AWARD NUMBER: W81XWH-20-1-0933

TITLE: Effects of Mild Traumatic Brain Injury on Retinal Ganglion Cell Light Adaptation

PRINCIPAL INVESTIGATOR: Andrew Hartwick

CONTRACTING ORGANIZATION: OHIO STATE UNIVERSITY, 1960 KENNY RD COLUMBUS OH 43210-1016

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2023	Annual Report	30SEPT2022 - 29SEPT2023

4. TITLE AND SUBTITLE

Effects of Mild Traumatic Brain Injury on Retinal Ganglion Cell Light Adaptation

5a. CONTRACT NUMBER

W81XWH-20-1-0933

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Andrew Hartwick

E-Mail:hartwick.4@osu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

AND ADDRESS(ES)

The Ohio State University
1960 Kenny Road
Columbus, OH USA 43210

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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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:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Goal 1: Work with collaborators at Diagnosys LLC (Lowell MA) to develop a device, along with associated 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 beable 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?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the

project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Goal 1: Although there were some initial delays in the manufacturing process of the prototype electroretinogram/pupillometry devices due to the global pandemic, we have continued to work with Diagnosys to develop a device, with custom software, that enables the concurrent recording of pupil size and retinal activity (electroretinograms or ERGs) in either eye. Light stimuli can be delivered to either eye independently, allowing each eye to be maintained at different levels of light/dark adaptation (Figure 1).

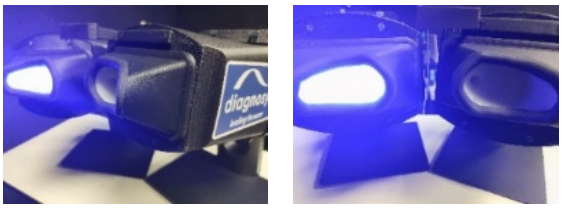


Figure 1. Novel ERG/pupilllometer developed for this project. Note the two eye-cups allowing light to be applied to either eye independently. Cameras located in the eye-cups are used to record pupil size, and a nearby amplifier connects to ERG electrodes.

Over the last year, we have enhanced the custom software and the dynamic range of the light stimuli used in the device, allowing us to better develop protocols that can assess the primary hypothesis of this work that there is an altered ability of retinal ganglion cells to adapt to light in individuals with increased light sensitivity after head injury. In particular, we are interested whether the function of a specific class of ganglion cell photoreceptors (intrinsically photosensitive retinal ganglion cells or ipRGCs) is altered in these individuals. As ipRGCs contribute to the regulation of pupillary light responses, and due to their sluggish responses to light that cause them to continue to respond for many seconds post-light offset, one can assess their function by monitoring the slow re-dilation that occurs after exposure to bright light stimuli. Due to the spectral sensitivity of these photoreceptors, blue light is especially effective at stimulating ipRGCs, as compared to similarly bright red light stimuli. Figure 2 illustrates pupil recordings obtained from the PI (AH) across multiple days using the novel device.

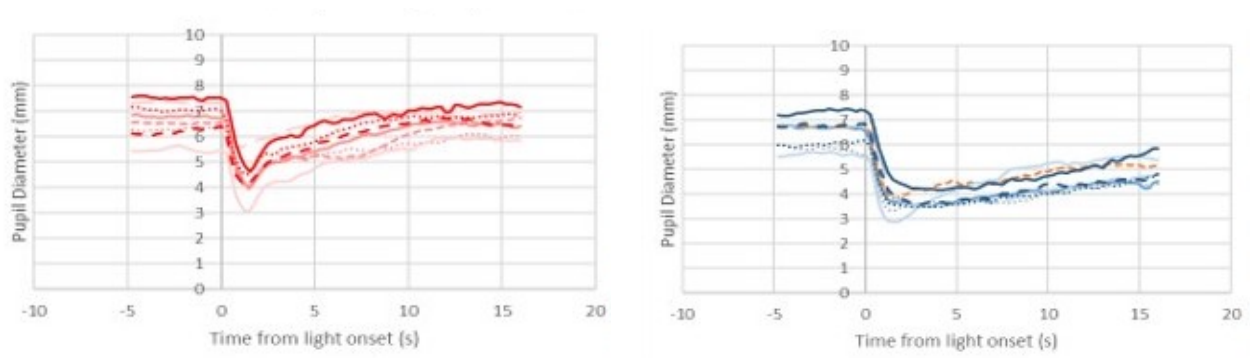


Figure 2. Pupil size (in mm), recorded in multiple trials on PI (AH) conducted across different days, after stimulation with a 1 sec pulse of (left) 200 cd/m^2 red light and a (right) 25 cd/m^2 blue light. After initial pupil constriction (decreased pupil size at time zero), note the slower re-dilation that occurs after blue light offset as compared to red. This is due to contribution of ipRGCs to the blue light-driven pupil responses.

We have developed a data analysis protocol that uses the raw pupil data that is recorded by the novel pupillometer to generate re-normalized traces during the post-light period that enables the rate of re-dilation to be assessed (Figure 3). These traces are then fit with exponential decay functions that reflect objective measurements of the speed of re-dilation. The rate of pupil re-dilation can then be used as a marker of ipRGC function.

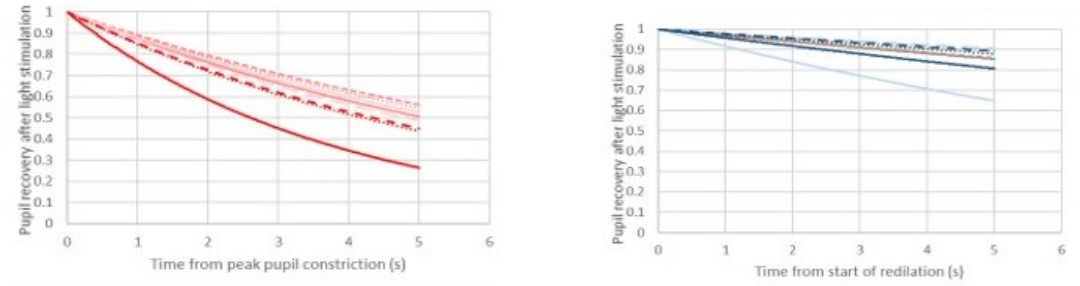


Figure 3. Re-normalized traces of pupil size post-light stimulation using the raw data shown in Figure 2. The generation of these graphs facilitates the fitting of the data with exponential decay curves. Higher values for the decay constant, as would occur for the red traces on the left, indicates faster re-dilation and less ipRGC contribution to these red stimuli as compared to the blue stimuli with lower decay constant values on the right.

The second major goal has been to optimize a protocol using the new device and software that can assess altered light adaptation in both regular ganglion cells and ganglion cell photoreceptors. We have been piloting different protocols, with the PI and co-PI (AH and SV) serving as the test participants to develop approaches that take advantage of the novel characteristics of the new device/software.

In one protocol, the background is filled with 2 min of full-field blue light in increasing intensity steps, with 2 minutes of darkness interleaved between the steps (Figure 4). This light is applied only to the left eye, while the consensual pupil response is monitored in the dark-adapted right eye. We can then examine the ability of the pupil to maintain constriction during the 2 min light steps (measured as the difference in pupil constriction at the end of the light step versus the peak at the beginning), and the rate or recovery during the 2 min light pulse (fitting exponential decay curves after the period marked 'Rec' in Figure 4). Both of these measures provide information about ipRGC light adaptation, as altered adaptation is reflected by an inability of the pupil to maintain stable constriction and re-dilation rates.

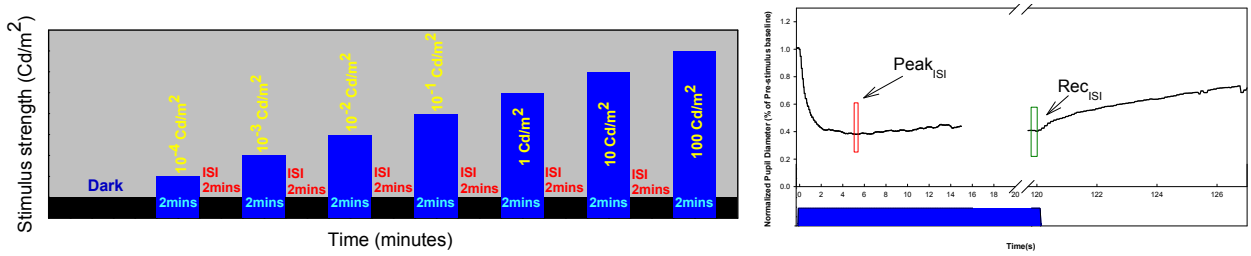


Figure 4. This protocol utilizes 2 minutes of inter-stimulus interval (**ISI**) of darkness between steps of increasing intensities of blue light stimuli. The change in pupil constriction from peak constriction and the rate of recovery post-light stimulus are monitored and determined, and can be used to assess the ability of ipRGCs to adapt to the increasing light steps.

In a second protocol, increasing steps of blue light are applied to the right eye with no periods of darkness interleaving the light steps (Figure 5). Due to the separate eye-cups in the pupillometer, which enable light stimulation to be applied independently to the two eyes, the right eye remains dark adapted during application of the first protocol (Figure 4). The unique design of the novel pupillometer enables these two protocols to be measured in the same session without concern that the light exposure in the first protocol influences the responses to the light in the second protocol. During this second protocol, while the right eye receives the stimulation, the consensual pupil response is measured in the left eye.

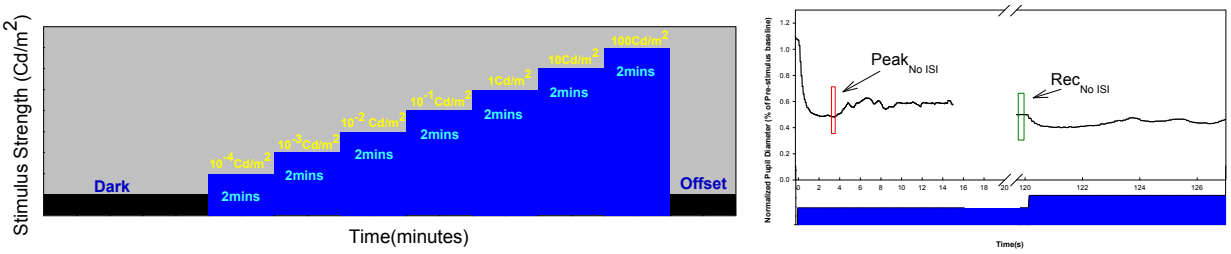
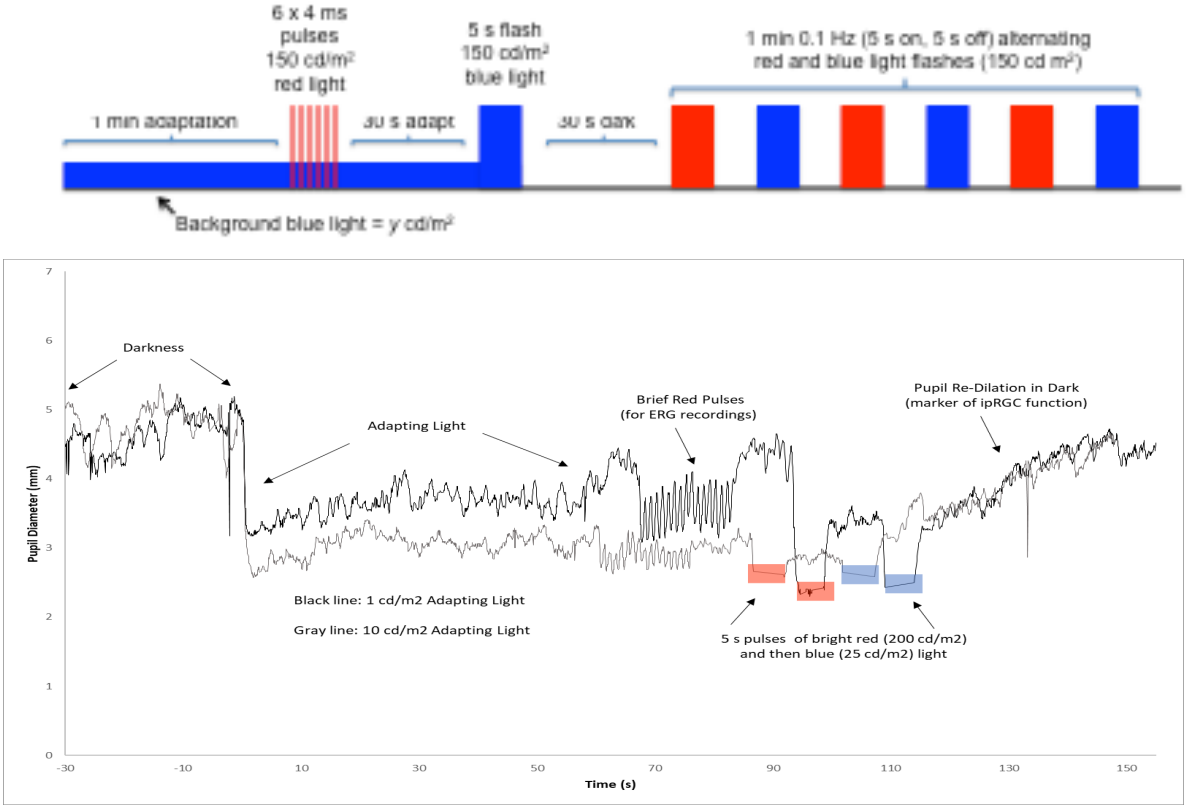


Figure 5. This second protocol does not utilize periods of darkness spacing between the light steps (no inter-stimulus interval [ISI]). The change in pupil constriction from peak constriction during each light step and the change in constriction elected by subsequent light steps will be calculated and compared to the results obtained in the first protocol.

Participants will then return for a second session in which both electroretinograms (ERG) and pupil responses will be measured. During this session, the pupil in the left eye will be pharmacologically dilated and a wire electrode will run along the lower eyelid. Light will be applied to the left eye during this session, with the electrode relaying gross retinal activity into an amplifier that is fed into a custom software program, while the consensual pupil response is monitored in the right eye concurrently. In the first protocol of this session, the left eye will be exposed to a background adapting blue light, which brief pulses of red light applied on top of the adapting light to generate the ERG recordings (Figure 6).



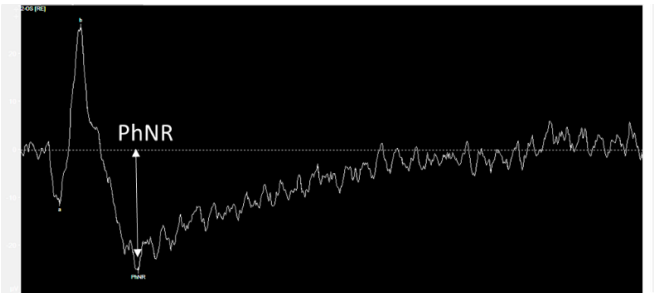


Figure 6. (Top) Outline of protocol used in the second session, which involves 5 trials. The intensity of the blue background light varies across the 5 trials. (Middle) The consensual pupil response is measured across the entire paradigm, while the (Bottom) ERG recordings are saved during the brief red light pulses applied on top of the blue background. The PhNR reflects function of non-photosensitive RGCs.

We have finalized the development of the devices and tested these protocols with the PI (AH)m and the co-PI (SV) serving as the test participants in these pilot studies. Both devices have been calibrated to ensure that the light intensities used for the different stimuli are identical between the two sites (OSU and SUNY). Thus, the second major goal has been completed and the final stage of the project will focus on completing the third major goal - using this novel device and these protocols to assess the ability of ganglion cell photoreceptors and regular RGCs to adapt to changing light levels in individuals with photophobia due to head injury.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. 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 (October 2021). The think tank was limited to roughly 40 invited participants and a summary of this meeting was provided to American Academy of Optometry leadership. Discussion of funding opportunities from the DoD for head injury-related research was included at meeting.

Although they do not receive direct funding from this DoD grant, two trainees (Ashwin Pothiadia-Irungovel at SUNY and Rachel Fenton at OSU) have assisted in developing the software and data analysis techniques, which has facilitated their development and growth as emerging vision scientists.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

See above regarding the Think Tank at the American Academy of Optometry meeting. Dr. Hartwick provided an invited seminar at Dalhousie University (Halifax NS) in October 2023, in which the overall design and goals of project were discussed in order to receive feed-back and to promote future collaborative work involving the newly developed pupillometer/ERG device. The goal is to submit manuscripts at the end of the current no-cost extension year, when data collection from TBI participants is completed.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

With the development of a finished prototype of the pupillometer/ERG device, improvements to the software program and data analysis tools, and optimization of the two-session protocol designed to test the original hypothesis, the next year will be dedicated to collecting data from individuals with TBI-associated photophobia. The revised protocols have been submitted to the OSU IRB and we are awaiting final approval.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Upon completion of this project, we expect there will be considerable interest in the vision research community in a device that is capable of concurrently measuring ERGs and pupil responses while maintaining the two eyes in separate states of light/dark adaptation. This device will enable researchers to compare function of different retinal ganglion cells types in one eye and between eyes.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report yet, but we see promise is this device and the new optimized protocols in being of interest to vision researchers and clinicians caring for TBI patients.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Photophobia is a frustrating condition because there are currently no standardized approaches to diagnosing, monitoring or treating the condition. Finding altered ganglion cell adaptive functions, as hypothesized that these results will show, will provide verification that the condition is associated with biological change in the retina. This will be of considerable importance to both patients and clinicians as it will confirm that the increased light sensitivity after head injury is not psycho-somatic. This research should then provide new avenues for more objective diagnostic testing of photophobia and could offer methodologies that enable recovery from potential treatments to be monitored.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

There were some initial production delays due to the global pandemic and some family health issues experienced by the PI (AH) over the last year which slowed down the progress of the project to the final phase of testing the protocols in patients with TBI-associated photophobia.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The device and software development are now considered complete and the protocol has been tested on the PI's to ensure that both sites are ready to commence the final phase. We have been granted a no-cost extension and are awaiting final IRB approval, but we are on track to complete the project during the final no-cost extension year.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No changes.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes.

Significant changes in use of biohazards and/or select agents

No changes.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report as of yet. The data is expected to be presented at American Academy of Optometry 2024 and ARVO 2025 meetings.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

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)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

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

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

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

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?

Organization Name: Diagnosys LLC
Location of Organization: Lowell MA
Partner's contribution to the project (identify one or more)

- *Collaboration (e.g., partner's staff work with project staff on the project); Jeff Farmer worked with us in terms of the structural build of the device and the development of the software.*
- *Jeff Farmer came to OSU and SUNY at his own company's expense to assist with the implementation of the new prototype devices.*

8.

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: ***N/A***

9. **APPENDICES: N/A**