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PRINCIPAL INVESTIGATOR: Andrew Hartwick

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Andrew Hartwick

E-Mail: Hartwick.4@osu.edu

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14. ABSTRACT

Many people develop an intolerance to light after a head injury. We do not understand why this occurs and how it is linked to head injuries. It is our speculation that cells in the retina of the eye become unable to adjust appropriately to changes in environmental light levels, with the result being that they signal the brain that it is brighter than it actually is. In this work, we will study individuals who had a recent brain injury and developed light intolerance, individuals with a recent brain injury who do not experience light intolerance, and a comparison group of people who have never had a brain injury. We will measure the function of certain retinal cells by recording their electrical activity, which can be detected with a probe placed near the eyelid, when the eye is stimulated with light. We will also measure the function of a different group of cells in the retina by measuring how the pupil changes size in response to different light exposures. These techniques could provide new approaches for clinicians to use, allowing them to quantify the magnitude of the light intolerance experienced by these patients.

15. SUBJECT TERMS					
Pupillometry, electroretinogram, melanopsin, retinal ganglion cell, adaptation, traumatic brain injury, photophobia					
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1. INTRODUCTION:

The initial goal of this work is to develop a device and protocol that enables the concurrent recording of light –evoked pupil responses and electoretinogram (ERG) recordings of ganglion cell activity. The ideal device would allow each eye to be independently stimulated with light, and allow full-field stimulation across a range of light intensities and wavelengths. Upon successful development of the device and protocol, the aims will be to: determine whether light adaptation properties of non-photosensitive retinal ganglion cells (RGCs) and RGC photoreceptors are altered in individuals with TBI, particularly in those with photophobia, as measured using ERG methods and pupillometry, respectively.

2. KEYWORDS:

Pupillometry, electroretinogram, melanopsin, retinal ganglion cell, adaptation, traumatic brain injury, photophobia

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Goal 1: Work with collaborators at Diagnosys LLC (Lowell MA) to develop a device, along with software, that enables concurrent recording of pupil size measurements and retinal neuron electrical activity (electroretinograms). The ideal device would enable light to be applied independently to either eye (in order to keep each retina at separate light adaptation levels) and be able to deliver light across a range of light intensities and wavelengths.

Major Goal 2: Develop a protocol using the device that assesses the ability of both regular non-photosensitive retinal ganglion cells (RGCs) and RGC photoreceptors (melanopsin-expressing neurons) to adapt to changing background light levels.

Major Goal 3: Test the hypothesis that retinal adaptation properties of ganglion cells, particularly the ganglion cell photoreceptors, are altered in individuals with TBI-associated photophobia.

What was accomplished under these goals?

Major Goal 1: Although there were some initial delays in the manufacturing process of the prototype ERG/pupillometry devices due to the global pandemic, we were able to work with Diagnosys to develop devices, along with custom software. There were some initial issues with 1) the ability of the pupillometry software to accurately find and measure pupil size, especially at brighter light levels and 2) electrical noise from the camera and light stimulation hardware that interfered with the ERG measurements. We are currently on the third version of these prototype devices, and we have identical devices at the two sites (OSU and SUNY).

The key advantages of this device (see Figure 1) compared to standard commercial equipment are that: 1) each eye is enclosed by separate eye-cups during recording which enables light to be administered to either eye, and allows the two retinas to be maintained in different, independent levels of light adaptation; 2) the software enables concurrent recording of ERGs and pupil size, and so the function of non-photosensitive RGCs (through ERGs) can be assessed at the same time as RGC photoreceptors (through pupillometry); and 3) a broad range of light stimuli, ranging from 0.00001 to 200 cd/m2 in intensity and 450 (blue) through 620 (red) nm in wavelength, to be assessed.

The current version of the devices and the software have the capability to facilitate the experiments outlined in this proposal and so this Major Goal is complete. The third version of the devices were in place at each of the two sites in June, 2022.

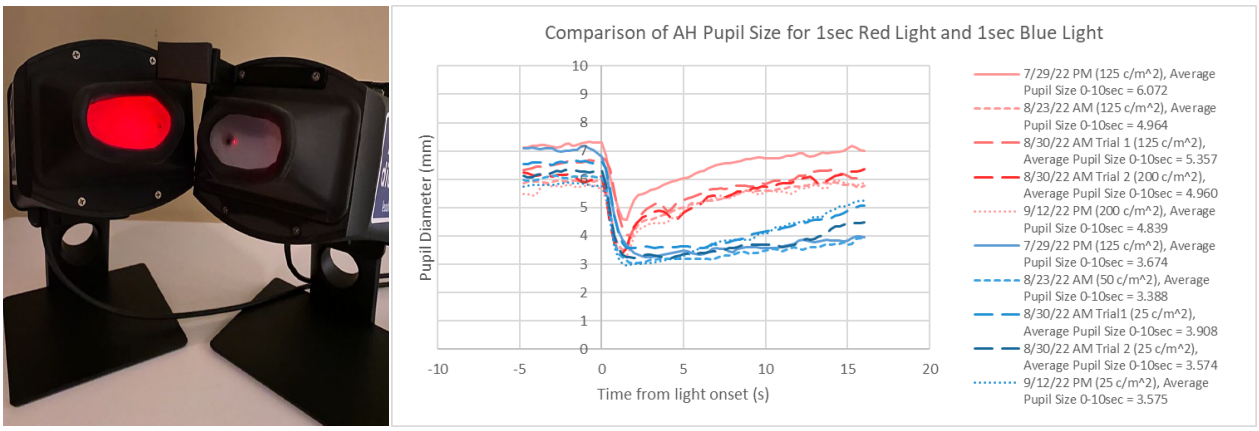

Figure 1. Left: custom pupillometer/electroretinogram device designed with two independent eye cups. Light can be administered monocularly while the other eye remains dark adapted. Right: Example pupil recordings from PI (Hartwick) evoked by 1 s pulses of red and blue light. Note the repeatability and the slower re-dilation evoked by the blue light, consistent with the involvement of ganglion cell photoreceptors in the response to the blue light, but not the red light.

Major Goal 2: We have developed and tested (the co-PIs served as the subjects in these experiments to confirm the validity and repeatability of the instrument) a protocol for measuring adaptation of both non-photosensitive (evaluated using ERGs) and photosensitive (evaluated using pupillometry) RGCs. During this work, we realized that we needed to improve the software for pupil tracking and decrease the electrical noise in the pupil recording system that was interfering with the ERG electrical recordings. The current version of the software exhibits excellent tracking of the pupil, and additional grounding of the cables connecting the camera and light sources (LEDs) to the main console have been moved and re-grounded. Figure 2 shows one of the proposed protocols. Figure 3 displays the pupil data and Figure 4 the ERG data collected at two example light intensities that was performed with Hartwick serving as the subject.

Figure 2. Outline of light stimulation protocol proposed for use during the first 5 trials of second study session. The intensity of the background light varies across the 5 trials. After each trial, participants will sit in dark room for 10 minutes, with head pulled away from instrument.

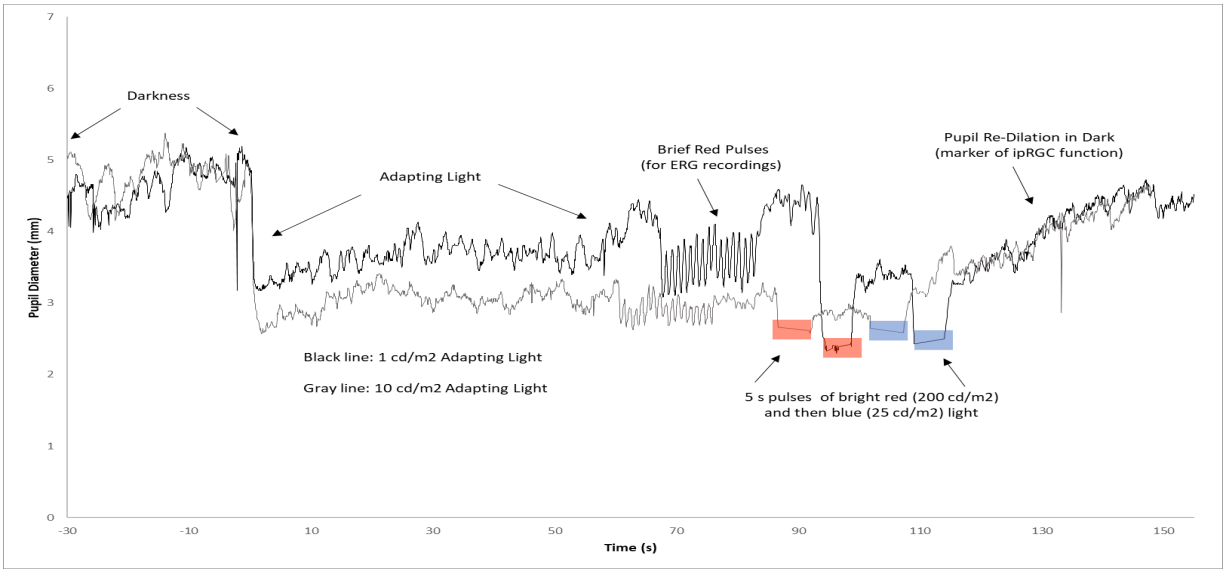

Figure 3. Pupil size recording using similar protocol as shown in Figure 2 (fewer pulses of red/blue at end during trial) with PI (Hartwick) serving as subject. Note that the device enables the measurement of pupil size during the adaptation light exposure, providing an objective measure of differences in retinal adaptation. Regulare RGC function is then assessed using the ERG recordings to multiple, brief red flashes of light. RGC photoreceptor function is then assessed by comparing the responses of the 5 s bright red flashes to the bright blue and assessing the rate of pupil re-dilation at end of trial.

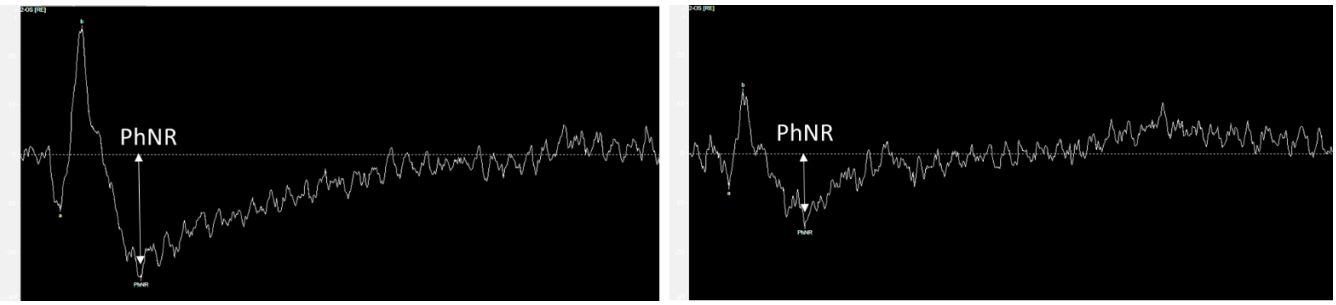

Figure 4. Averaged ERG recordings to the brief red flashes during the trial shown in Figure 3 (subject: Hartwick) after adapting to 1 cd/m^2 (left) and 10 cd/m^2 (right). The photopic negative response (PhNR) can be used as a marker for regular RGC activity. Note the PhNR decreases when the brighter adapting light is used.

In the above cases, light was administered to only the left eye, which was pharmacologically dilated to aid the ERG recordings. The advantage of this custom-design device is that we can then immediately assess an intensity-response function for pupil responses evoked by increasing intensities of blue light in the right eye, which has remained dark adapted through the initial trials. The data below (Figure 5) was collected with the co-PI (Viswanathan) serving as the subject using this paradigm.

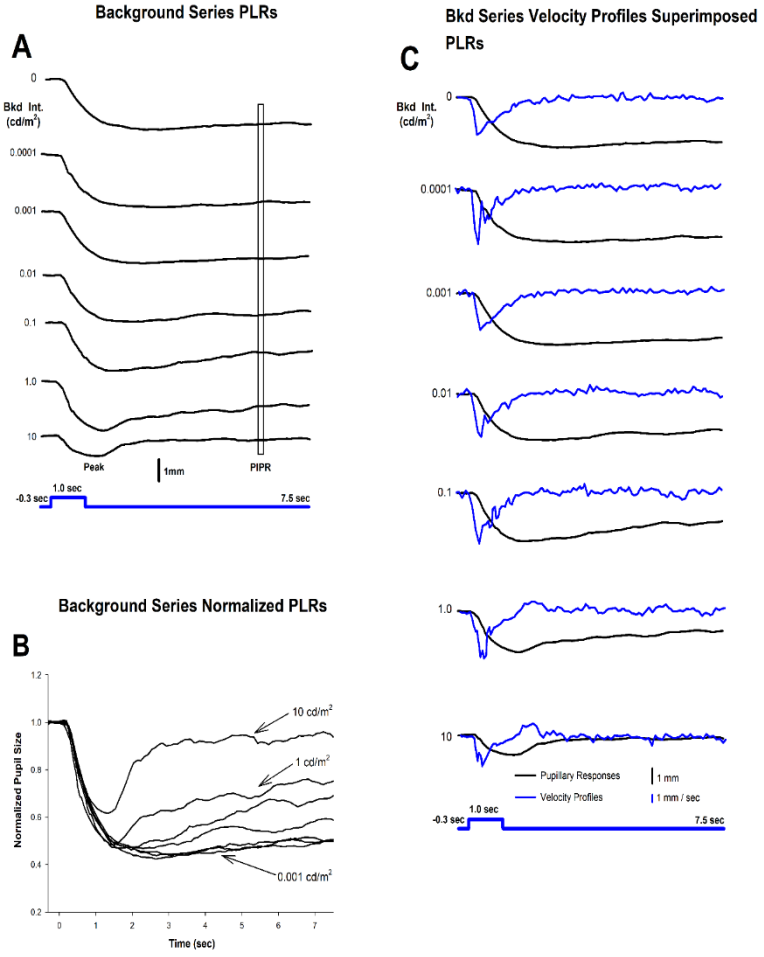

Figure 5. A. Pupil responses to 100 cd/m^2 blue light after 1 minute exposure to backgrounds of varying intensities (darkness: top trace , increasing to 10 cd/m^2 blue light for bottom trace. B. Same traces superimposed on top of each other for reference. C. Same traces in black, the blue traces show the velocity of the pupil constriction at the same timepoints. Co-PI Viswanathan served as the subject for these trials to test the capabilities of the instrument.

In both the protocols used in Figures 2 and 5, a 60 s duration was employed for the adapting light. This was based on pilot studies (Figure 6) performed with the two PIs serving as test subjects during a site visit that occurred in August, 2022 in which Dr. Viswanathan visited OSU (Figure 7). Due to travel restrictions during the first year of this grant period, this was the first in-person meeting of the two PIs for this project. After this visit, we felt that the device and protocol was sufficient to proceed with testing the hypothesis outlined in Major Goal 3.

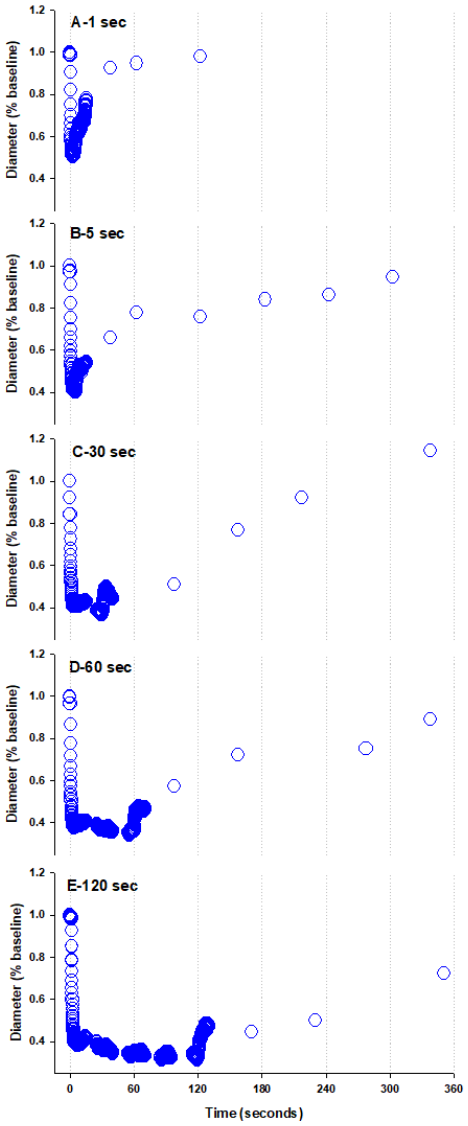

Figure 6. Pupil size recordings to a 100 cd/m^2 blue test flashes of 1, 5, 30, 60 and 120 s duration (A-E) expressed as a percent of the pre-stimulus baseline diameter following 5 minutes of dark-adaptation from a 53 year old male (Viswanathan). Note the slower return to baseline pupil size for the longer duration light exposure. Based on these preliminary test findings we will use a 60 s duration for light adaptation at each background with a 6 minute dark-adaptation between successive backgrounds that are near or above the threshold for melanopsin activation.

Figure 7. Photos of Drs. Viswanathan and Hartwick (co-PIs) at a site visit at OSU in August 2022 to trial the protocols and assess the capabilities of the prototype device for the proposed experiments. Left: Dr. Viswanathan prior to ERG recordings; Right: Dr. Hartwick prior to pupil recordings.

Major Goal 3: We are now proceeding to the final phase of this project in testing the protocols outlined in the figures above on individuals with traumatic brain injury with and without photophobia, and comparing these results to age-matched controls. The protocol has been submitted to OSU IRB and will be submitted to HRPO upon approval. The goal is to start recruiting participants spring 2023.

What opportunities for training and professional development has the project provided?

Drs. Viswanathan and Hartwick served as co-organizers of a Think Tank on "Traumatic Brain Injury" that was held on the day following the American Academy of Optometry Annual Meeting in Boston MA in October 2021. The think tank was limited to roughly 40 invited participants and included Drs. Randy Kardon (Iowa), Tawna Roberts (Stanford), Kathy Weise (UAB), Nick Port (Indiana), Aparna Raghuram (Harvard) and others. Drs. Viswanathan and Hartwick acknowledged DoD funding and provided invitees more information about DoD funding mechanisms. A summary of this meeting was provided to American Academy of Optometry leadership and a summary paper is under revision for submission to *Optometry & Vision Science.*

How were the results disseminated to communities of interest?

See above regarding the Think Tank at the American Academy of Optometry meeting. The goal of this meeting was to bring stakeholders in brain injury together to identify gaps in our knowledge base. No manuscripts from this project have been published as of yet.

What do you plan to do during the next reporting period to accomplish the goals?

With the successful development of the prototype of the custom pupillometer/ERG device and the trial of the protocols in pilot studies on the co-PIs to ensure feasibility and confirm the functional capabilities of the device, we are ready to move on to the recruiting individuals with traumatic brain injury and collecting data to test the hypothesis that those individuals have altered adaptation properties in their ganglion cells, particularly the RGC photoreceptors. The immediate goal is to obtain IRB approval from the tested protocol described here. We have already met with rehabilitation clinics at our respective institutions and they have agreed to assist in participant recruitment.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The innovation of this project has been our collaboration with a private company (Diagnosys) to assist them in the development of a novel pupillometer/ERG device. This device is optimal, compared to commercial devices currently on the market, for testing the adaptive properties of retinal ganglion cells through combined ERG and pupillometer recordings.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report yet, but we see promise in this device being of interest to both clinicians and vision researchers.

What was the impact on society beyond science and technology?

Nothing to report yet, but the hope is that the results obtained going forward will provide information on why photophobia is such a common symptom that occurs after brain injury. These individuals are often frustrated that everything seems 'normal' at their regular eye examinations. This work could lead to a better understanding of how to diagnose and monitor photophobia (measure changes in pupil and ERG markers that occur when background lighting is altered).

5. CHANGES/PROBLEMS:

No changes in the direction of the project. There were delays in moving onto the final phase of the project involving the recruitment/recordings of individuals with traumatic brain injury due to a family health issue encountered by the PI (Hartwick) over the last few months.

Actual or anticipated problems or delays and actions or plans to resolve them

The family health issue is moving in a positive direction and is less likely to cause delays going forward.

Changes that had a significant impact on expenditures

No changes.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No changes.

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

No changes.

PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report as of yet. The goal is to present data from the individuals with traumatic brain injury at ARVO 2024 with publications to follow in peer-reviewed journals.

Books or other non-periodical, one-time publications.

Nothing to report as of yet

Other publications, conference papers and presentations.

Presentation at the Think Tank on Traumatic Brain Injury at the American Academy of Optometry meeting in which the potential hypothesis for TBI-related photophobia was presented.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

We collaborated with a company (Diagnosys) to help develop and refine the custom pupillometer/ERG device and will be using the device the run the outlined testing protocol.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Andrew Hartwick*
Project Role:	*Principal Investigator*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Hartwick has led the project and conducted regular meetings with Dr. Viswanathan to provide input to Diagnosys LLC in the design and build the new combined pupillometer/ERG device. Dr. Hartwick has taken the lead on writing and testing the protocol and in writing the regulatory documents.*

Name: *Suresh Viswanathan*
Project Role: *Co-Investigator*
Nearest person month worked: *1.2*

Contribution to Project: *Dr. Viswanathan has conducted regular meetings with Dr. Hartwick to provide input to Diagnosys LLC in the design and build of a new combined pupillometer/ERG device. Dr. Viswanathan has assisted Dr. Hartwick with the writing and testing of the protocols and the writing of the regulatory documents.*

Name: *Catherine McDaniel*
Project Role: *Key Personnel (Clinical Optometrist, OSU)*
Nearest person month worked: *1*

Contribution to Project: *Dr. McDaniel had helped to outline the flow of procedures that will make up the first study visit. In this visit, the participants will receive a comprehensive eye exam. She has assisted in facilitating communication with neurological centers necessary for recruitment at OSU.*

Name: *Pat Modica*
Project Role: *Key Personnel (Clinical Optometrist, OSU)*
Nearest person month worked: *1*

Contribution to Project: *Dr. Modica had helped to outline the flow of procedures that will make up the first study visit. In this visit, the participants will receive a comprehensive eye exam. She has assisted in facilitating communication with neurological centers necessary for recruitment at SUNY.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

8. APPENDICES: