Feasibility and applicability of a clinical assessment of both the ON and OFF pathways in patients with glaucoma and controls.

By
Veronica Moore-Stoll

Thesis
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Approved by Master’s Thesis Committee:

Mitchell W. D. OD, MS (graduate research advisor)

Jose Manuel Alonso, M.D. PhD (committee chair)

Çagir Zaler, PhD (committee member)

Associate Dean’s Signature:

Stewart Bloomfield, PhD
Associate Dean for Graduate Studies and Research
Abstract

**Purpose:** To assess the feasibility and clinical utility of a head-mounted, On/Off perimetry test and to investigate the effect of early to moderate glaucoma on reaction time and accuracy to ON and OFF perimetric stimuli.

We were able to directly investigate the observer’s responses within their scotomas by restricting the eye movements and placing targets from 5–30˚ eccentricity.

**METHODS**

We tested one eye each of 9 patients with early to moderate primary open angle glaucoma (mean = 71.88 years, std = 5.17), 9 visually-normal control patients of a similar age (mean = 63.88 years, std = 5.17), and 9 visually-normal optometry students (ages 22-25 years). Subjects included 12 patients with early to moderate primary open angle glaucoma (mean=69.75 years, std=7.64), and 12 controls (mean=63.67 years, std=4.25). We used a head mounted display equipped with an eye tracker (HTC VIVE embedded Tobii). Custom software (Unity, version 2017) was used to create the stimuli and a library provided by Tobii Pro was used to measure eye movements at 120 Hz.

Stimulus size changed as a function of eccentricity using a power law relationship: stimulus size= minimum scale*(eccentricity/5)^α. Eye movements were restricted to a central circle with a 2.5 degree radius. **Stimulus contrast was**
initially set to 100%. A single test comprised of 579 trials, including 51 catch trials, presented at 90 different positions in the visual field. Each test location was repeated 3 times for both light and dark stimuli, with 6 repeats in each of two blind spot positions.

Observers were given hand-held response buttons in each hand and were instructed to respond with the response button that corresponded with the side of the visual field on which the stimulus was presented. If the observer did not see a stimulus they were instructed to not respond and wait for the next stimulus to appear. A total of 528 trials, including 24 catch trials, were tested in 84 locations with 3 repeats for both increment and decrement stimuli.

Methods: We asked nine glaucoma patients, nine age-similar controls, and nine young controls to respond to black or white squares presented on a binary noise background while maintaining fixation within the central 5° of the visual field. The targets were presented within 30° of eccentricity from central fixation and the subjects responded to the side of the field on which the target was presented.

RESULTS

Our results demonstrate asymmetry between the two achromatic visual transduction pathways. These results support previous findings that dark targets elicit a faster and more accurate response than light targets, when presented on binary background noise. Our results extend previous work by demonstrating that the two pathways remain asymmetrical in eccentricities up to 30 degree from fixation. We also show that the relationship between the percentage of correct
responses for ON pathway and OFF pathway stimuli follows a power function, wherein glaucoma and controls overlap ($R^2=0.842$) (Figure 1a). This overlap decreases when we quantify only the subthreshold (unseen) increment targets in a linear relationship ($R^2=0.7074$) (Figure 1b). All controls had less than 12% of subthreshold increment targets whereas the percentage of subthreshold targets was higher for 75% of the glaucoma subjects, even in early stages of the disease. (~1.72 dB of visual sensitivity loss). Increment/decrement ON/Off perimetry measured large increment visual deficits in patients with limited loss of visual sensitivity (Figure 1c), which may improve detection of early disease.

Results and Conclusion: We have confirmed asymmetry between the ON and OFF pathways in patients with glaucoma and in control patients. We were also able to refute claims that this asymmetry is found only in the central 5° of the visual field.

CONCLUSION
We have demonstrated that ON/OFF perimetry is feasible in a VR environment and we have confirmed an asymmetry between the ON and OFF pathways in patients with glaucoma and in control patients in both central and peripheral visual fields. We measured on-pathway deficits in patients with limited loss of visual sensitivity which may improve detection of early disease. We were also able to refute claims that this asymmetry is found only in the central 5° of the visual field. On/Off perimetry is feasible in a VR environment. Future work will focus on optimizing stimulus parameters to improve the sensitivity and specificity of this test.

Introduction

There are two segregated pathways that transmit achromatic visual information from the retina to the visual cortex: the ON pathway which responds to light increments in local visual space, and the OFF pathway which responds to light decrements. The pathways do not integrate until the primary visual cortex, where the input layers are comprised of approximately equal quantities of ON-center neurons and OFF-center neurons. The output layers of the visual cortex, however, contain a significant majority of OFF center neurons. Additionally, it has been shown that in the cortical region representing area centralis, 0-5° eccentricity from central fixation, there is a significantly greater number of OFF pathway neurons than ON neurons. Literature has previously shown that this is not the case for cortical regions representing greater than 5°.
eccentricity. This asymmetry in the visual cortex leads to greater accuracy and faster reaction time in response to dark targets than to light targets when tested in a psychophysical model. There is evidence that, in regions of visual cortex representing regions peripheral to 5°, this OFF dominance may be altered.

In the retina, it has been shown that more resources are allocated to OFF retinal ganglion cells than to ON ganglion cells. This asymmetry corresponds with the bias toward light decrements in natural scenery. It has been found that the segregation and asymmetry in the retina may originate as early as the photoreceptor layer of the visual transduction system.

The effects of retinal ganglion cell dysfunction, due to glaucoma, has been previously studied. It was shown that there was a significant difference between the ON and OFF response pathways in both the glaucoma subjects and the control subjects; however, significant differences between the glaucoma group and the control group were not found.

Virtual Reality (VR) technology can be used to present stereoscopic stimuli at different spatial positions of the visual field while carefully monitoring eye position. In this study, this technology is used to investigate asymmetries in responses of the on and off pathways using to-increment (on-pathway oriented) dark and decrement light stimuli (off pathway oriented).

The purpose of this study was to measure the effect of glaucoma on ON and OFF pathway asymmetry in the retina from 5° of eccentricity to 30° of eccentricity. We asked observers to respond to dark-black and light-white targets on a binary noise background in the virtual reality environment. To assure
accurate fixation and consequently, assurance of the retinal location of the 
presented stimuli. **In this model,** the observer’s fixation was restricted to the 
central 5° of vision, **this allowed for less necessary trials for each valid test result** 
and accuracy in the presentation of the targets. **If the patient changed fixation** 
beyond this boundary, the trial associated with this aberrant eye movement was 
not included in the final analysis of the data.

Our results demonstrate that there is a statistically significant difference in 
reaction times between the control subjects and the glaucoma subjects, within 
regions with scotomas. The accuracy of the responses seems to also be affected by 
 glaucoma, although to a less significant extent. Our results also show trends that suggest that the scotomatous regions in the glaucoma patient’s visual 
field showed greater asymmetry between the ON and OFF pathways than the non-
affected areas of the visual field.

**Materials and Methods:**

**Subjects**

We recruited 9 patients with early to moderate primary open angle 
 glaucoma (mean = 71.88 years, std = 5.17), 9 visually-normal control patients of 
a similar age (mean = 63.88 years, std = 5.17), and 9 visually-normal optometry 
students (ages 22-25 years). Only one eye was tested for each subject. The study 
was performed following the principles outlined in the Declaration of Helsinki. 
The inclusion criteria required an absence of known eye disease, except in the 
glaucoma group. For the glaucoma group, the inclusion criteria required a visual 
field recorded within six months of the study.
Apparatus

This study utilized a head mounted display equipped with an eye tracker (HTC VIVE embedded Tobii), which has a refresh rate of 90 Hz, a max luminance of 200 cd/m² and a horizontal field of view of 100°. Customized software (Unity, version 2017) was used to create the stimuli and a library provided by Tobii Pro was used to measure eye movements at 120 Hz. The stimuli created are light or dark squares superimposed on a spherical background.

Procedures

The experiment began with a calibration sequence, during which the observer was instructed to move their fixation to five different presentation positions of a red circle. This calibration enabled the eye tracker to accurately track the participant’s gaze. The eye movement of the observer was restricted to the center of the visual field, within a circle with 2.5° radius. If the participant’s fixation moved outside of the central 5° of the visual field the program ceased to record any responses. Observers were given two hand-held response buttons, one for each hand, and were instructed to respond using the button that corresponded with the side of the visual field on which the light or dark stimulus was presented. If the observer did not see a stimulus, they were instructed to press both response buttons simultaneously or to simply wait two seconds for the next stimulus to appear. Non-seeing trials were included in the experiment in order to ensure that the observer was responding as instructed.
A blue dot (fixation point) and blue circle (fixation window, radius: 2.5 deg) were presented at the center of the visual field. The blue circle turned red and the stimulus trial was aborted when the eye position moved out of the fixation window. While maintaining fixation, a large square target (dark or light) was randomly presented on one out of 90 different positions in the visual field. Target eccentricities were arranged within 5 to 30 degrees in the nasal visual field and 5 to 25 degrees in the temporal visual field (5 degree step size). Participants were instructed to indicate within 2 seconds (press bottom) whether the target appeared in the left or right visual field.

Image 1: The design of our device’s test including the target, fixation window in which a subject’s fixation would still illicit a response, and the fixation point that the subject was instructed to view. Note, this image shows a lower contrast than used for this study.
Observers were given hand held response buttons in each hand and were instructed to respond with the response button that corresponded with the side of the visual field on which the stimulus was presented.

Image 2: A lab member demonstrating proper use of the device.

The background is centered on the cyclopean eye of the observer and is made of dark and light equilateral triangles (0.5°/side). The size of the stimuli changes as a function of eccentricity using a power law relationship:

$\text{stimulus : minimum scale} \times \left( \frac{\text{eccentricity}}{5} \right)^\alpha$

Where the minimum scale is the size of the stimulus, in degrees, at 5° from the fovea, the most central point measured, eccentricity is the distance from foveal vision in degrees, and alpha is the exponential scale in which the size of the
stimulus increases 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 a number of control patients. The stimulus size parameters were then changed to a minimum scale of 2.5° and an alpha of 0.26.

**Image 3:** This is an example image of both ON pathway (left) and OFF pathway (right) stimuli shown to the patient during the test. The central blue circle marks the area of fixation. The green dots represent the gaze tracking mechanism.

A single test comprised of 579 trials, including 51 catch trials, presented at 90 different positions in the visual field (figure 2). Each test location was repeated 3 times for both light and dark stimuli, with 6 repeats in each of two blind spot positions.
The stimuli were shown in 528 trials, including 24 non-seeing trials, at 84 different positions in the visual field, each position repeated three times for each the light and dark trials.

The results were categorized into “Glaucoma Scotoma,” “Glaucoma Normal,” and “Control”. These designations were determined based on the glaucoma subject’s clinical visual field, where “Glaucoma Scotoma” was the quadrant in which the subject was shown to have the most field loss. This quadrant was paired with the corresponding quadrant of the age-similar control eye and was labeled the “Control” region. A third designation, “Glaucoma Normal”, represented a quadrant with little or no clinically significant field loss. All regions where then grouped based on whether the response was to a light or dark trial. The control and glaucoma subjects were paired based on the closest age match available in our sample. Matlab was used to construct all figures of the results and to complete the statistical analyses.
**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.

**Procedures**

The experiment began with a calibration sequence, during which the observer was instructed to move their fixation to five different presentation positions of a red circle. This calibration enabled the eye tracker to accurately track the participant’s gaze. The eye movement of the observer was restricted to the center of the visual field, within a circle with 2.5˚ radius. If the participant’s fixation moved outside of the central 5˚ of the visual field the program would cease to record any responses. Observers were given two hand-held response buttons and were instructed to respond with either the right or left response button that corresponded with the side of the visual field on which the light or dark stimulus was presented. If the observer did not see a stimulus they were instructed to press both response buttons simultaneously or to simply wait two seconds for the next stimulus to appear. Non-seeing trials were included in the experiment in order to ensure that the observer was responding as instructed. The stimuli were...
shown in 528 trials, including 24 non-seeing trials, at 84 different positions in the visual field, each position repeated three times for each the light and dark trials.

**Results** Matlab was used to construct all figures of the results and to complete the statistical analyses. The results were categorized into “Glaucoma Scotoma,” “Glaucoma Normal,” and “Control” based on the phi region of the trials in the experiment. The regions were determined based on the glaucoma subject’s clinical visual field, where “Glaucoma Scotoma” is the quadrant in which the subject was shown to have the most field loss, and “Glaucoma Normal” is a corresponding quadrant where there is very little field loss. The “Control” region is the corresponding “Glaucoma Scotoma” quadrant in the control patient. All regions are then grouped based on whether the response was to a light or dark trial. The control and glaucoma subjects were paired based on the closest age match available in our sample.

Accuracy, recorded as a percent, was compared between each region within the ON and OFF groups, and between the ON and OFF groups between region pairs (Figure 1).
Figure 1: This figure represents accuracy, reported as a percent, for each of the three regions and in response to OFF (blue bars) and ON (red bars) targets. Comparisons were made between each sector within the ON and OFF pathways, then between the ON/OFF pairs of sectors. Significant differences between pairings are based on confidence intervals found with the Clopper-Pearson method with $\alpha=0.05$.

The comparisons that showed statistically significant differences in accuracy - significance for accuracy - for only three of the eight pairs of subjects, are between: OFF Glaucoma Scotoma and OFF Control, ON Glaucoma Scotoma and ON Control, ON Glaucoma Scotoma and OFF Glaucoma Scotoma. All other comparisons were significant in less than three pairs, if at all. These results do suggest a slight trend that the scotoma regions of the glaucoma patients show less accuracy when compared to - are more different from - the corresponding region in the control patients. However, but these findings do not reach statistical significance than between the scotoma regions and the normal regions within-
the glaucoma observers. We have also plotted the differences between the percent accuracy of the OFF and ON pathways within each sector (Figure 2).
The positive values of all but one of these comparisons upholds the-
previous findings that the responses of the OFF pathway are more accurate than the responses of the ON pathway. These results also show a slight trend that the Glaucoma Scotoma sectors have a greater asymmetry between the ON and OFF pathways than the Glaucoma Normal and Control sectors, in which OFF responses tend to be more accurate than ON responses.
Figure 1: This figure represents accuracy, reported as a percent, for each of the three regions and in response to OFF (blue bars) and ON (red bars) targets. Comparisons were made between each sector within the ON and OFF pathways, then between the ON/OFF pairs of sectors. Significant differences between pairings are based on confidence intervals found with the Clopper-Pearson method with $\alpha=0.05$. 
Figure 2: Differences between the accuracy of the OFF pathway and the ON pathway within each analyzed region. The plots represent OFF Accuracy – ON
Accuracy, meaning that a higher OFF response accuracy would result in a positive value in the plot. Statistically significant differences are marked with a star based on confidence intervals.

The positive values of all but one of these comparisons support the previous findings that the responses of the OFF pathway are more accurate than the responses of the ON pathway. These results also suggest a slight trend that the Glaucoma Scotoma sectors have a greater asymmetry between the ON and OFF pathways than the Glaucoma Normal and Control sectors, in which OFF responses tend to be more accurate than ON responses.

Reaction time was plotted as the average time, in seconds, for the given sector of the visual field (Figure 3). Statistically significant differences were measured in six of the eight pairs between OFF Glaucoma Scotoma and OFF Control, and in five of the eight pairs between ON Glaucoma Scotoma and ON Control. In addition, six of the eight pairs showed significant differences in reaction time between Glaucoma Normal and Control sectors in both the ON and OFF responses. There are also four and three pairs with significant differences between the ON and OFF responses for the scotoma region of the glaucoma observers and the control observers, respectively. The differences between the average reaction times of the OFF responses and the ON responses were also plotted (Figure 4). A majority of the differences plotted in this figure have negative values, meaning that the average reaction time for the OFF pathway was typically faster than the reaction time of the ON pathway.
Figure 3: This figure represents average reaction time, in seconds, for each of the three regions and in response to OFF (blue bars) targets and ON (red bars) targets. Comparisons were made between each sector within the ON and OFF pathways, then between the ON/OFF pairs of sectors. Significance was found based on the Wilcoxon Sum Rank test using $\alpha=0.007$. 
Figure 4: In this figure the differences between the average reaction times for the responses in the OFF and ON pathways of each sector are plotted. The plots represent OFF Reaction Time – ON Reaction Time, meaning that a faster OFF response reaction time would result in a negative value in the plot.
In a subsequent analysis of 12 patients with early to moderate primary open angle glaucoma (mean=69.75 years, std=7.64), and 12 controls (mean=63.67 years, std=4.25), we demonstrated a relationship between the percentage of correct responses for ON and OFF pathway stimuli follows a power function, wherein glaucoma and controls overlap ($R^2=0.842$) (Figure 5a). This overlap decreases when we quantify only the subthreshold (unseen) increment targets in a linear relationship ($R^2=0.7074$) (Figure 5b). All controls had less than 12% of subthreshold increment targets whereas the percentage of subthreshold targets was higher for 75% of the glaucoma subjects, even in early stages of the disease (-1.72 dB of visual sensitivity loss). ON/Off perimetry measured large increment visual deficits in patients with limited loss of visual sensitivity (Figure 5c), which may improve detection of early disease.

**Figure 5:** A) Correlation of percent of correct responses to either light or dark stimuli by both control subjects and subjects diagnosed with glaucoma. ($R^2=0.842$). B) Correlation of percent of missed light or dark stimuli by both control subjects and subjects diagnosed with glaucoma. ($R^2=0.7074$). C) This figure represents the percentage of missed light or dark stimuli by patients diagnosed.
with glaucoma compared to the patients’ visual sensitivity loss determined by a traditional Humphrey Field Analyzer test.

Discussion

Our results demonstrate the asymmetry between the two achromatic visual transduction pathways. These results support previous findings that dark targets elicit a faster and more accurate response than light targets, when presented on binary background noise. This asymmetric trend was seen in sectors containing a scotoma in glaucoma patients, visually normal sectors in glaucoma patients, and in age-similar control patients. We also see a trend that there may be more asymmetry in the sectors containing a scotoma than in other sectors. Additionally, our results do not support previous findings from Jin et al., 2008 that suggested the asymmetry between the ON and OFF pathways is restricted to only the central 5° of the visual field. Every target in the present experiment were as-presented to the observer in the regions varying from of 5-30° eccentricity, therefore, our results suggest how that the two pathways are acting asymmetrically even in peripheral vision.

The lack of significance between glaucomatous and non-glaucomatous sectors of visual field may reflect the limits of the use of a single high-contrast stimulus.

Future Directions:

In the future we will be using this technology to analyze the applicability functionality of the ON and OFF perimetry pathways across a wider array of glaucoma patients and controls within large scotomas of patients with moderate to severe glaucoma. We will also explore the influence of reducing
contrast of the stimulus. With a larger sample size we hope to see more evidence of the trends that are emerging in these results.

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