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14. ABSTRACT

The purpose of this grant is to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. The studies performed in the previous year have shown that blast exposure (approx. 225 kPa magnitude) in a rat model leads to time-dependent ocular pathology changes over the course of eight weeks. Specifically, we have found that the behaviorally assessed visual acuity of blast exposed animals is significantly degraded following blast exposure. The decrease in visual ability is statistically significant when comparing blast-exposed animals to their baseline, pre-blast visual ability results. The decrease is also significant when comparing control and blast exposed animals at each time point after exposure. These deficits first become significant at two weeks after blast, and do not resolve by the end of the study. The visual acuity findings appear to be initially attributed to immediate retinal damage following blast exposure, but corneal injury also contributes to vision degradation several weeks after the initial blast exposure. We also found early biomarkers of corneal damage that could lead to treatment opportunities for corneal scarring.

15. SUBJECT TERMS
Biomechanics, ocular trauma, blast, rats, visual acuity

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INTRODUCTION

Ocular trauma during military conflicts has steadily increased from 0.5% in the civil war to 13% in present day. This increase is likely associated with the advancement of weaponry and the increased use of explosive devices. The majority of eye injuries from an explosion can be classified as either open globe or closed globe. Open globe injury is often readily identifiable and typically undergoes urgent surgical repair. However, closed globe injury may not be detected immediately and can result in a series of sequelae that lead to visual dysfunction months after the blast. The progression of closed globe eye injury and visual degradation following blast exposure has not been well characterized. Furthermore, it is unknown if there are early indicators that denote an increased risk for developing visual dysfunction following blast exposure. Therefore, the objectives of this proposal are to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. We propose to accomplish these objectives by first identifying the probability of military personnel developing visual system injury after blast exposure, and determining the time point after blast exposure that visual system injury becomes identifiable. Next, we propose to systematically evaluate the time course of visual system injury from blast exposure using our existing rat model for blast traumatic brain injury. From these experimental studies we can identify early predictors of visual dysfunction. Finally, we will evaluate these early predictors in a clinic setting to verify their usefulness in real-world scenarios. By understanding the temporal and chemical progression of eye injury from blast exposure, we can establish early identifiers of visual system injury. This will enhance our diagnostic capabilities and lead to the development of time-dependent treatment strategies to mitigate the loss of vision in military personnel.

KEYWORDS: blast, vision loss, biomarkers, pressure, ocular trauma, animal model, clinical study

OVERALL PROJECT SUMMARY

Aim 1: Investigate the progression of visual system injury in service members exposed to a blast.

Current Objectives

- Complete a retrospective analysis of the military personnel with visual system injury attributed to blast exposure but not immediately identifiable at the time of the blast. (SOW 1)
- Use the data to identify the probability of eventual visual system injury from blast exposure with and without an associated traumatic brain injury. (SOW 2)
- Statistically determine the time after the blast exposure that visual dysfunction is identifiable. (SOW 3)
- Identify local cases of military personnel with exposure to blast injury and no identifiable signs or symptoms of visual injury (SOW 4)

Key Methodology

University of Utah health records were searched for the following ICD9 codes: E993, E921, E923, E803, E837, E993.4, E890.0, E923.9. These ICD9 codes involve injuries from multiple types of explosions. The target date range was from 2005 to present. Inclusion criteria for this study are (1) No obvious sign of open globe trauma (e.g, facial burns, shrapnel to the eye, etc.) (2) Eye examination following blast exposure. Our control group consists of people involved in other traumatic injuries that would not affect the visual system (e.g., accidental or inflicted trauma to the extremities or torso without an associated head impact).

Medical records will be evaluated for information that may provide insight into the severity of the blast. Any history associated with the blast exposure will be investigated for signs of stand-off distance, height of the explosive, and the type of the explosive. In addition, injuries related to the initial blast exposure will be identified and given an assessment score based on the Abbreviated Injury Scale (AIS) which is an anatomical scoring system for classifying the severity of the injury. An increased injury severity score will be assumed to indicate an increased severity of blast exposure. To maximize efficiency with data collection, we have designed a database within REDCap at the University of Utah. REDCap is a secure, web-based application for building and managing online surveys and databases. This database also allows us to share data with all the IRB approved investigators on the grant. The data entry form created for the database is provided in **Appendix A**.

All statistical analyses will be performed using SAS statistical software (JMP 10.0, Cary,NC). Descriptive and univariate analyses will first be performed to identify the occurrence of delayed visualsystem injury after blast exposure. Of the cases with delayed visual system injury, the time between the blast exposure and diagnosis will be collected. Significant differences with age, gender, the presence of absence of traumatic brain injury, and blast severity will be evaluated. Statistical significance will be set

at a p-value of < 0.5. Logistic regression will also be used to determine the probability for developing visual system injury following blast exposure given age, gender, blast severity, and the presence/absence of traumatic brain injury. In addition, a survival analysis will be performed using Cox's proportional hazards regression model to determine the time post blast exposure that visual system injury is most likely to be identified. Multiple regression analysis will be used to determine the effect of participant age, gender and blast severity on the survival analysis.

Results

The retrospective review at the University of Utah resulted in 535 unique medical records. **Table 1** provides demographics for the cases. The majority of these (n=431) did not have a record of an eye exam and have been excluded from the study. Another subset of cases (n=75) were eliminated from the study due to open globe trauma. These include corneal abrasions, corneal lacerations, corneal burns, and foreign body to the eye. We have elected to keep one case of corneal abrasion because it resolved and there was a follow-up exam monitoring vision. The remaining 29 cases are currently being evaluated for details regarding each of the events to determine whether they meet the remaining criteria of the study or will be excluded. No statistics have been performed to date because of the limited sample size.

Gender		
	Male	(81.5%) 436
	Female	(18.5%) 99
Age		
	Mean	33.6±17.5 years
	Min	3 months
	Max	88 years
Race		
	American Indian/Alaska Native	0.7% (4)
	African American	0.6% (3)
	Hispanic or Latino	9.5% (51)
	Native Hawaiian/Pacific Islander	0.9% (5)
	Caucasian	62.4% (344)
	Unknown	24.9% (133)
	Other	0.4% (2)
Common Causes		
	Fireworks	15.7% (84)
	Propane-related explosion	11.4% (61)
	Smoking while on oxygen	3.9% (21)
	Cooking incidents	3.6% (19)
	Gun-related incidents	3.0% (16)
	Tire explosion	2.8% (15)

Table 1. Demographics of civilians treated for injuries related to an explosion.

We have continued the retrospective review of the Polytrauma group at the Salt Lake City VA. 25 cases have been evaluated to date. Table 2 describes the summary characteristics of this group.

Gender		
	Male	(100%) 25
	Female	(0%) 0
Age		
	Mean	33.6±17.5 years
	Min	3 months
	Max	88 years
Race		
	American Indian/Alaska Native	0% (0)
	African American	0% (0)
	Hispanic or Latino	12.0% (3)
	Native Hawaiian/Pacific Islander	4.0% (1)
	Caucasian	76.0% (19)
	Unknown	8.0% (2)
	Other	0% (0)
Blast Mechanism		
	Improvised Explosive Device	76.2% (16)
	Rocket Propelled Grenade	42.9% (9)
	Mortar	33.3% (7)
	Other	20.0% (5)
TBI Diagnosis		
	Yes	100% (24)
	No	0% (0)
Vision Complaint Severity (blurring, troubles seeing, etc)	Mild	39.1% (9)
	Moderate	39.1% (9)
	Severe	8.7% (2)
	None	13.0% (3)

Table 2. Demographics of veterans treated for injuries related to an explosion.

Progress and Accomplishments

The retrospective review of the University of Utah records was completed, but resulted in minimal viable records. Out of the 535 cases, 33 were found that met our inclusion criteria. Six of these cases involved corneal abrasion (which may affect vision and were thus excluded). The remaining 27 cases will be evaluated and analyzed. No statistics have been performed because of the small sample size. We will combine this dataset (if appropriate) with the data set from the VA. There have been personnel issues that have prevented substantial progress to be made in the retrospective review of the VA dataset. We have been hiring medical students to perform the review.

However, access to the records takes 3 months. By the time the medical students have access they have either lost interest or no longer have time available. Therefore, we have only collected 25 cases to date. To solve this problem, I have posted an ad for a health data analyst. This person will be able (expected) to devote much more time to the position. I currently have 3 viable applicants that I am selecting from. One is a retired Nurse who has clinical research experience in Ophthalmology. I will wrap up interviews in the next week and select the new personnel.

For the prospective study, the University of Utah and Salt Lake City VA have approved our IRB protocols. We submitted the approved protocols to HRPO on April 24, 2015. We have followed up several times with HRPO, but have not received approval to move forward. We will continue to contact them to determine the status.

This year, we made contact with Tom Vivace, who is working with the Vision Center for Excellence. They maintain several large DOD databased on ocular trauma in the military and are looking for people who can utilize the data. I had several meetings with them and we discovered their interests on this project are closely aligned with ours. Therefore, we have begun the steps to get access to their databases which includes data from the theaters and VA medical records across the country. This will greatly expand our search and result in a much stronger statistical analysis.

Aim 2: Investigate the progression of visual system injury following blast exposure in an animal model and identify early indicators of visual dysfunction.

Current Objectives

- Complete final animal studies
- Analyze collected tissue samples
- Perform Statistical Analysis
- Measure IOP during blast

Key Methodology

Briefly, adult Long Evans rats were administered carprofen one day before the blast for pain management. A baseline of vision functionality was established before the blast using the custom optokinetic tracking device we developed in Year 1 (**Figure 1A**). For increased accuracy, each animal is tested three times on each testing day and an average acuity is used for the final measurement. In Year 3, we updated our behavior code to separately assess visual ability in each eye.

On the day of the blast, the animal is anesthetized using inhaled isoflurane followed by an injection of ketamine and dexmetomedine administered IP. The anesthetized animal is placed in the custom rat holder also designed in Year 1 (**Figure 1B**) to provide a side-on blast exposure while preventing injury to the animal torso. After blast exposure, the animal is removed from the device, allowed to recover from the anesthesia, and then returned to the animal facility. While animals do not show signs of pain

following the blast exposure, carprofen is administered the next day as a precaution. The vision metrics (vision behavior, OCT) are then repeated the day after the blast and every subsequent week following the blast until sacrifice. At sacrifice, the eyes and brain are harvested for later analysis. The length of the survival period was increased to 8 weeks and the number of blast levels investigated was decreased to two.

In addition to monitoring retinal thickness over time, we added an evaluation of the corneal damage following blast exposure. Corneal damage was assessed by measuring corneal thickness via OCT and H&E microscopic staining. For OCT image analysis, we developed two MATLAB image

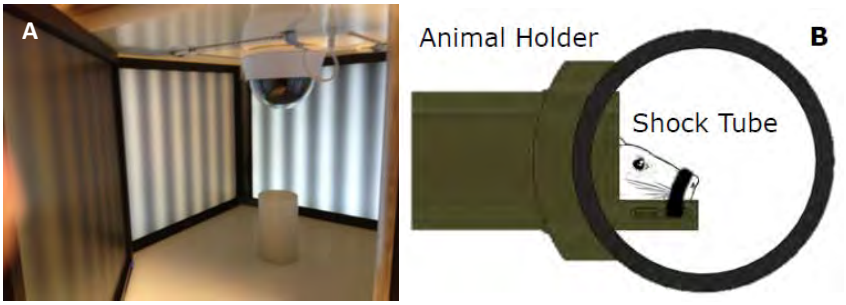


Figure 1. (A) Custom optokinetic device designed to test visual acuity in rats. (B) Rat mounted in the shock tube for side-on blast exposure.

processing programs to evaluate the thickness of the retina and the cornea. The retina image processing program measures the thickness of the retina and RPE layers as shown in **Figure 2**. Thickness of both the retina and RPE was measured for forty pixel columns and averaged. The average for each image was then averaged with other images in the same retina region. The regions are defined in relation to the optic nerve: superior medial/distal (SM/SD), inferior medial/distal (IM/ID), nasal medial/distal (NM/ND), and temporal medial/distal (TM/TD).

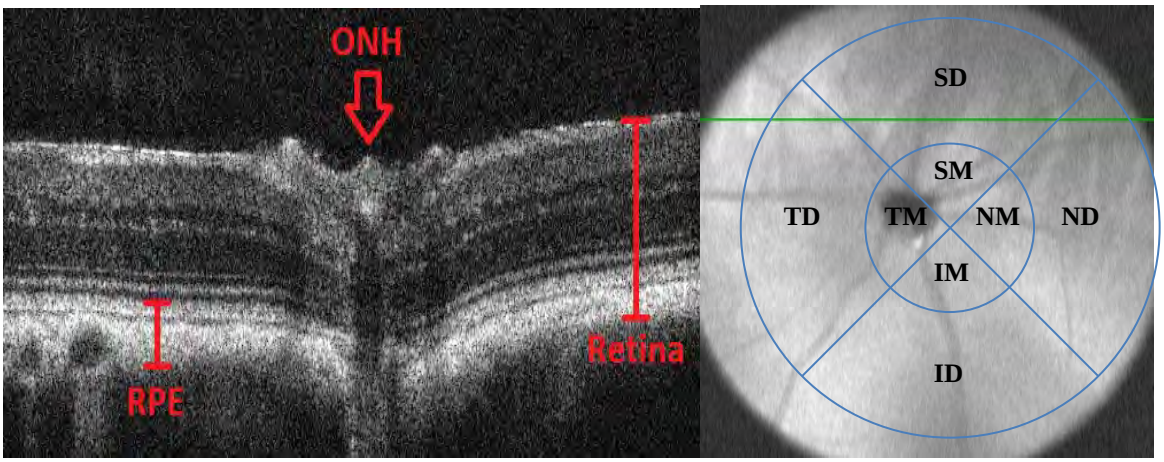


Figure 2. (A) Optical coherence tomography (OCT) is used to monitor changes in the retinal thickness. (B) The thickness of the RPE and retina are averaged across regions defined by a radial pattern around the optic nerve of the eye.

The corneal thickness program is semi-automated. Each frame of an OCT region is presented and a user selects a region of the cornea to analyze (**Figure 3**). The cornea thickness is determined by calculating the distance of every pixel point on the top of the cornea image to the corresponding

point on the bottom edge. Pixel distances are converted to millimeters. An average thickness is determined by averaging these pixel distances for each frame. Subsequent frames from the same eye are analyzed and all the frames for a single cornea are averaged.

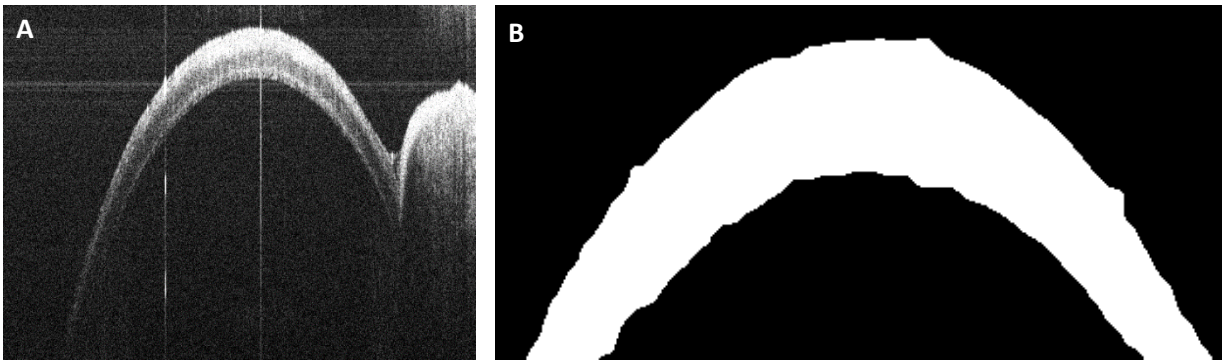


Figure 3. (A) Optical coherence tomography (OCT) is used to monitor changes in the corneal thickness. (B) A custom image processing script removes any skew from the original OCT image and then creates a binary image of the cornea. Thickness is averaged across the entire cornea for each frame.

Results

To date, 90 animals have been evaluated. There was a significant initial drop in visual acuity following blast compared to controls. Over a period of 8 weeks, visual acuity decreased and never recovered to baseline levels (**Figure 4**). In fact, the visual acuity from control animals improved slightly, likely due to increased familiarity with the optokinetic device and less distraction by surroundings during testing. Of the 90 animals tested, only 2 mortalities occurred. It should be noted that two of the 90 animals tested were actually used to determine the effect of the blast on intraocular pressure (IOP). There were complications during those tests and we have designed a new setup to try during the next quarter.

The corneal OCT analysis resulted in significant, time-dependent changes in corneal layer thickness (**Figure 5,6**). Stromal thickness significantly increased at one week post-injury in the eye contralateral to the blast. This thickness change resolved by week 2. In the eye ipsilateral to the blast, the stromal thickness significantly increased at week 2 and stayed increased until week 5. The epithelium significantly increased at week 5 and remained increased until week 6. This increase only occurred in the eye ipsilateral to the blast. A small subset of eyes were examined immediately post-blast using fluorescein staining to ensure that corneal injuries were not induced by sporadic particles caused by membrane rupture. We are confident that these injuries are truly caused by the blast pressure wave.

Retinal thickness was measured using the semi-automated MATLAB program. All data has been collected from the images, and statistical analysis of these data is underway.

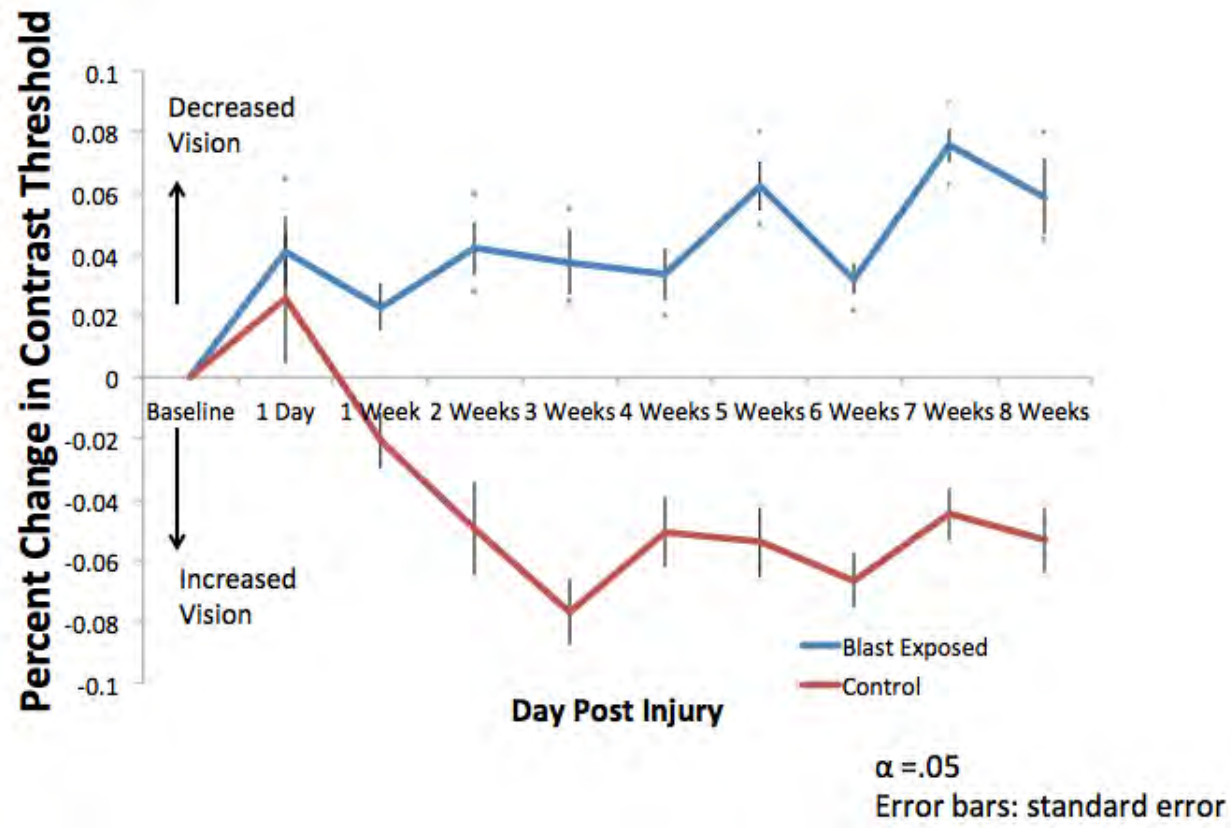


Figure 4. Behavior test results from control and blast exposed. *indicates significant differences between control and animals experiencing a low-level blast pressure ($p<0.05$).

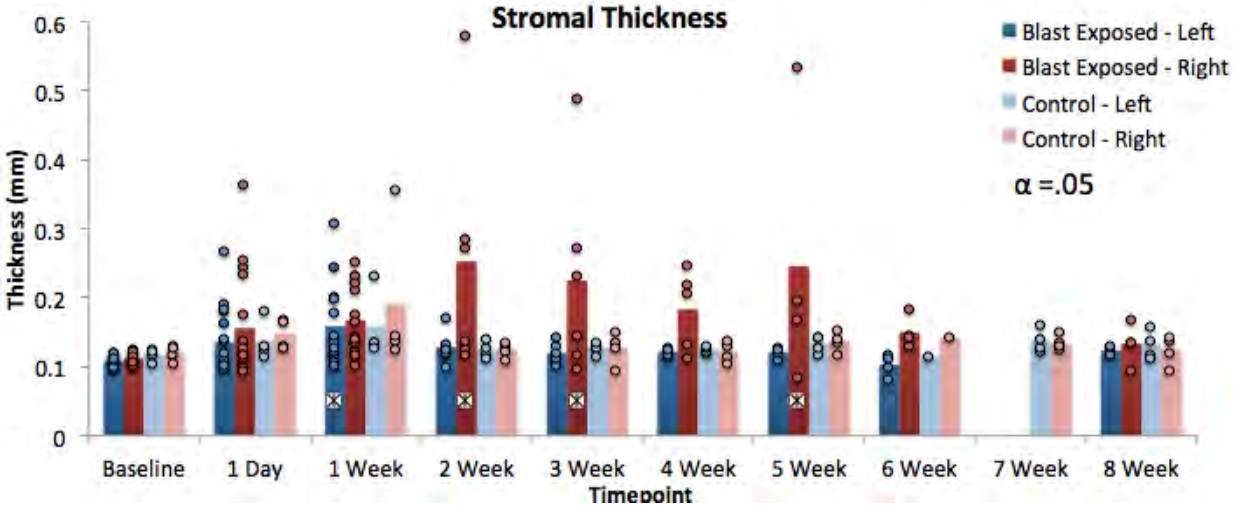


Figure 5. Analysis of cornea stromal thickness following blast exposure. Stroma significantly thickened from baseline at weeks 1-3 and 5. The majority of the changes were seen in the blast exposed (ipsilateral) eye.

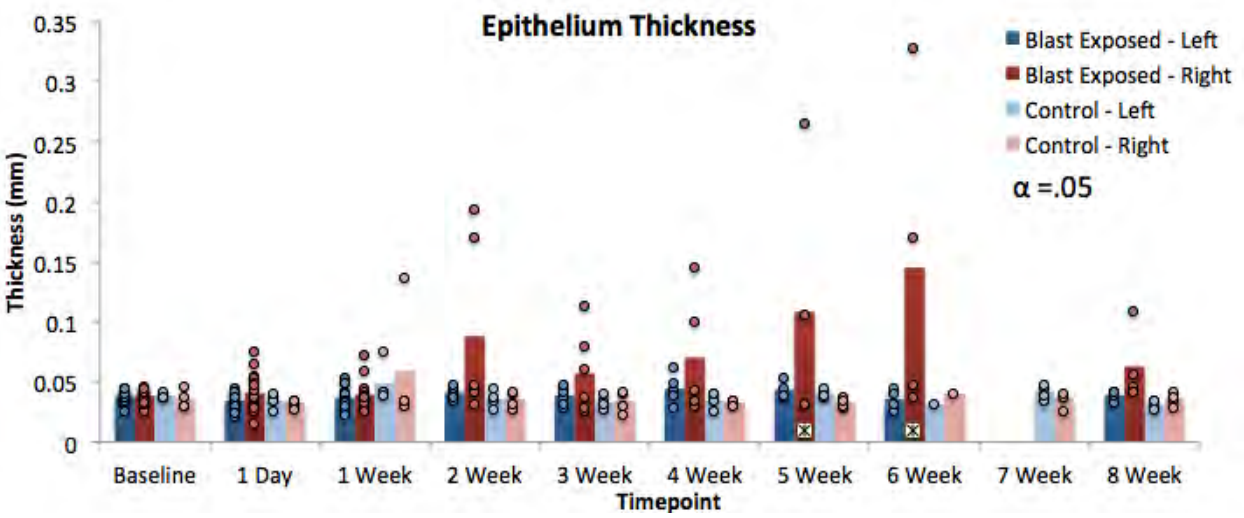


Figure 6. Analysis of epithelium thickness following blast exposure. Epithelium significantly thickened from baseline at weeks 5 and 6. No significant changes were seen in any control groups or in the left (contralateral) eye.

Progress and Accomplishments

This year, we completed our proposed animal studies. Head motion during blast was recorded using a high speed camera in a subset of blasts. A still from this recording can be seen below (**Figure 7**). These recordings show some head movement during the blast. To alleviate concern that the eye impacted the animal holder, four animals were evaluated by an ophthalmologist directly after blast exposure. Fluorescein staining was used to visualize cornea defects. The animals examined showed no signs of immediate ocular trauma, so impact trauma for the holder or membrane shrapnel was ruled out as a confounding injury mechanism. OCT imaging of the cornea showed significant changes in corneal thickness after blast exposure in both the stroma and epithelium. These changes were not immediate, but rather took weeks to manifest. Visual acuity behavior testing resulted in a more immediate change that may be related to immediate retinal dysfunction rather than corneal injury. Although, corneal injury likely contributes to vision loss later post-injury. Future work should further explore the delineation between these injuries. To assist in this, we have modified our behavior test apparatus to independently test the visual acuity in the left and right eye.

Our research from Aim 2 was presented at two conferences this year. At ARVO (held in Denver, CO) we presented our experimental design and the behavioral changes that we have found. These findings were presented in a podium presentation. At the Summer Biomechanics, Bioengineering, and Biotransport Conference we had another podium presentation, in which we presented our experimental overview, behavior results, and cornea OCT findings. This podium presentation was a finalist in the PhD competition (15% acceptance rate). We envision a series of three papers describing our findings. The first paper will cover the design and development of the shock tube. The second paper will report the visual behavior device and retinal

findings (histology and OCT). The third paper will present the corneal injury patterns as determined from histology and OCT. We have begun writing the first of these papers.

We have also begun a collaboration with Jade Therapeutics, a local biomedical company developing drug delivery systems for the eye. Jade has developed a hyaluronic acid treatment designed to aid wound healing in the cornea. The pilot study on a group of eight animals has been completed. Findings will be used to drive a future grant submission.

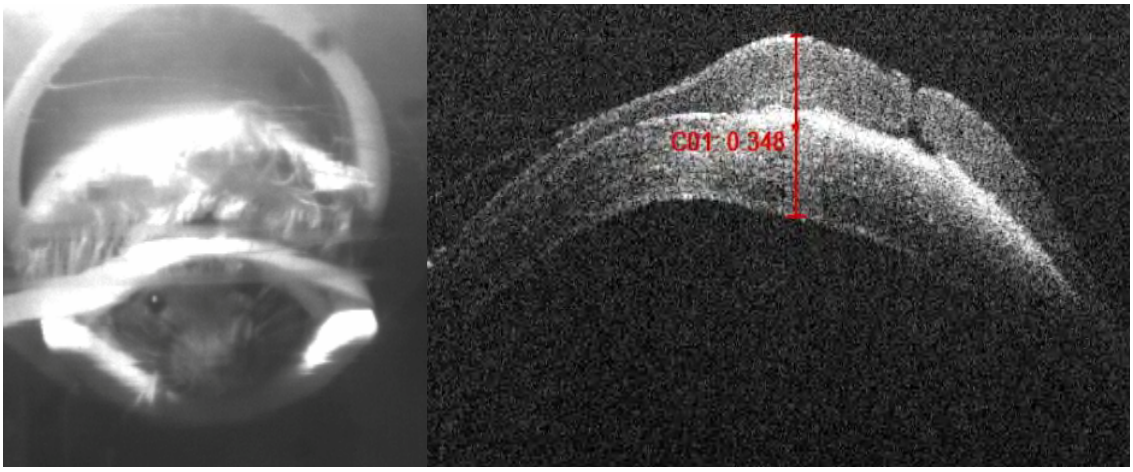


Figure 7. (Left) Image still from recorded blast impact. (Right) Corneal damage at 5 weeks after blast.

Aim 3: Identify changes in vitreous protein expression that correlate with visual system injury

Current Objectives

- Collect vitreous samples following every animal experiment in Aim 2. (SOW 1)
- Assay samples for NfH, VEGF, IL-10, MCP-1, and MIP-3 (SOW 2)

Key Methodology

The vitreous from half of the animals in each group at 1 day and 1, 4 and 8 weeks post-blast were evaluated for biomarkers of ocular trauma. VEGF and other cytokines wer measured using a commercially available antibody array (RayBio Rat Cytokine Antibody Array G, RayBiotech, Norcross, GA). Signal intensities were evaluated using an ELISA plate reader at an excitation frequency of 532 nm. Positive and negative controls in the array allowed comparison between different array analyses. All samples were tested in duplicate on a single plate and the average intensity was recorded for statistical analysis. To evaluate changes in neurofilament-heavy chain (NfH) following blast injury, a method similar to that presented by Petzold et al. was used. All samples were tested in duplicate on a single plate and the average intensity is recorded for statistical analysis.

Results

We have analyzed all of the vitreous biomarkers from the experimental studies, with the exception of a small supplemental study group to improve sample sizes. To date, we found significant decreases in contralateral LIX, and TNF-α up to four weeks post-injury (**Figure 8**) and a significant increase in ipsilateral LIX and TNF-α compared to the contralateral eye, but only at four weeks. No significant changes were found in VEGF. LIX (CXCL5/LPS-induced chemokine) is involved in neutrophil recruitment to the corneal stroma. Corneal damage has been seen in the contralateral eye at 1 week, but it resolves at 2 weeks. This could explain the decrease in LIX at 4 weeks compared to 1 week post-blast in the contralateral eye. Corneal injury in the ipsilateral eye appears at 2 weeks in the stroma and at 6 weeks in the epithelial layer. For this reason, it makes sense that LIX is significantly higher in the ipsilateral eye at 4 weeks compared to the contralateral eye. TNF-α is a proinflammatory cytokine that is associated with many diseases. It is not clear whether injury to the cornea initiated the increase of this protein, or injury elsewhere to the eye. The response is very similar to LIX, so we believe it is related to the corneal damage.

Neurofilament Heavy Chain (NfH) significantly increased immediately after injury and was maintained up to 4 weeks after injury and began to decline at 8 weeks (**Figure 9**). NfH is believed to be a marker of retinal degeneration. This implies that the retina from the blast has sustained breakdown until after 4 weeks. Behaviorally, we see immediate and sustained vision deficits which correlate with these biomarker findings. Vision deficits seen after 4 weeks could be due to the manifestation of corneal injury by that time point.

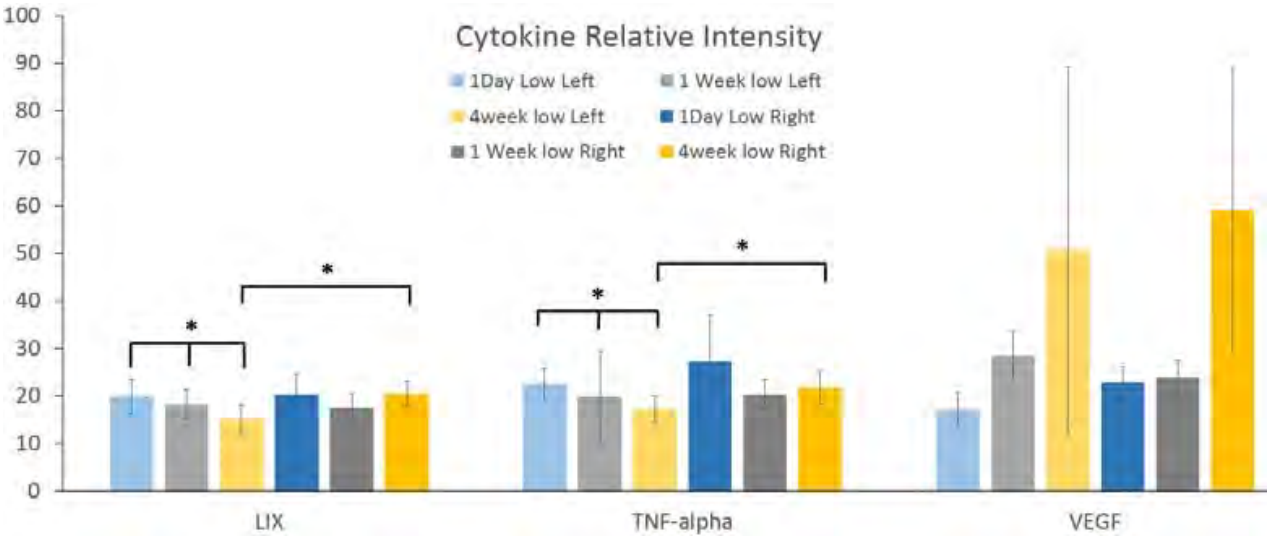


Figure 8. Results of protein biomarker analysis 1 day, 1 week, and 4 weeks after blast exposure. LIX and TNF-alpha significantly decreased in the contralateral blast eye, but were significantly increased in the ipsilateral eye at 4 weeks. This is likely related to the temporal response of the corneal injury. VEGF showed some trends, but these are not significant at this time.

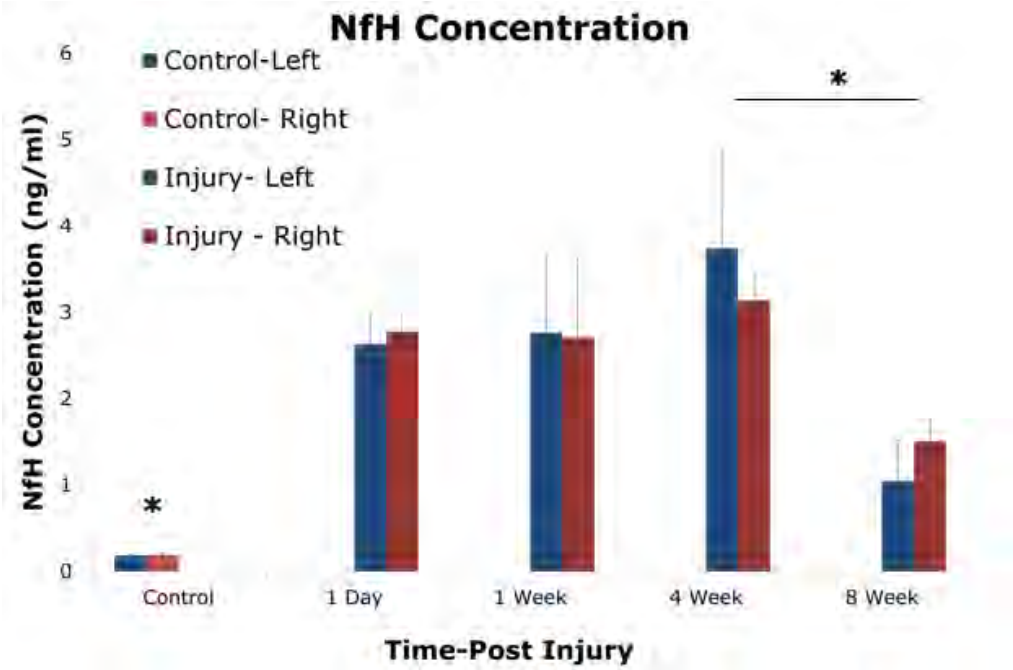


Figure 9. Neurofilament heavy chain (NfH) significantly increased in both the left and right eyes following blast exposure and was sustained for 4 weeks post injury.

Progress and Accomplishments

All vitreous cytokine studies are complete except for a small subset added at the end to boost our sample size at 8 weeks. These experimental studies are complete and the vitreous will be analyzed in the next quarter. The results from the vitreous biomarker studies correlate well with the injury findings in Aim 2. The significant changes in LIX and NfH are exciting as they may be early predictors of retinal and corneal injury, respectively. Future studies should correlate NfH findings with electrical output in the retina. Furthermore, drug treatments targeting LIX may have some influence in corneal healing. Both of these aspects will be explored in future proposal submissions.

All the data shown in this section were presented in a poster presentation at SB3C 2015. The poster was awarded second prize in the undergraduate research competition. A manuscript related to these results is currently being drafted.

KEY RESEARCH ACCOMPLISHMENTS:

- Identified immediate decrease in vision following a low-level blast exposure that remains steady until 8 weeks post injury. This was significantly different than control animals which actually improved with time.
- Characterized significant temporal corneal changes following blast exposure. In eyes not directly exposure to a pressure wave, these changes appear to occur early on, but resolve. In eyes directly

exposed to a pressure wave, the stroma thickens after 2 weeks, then the epithelial layer thickens at 5 weeks. Eventual corneal scarring occurs in many of the animals. The initial identification of corneal thickening provides a window of opportunity for drug treatment that may prevent eventual scarring.

- There is a significant increase in LIX and TNF-a at time points correlating to structural changes in the cornea. These protein changes may be influential in identifying appropriate drug treatment targets.
- A significant increase in NfH immediately post-blast correlates well with the findings of immediate visual acuity loss post-blast. This suggests that retinal damage may be responsible for immediate changes in vision, but subsequent vision loss may be due to both retinal and corneal injury. Future studies should investigate the contribution of each of these injuries to vision degradation.

CONCLUSION:

The successful completion of the studies proposed in this 4 year project will form the basis for understanding the temporal and chemical progression of visual system injury following blast exposure. In the first year, all the infrastructure and product development was completed to successfully achieve the stated goals of the study. In Year 2, the bulk of the experimental work was performed. Several modifications to the blast device were made. In year 3, all animal studies were completed and resulted in some remarkable findings regarding the time course of retinal and corneal injury following a blast exposure. The results from these studies will be critical to the development of treatment strategies to prevent vision loss in military personnel following blast exposure. The last year of this project will be focused on ramping up clinical efforts to develop translational metrics between the clinical and experimental findings.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Abstract & Presentation

Shedd DF, Jones J, Coats B. Ocular Injury following Low Level Blast Exposure in Rats. Association for Research in Vision and Ophthalmology Annual Meeting 2015. Denver, CO. May 2015.

Jones J, Shedd DF., Coats B. The Temporal Change in Protein Biomarkers in the Vitreous Humor following Blast Trauma. Summer Biomechanics, Bioengineering and BIotransport Conference 2015. Park City, UT. June 2015

Shedd DF, Jones J, Zaugg B., Coats B. Cornea Damage Progression following Blast Exposure. Summer Biomechanics, Bioengineering and BIotransport Conference 2015. Park City, UT. June 2015

Shedd DF and Coats B. Temporary visual dysfunction following low-level blast exposure. 7th World Congress of Biomechanics. Boston, MA July 2014

Book Chapter
Coats B. and Shedd DF. Biomechanics of Eye Injury in the Military. In A. Gefen & Y. Epstein (Eds) *Mechanobiology and Mechanophysiology of Military-Related Injuries.* New York: Springer (*in press 2016)*

INVENTIONS, PATENTS AND LICENSES:

Nothing to report.

REPORTABLE OUTCOMES:

- Silencer and dump tank developed for 12" diameter shock tube. Results in minimal change to the resulting pressure profile and results in a 15% reduction in decibel level.
- Designed a clamping system to pressurize shock tubes to high pressures and reduce early membrane failure.
- Developed semi-automated image processing tools for analyzing the thickness of the retina and cornea from OCT data.
- Developed automated image processing tools for analyzing cytokine biomarkers and NfH protein assays.
- Identified the time course of corneal injury following blast exposure.
- Identified initial biomarkers for corneal scarring following blast exposure.
- Identified the time course of cytokine and neurofilament heavy chain changes in the vitreous following blast exposure which help explain the mechanisms of vision loss.

OTHER ACHIEVEMENTS:

Nothing to report.

REFERENCES:

Petzold et al. A specific ELISA for measuring neurofilament heavy chain phosphoforms. *Journal of Immunological Methods.* 278, 179-190 (2003).

APPENDICES:

Appendix A: Data Collection Form on REDCap
Appendix B: 2014 World Congress of Biomechanics Abstract
Appendix C: 2014 World Congress of Biomechanics Poster

Appendix D: Research Highlight of this proposal included in the FY14 Report to the executive Agent for Prevention, Mitigation, and Treatment of Blast Injuries.
Appendix E: 2015 ARVO abstract – Podium presentation
Appendix F: 2015 Summer Biomechanics, Bioengineering and Biotransport Conference Abstract (PhD Competition Finalist)
Appendix G: 2015 Summer Biomechanics, Bioengineering and Biotransport Conference Abstract (2nd place winner in the undergraduate research competition)
Appendix H: Brittany Coats (PI) CV
Appendix I: Quad Chart

Initial Pass Data

Record ID _____

MRN _____

Gender
- ☐ Male
- ☐ Female
- ☐ Unknown

Age at Presentation _____

Height _____
(In inches)

Weight _____
(In pounds)

BMI _____

Race/Ethnicity
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Hispanic or Latino
- ☐ Native Hawaiian or Other Pacific Islander
- ☐ White
- ☐ Unknown
- ☐ Other

Other Race _____

History of Present Illness (HPI)

Date of Presentation _____

Date of Explosion (if different than admission date) _____

Burns Present
- ☐ Yes
- ☐ No

Description of Burns _____

Glasgow Coma Score _____

HPI Description _____

Head Injury Diagnosis
- ☐ TBI Present
- ☐ TBI Absent
- ☐ No Diagnosis of TBI Recorded

Description of Head Injury Diagnosis _____

Initial Eye Exam

Fluorescein Administered
- ☐ Yes
- ☐ No

Result of Fluorescein Test _____

Visual Acuity - Right Eye (OD)

- ☐ 20/15
- ☐ 20/20
- ☐ 20/25
- ☐ 20/30
- ☐ 20/40
- ☐ 20/50
- ☐ 20/70
- ☐ 20/80
- ☐ 20/100
- ☐ 20/200
- ☐ 20/400
- ☐ Count fingers
- ☐ Light perception
- ☐ No light perception

Visual Acuity - Left Eye (OS)

- ☐ 20/15
- ☐ 20/20
- ☐ 20/25
- ☐ 20/30
- ☐ 20/40
- ☐ 20/50
- ☐ 20/70
- ☐ 20/80
- ☐ 20/100
- ☐ 20/200
- ☐ 20/400
- ☐ Count fingers
- ☐ Light perception
- ☐ No light perception

Visual Acuity - Both Eyes (OU)

- ☐ 20/15
- ☐ 20/20
- ☐ 20/25
- ☐ 20/30
- ☐ 20/40
- ☐ 20/50
- ☐ 20/70
- ☐ 20/80
- ☐ 20/100
- ☐ 20/200
- ☐ 20/400
- ☐ Count fingers
- ☐ Light perception
- ☐ No light perception

Pupillary Defect - OD

- ☐ Yes
- ☐ No (PERRLA)

Description of Defect _____

Pupillary Defect - OS

- ☐ Yes
- ☐ No (PERRLA)

Description of Defect _____

Extraocular Motility

- ☐ EOMI
- ☐ OD deficit
- ☐ OS deficit
- ☐ OU deficit
- ☐ Other

Other extraocular motility findings not listed above _____

Visual Field Defect- OD

- ☐ Yes
- ☐ No

Description of Defect _____

Visual Field Defect - OS
☐ Yes
☐ No

Description of Defect _____

Periorbital Edema Present - OD
☐ Yes
☐ No

Periorbital Edema Present - OS
☐ Yes
☐ No

Conjunctiva Damage - OD
☐ Yes
☐ No

Conjunctiva Damage - OS
☐ Yes
☐ No

Initial Ophthalmic Findings - Additional pertinent findings not discussed elsewhere _____

Follow-Up Information

Follow-up at Moran
☐ Yes
☐ No
☐ Unknown

Exclude from study?
☐ Yes
☐ No

Reason for Exclusion _____

Need PowerChart Access (chart unavailable in Epic)
☐ Yes
☐ No

Follow-Up

Visual Acuity - Right Eye (OD)

- ☐ 20/15
- ☐ 20/20
- ☐ 20/25
- ☐ 20/30
- ☐ 20/40
- ☐ 20/50
- ☐ 20/70
- ☐ 20/80
- ☐ 20/100
- ☐ 20/200
- ☐ 20/400
- ☐ Count fingers
- ☐ Light perception
- ☐ No light perception

Visual Acuity - Left Eye (OS)

- ☐ 20/15
- ☐ 20/20
- ☐ 20/25
- ☐ 20/30
- ☐ 20/40
- ☐ 20/50
- ☐ 20/70
- ☐ 20/80
- ☐ 20/100
- ☐ 20/200
- ☐ 20/400
- ☐ Count fingers
- ☐ Light perception
- ☐ No light perception

Pupillary Defect - OD

- ☐ Yes
- ☐ No (PERRLA)

Description of Defect _____

Pupillary Defect - OS

- ☐ Yes
- ☐ No (PERRLA)

Description of Defect _____

Intraocular Pressure - OD _____

Intraocular Pressure - OS _____

Visual Field Defect - OD

- ☐ Yes
- ☐ No

Description of Defect _____

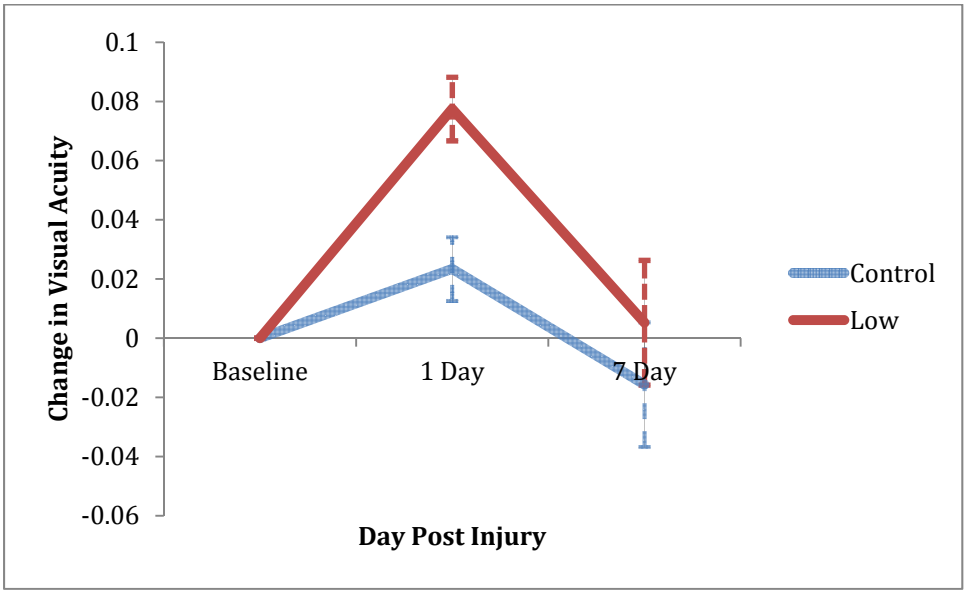
Visual Field Defect - OS

- ☐ Yes
- ☐ No

Description of Defect _____

Title: Temporary Visual Dysfunction following Low Level Blast Exposure

Blast exposure is a leading cause of eye injury for the US Army. Open globe ocular trauma, including shrapnel or debris to the eye, is easily identified and rapidly treated. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, we have developed a rodent model to characterize the time-dependent changes in visual acuity after blast exposure. To assess visual acuity in rodents, a custom vision behavioral device was built to measure the threshold for the natural optokinetic nystagmus reflex. The test animal is placed in the center of the device and a cylindrical sine wave grating is displayed on four surrounding computer monitors. The grating rotates around the animal, which causes the animal to reflexively track the grating motion with head movements. The level of grating contrast at which the direction of drift is correctly tracked by the animal represents the level of functional visual acuity. An increase in visual acuity indicates a decrease in vision functionality. For the present study, anesthetized Long-Evans rats were exposed to 230 kPa pressure waves using a compressed-air shock tube. Control animals were anesthetized and placed in the shock tube, but no pressure wave was activated. Visual acuity was assessed three times in each animal at three time points: before blast exposure, one day after exposure, and one week after exposure. Relative to baseline measurements, animals exposed to the blast pressure wave had a significant increase from visual acuity one day after the blast and then returned to pre-injury levels one week after the blast. No increase was found in control animals. This suggests that a low level blast may cause temporary visual dysfunction, but it is not sufficient to cause long-term injury. Future studies will investigate visual functionality at more severe levels of blast exposure and for later time periods after blast exposure.



Temporary Visual Dysfunction following Low Level Blast Exposure

Daniel F. Shedd and Brittany Coats, PhD
Mechanical Engineering, University of Utah, Salt Lake City, UT USA

THE UNIVERSITY OF UTAH

PEDIATRIC HEAD TRAUMA
Injury Biomechanics Lab

Introduction

Blast exposure is a leading cause of eye injury for the US Army [1]. Typically, ocular injury occurs from explosive shrapnel and debris, but recently many soldiers have developed vision deficits 6-12 months following a blast exposure without any signs of injury [2]. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, we developed a rodent model to characterize the time-dependent changes in visual acuity after blast exposure using behavioral vision testing and optical coherence tomography (OCT).

Methods

Anesthetized Long-Evans rats (300-350g, n=12) were exposed to 230 kPa pressure waves using a compressed-air shock tube (Fig. 1). Control animals (n=12) were anesthetized and placed in the shock tube, but no pressure wave was activated. Animals were euthanized at 1 day, 1 week, 4 weeks, or 8 weeks post-blast.

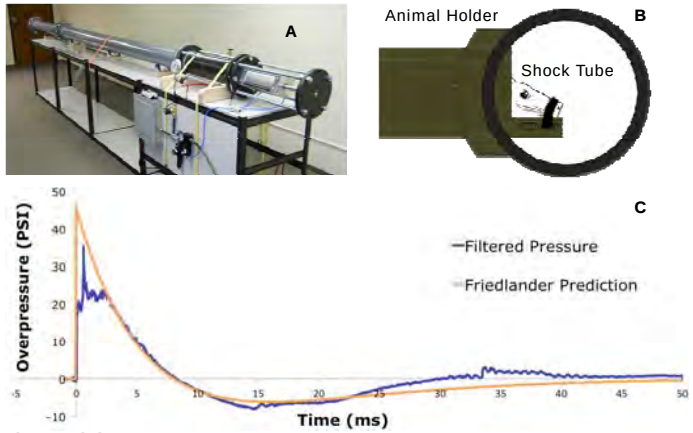


Fig. 1. (A) The 6" experimental shock tube was triggered via rupturing BoPET membranes and instrumented with 1 MS/s pressure sensors (PCB 113B26) along the length of the driven section. **(B)** Animal placement within shock tube. **(C)** Representative filtered pressure profile used to apply blast insult. Comparison to ideal Friedlander waveform shown. R^2 = .92

A custom vision behavior device (Fig. 2) was built to measure the visual acuity threshold using the optokinetic nystagmus reflex. Test animals were placed in the center of the device and a cylindrical sine wave grating was displayed on four surrounding computer monitors. The grating rotated around the animal, which caused the animal to reflexively track the grating motion. The grating contrast at which the direction of drift was tracked by the animal represented the level of functional visual acuity (Fig. 3). Visual acuity was assessed three times in each animal at up to eight time points. A two-tailed matched-pair test with p=.05 was used to find significant vision changes. OCT imaging (Fig. 4) was performed using Bioptigen Envisu™ R2200 OCT scanner with an ultra-high resolution (UHR) light source and a rat retina lens. The scan settings were: 1000 A-scans per B-Scan, 100 B-scans over a field of view of 2.6 mm by 2.6 mm. Images were processed and analyzed using MATLAB [3] to find total retinal thickness and RPE thickness.

More information about the Utah Laboratory of Pediatric Injury Biomechanics is available at our lab website: pedtrauma.mech.utah.edu

Methods

Figure 2. Visual acuity behavior test device

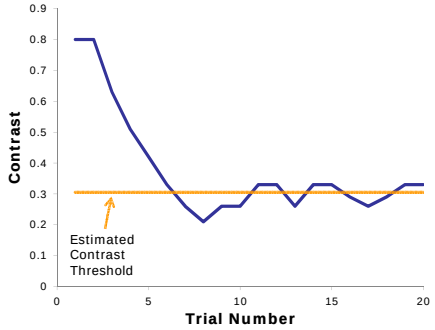


Fig. 3. Representative plot identifying visual acuity in a rat following blast exposure.

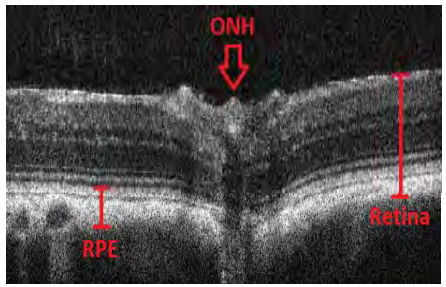


Fig. 4. Representative B-scan centered at optic nerve head (ONH). Total retinal thickness and RPE thickness were extracted across retina width and averaged. For the purposes of analysis, RPE included the photoreceptor inner/outer segments.

References

[1] DeFraites, R., *et al.*, Medical Surveillance Monthly Report, May 2011: 2-6. 2011.
[2] Cockerham, G., *et al.* Eye and visual function in traumatic brain injury. *Journal of Rehabilitation Research and Development* 46, 811-818. 2000.
[3] MATLAB and Image Processing Toolbox Release 2012b, The MathWorks, Inc., Natick, MA.

Results

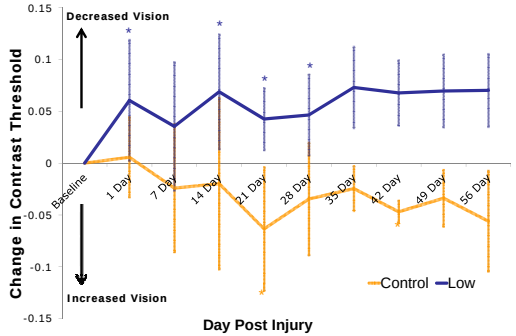


Fig. 5. Change in contrast threshold from baseline over time for control and low-level blast-exposed animals. * p<.05

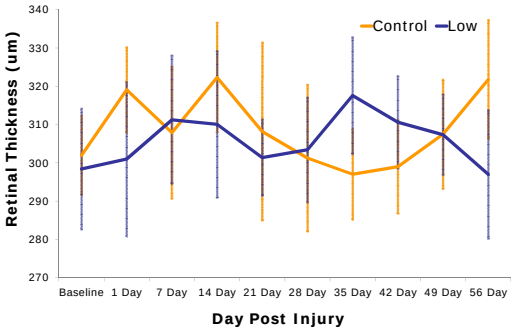


Fig. 6. Thickness of retinal layer over time, representing blast animals (n=3) and control animals (n=3). All data was gathered from eyes ipsilateral to blast insult. No significant trends were found.

Conclusions

- **Blast-exposed animals exhibited decreased visual acuity at one day, two week, three week, and four week time points as measured by behavior testing.**
- **Control animals exhibited unchanged visual ability, with the exception of increased visual ability at three week and six week time points. This may be due to increased comfort with the behavior system.**
- **Retinal thickness did not significantly change in either group at any time point.**

Acknowledgements

We would like to thank *USAMRMC #W81XWH-12-1-0243* for support of this project .

Contact Information

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Brittany Coats, PhD
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APPENDIX C - Poster for 7th World Congress of Biomechanics

APPENDIX D - Research Highlight included in the FY14 Report to the executive Agent for Prevention, Mitigation, and Treatment of Blast Injuries.

DATA CALL SUMMARY

Please review and approve

Temporal Progression of Visual Injury from Blast Exposure

Dr. Brittany Coats from the University of Utah's Department of Mechanical Engineering is conducting research funded by a U.S. Army Medical Research Acquisition Activity (USAMRAA) grant W81XWH1210243 to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. Although ocular trauma is not uncommon in modern day military conflicts, closed globe injury may not be detected immediately, and can result in sequelae that lead to visual dysfunction months after the blast exposure. Furthermore, the progression of closed globe eye injury and visual degradation following blast exposure has not been well characterized, and it is unknown if there are early indicators that denote an increased risk for developing visual dysfunction following blast exposure. Two studies comprise Coats' current work on the progression of visual system injury: (1) a retrospective and prospective analysis of Service Members exposed to a blast, and (2) an experimental study using a rat model to evaluate retinal and corneal damage as well as vitreous protein expression. The first study is ongoing. The results of the second study using the rat model indicate that there is an immediate decrease in vision following a low-level blast exposure that remains steady until 8 weeks post injury. Corneal damage resulted from blast pressure alone, but wasn't identifiable until 3 weeks after the blast. The work from this project has resulted in a collaboration with Dr. Barbara Wirostko, CSO of Jade Therapeutics, Inc., who is also funded by the USAMRAA to develop biodegradable biofilms that can be placed in the eye for drug delivery. It is Coats' and Wirostko's hope that Jade's novel crosslinked hyaluronic acid polymer can prevent or treat corneal damage resulting from blast exposure. The successful completion of these studies will expand our understanding of the time-dependent response of the visual system to blast, enhance current diagnostic capabilities, and lead to the development of time-dependent treatment strategies to mitigate the loss of vision in military personnel.

Please complete below so that the summary can be finalized

Performing organizations:	University of Utah, Department of Mechanical Engineering
Sponsoring organizations:	USAMRAA grant W81XWH1210243
Approved by:	Brittany Coats
Date:	March 26, 2015
Comments:	

CONTROL ID: 2178343
SUBMISSION ROLE: Abstract Submission

AUTHORS

AUTHORS (LAST NAME, FIRST NAME): Shedd, Daniel[1]; Coats, Brittany[1]
INSTITUTIONS (ALL):
1. Mechanical Engineering, University of Utah, Salt Lake City, UT, United States.
Commercial Relationships Disclosure (Abstract): Daniel Shedd: Commercial Relationship: Code N (No Commercial Relationship) | Brittany Coats: Commercial Relationship: Code N (No Commercial Relationship)
Study Group: Developmental Head Injury Biomechanics Laboratory

ABSTRACT

TITLE: Visual Dysfunction Following Low Level Blast Exposure in Rats

ABSTRACT BODY:
Purpose: Blast exposure is a leading cause of eye injury for the US Army. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, a rodent model was developed and used to characterize the time-dependent changes in visual acuity after blast exposure using behavioral vision testing and optical coherence tomography (OCT).
Methods: Anesthetized Long-Evans rats (300-350g, n=26) were exposed to 230 kPa pressure waves using a 6 inch diameter compressed-air blast tube. Animals were evaluated at 1 day post-blast and weekly up to 8 weeks post-blast. A custom vision behavior device was built to measure the visual acuity threshold using the optokinetic nystagmus reflex. Test animals were placed in the center of the device and a cylindrical sine wave grating was displayed on four surrounding computer monitors. The grating rotated around the animal, which caused the animal to reflexively track the grating motion. The contrast of the grating at which the direction of drift was tracked by the animal represented the level of functional visual acuity. Three trials were completed for each animal at each time point. A two-tailed matched-pair test with p=.05 was used to find significant vision changes. OCT imaging (Bioptigen Envisu™ R2200) with an ultra-high resolution (UHR) light source was used to identify changes in retinal thickness in 8 regions around the optic nerve.
Results: There was a significant reduction in visual acuity in all rats 1 day after blast exposure (Figure 1). This reduction was sustained for the duration of the study. The visual acuity in control animals (n=26) increased after day one and remained stable up to 8 weeks. Retinal thickness was normalized to baseline values and compared with controls. Several regions of the posterior retina thickened slightly (~8%) at week 2, but was resolved by week 8.
Conclusions: Low level blast exposure results in an acute decrease in visual function that was sustained up to 8 weeks. The blast also resulted in delayed changes in retinal thickness which resolved over a month. Additional studies are underway to evaluate the electrical physiology of the retina to support theh findings of decreased functional visual acuity.

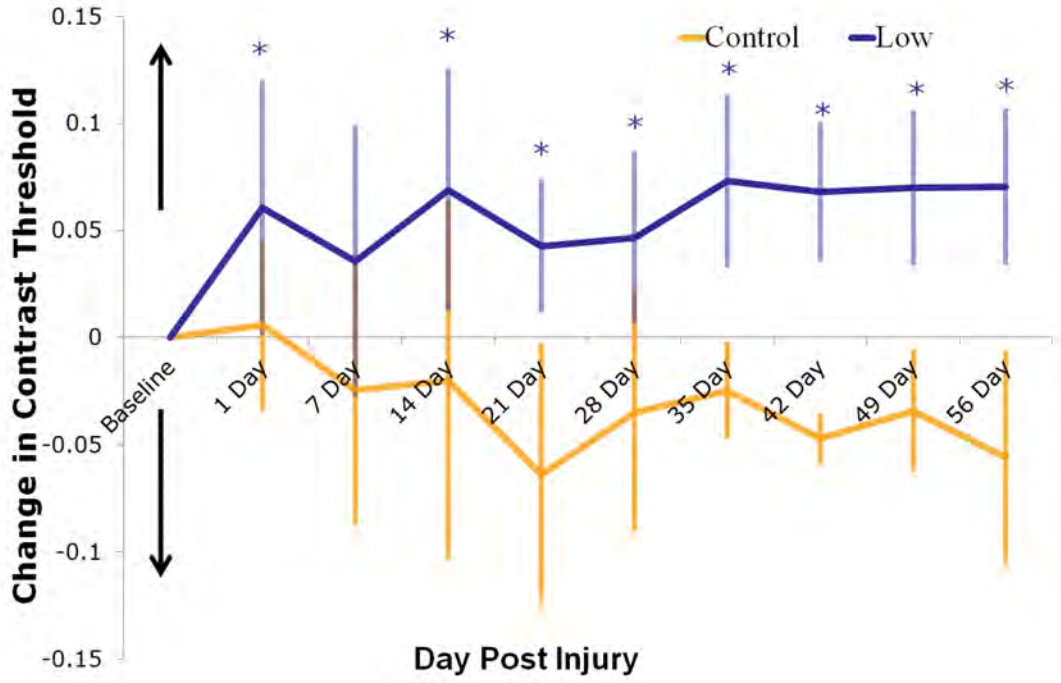

Figure 1. Change in contrast threshold from baseline over time for control and low-level blast exposed animals. *p<0.05.

DETAILS

PRESENTATION TYPE: #1 Paper, #2 Poster
CURRENT REVIEWING CODE: 3720 trauma: posterior segment, clinical - RE
CURRENT SECTION: Retina
KEYWORDS: 742 trauma, 688 retina, 730 temporal vision.
Clinical Trial Registration (Abstract): No
Other Registry Site (Abstract):
Registration Number (Abstract):
Date Trial was Registered (MM/DD/YYYY) (Abstract):
Date Trial Began (MM/DD/YYYY) (Abstract):
Grant Support (Abstract): Yes
Support Detail (Abstract): USAMRMC #W81XWH-12-1- 0243

TRAVEL GRANTS and AWARDS APPLICATIONS

AWARDS: ARVO and ARVO Foundation Travel Grants|ARVO 2015 Members-in-Training Outstanding Poster Award

SB^{3}C2015
Summer Biomechanics, Bioengineering and BIotransport Conference
June 17-20, 2015,Snowbird Resort, Utah, USA

CORNEA DAMAGE PROGRESSION FOLLOWING BLAST EXPOSURE

Daniel F. Shedd (1), Justin A. Jones (2), Brian Zaugg (3), Brittany Coats (1)

(1) Department of Mechanical Engineering
University of Utah
Salt Lake City, Utah, USA

(2) Department of Bioengineering
University of Utah
Salt Lake City, Utah, USA

(3) John A. Moran Eye Center
University of Utah
Salt Lake City, Utah, USA

INTRODUCTION

Blast exposure is a significant cause of injury for the US Army [1]. The time course of eye injury subsequent to exposure is not well understood, especially for cases of closed globe trauma. Several studies have investigated visual impairment in soldiers with traumatic brain injury from blast exposure. They report ~75% of soldiers with traumatic brain injury also have visual dysfunctions [2,3]. One study in particular performed complete ocular exams on all soldiers with a history of traumatic brain injury from blast exposure and found retinal injuries in several military personnel that were unaware they had any ocular or visual problems [4].

To investigate the time course of blast induced vision loss, we developed a high-pressure blast injury model in the rodent. Our objective in this study was to evaluate the long-term (8 week) time course of corneal injury following blast exposure.

METHODS

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Utah. Long-Evans rats (300-350g, n=38) were anesthetized using IP injection of a ketamine-dexmedetomidine mix and exposed to blast waves (peak overpressure = 230 kPa) using a compressed air driven shock tube (Fig. 1). Firing of the shock tube was controlled by material failure of a biaxially oriented polyethylene (BoPET) membrane (.01" thickness), which occurred at a driver section overpressure of 650-750 kPa. A representative blast wave generated at the location of the animal is shown in Fig. 2.

The animals were placed inside of the tube using a 3D-printed mount exposing the head and eyes to a side-on blast insult, while protecting the body and lungs from the injury. Additional protection of the body was achieved by wrapping the anesthetized animal in a Kevlar shroud. The right eye of the animal was always ipsilateral to the oncoming pressure wave throughout the study. A subset of blast studies were videotaped using a Phantom high speed camera at 5000 fps to assess head motion induced by the blast wave. The video data was also used to ensure that the rupturing membrane did not generate any shrapnel, as this could cause additional injuries to the animal.

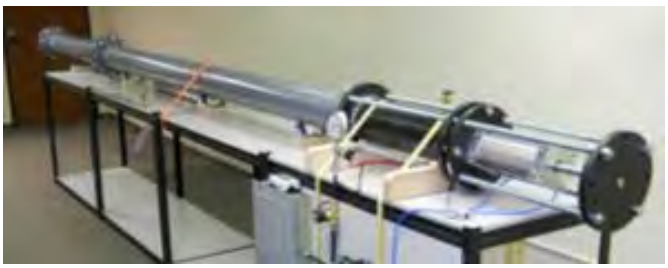

FIGURE 1. 6-IN INTERNAL DIAMETER SHOCK TUBE USED TO GENERATE FRIEDLANDER SHAPED BLAST WAVES.

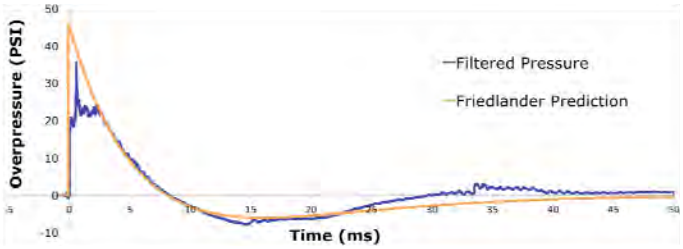

FIGURE 2. BLAST WAVE MEASURED 1-IN IN FRONT OF ANIMAL LOCATION COMPARED TO PREDICTION OF BLAST FROM FRIEDLANDER WAVE EQUATION

At time points 1 day before blast, 1 day post blast, and every week after blast up to 8 weeks, corneal imaging of both left and right eyes was performed using a Bioptigen Envisu R2200™ OCT scanner with an ultra-high resolution light source (Telecentric lens, 4.0 x 4.0 FOV, 100 B scans, 1000 A scans).

Gross ocular examinations were performed at each time point to determine the presence of any easily identifiable injury or corneal defects. When possible, an ophthalmologist (BZ) performed ophthalmic exams of the eyes. Fluorescein staining was used to aid in visualization of superficial corneal epithelium injuries.

Overall corneal thickness was measured regionally using a MATLAB code created using the Image Processing Toolbox. Stromal and epithelium thickness were measured manually using InVivo Vue software (Bioptigen, North Carolina).

A Dunnett's test was used to compare the corneal, stromal, and epithelial thicknesses at every time point to the baseline (pre-injury) measurement (JMP, SAS Institute, North Carolina). A p-value < 0.5 was considered significant.

RESULTS

The superficial epithelium layer and the underlying stroma layer could be clearly resolved in all images (Figure 3A).

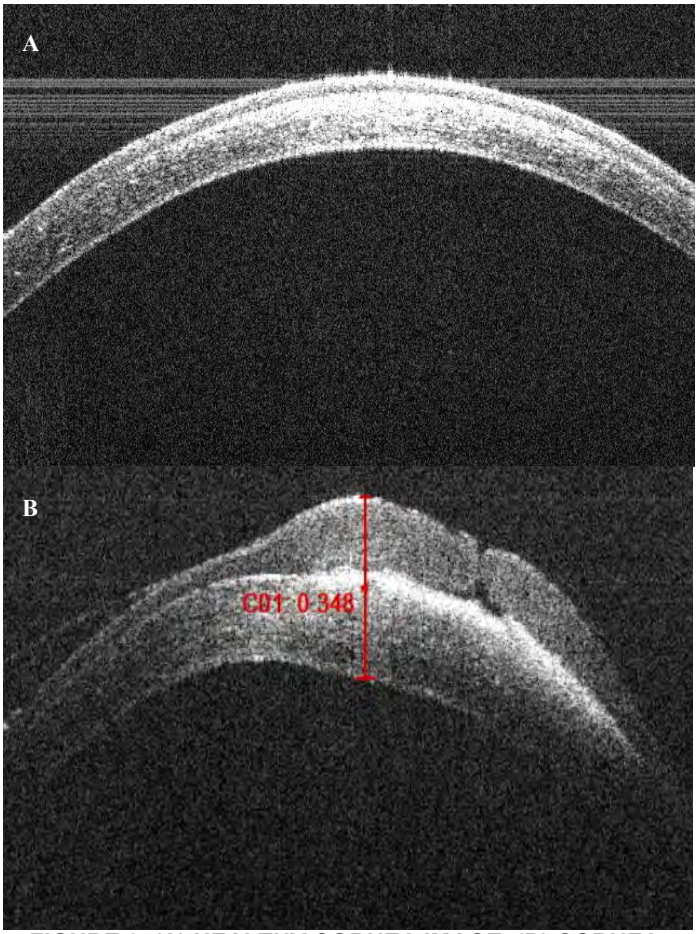

FIGURE 3. (A) HEALTHY CORNEA IMAGE. (B) CORNEA EXHIBITING THICKENING OF EPITHELIUM INDICATIVE OF INFLAMMATION. THE CORNEAL STROMA IS ALSO SLIGHTLY ENLARGED, AND THE BORDER BETWEEN LAYERS IS UNHEALTHY.

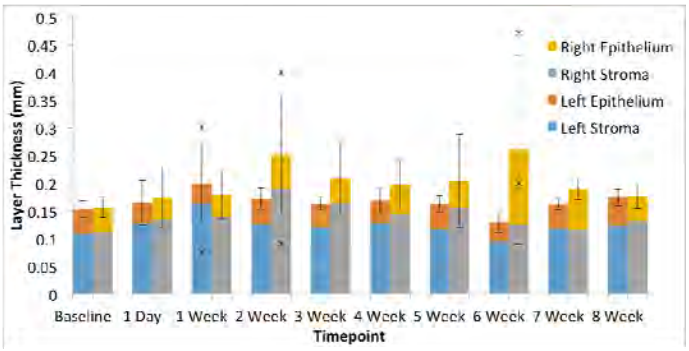

FIGURE 4. AVERAGE THICKNESS FOR TOTAL CORNEA AS WELL AS STROMA AND EPITHELIUM. STARRED COLUMS INDICATE STATISTICAL SIGNIFICANCE COMPARED BASELINE (PRE-INJURY).

In the eye contralateral to the blast (left), significant changes in overall corneal thickness and stromal thickness (p=.0105) occurred at one week (Figure 4). These changes resolved by two weeks after blast. The eye ipsilateral to the blast (right) had significant increases in corneal thickness at two (p=.0066) and six weeks (p=.0167). At two weeks, the increased thickness came from welling of the stroma, while at six weeks the thickening was due to epithelial changes. These data points correlated with the appearance of visible gross injury to the cornea at 3-4 weeks followed by scarring at 6-8 weeks, as well as neovascularization.

DISCUSSION

The blast exposure appears to have caused a structural injury in the stroma of the right eye. This defect develops into a measurable change in thickness of the stroma which is at first not visible on the surface of the eye. This injury may be caused by a disorganization or disruption of collagen plates that make up the corneal stroma. The stroma eventually heals, returning to pre-injury thickness, but leaves behind unhealthy epithelial tissue, which presents as a superficial hemorrhage and eventual scarring. Interestingly, there was transient thickening of the left cornea which was contralateral to the blast. The thickening resolved quickly and did not result in gross indications of injury.

These data show that blast exposure can cause delayed expression of injury to the cornea. The injury is not limited to the ipsilateral side of blast exposure, but may also be present contralateral to a lateral blast. These injuries may also be measurable by other types of collected data, such as vision behavioral studies or protein expression, which are not presented in this work. A better understanding of these injuries and their time course will aid in the detection and treatment in blast-exposed individuals.

ACKNOWLEDGEMENTS

We would like to thank USAMRAA #W81XWH-12-1-0243 for support of this project. We'd also like to thank Krishna Womack for her assistance with data analysis.

REFERENCES

[1] Ari AB., *Optometry*, 77:329-339, 2006.
[2] Kapoor, N. et al., *Neurology* 4:271-280, 2002.
[3] Brahm, K. et al., *Optometry and Vision Science* 86:817-825, 2009.
[4] Cockerham, G, *et al. J Rehabil Res Dev* 46: 811-818, 2009.

SB^{3}C2015
Summer Biomechanics, Bioengineering and BIotransport Conference
June 17-20, 2015,Snowbird Resort, Utah, USA

The Temporal Change in Protein Biomarkers in the Vitreous Humor following Blast Trauma

Justin A. Jones (1), Daniel F. Shedd (2), Brittany Coats (2)

(1) Department of Bioengineering
University of Utah
Salt Lake City, Utah, United States

(2) Department Mechanical Engineering
University of Utah
Salt Lake City, Utah, United States

INTRODUCTION

Ocular injuries due to blast exposure have increased in occurrence over the last several decades. Between 1983 and 2002, 36,110 bombings occurred in the United States, resulting in 5931 injuries [1]. The incidence of eye injury due to blast trauma with soldiers has increased from 9% to 13% since the Vietnam War [2]. Progression from ocular injury to ocular disease is not adequately characterized; and currently, indicators of injury progression to vision loss are largely unknown. Common closed globe injuries, including, retinal detachment, retinal tears, and optic nerve fiber degeneration [3], can elicit a cellular inflammatory response that releases proteins into the vitreous humor of the eye.

One prominent ocular protein biomarker is the neurofilament heavy chain (NfH). It is believed that NfH is released from degenerating retinal ganglion cells and their axons into the vitreous [4]. Other protein biomarkers of importance are cytokines. Cytokines are signaling proteins present in the inflammatory cascade. A particularly well known cytokine is vascular endothelial growth factor (VEGF) and has importance to pathologic angiogenesis [5]. Its subcomponents are said to be involved in endothelial cell migration, proliferation, survival and permeability and are typically present any time there is an inflammatory response [6].

The goal of this study was to discover if protein biomarkers known to reflect ocular injury can be used as reliable early identifiers of vision loss due to blast exposure.

METHODS

All testing procedures were approved by the University of Utah Institutional Animal Care Use Committee (IACUC). Male Long Evans rats (n = 24; 300-350g) were placed in a 6 meter long by 15.24 cm internal diameter blast tube, and exposed to a 30 psi overpressure blast with a 7 msec duration (Figure 1). Experimental animals were separated into three survival time groups: 1 day, 1 week and 4 week. Before the blast exposure was performed, each animal was weighed and anesthetized using a mix of ketamine and dexmedetomidine with a dosage of 65mg/kg and 0.14mg/kg, respectively. A visual eye examination was then performed by an ophthalmologist. The animals were exposed to a blast perpendicular to the sagittal plane from right to left. After the blast, the animal was removed and another visual eye examination was performed.

After the respective survival time was reached, the animals were sacrificed by formalin perfusion fixation through the heart using standard practices [7]. The whole globe eyes and brains were harvested at necropsy. The eyes were then eviscerated and the vitreous and lens removed. The vitreous and lens were separated by placing them into a filter centrifuge tube and spun down (10k rpm, 10 min). The separated vitreous (approx. 50 µL) was diluted with phosphate buffer saline (PBS) until a total volume of 150µL was reached. The sample was then separated into three equal tubes.

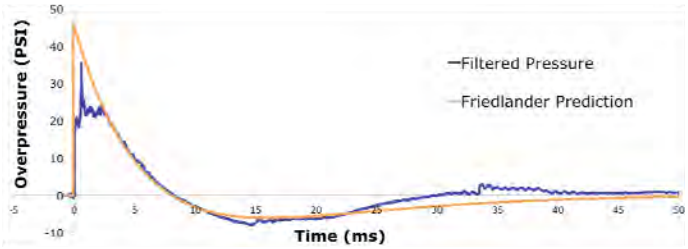

FIGURE 1. PRESSURE-TIME HISTORY AT LOCATION OF ANIMAL PLACEMENT WITHIN BLAST TUBE

To analyze the NfH content, an ELISA protocol was used according to an ELISA technique previously developed by Petzold et al [8]. The microtitre plates were coated overnight at 4°C with 100 µL of capture antibody, SMI35. The plates were then washed three times for 10 minutes using a barbitone buffer wash containing 0.1% BSA, and 0.05% Tween 20. After washing, 250µL of barbitone block with 1% BSA was added to each well and the plate was incubated at room temperature (RT) for 1 hour. After another wash cycle, 50µL of sample, standard, or negative control was added to each well of the plate in duplicate. After one hour incubation at RT the wash processes was repeated. After washing, 100µL of second antibody was added to each well of the plate and incubated for 1 hour at RT. Following a third wash cycle 100µL HRP-labeled swine anti-rabbit antibody was added to the plates and incubated for one hour at RT. After a final wash 100µL TMB substrate was added and incubated for 20 minutes in a dark room, the reaction was stopped by adding 50µL of 1 M HCL. The absorbance was then read using an ELISA plate reader at 450nm with 750nm reference wavelength.

The analysis of inflammatory cytokines was performed using a commercially available kit (RayBio® Rat Cytokine Antibody Array G). These kits tested for the 19 cytokines including VEGF, LIX and TNF-α. The methods to develop these kits were done according to the manufacturer's instructions and can be found on RayBio® Tech website. Once the glass chip was developed, the intensities were read using a GENEPIX™ 4000A microarray scanner at an excitation frequency of 532nm.

At this point in time, sample size is small (n=4 per group), so 5 one-way ANOVAs were performed. Two assessed significant changes across time points within each eye side, and three assessed significant differences between the right and left eye at each time point. Collection of control data is ongoing and is not included in this abstract. All analyses were performed using JMP® software with a p-value < 0.05 considered significant.

RESULTS

Both the left and right eyes showed a general increase in NfH concentration, but this increase was only significant in the right eye between 1 day and 4 weeks (p=0.044, Figure 2). There were no significant differences between NfH concentration of the ipsilateral (0.126±0.02 ng/ml) and contralateral (0.137±0.05 ng/ml) eyes at the 4 week time point.

Several cytokine proteins in the eye contralateral to the injury significantly decreased over time post-injury (GM-CSF, IL-1β, IL-4, IL-10, LIX ,TNF-α), but remained relatively constant in the eyes ipsilateral to injury (Figure 3). At 4 weeks after the injury, LIX and TNF-α were significantly higher in the eye ipsilateral to the injury

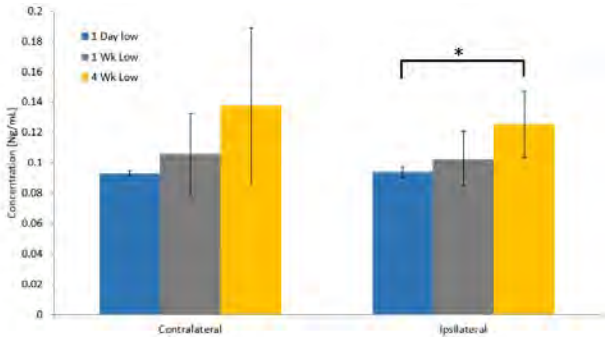

FIGURE 2. CHANGES IN NFH CONCENTRATION POST INJURY IN IPSILATERAL (RIGHT) AND CONTRALATERAL (LEFT) EYES. *p<0.05

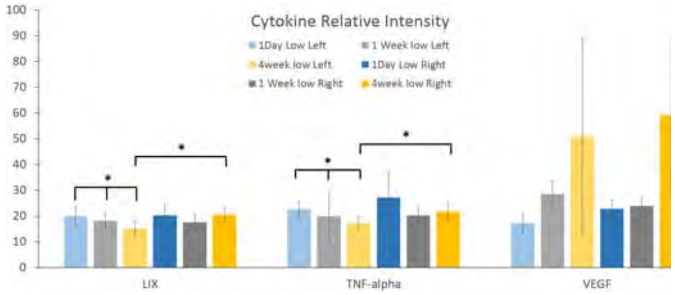

FIGURE 3. CYTOKINE RELATIVE INTENSITIES

compared to the contralateral eye (p = 0.015 and 0.043, respectively). No significance differences were seen in either of the eyes the remaining proteins of the cytokine array, including VEGF (shown above).

DISCUSSION

The significant decrease in NFH and cytokine proteins with time suggests that there was an acute (1 day) inflammatory response that occurred in both eyes, and only significantly decreased in the eye contralateral to the blast pressure. NfH is hypothesized to release from degenerating retinal ganglion cells and their axons. Previous research has shown that decreased axonal transport is preceded by cytoskeletal changes and degradation of NfH [4]. The sustained elevation of NfH in the ipsilateral eyes in this study suggest that cytoskeletal changes in the retina are ongoing at 4 weeks after injury. This long-term injury may lead to future vision degradation. Longer-term analysis, vision assessment and control group evaluation needs to be completed before making this assertion.

We found no significant change in VEGF in either eye. This was surprising as significant increases in VEGF have been reported in many ocular disorders including diabetic retinopathy, diffuse macular edema, retinal vein occlusion and retinal detachment [5]. Instead, we found several changes in interleukins, TNF-α, and LIX. The significance of these proteins in the blast injury response will be explored further do determine if they are merely a generic inflammatory response to the blast that is quickly resolved.

In summary, the blast pressure in this study appears to create some damage to the retina that is potentially recovered quickly in the contralateral eye, but not in the ipsilateral eye. Longer time points are currently being explored to determine the resolution of NfH to baseline levels post injury. Future work will include completion of the control groups as well as performing higher blast pressures to perhaps create higher levels of injury. We are also working to combine the findings of this study with changes in visual acuity and histology in the same animals.

ACKNOWLEDGEMENTS

We would like to thank USAMRAA #W81XWH-12-1-0243 for support of this project.

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[8]Petzold A., et al. *J Neural Transm* 116:1601-1606, 2009.

Temporal Progression of Visual Injury from Blast Exposure

Proposal Number: 11257006
Award Number: W81XWH-12-1-0243

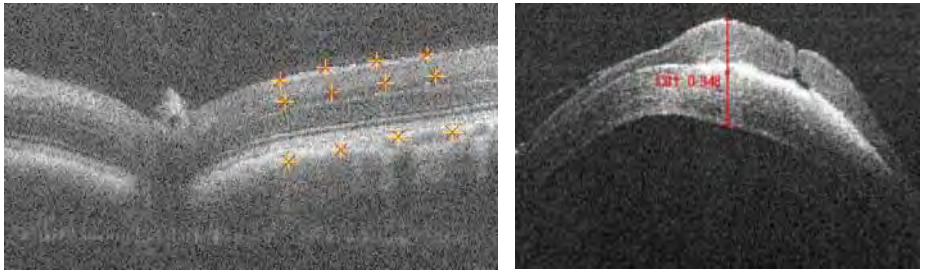
PI: Brittany Coats **Org:** University of Utah **Award Amount:** $997,528

Study/Product Aim(s)

- Investigate progression of visual system injury in service members exposed to blast
- Investigate progression of visual system injury following blast exposure in an animal model and identify early indicators of visual dysfunction
- Identify changes in vitreous protein expression that correlate with visual system injury

Approach

1) Retrospective chart review of service members exposed to blast as well as prospective study.
2) Track visual acuity in rat model with optokinetic testing and OCT subsequent to blast injury simulated by shock tube.
3) Collect vitreous samples following animal studies; assay for NfH, VEGF, IL-10, MCP-1, MIP-3.



Accomplishments: Completed all blast and control animal groups. Behavior, OCT, and biomarker data collection is complete, and final data analysis is underway. Sample OCT images are shown above.

Timeline and Cost

Activities FY	13	14	15	16
Investigate the progression of visual system injury in service members exposed to blast				
Investigate the progression of visual system injury following blast exposure in animal model				
Identify changes in vitreous protein expression that correlate with visual system injury				
Estimated Budget ($K)	301	239	266	191

Updated: September 30, 2015

Goals/Milestones

CY13 Goal – IRB/IACUC Approvals, system acquisition, initial testing
☑ Obtain equipment required for experimental setup
☑ Get IRB and IACUC approvals Identify service members exposed to blast between 2007-12
☑ Complete first set of 40 animal blast experiments
CY14 Goals – Animal testing, service member studies
☑ Complete data analysis from retrospective study
☐ Enrollment, interviews, and ocular examination of service members
☑ Complete animal blast experiments
☑ Complete first set of protein assays
CY15 Goal – Data analysis, prospective studies
☐ Complete enrollment of service members for prospective studies
☑ Complete data analysis for experimental studies, protein assays
CY16 Goal – Clinical ocular exams, data analysis
☐ Complete clinical exams for participants and data analysis
☐ Complete data analysis of protein assays

Comments/Challenges/Issues/Concerns: Still waiting for HRPO approval of prospective studies. Waiting to get database access for new personnel.

Budget Expenditure to date

Projected Expenditure: 806,000 Actual Expenditure: 660,170