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TITLE: Role of Endoplasmic Reticulum Stress and Systemic Inflammation in Blunt TBI-Induced Optic Nerve Injury

PRINCIPAL INVESTIGATOR: Nathan K. Evanson

CONTRACTING ORGANIZATION: Children's Hospital Medical Center, Cincinnati, OH

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Role of Endoplasmic Reticulum Stress and Systemic Inflammation in Blunt TBI-Induced Optic Nerve Injury

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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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14. ABSTRACT

Traumatic brain injury (TBI) is a major cause of injury in American military personnel. TBI caused by blunt head trauma is often associated with vision loss, due to injury of the optic nerve. Such vision loss can be severe. Up to approximately 75% of military personnel with TBI report visual symptoms, and such symptoms lead to significantly reduced quality of life measures. In spite of the magnitude of this problem, there are currently no treatments for optic nerve trauma that are backed by scientific evidence.This work is focused on better understanding pathological mechanisms active in a murine model of traumatic optic neuropathy from head trauma. Specifically, we aim to better understand the roles of endoplasmic reticulum stress and peripheral cytokine-mediated inflammation on retinal injury resulting from blunt head trauma. We are approaching the goals of this application via the following specific aims: Aim 1: To determine which element(s) of the endoplasmic reticulum stress response are most relevant to degenerative injury after optic nerve trauma. Aim 2: To determine the degree to which the peripheral inflammatory response contributes to outcomes after optic nerve trauma. This research will help clarify what should be targeted after blunt head injury to improve visual outcomes after TBI.

15. SUBJECT TERMS
Traumatic brain injury; traumatic optic neuropathy; head trauma; endoplasmic reticulum stress; inflammation; cytokines; mice

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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TABLE OF CONTENTS

1. INTRODUCTION:

This work is focused on better understanding pathological mechanisms active in a murine model of traumatic optic neuropathy from head trauma. Specifically, we aim to better understand the roles of endoplasmic reticulum stress and peripheral cytokine-mediated inflammation on retinal injury resulting from blunt head trauma. We are approaching the goals of this application via the following specific aims: Aim 1: To determine which element(s) of the ER stress response are most relevant to degenerative injury after optic nerve trauma. Aim 2: To determine the degree to which the peripheral inflammatory response contributes to outcomes after optic nerve trauma.

2. KEYWORDS:

Traumatic brain injury; traumatic optic neuropathy; head trauma; endoplasmic reticulum stress; inflammation; cytokines; mice

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Test activators and inhibitors of arms of the ER stress response for effect on visual outcomes after blunt TBI. Months 1-16
Percentage completion: 25%

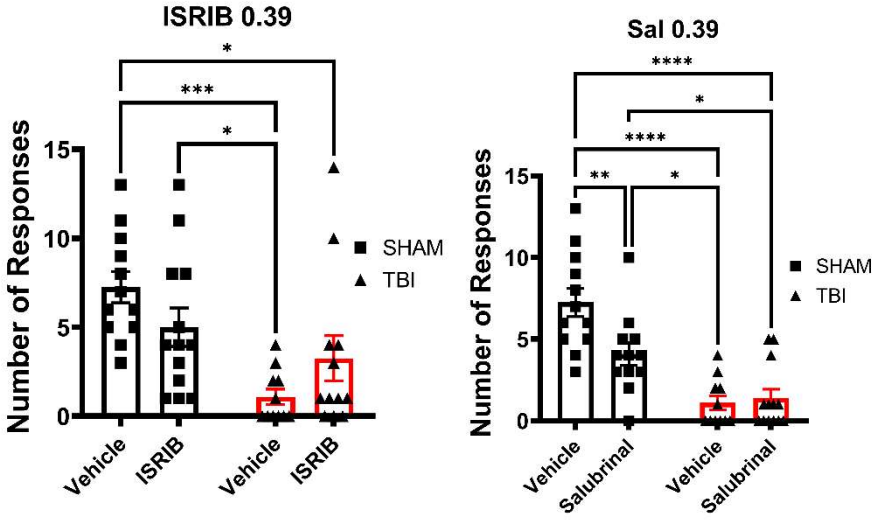
Major Task 2: Determine blunt head TBI visual outcomes after treatment with immune modulating drugs. Months 12-24
Percentage completion: 75%

What was accomplished under these goals?

Aim 1: To determine which element(s) of the ER stress response are most relevant to degenerative injury after optic nerve trauma.

For objectives under this aim we used a murine head trauma model. Mice are treated 24 hours after injury with inhibitors and activators of three major endoplasmic reticulum (ER) stress pathways (IRE-1, PERK, and ATF-6). Experimental endpoints include behavioral vision assessment (optokinetic nystagmus), retinal protein expression (Western Blotting), and histologic analysis of retinas and brains.

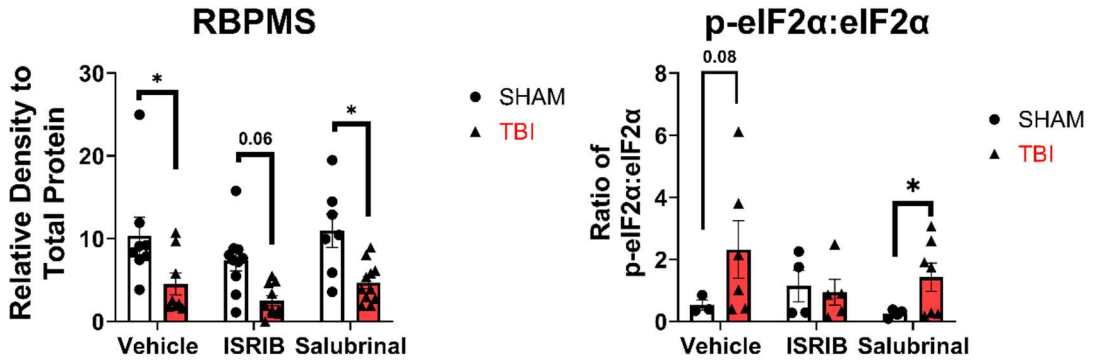
Major Task 1: Test activators and inhibitors of arms of the ER stress response for effect ovisual outcomes after blunt TBI
Subtask 1: regulatory approvals: completed
Subtask 2: Functional visual assessment: completed for the PERK pathway. IRE-1 and ATF6 pathways will be completed in year 2 of the award. Results so far suggest that activation of the PERK pathway using salubrinal may reduce optomotor activity in sham-treated animals, and that inhibition using ISRIB improves optomotor activity.



Subtask 3: Histologic evaluations: These are in progress for the PERK pathway, and are expected to be completed in the next few months. Analyses for IRE-1 and ATF-6 pathways will be completed in Year 2 of the award.

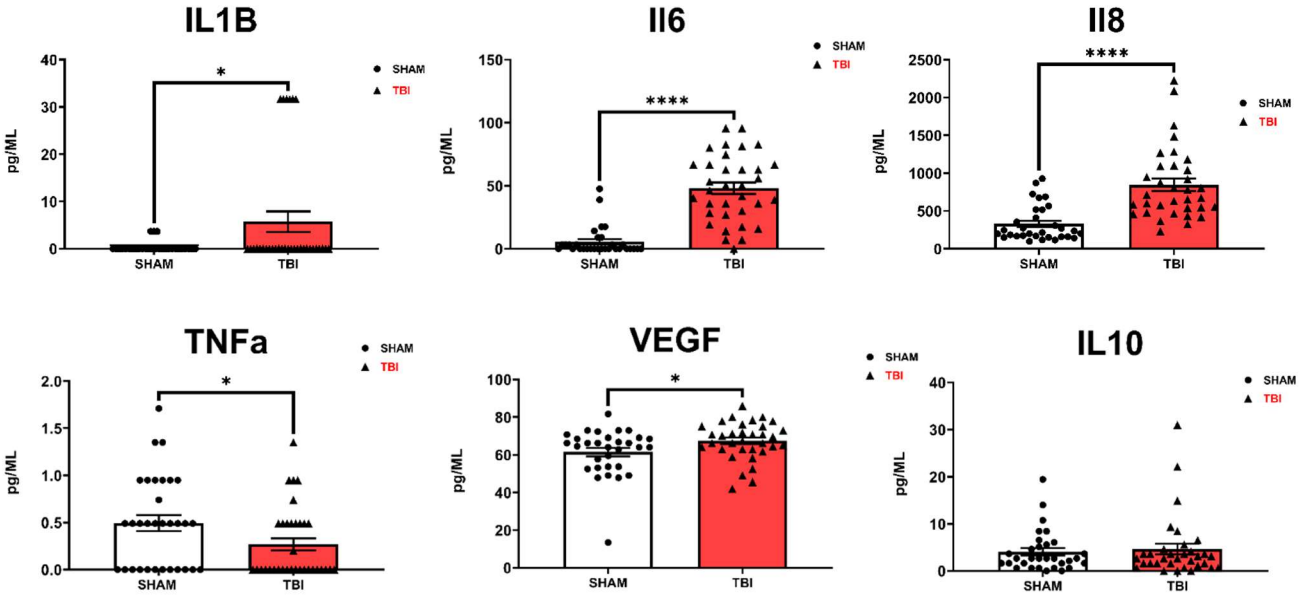
Subtask 4: Retinal Western Blotting assessment: these are mostly completed for the PERK pathway. Analyses for IRE-1 and ATF-6 pathways will be completed in Year 2 of the award. Results so far suggest that neither blocking nor activating the PERK pathway resulted in significant preservation of retinal ganglion cells (as measured by RBPMS expression. However, activating the PERK pathway using Salubrinal did increase the ratio of phosphorylated to total eIF-2alpha expression in retinal samples, suggesting this treatment maintained activation of this pathway for at least a week after treatment. We did not find significant changes in expression of other retinal

markers evaluated (including PERK, Cas 3, Cas 12, CHOP), although not all of these have been completed at this point.

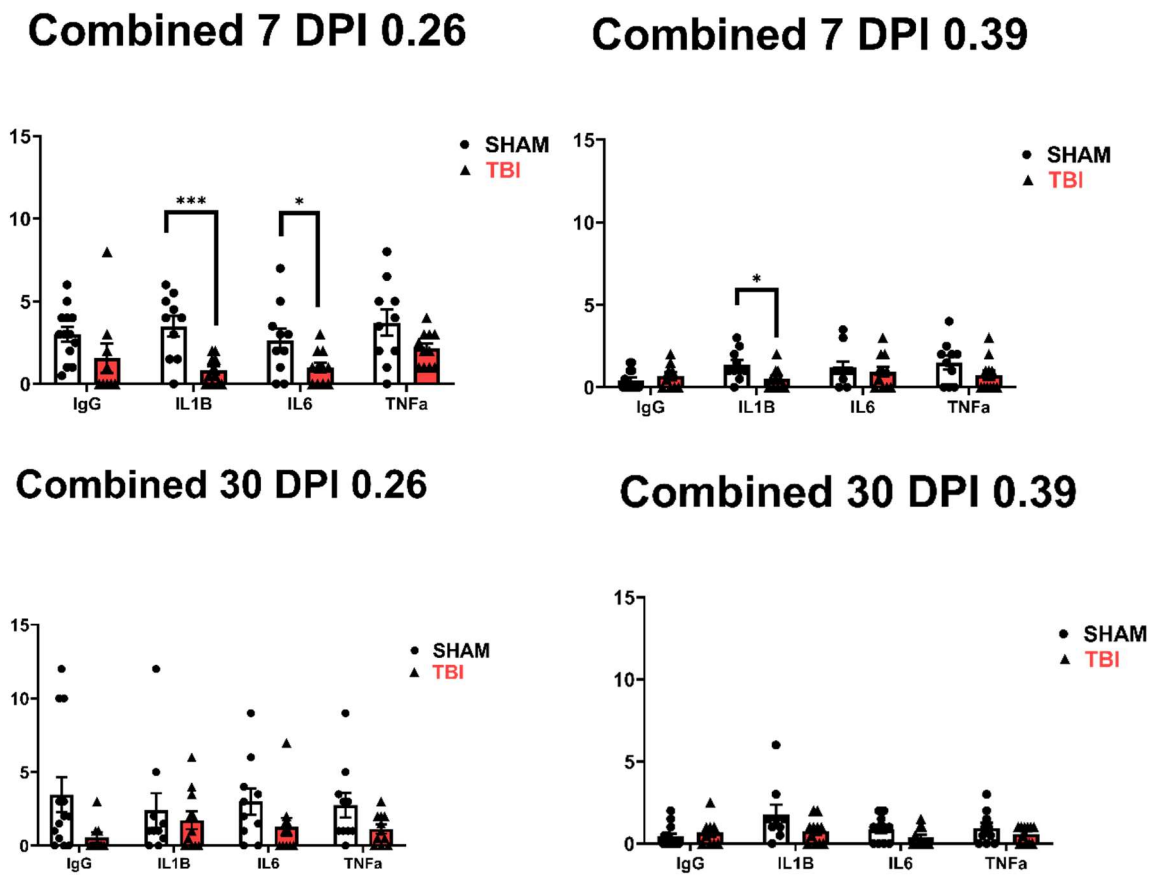


Aim 2: Ascertain the role peripheral inflammatory responses in visual deficits and retinal injury after blunt-head TBI.
For objectives under this aim, we also used a murine head trauma model. Serum samples were obtained 6 hours after injury for measurement of peripheral cytokine levels, and neutralizing antibodies against IL-1beta, TNF-alpha, or IL-6 were given 24 hours after injury. Optokinetic nystagmus was measured as a behavioral assessment of visual function. Retinal samples were taken for histologic and Western Blot analysis, and brains obtained for histologic analysis.

Major Task 2: Determine blunt head TBI visual outcomes after treatment with immune modulating drugs.
Subtask 1: Peripheral immune response to injury: completed. Head trauma led to increased serum IL-1 beta, IL-6, IL-8, and VEGF levels. TNF-alpha levels were significantly decreased, and there was no change in IL-10 levels. These results overall are consistent with activation of peripheral inflammatory response, as hypothesized.



Subtask 2: Functional visual assessment: Behavioral assessment videos have been completed, and most of the behavior analysis has also been completed. Results to this point suggest that TNF-alpha neutralization leads to improvement of optomotor function at 7 days after injury. This is an interesting finding given that early TNF-alpha levels are actually lower in mice with head trauma, compared to shams. At 30 days after injury, there are no significant differences between groups, although some of the differences may turn out to be significant once all data are included. This is expected to be completed in the next 1-2 months.



Subtask 3: Histologic and protein expression analysis: These analyses are in progress, and are expected to be completed in the next few months.

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

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

We plan to complete the remainder of the goals and objective during the next reporting period. Specifically, we will complete remaining analysis for PERK pathway data, perform experiments and data analysis for IRE-1 and ATF-6 pathways, and complete the optomotor and histologic analyses for cytokine neutralization experiments in Aim 2.

4. **IMPACT:**

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

There appears to be a divergence in the effect of different peripheral inflammatory cytokines on optomotor functioning in the model (as noted in Section 3, above). Specifically, blocking TNF-alpha appears to be beneficial. We expect to complete experiments and publish these results in the first part of the next reporting period. This finding may open new research avenues in traumatic optic neuropathy.

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:

Nothing to report

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

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

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

Nothing to report

Significant changes in use or care of vertebrate animals

No changes.
IACUC approval dates: 4/16/2020 to 4/16/2023

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS

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

Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers and presentations.

1. Deckard, K., Hetzer, S.M., and Evanson, N.K. Role of systemic inflammation in TBI-induced optic nerve injury. [oral presentation] Presented at University of Cincinnati Capstone Event. 4 Aug 2022, Cincinnati, OH. 2. Bellary, R., Hetzer, S.M., and Evanson, N.K. Examining the effects of salubrinal and ISRIB intervention on the endoplasmic reticulum stress response in traumatic brain injury induced optic neuropathy. [oral presentation] Presented at University of Cincinnati Undergraduate Scholarly Showcase. 21 April 2022, Cincinnati, OH

- **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**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Nathan K. Evanson MD, PhD
Project Role: PI
Researcher Identifier: 0000-0003-2474-9764
Nearest person month worked: 2.4
Contribution to Project: Dr. Evanson is the PI of the project and has supervised recruitment of students, supervised and directed performance of the experimental work, and interpreted results.

Name: Shelby Hetzer
Project Role: Graduate Student
Researcher Identifier: 0000-0002-6497-378X
Nearest person month worked: 9
Contribution to Project: Ms. Hetzer performed initial experiments on the salubrinal and ISRIB (Aim 1) studies, and assisted with/supervised undergraduate students who have helped with the Aim 2 studies.

Name: Jordyn Torrens
Project Role: Research Associate
Researcher Identifier: 0000-0002-3502-0680
Nearest person month worked: 3
Contribution to Project: Ms. Torrens is completing the experiments on peripheral inflammation (evaluation of tissue samples) and is now running the endoplasmic reticulum stress experiments.

Name: Kamden Deckard
Project Role: Undergraduate research fellow
Researcher Identifier: none
Nearest person month worked: 2
Contribution to Project: Ms. Deckard worked on the initial portion of the peripheral inflammation (Aim 2) studies as part of her summer undergraduate research fellowship.
Funding Support: University of Cincinnati RISE-Up program (R25)

Name: Rohan Bellary
Project Role: Undergraduate Student
Researcher Identifier: 0000-0002-5360-5818
Nearest person month worked: 2
Contribution to Project: Mr. Bellary helped with performing the experiments on salubrinal and ISRIB (Aim 1), including tissue analysis and behavior analysis.
Funding Support: Rotation for course credit

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

RIP Award **6/15/21 – 6/14/22**
Sponsor: Cincinnati Children's Hospital Research Foundation
Title: Mechanistic studies on brief oxygen exposure in head trauma-associated traumatic optic neuropathy.
Objective: To perform pilot studies on the mechanism of brief oxygen exposure after head trauma on ameliorating markers of optic nerve injury
Role: PI
Effort: no salary support
Performance period completed, so no longer part of other support.

WX81XWH2211075 VR210102 **9/15/22 – 9/14/2025**
Sponsor: Department of Defense Vision Research Program
Title: Delayed outcome mechanisms in chronic traumatic optic neuropathy
Objective: To determine mechanisms underlying divergent long-term outcomes after traumatic optic neuropathy at chronic time points, particularly including those that cause some mice to have improved recovery, and to determine whether early ameliorative effects of oxygen treatment lead to long-term improvements, or lead to oxygen toxicity.
Role: PI
Effort: 30%
New grant support which just started; no effect on PI effort for the current project.

What other organizations were involved as partners?

Organization name: University of Cincinnati
Location of organization: Cincinnati, OH
Contribution: facilities and students

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

See attached updated Quad Chart

Role of endoplasmic reticulum stress and systemic inflammation in blunt TBI-induced optic nerve injury

W81XWH2110907-VR200166

PI: Nathan K. Evanson **Org:** Cincinnati Children's Hospital Medical Center **Award Amount:** $260,000

Study/Product Aim(s)

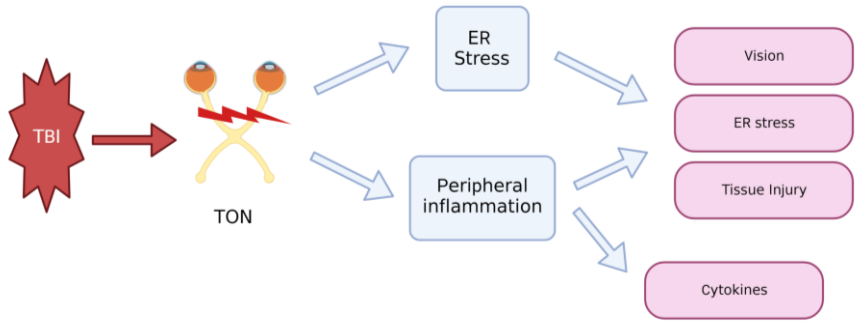
- Aim 1: To determine which element(s) of the ER stress response are most relevant to degenerative injury after optic nerve trauma.
- Aim 2: To determine the degree to which the peripheral inflammatory response contributes to outcomes after optic nerve trauma.

Approach

We are using a murine closed head weight drop model of head trauma, which results in bilateral optic nerve injury.

- Optokinetic nystagmus for visual function
- Histologic measures of tissue injury (brain and retina)
- Western Blotting for ER stress measures
- Serum cytokine measures for Aim 2
- Antibody-mediated neutralization of peripheral cytokines

Endoplasmic reticulum stress and peripheral inflammation in traumatic optic neuropathy.



TNF-alpha appears to be most important peripheral cytokine for driving visual deficits early after injury

Timeline and Cost

Activities	CY 21	22	23	
Specific Aim 1				
Specific Aim 2				
Data analysis and reporting				
Estimated Budget ($K)	**$32K**	**$130K**	**$98K**	

Updated: 12 Oct 2022

Goals/Milestones (Example)

CY21 Goal – Initiation of project

☑ Obtain regulatory approval, hire staff, get first cohorts

CY22 Goals –

☑ Initiate experimental procedures Aim 1

☑ Initiate experimental procedures Aim 2

CY23 Goal –

☐ Complete experiments and data analysis

☐ Submit results for publication

.

Budget Expenditure to Date

Projected Expenditure: $135K

Actual Expenditure: $135,608