellen best-corrected visual acuity (BCVA) of 20/20, and under the age of 35 years old (mean 25.90 years old ± 2.77). All subjects had to have
a comprehensive eye exam within the past two years prior to the start of the study and performed testing in habitual distance soft contact lenses. All experiments were approved by the Institutional Review Board at the State University of New York College of Optometry and followed the principles outlined in the Declaration of Helsinki.

**Procedures**

*ON-OFF perimetry*

ON-OFF perimetry is a test developed in our lab (Rahimi-Nasrabadi et al., 2023) to measure ON and OFF pathway function at various positions of the visual field.

Prior to perimetric testing, all observers were seated comfortably, received instructions to properly adjust the headset position, inter-pupillary distance, and perform the ON-OFF perimetry test. The observer’s saccades were measured in response to a moving target presented at five different locations in the display to calibrate eye position. The headset adjustments and eye-tracking calibration lasted less than one minute and were followed by approximately 30 seconds of practice trials and adaptation to background luminance.

The observer wore a head-mounted visual display (HTC Vive Pro Eye, refresh rate: 90 Hz, maximum luminance: 112 cd/m2) that presented stimuli in various parts of the visual field (image 1). All stimuli were presented monocularly in one of the two independent screens in the head-mounted display, while the other eye was presented with a black screen. Observers responded to stimuli presented in the right or left hemifield by pushing a button with their right hand or left hand, respectively. All myopic subjects underwent testing with habitual soft contact lens correction.
The stimuli were generated by Unity 2018 custom software and were light or dark squares. The stimulus size varied as a function of visual eccentricity (minimum of 2.5 degrees/side at 5 degrees and maximum of 4 degrees/side at 30 degrees) (image 3). The following power-law function was used to determine the size of the stimuli as eccentricity increased:

\[ \text{stimulus size} = \text{minimum scale} \times (\text{eccentricity}/5)^\alpha \]

Stimulus size was measured in degrees per side and visual eccentricity (distance from foveal vision) was measured in degrees. Alpha is the exponent which sets the increase in the size of the stimulus as the target is presented at points further from foveal focus. For the control and full visual field analyses, the minimum scale was initially set at 2.0° with an alpha of 0.4, however, the central stimuli with these parameters were determined to be too small after several control patients. The stimulus size parameters were then changed to a minimum scale of 2.5° and an alpha of 0.26. Stimuli were superimposed over a noisy background made of 131,072 equidistant triangles with 0.5 degree/side that tiled a sphere centered around the observer and changed randomly every trial. The absolute luminance difference from mid-gray was the same for dark and light stimuli.

Observers were told to fixate on a central blue dot while peripheral stimuli were presented at random locations in the visual field. The observer’s fixation had to be maintained within a central circular radius of ± 2.5 degrees. If the observer looked outside of this window, the blue circle turned red, stimulus was aborted (response not recorded), and repeated later in the test (images 2 and 3). If the observer did not see a...
stimulus, they were instructed to wait until the next stimulus appeared. Non-seeing trials (catch trials) were included to measure the rate of false positive responses. Target eccentricities were arranged within 5 to 30 degrees in the nasal visual field and 5 to 25 degrees in the temporal visual field in 5-degree steps.

Both superonasal and inferonasal quadrants were sampled with more stimulus positions (24 each) than superotemporal and inferotemporal quadrants (20 each) to allow future comparisons with traditional clinical perimetry. The blind spot and its mirror nasal hemifield position was sampled with a small stimulus size (2 degrees). In total, 90 different stimulus positions were sampled (20x2 temporal +24x2 nasal +1x2 blind spot/mirror =90) (image 4). Each visual field point was sampled six times, three times with a dark stimulus and three times with a light stimulus. The blind spot was sampled six times for each of the light and dark stimuli. Therefore, the test included a total of 579 trials (90x6 test points + 2x6 blind spot/mirror+ 27 catch trials = 579 trials).

Each subject ran the ON-OFF perimetry test over several visits at eight different Michelson contrasts (5%, 6%, 7%, 8%, 9%, 10%, 15% and 20%), starting with the highest stimulus contrast (20% contrast). As the subjects became familiar with the test, they were tested at increasingly lower contrasts. Most subjects completed two contrasts per visit (approximately one hour per visit) and completed the test for the 8 different contrasts in approximately 4-5 visits.

Refractive measurements

Residual central and peripheral refractive error was measured in the test eye of each subject using a Grand Seiko WAM-5500 autorefractor. Peripheral refractive error measurements were considered for the impact of peripheral blur on ON-OFF perimetric
testing. Subjects sat 10 feet away at eye level from a central fixation target wearing habitual distance contact lens correction. Fixation targets were arranged in primary gaze as well as four peripheral targets 22.5° away from the central target at 45°, 135°, 225°, and 315° quadrants (image 5). Ten autorefraction measurements were taken while the subject was fixating on each of the 5 fixation targets. An average of all refractive measures was provided by the WAM-5500 autorefractor printout for each location.

Visual field testing & axial length

Subjects underwent static automated perimetric testing using the 30-2 threshold program (stimulus: White White, background: 31.5 asb, strategy: SITA-Standard) of the Humphrey 750i Visual field device (Carl Zeiss Ophthalmic System, Humphrey Division, Dublin, California USA) wearing habitual distance soft contact lens correction. Reliability criteria included, <30% fixation losses and < 20% for false positive and false negative responses. Visual field mean sensitivity (VFMS) was calculated for three eccentric rings of 0-10 degrees, 11-20 degrees and 21-30 degrees. Further analysis of peripheral visual field mean sensitivity included isolating 21-25 degrees eccentricity for comparison between ON-OFF perimetry and 30-2 visual field.

The central axial length (AL) of each subject’s test eye was measured using the Lenstar LS900 (Haag-Streit USA, Mason OH).

Optical coherence tomography & segmentation

Wide field (55° diameter) spectral domain optical coherence tomography (OCT) scans were measured for peripheral retinal thickness analysis. Subjects were wearing their habitual distance contact lenses during image acquisition. Scans were acquired using the OCT Spectralis OCT and an Ultra-Wide Field lens attachment (Heidelberg...
Eight high-resolution, dense preset radial macular scans were acquired on the test eye for each subject. Each radial scan was separated by 22.5 degrees.

Retinal layer segmentation was performed using neural networks, each trained on a DeepLab v3+ network based on Resnet-18 (Srinivasan, Das, & Patel, 2022). The two best quality scans for each test eye were chosen and the data was extrapolated from the segmentation in an excel database. Segmentation and scan quality at the further eccentricities, especially in those with higher levels of myopia, was poor. Because of this, the furthest eccentricity that could be consistently segmented accurately was approximately 20 degrees (either 19.31 or 20.45 degrees depending on the eye and nasal versus temporal quadrants) away from the fovea (image 6).

The measurement of interest using this segmentation software was the total retinal thickness (TRT), defined as the distance between the inner limiting membrane (ILM) and Bruch’s membrane (Srinivasan et al., 2022). The TRT at 20 degrees eccentricity of the three radial scans taken in the superonasal (SN), inferonasal (IN), superotemporal (ST), and inferotemporal (IT) were averaged. This was done twice, once for each of the two best quality scans. The average of the two SN, IN, ST, and IT TRT was taken for a final TRT of each of the four quadrants for the subject’s test eye.

RESULTS:

Central and Peripheral Refractive Error

Average spherical equivalent residual peripheral and central RE in diopters (D) was -0.32 +/- 0.92 and -0.10 D ± 0.39 respectively (figure 1). The average spherical
equivalent RE in upper-right gaze, upper-left gaze, lower-right gaze, and lower-left gaze was -0.05 D, -0.23 D, -0.38 D, and -0.61 D, respectively.

**Visual Field 30-2 Mean Sensitivity**

The relationship between VFMS and both AL as well as spherical equivalent RE was analyzed (figure 2, middle and right). No significant correlation between VFMS and AL (p=0.4513) or RE (p=0.5412) at the 21-30° eccentric range was found. There was a significant negative correlation between all errors in percent of ON and OFF responses of ON-OFF perimetric testing and VFMS at 21-30° eccentricity (p=0.0214) (figure 2, left).

**ON-OFF Perimetry and Myopia**

The relationship between higher levels of myopia (both axial length and spherical equivalent RE) and error rates of ON-OFF perimetric testing was analyzed (figure 3). There was a statistically significant positive correlation between AL and combined light and dark errors across the entire testing area of 5-30° (p=0.0019) as well as each eccentric range (5-10° p=0.0389; 11-20° p=0.0015; 21-30° p=0.0008). There was a statistically significant positive correlation between errors to light stimuli as a function of AL across the entire testing area of 5-30° (p=0.0251), 11-20° (p=0.0207) and 21-30° (p=0.0178). There was a statistically significant positive correlation between AL and dark stimuli errors across the entire testing area of 5-30° (p=0.0461), 11-20° (p=0.0424) and 21-30° (p=0.024). There was no statistically significant correlation when analyzing errors to light and dark stimuli separately at the 5-10° eccentricity.

The results of comparing ON-OFF perimetry with refractive error (figure 4) display a statistically significant negative correlation between RE and combined light and
dark errors across the entire testing area of 5-30° (p=0.0444) and a statistically significant negative correlation at the most peripheral eccentric range of 21-30° (p=0.0128). When analyzing errors to light and dark stimuli separately as a function of RE, there was no statistically significant correlation (entire field dark p=0.19, light p=0.1519; 5-10° dark p=0.429, light p=0.4421; 11-20° dark p=0.2449, light p=0.1529; 21-30° dark p=0.0823, light p=0.0871).

When comparing responses to light versus dark stimuli (figure 5), subjects displayed higher errors to light stimuli when compared to dark stimuli over the entire testing area 5-30° (p=0.0166) and 21-30° (p=0.0007), but not at 5-10° or 11-20° (5-10° p=0.7043; 11-20° p=0.2572).

Retinal thickness analysis

At 20° eccentricity, the average TRT ranged from 265.44 ± 18.85 microns in the IT quadrant to 213.28 ± 11.34 microns in the SN quadrant (p = 3.81 x 10^-7, Wilcoxon test for IT vs SN comparison). A significant relationship existed between TRT and both ON-OFF errors and MS. The quadrant with the greatest average retinal thickness (IT) was associated with the lowest %errors (6.45 ± 6.56) and highest MS (30.9 ± 1.08 dB), whereas the quadrant with the least average retinal thickness (SN) was associated with the highest % errors (22.77 ± 15.93, p=8.91 x 10^-9 for IT vs SN comparison) and among the lowest MS (29.43 ± 1.34, p=0.0020 for IT vs SN comparison). A significant negative correlation exists between MS and % errors only between 21-30° (r = -0.38, p=0.0214) (figures 6-7).
DISCUSSION:

The results from this study show that individuals with longer axial lengths and higher levels of myopia displayed higher rates of error to ON-OFF perimetric testing across the entire testing area and most pronounced at the furthest eccentric range tested (21-30°). Higher myopes also had less sensitive to light targets compared to dark targets. This asymmetry was observed over the entire testing area; however, it was most pronounced in 21-30° eccentricity of the visual field. These findings suggest that there may be a decreased sensitivity of the ON pathway compared to the OFF pathway that becomes more significant with higher levels of myopia and at more peripheral retinal areas.

We also show higher rates of error during ON-OFF perimetric testing in visual field quadrants with thinner total retinal thickness. Highest rates of error in superonasal visual space, which corresponded to a thinner average inferotemporal total retinal thickness. Better understanding of asymmetries in ON-OFF pathway functioning and retinal structure may shed light on the cellular mechanisms involved in the development and progression of myopia.

Minor amounts of refractive error were measured both centrally and peripherally, ruling out a potential impact of blur on peripheral stimuli with ON-OFF perimetric testing. Stimuli were scaled as a function of eccentricity using a power-law relationship, suggesting sensitivity should be similar centrally and peripherally. There was a statistically significant relationship between ON-OFF perimetry and standard perimetry at the furthest eccentricity analyzed, which supports that the two tests should provide comparable results. However, there was no relationship between axial length or refractive
error with standard perimetry at the furthest eccentricity measured. This may be due, in part, to differences in the psychophysical task associated with each test. For example, the ON-OFF perimetric stimuli increased in size as a function of eccentricity and is tested at different contrast levels, whereas static automated perimetric stimuli size remains constant, does not include luminance decrement stimuli (OFF pathway) and luminance of stimuli change to measure sensitivity.

ON pathway functioning has been suggested to be affected in several ocular conditions, including myopia (Chakraborty et al., 2015; Pardue et al., 2008; Zhang et al., 2007). Neuronal blur, a characteristic of ON pathways functioning, increases under visual conditions that also are known risk factors for myopia progression (i.e. dim illumination and optical blur), suggesting a possible neuronal mechanism linking myopia progression and ON pathway functioning (Pons et al., 2017). Time spent outdoors is an established protective factor against myopia and may be related to stimulation of retinal dopamine release (Jin et al., 2019; Zhang et al., 2007; Zhou et al., 2017). It has also been postulated that retinal dopamine release is primarily mediated by ON pathway stimulation (Pons et al., 2017; Zhang et al., 2007). Therefore, environments that generate weak ON responses may reduce retinal dopamine release and cause myopia progression (Pons et al., 2017). There is also evidence that certain genetic mutations associated with ON pathway inhibition are also associated with high myopia and have shown decreased retinal dopamine and increased risk for form deprivation myopia (Pardue et al., 2008; Zhang et al., 2007). This evidence further supports the notion that deficits in ON pathway function may play a role in myopia development.
Our results also demonstrate mild changes between central and peripheral (out to 22.5 degrees) spherical equivalent refractive error, however the measurements may be more variable with higher levels of myopia. Other studies have examined the asymmetric refractive profile of myopic eyes, especially with implementation of myopia control methods that target peripheral defocus. One study displayed relative peripheral hyperopia 30 degrees temporally and relative peripheral myopia 30 degrees nasally (Yelagondula et al., 2022). Other studies have consistently found that emmetropes show myopic shifts in the periphery, where most myopes greater than two diopters show relative hyperopic shifts (Atchison et al., 2005; Logan et al., 2004). Atchison et al. (2005) reported that the degree of myopia had more effect on peripheral refraction along the horizontal axis as compared to the vertical.

Several studies exist examining structural differences in myopic eyes. Choroidal thickness has been of particular interest, as thinner choroids have been demonstrated to precede myopia onset and progression (Read et al., 2013). In a longitudinal study, Yelagondula et al. found that as myopia progresses in a pediatric population, there is a decrease in choroidal thickness in all regions within the perifoveal area while the retinal thickness remains unchanged or was thickened (Yelagondula et al., 2022). There are conflicting reports of changes seen in regional retinal thickness of the macula of myopes compared to emmetropes, with some showing no significant change between the two groups as well as thinning in myopic eyes compared to emmetropes (Lam et al., 2007; Wu et al., 2008; Yelagondula et al., 2022). One study of high myopes using spectral-domain OCT found an increase in foveal thickness and a decrease in the inner/outer macular thickness with longer axial lengths. There were also differences between male
and female foveal thickness, with females displaying thicker average foveal thickness, and thinner macular thickness (Song et al., 2014). A more recent study suggests that highly myopic eyes with pathological changes show relevant retinal thinning in the macular region (Kim et al., 2020).

CONCLUSION:
Higher levels of myopia are associated with greater response errors during ON-OFF perimetric testing with higher error rates in response to light targets compared to dark targets. Both findings are most pronounced at the 21–30-degree eccentricity and have a stronger correlation with axial length compared to refractive error. This suggests that reduced ON pathway sensitivity may be related to increases in myopic refractive error. When looking at specific quadrants of ON-OFF perimetric testing, higher rates of error correspond to thinner retinal thickness in the corresponding retinal quadrant. The highest average percent errors on ON-OFF perimetric testing were present in the superonasal visual field, which coincides with the thinnest total retinal thickness in the corresponding region of the retina (interotemporal). Better understanding of the structural and corresponding functioning relationship between ON-OFF perimetric testing and retinal thickness may further enhance our understanding of myopic refractive development.
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Figures/Images

**Image 1:** A lab member demonstrating proper use of the device.

**Image 2:** The device’s visual display during ON-OFF perimetry including the stimulus target, 2.5-degree radius fixation window, and the fixation point that the subject was instructed to view. Note, this image shows a higher contrast than used for this study.
Image 3: An example image of both ON pathway (left) and OFF pathway (right) stimuli shown to the patient during the ON-OFF perimetric test. The observer had to maintain fixation within the 2.5-degree radius circle for stimuli to be presented. The green dots represent the gaze tracking mechanism. Note, this image shows a higher contrast than used for this study.
Image 4: This is a representation of the final data acquired via MatLab. White units represent areas where all tests at that position were responded to accurately. A grey scale represents if either one or two tests were responded to accurately at that position. A black unit represents that zero tests were responded to accurately at that position.
Image 5: Representation of the fixation targets for the subjects during central and peripheral refractive measurements with open-field auto-refractor. The subject would fixate in primary gaze (PG), then at the upper-right (UR) target, upper-left (UL) target, lower-right (LR) target, and the lower-left (LL) targets as refractive measurements were taken while being fully corrected in soft contact lenses.

Image 6: An example of a radial scan taken and segmented on a myopic subject. The radial scans were acquired and segmented using custom MATLAB software to segment the total retinal thickness (TRT), retinal nerve fiber layer (RNFL), and ganglion cell-inner plexiform layer (GCIPL) complex.

<table>
<thead>
<tr>
<th>Avg of each quadrant at 22.5 deg</th>
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<tbody>
<tr>
<td>-0.23</td>
</tr>
<tr>
<td>-0.10</td>
</tr>
<tr>
<td>-0.61</td>
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<tr>
<td>-0.38</td>
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Avg of all peripheral quadrants = -0.32 (std. dev. = 0.92)
Avg of all central = -0.10 (std. dev. = 0.39)
Figure 1: Average residual refractive error in primary gaze as well as the residual refractive error for the upper right, upper left, lower right, and lower left eccentric quadrants 22.5 degrees away from fixation.

Figure 2: Errors in percent of light targets (red), dark targets (blue), and combined light and dark targets (black) as a function of visual field mean sensitivity using a 30-2 Humphrey Sita Standard algorithm at 21–30-degree eccentric range (left). Visual field means sensitivity using a 30-2 Humphrey Sita Standard algorithm at 21–30-degree eccentric range as a function of each subject test eye’s axial length (middle) and spherical equivalent refractive error (right).

Figure 3: Errors in percent to light targets (red), dark targets (blue), and combined light and dark targets (black) as a function of each subject test eye’s axial length over the entire ON-OFF perimetry test area of 0-30 degrees (left), 0-10 degrees (middle left), 11-20 degrees (middle right), and 21-30 degrees (right).

Figure 4: Errors in percent to light targets (red), dark targets (blue), and combined light and dark targets (black) as a function of each subject test eye’s spherical equivalent...
refractive error over the entire ON-OFF perimetry test area of 0-30 degrees (left), 0-10 degrees (middle left), 11-20 degrees (middle right), and 21-30 degrees (right).

**Figure 5**: Comparison of errors in percent to light targets (red) and dark targets (blue) in ON-OFF perimetry over the entire testing area of 5-30 degrees (left), 5-10 degrees (middle left), 11-20 degrees (middle right) and 21-30 degrees (right).

**Figure 6**: Comparison of errors in percent at 21-25 degree from ON-OFF perimetry in superonasal, superotemporal, inferonasal, and inferotemporal visual space as a function of total retinal thickness (in microns) of the corresponding retinal quadrant 20 degrees away from the macula. Horizontal red arrows represent the average of all ON-OFF perimetric errors and the vertical red arrows represent the average OCT total retinal thickness.
Figure 7: Comparison of errors in percent at 21-25 degree from ON-OFF perimetry in superonasal, superotemporal, inferonasal, and inferotemporal visual space as a function of visual field mean sensitivity (in decibels) of the same quadrant.