AWARD NUMBER: W81XWH-15-1-0074

TITLE: Torsion-Induced Traumatic Optic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Injuries to the Central Nervous System

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REPORT DATE: June 2022

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
June 2022	Annual	22Mar2021-21Mar2022

4. TITLE AND SUBTITLE

Torsion-Induced Traumatic Optic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Injuries to the Central Nervous System

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0074

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Deployment-related injuries to the central nervous system (CNS) have increased in frequency since World War II. The increasingly frequent use of improvised explosive devices over the past decade has dramatically increased the rate of these injuries. Unfortunately, rapid diagnosis of these injuries during the window of opportunity for significant CNS regeneration remains problematic. Further, no treatments have yet been developed to the point of clinical applicability which successfully regenerate CNS tissues. We have therefore developed and will use a unique and novel animal model of traumatic optic neuropathy (TON) which allows ready access to the central nervous system for studies on CNS regeneration.

15. SUBJECT TERMS
Nerve injury modeling; traumatic optic neuropathy; diagnosis; neural regeneration

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Traumatic optic neuropathy (TON) is a blinding injury to the optic nerve. While it is known to occur in a significant number of military and civilian injuries each year, no early diagnostic criteria or treatments are currently available. This critical gap is due, at least in part, to the lack of a suitable animal model. We have therefore developed a non-surgical model of TON, as well as several methods which may allow early diagnosis. Phase I of this study will establish those diagnostic criteria, while Phase II will use them to quantify changes in optic nerve structure and function following one of several candidate treatments.

2. KEYWORDS:

Nerve injury modeling; traumatic optic neuropathy; diagnosis; neural regeneration

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Develop injury diagnostics	Target Date	Completion
Milestone 1: ACURO approval	June 2019	100%
Milestone 2: Co-author manuscript on diagnostic methods for TON	Dec 2022	80%
Major Task 2: Develop vehicles for delivery of neuroprotective agents		
Milestone #3: Co-author manuscript on hydrogel "cast" for drug delivery and mechanical assistance for neuroprotection	July 2022	100%
Major Task 3: Characterization of optic nerve rescue by proposed treatments in vivo		
Milestone #4: Co-author manuscript on changes in optic track following treatment	Mar 2021	0%

What was accomplished under these goals?

Major Task 1: Develop injury diagnostics

We have collected preliminary evidence that photopic negative responses (PhNRs) are diagnostic for TON as little as 24 hours after injury. Other electrophysiological biomarkers, including Briefly, flash electroretinograms (FERGs), flash oscillatory potentials (FOPs), and flash visual evoked potentials (FVEPs) appear unchanged at this early time point. Since PhNR primarily originates in the retinal ganglion cells (RGCs) and RGCs are those thought to be injured in TON, this finding is exciting for several reasons. First, it implies that our animal model isolates injury to the RGCs and is therefore a specific model of TON rather than a non-specific retinal injury model. Second, PhNR measurements may be diagnostic for TON. These measurements may be readily performed in human subjects and, by comparing the amplitude in one eye to the other, a deficit will be at least suggestive of TON. Additional measurements, as well as correlation with histology and immunohistochemistry, are underway. These additional experiments will be required to demonstrate statistical significance of these preliminary findings.

Methods

Rats received intraperitoneal injection of ketamine (0.8 mL/kg ketamine; West-Ward; Eatontown, NJ) and xylazine (0.375 mL/kg xylazine; Akorn). Once pain reflex was absent, whiskers were cut back, and the eyes were dilated with 1% Tropicamide. The rat was then placed on a temperature-controlled electrophysiology system (Celeris Testing System with Espion V6 software; Diagnosys LLC; Lowell, MA). A platinum subdermal needle electrode (Natus Manufacturing Limited; Gort, CO. Galway Ireland) was placed in the tail while another was placed at bregma as ground and active electrodes respectfully. One 6 mm gold surface cup electrode (Natus) was placed on top of the tongue to serve as a reference. The eyes of the animal were numbed with 0.5% Tetracaine Hydrochloride Ophthalmic Solution (Bausch & Lomb Inc.; Tampa, FL). The eyes were then lubricated with Systane gel (Alcon Laboratories Inc.; Fort Worth, Texas) or GentleTears gel (Alcon). The eye gel lubricant was then used on the cup of the Diagnosys flash stimulators. The stimulators were aligned in the center of axis of vision and then placed lightly on the corneal surface. FVEPs, FERGs, FOPs, and flickers were obtained in light adapted conditions with a 200 cd.s/m^2 flash. Three PhNR responses were recorded in light adapted conditions using the following stimuli: 200 cd.s/m^2 white flash with a 20 cd.s/m^2 green background, 200 cd.s/m^2 white flash with a 40 cd.s/m^2 green background, and a 200 cd.s/m^2 green flash with a 40 cd.s/m^2 green background. PhNR values were taken from measuring baseline to trough of the resultant wave. After testing was complete, the stimulators were removed from the eyes and cleaned with saline. The electrodes were removed from the animal, and the animal's eyes were cleaned with saline to remove excess eye lubricant. The animal was then injected with an IP dose of Antipamizole (0.4 mL/kg per kg of body weight) to reverse the xylazine and ease the recovery of the animal. This process was utilized to establish baseline values for each animal prior to injury, then repeated 24 hours after injury to determine the diagnostic value of electrophysiology measurements. Finally, measurements were repeated 7 days after injury prior to humane euthanasia and tissue harvesting for histology.

Rats were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde, and the brain and optic nerve were dissected and incubated overnight at 4°C in 4% paraformaldehyde. Samples were transferred into PBS and shipped to Vanderbilt University Medical Center where the optic nerves and chiasm were dissected away from the brain and cryo-preserved in 20% sucrose in PBS for 2 hrs at 4°C followed by 30% sucrose in PBS overnight at 4°C. The optic nerves were then embedded in OCT (Fisher Scientific, Waltham, MA) and longitudinally sectioned on a Microm HM550 cryostat (Fisher Scientific) at 10 μm thickness. For immunohistochemistry, sections were incubated in PBS to remove the OCT and then incubated in 1:20 normal donkey serum in PBS plus Triton-X-100 (PBT) at room temperature for 2 hrs. Sections were then incubated with specific antibodies as follows: anti-β-tubulin (1:1000; MAB5564; Millipore, Burlington, MA) and anti-glial fibrillary acidic protein (GFAP; 1:50; Z0334; DAKO, Santa Clara, CA) in PBT overnight at 4°C, rinsed with PBS, and incubated in donkey anti-mouse Alexa 488 and donkey anti-rabbit Alexa 594 (1:200; Fisher Scientific) in PBT overnight at 4°C. Finally, the sections were rinsed, mounted in Vectashield plus DAPI (Vector laboratories, Burlingame CA) and imaged on a wide-field fluorescence microscope (Nikon Eclipse, Melville, NY) using consistent settings.

Results

FERG and PhNR responses in the injured eyes of these animals were smaller in magnitude 24 hours after injury. PhNR, but not FERG, was also decreased in the contralateral eye. FOPs and VEPs remained largely unchanged. PhNR was lowest 24 hours after injury. PhNRs had smaller amplitude and longer latencies relative to baseline both one day after injury. Immunohistochemical analysis found decreased labeling intensity for both β-tubulin and GFAP in injured nerves relative to sham. Changes were observed bilaterally, though not symmetrically, suggesting that the biomechanical mechanism of injury employed must transmit stresses through the optic chiasm.

Major Task 2: Develop vehicles for delivery of neuroprotective agents

In this performance period, we revised and resubmitted a manuscript detailing the delivery vehicle. These findings form the basis of a manuscript which has been published in the *Journal of Biomedical Materials Research Part A* (impact factor: 4.854).

Major Task 3: Characterization of optic nerve rescue by proposed treatments in vivo

No progress to report.

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

Graduate Research Assistant Annie Ryan has received extensive training from Dr. Julie Racine with regard to rodent ERG, ERG, VEP, and PhNR; development of protocols relevant for diagnosing TON in our model system; and data analysis procedures. Annie has demonstrated her ability to conduct each aspect of the experiments in rats, producing the preliminary results described above.

How were the results disseminated to communities of interest?

Nothing to Report.

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

Major Task 1: We are in the process of conducting the experiments required to develop electrophysiological diagnostic markers in Major Task 1. This is essentially iteration of the preliminary results presented above using a larger number of animals. We hope to complete data collection for this Task within the next several months. Major Task 2: Nothing to Report.
Major Task 3: Nothing to Report.

4. IMPACT:

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

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report.

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

Prior setbacks related to grant transfer, ACURO approval, and the COVID-19 pandemic have significantly impacted this project's timeline and budget. We have therefore focused our efforts on Major Tasks 1 developing diagnostic criteria for TON) and 2 (developing a drug-eluting cast for TON). We have recently obtained additional funding related to Major Task 3 (determination of safety and efficacy of candidate TON treatments).

Changes that had a significant impact on expenditures

Nothing to report.

COVID-19 resulted in the sudden closure of all laboratories at Ohio State at a critical time in this project without significantly altering personnel-related expenditures. This loss of time, money, and highly trained personnel have constrained the project's operating budget. We have obtained internal funding for replacement personnel during the training period.

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 human subjects research will be performed to complete the Statement of Work.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:**

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

Journal publications.

- Maxwell, C.J., Soltisz, A.M., Rich, W.W., Choi, A., Reilly, M.A., Swindle-Reilly, K.E., Alginate Hydrogels as Injectable Drug Delivery Vehicles for Optic Neuropathy Treatment. *Journal of Biomedical Materials Research A*, 2022;110:1621-1635.

Books or other non-periodical, one-time publications.
Nothing to Report.

Other publications, conference papers and presentations.

- Ryan, A.K., Racine, J., Reilly, M.A., An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model. *Biomedical Engineering Society*, Annual Meeting, San Antonio, Texas, October 2022. Accepted.
- Reilly, M.A., Biomechanical Engineering Approaches in Presbyopia and Ocular Trauma. *Biomedical Engineering Society*, Annual Meeting, San Antonio, Texas, October 2022. Accepted.
- Ryan, A.K., Racine, J., Reilly, M.A., Diagnostic Electrophysiology in a Small Animal Model of Traumatic Optic Neuropathy (TON). *Association for Research in Vision and Ophthalmology*, Annual Meeting, Denver, Colorado, 2022. Accepted.
- Swindle-Reilly, K.E., Maxwell, C.J., Soltisz, A.M., Choi, A., Rich, W., Reilly, M.A., Injectable Alginate Hydrogels for Traumatic Optic Neuropathy, *Association for Research in Vision and Ophthalmology*, Annual Meeting, Virtual, May 2021. Published.

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

  Nothing to Report.

- **Technologies or techniques**

  Nothing to Report.

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

  Nothing to Report.

- **Other Products**

We have developed two novel computational mechanics models relevant to understanding the pathogenesis of traumatic optic neuropathy, as well as evaluation of mechanical aids to treatment. These finite element models allow thorough investigation of biomechanical mechanisms and, when supplemented with experimental data (as described above) may be useful for the study of additional diseases involving the optic nerve (e.g. glaucoma).

- Computational chemo-mechanical finite element model of hydrogel nerve cast
- Computational mechanical finite element model of ocular rotation

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Matthew Reilly
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0001-8029-0084
Nearest person month worked:	4
Contribution to Project:	Dr. Reilly has constructed mechanical models of ocular rotation and hydrogel casting.

Name:	Katelyn Swindle-Reilly
Project Role:	co-PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-1739-0263
Nearest person month worked:	1
Contribution to Project:	Dr. Swindle-Reilly has coordinated the hydrogel development, has attended meetings related to the research project, and has managed students performing experiments.

Name:	Julie Racine
Project Role:	co-PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4409-0936
Nearest person month worked:	1
Contribution to Project:	Dr. Racine has developed visual electrophysiology protocols, trained graduate students and employees to implement them, and analyzed electrophysiological data.

Name:	Courtney Maxwell
Project Role:	Research Assistant

Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3
Contribution to Project: Ms. Maxwell has performed experiments with hydrogels, drug release, swelling, pH, rheological and biocompatibility testing. She has been investigating optic nerve biomechanical testing and is first author of a manuscript describing the hydrogel cast.

Name: Wade Rich
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3
Contribution to Project: Mr. Rich has contributed to the development of biomechanical testing protocols for the optic nerve.

Name: Annie Ryan
Project Role: Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): 0000-0003-3645-8649
Nearest person month worked: 12
Contribution to Project: Ms. Ryan has developed electrophysiology protocols and carried out injury modeling in rats.

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

- Previous active grants have closed:
  - NEI 1R01EY029130-01, Structural, mechanical, and cell biological properties of the ciliary zonule: 2018-2022, Role: Co-investigator
  - NEI 5R01EY027399-02, Corneal biomechanics in ocular disease: 2017-2022, Role: Co-investigator

What other organizations were involved as partners?

Organization Name: Nationwide Children's Hospital
Location of Organization: Columbus, OH
Partner's contribution to the project: Collaboration Dr. Julie Racine, a visual electrophysiologist at Nationwide Children's Hospital, has been instrumental in training personnel for visual electrophysiology measurements and protocol development. She regularly comes to Ohio State for hands-on training and collaborative efforts.

Organization Name: Vanderbilt University
Location of Organization: Nashville, TN
Partner's contribution to the project: Collaboration Dr. Tonia Rex is an associate professor of ophthalmology at Vanderbilt. Her laboratory conducted immunohistochemistry analysis.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Torsional Indirect Traumatic Neuropathy (TITON): Animal Model for Diagnostics, Drug Delivery, and Therapeutics for Central Nervous System Injury

MR130235
W81XWH-15-1-0074

PI: Matthew A. Reilly **Org:** The Ohio State University

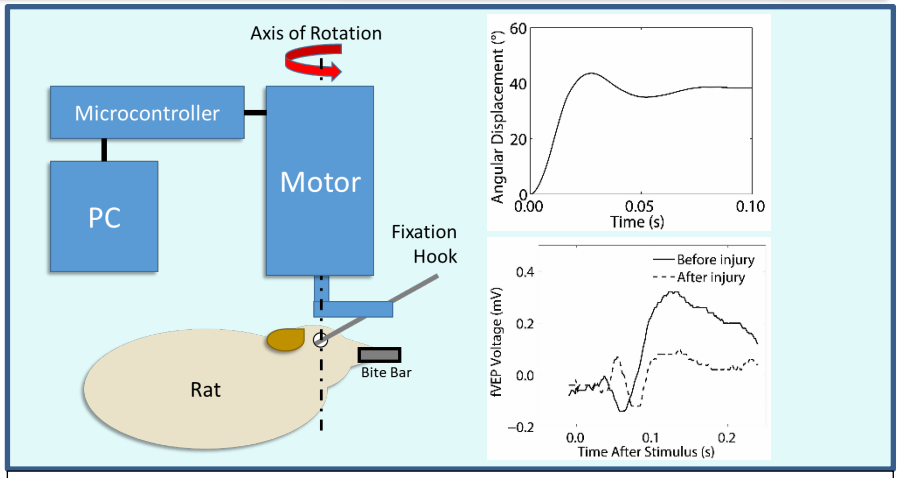
Award Amount: $902,737

Study Aims

- Diagnostics
  - Correlate TITON-induced changes using MRI, VEP, and ERG
  - Identify biomarkers with MALDI-IMS and scRNAseq
- Drug delivery vehicle
  - Develop and characterize drug reservoir hydrogel "cast"
- In vivo evaluation of candidate treatments
  - Treat with one or more therapeutic approach
  - Evaluate post-injury electrophysiology and histopathology

Approach

We have developed a new physiological model of indirect traumatic optic neuropathy (TON). This non-invasive technique achieves injury relevant to blast by rapidly rotating the eye to localize injury near the posterior insertion of the optic nerve. This model offers a simple platform for evaluating diagnostic and therapeutic modalities for TON. We will evaluate new local approaches to treatment including a novel hydrogel "cast" which also serves as a drug delivery reservoir.



Accomplishment: Preliminary measurements suggest electrophysiology will be diagnostic for early-stage TON.

Timeline and Cost

Activities CY	Lead	2019	2020	2021	2022
Develop injury diagnostics	Reilly				
Develop Tx delivery vehicles	Swindle-Reily				
Evaluate candidate Tx	Reilly				
Estimated Budget ($K)		**$382**	**$423**	**$87**	**$12**

Updated: 17 October 2022

Goals/Milestones

CY19 Goals – Development of novel materials and methods
☑ Formulate hydrogel cast candidates
CY20 Goal – Treatment studies
☑ Complete characterization of hydrogel cast candidates
CY22 Goal – Finalize diagnostic criteria
☐ Develop diagnostic criteria for TON
☐ Complete in vivo characterization of injury

Comments/Challenges/Issues/Concerns

- The COVID-19 pandemic has significantly impacted the project's timeline and budget.

Budget Expenditure to Date
Projected Expenditure: $902,737
Actual Expenditure: $890,363