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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

- Statistically determine the time after the blast exposure that visual dysfunction is identifiable. (SOW 3)

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 (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 visual system 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

In the previous year, the retrospective VA data was collected and evaluated in consultation with a biostatistician. Based on that consultation, we moved forward with hiring a MS-student to complete the project and will focus on the effect of blast exposure (type, # of exposures, and distance) and age on photophobia. Delays in seeking care by veterans, lack of serial examinations, and inaccurate or vague reporting of blast exposure dates prevented evaluation of the time post blast for development of photophobia. Therefore, DVIEVR data was added to the study (discussed later). The biostatistician evaluating the retrospective data has completed the descriptive analysis and is working on the multiple regression. Table 1 is a summary of the descriptive data.

Table 1. Descriptive statistics of veterans with photophobia following blast exposure.

Characteristic (Unit)	Statistics	None Photophobia	Mild Photophobia	Moderate Photophobia	Severe Photophobia	Very Severe Photophobia
Age (Years)	N	58	66	75	61	23
	Mean(SD)	28.5 (5.79)	31.1 (7.13)	31.6 (6.78)	31.9 (7.07)	33.7 (6.78)
	Median	27	29	30	30	32
	Min,Max	21 - 50	22 - 51	22 - 54	23 - 58	25 - 49
Blast Type	n(%)					
IED		34 (58.6)	42 (63.6)	50 (66.7)	47 (77.0)	17 (73.9)
RPG		10 (17.2)	11 (16.7)	20 (26.7)	11 (18.0)	6 (26.1)
Other		5 (8.6)	3 (4.5)	2 (2.7)	1 (1.6)	1 (4.3)
Unknown		4 (6.9)	4 (6.1)	7 (9.3)	3 (4.9)	1 (4.3)
Number of Blast	n(%)					
1 times		32 (55.2)	35 (53.0)	34 (45.3)	22 (36.1)	9 (39.1)
2 to 5 times		23 (39.7)	22 (33.3)	29 (38.7)	27 (44.3)	7 (30.4)
Greater than 1		2 (3.4)	5 (7.6)	10 (13.3)	10 (16.4)	7 (30.4)
Unknown		0	1 (1.5)	0	0	0
Distance	n(%)					
< 10ft		29 (50.0)	19 (28.8)	35 (46.7)	41 (67.2)	11 (47.8)
10-30ft		11 (19.0)	22 (33.3)	22 (29.3)	9 (14.8)	5 (21.7)
31-50ft		11 (19.0)	11 (16.7)	7 (9.3)	4 (6.6)	3 (13.0)
>50ft		4 (6.9)	7 (10.6)	5 (6.7)	3 (4.9)	1 (4.3)
Unknown		3 (5.2)	7 (10.6)	6 (8.0)	4 (6.6)	3 (13.0)
TBI Diagnosis	n(%)					
TBI Present		16 (27.6)	13 (19.7)	17 (22.7)	17 (27.9)	7 (30.4)
TBI-BH		32 (55.2)	38 (57.6)	40 (53.3)	38 (62.3)	16 (69.6)
TBI Absent		9 (15.5)	15 (22.7)	18 (24.0)	6 (9.8)	0
No Diagnosis of TBI Recorded		1 (1.7)	0	0	0	0

Data from the DVIEVR database was collected and has been evaluated to identify what data parameters to focus our efforts on. A data entry form has been created in RedCap and is included in Appendix A at the end of this report. We have continue to evaluate the DVIEVR data and have narrowed our focus to make the data more manageable. Specifically, our inclusion criteria are: (1) blast exposure, (2) no reported TBI, (3) no open globe

injury. From this, we expect to see what closed globe findings are present that may go undetected. However, when evaluating the data around these criteria, we found that visual acuity was rarely collected. We worked with the VCE to obtain this information as well as results of any testing that was performed. There are missing data throughout the dataset and we are working our way through each case to see what we have. All records with history of blast exposure have been compiled (n=3,395) with testing information and ICD9 codes being transferred to REDCap. 120 entries have been transferred into the REDCap system. Once we have entered the final patient population, we will reach out to the VCE to hopefully get access to the blast mechanics associated with these cases.

The first participants of the prospective study were enrolled and examined. For the initial round of the study, 30 participants were invited to join the study, 7 responded, 4 enrolled, and followed through with all testing. The prospective study initial visit consisted of an informed consent presentation, followed by consent. Participants were given Diplopia and 10-Item Neuro-Ophthalmic NEI-VFQ-25 questionnaires to complete. Following completion of the questionnaires, each participant was dilated by an ophthalmologist and visual acuity, visual field examinations, and OCT imaging of the retina were performed. We obtained permission to use the Moran Eye Center specular microscopy system in our clinical study. A graduate student was trained to use the system to image the endothelial cellular density of the cornea. Issues with billing substantially delayed progress, and roadblocks in recruitment were met. A decision was made to cease further recruitment and focus efforts on the retrospective analysis.

Progress and Accomplishments

During this period of the grant, the VA data was collected and analyzed. Current efforts are underway to publish results. The DVIEVR dataset was added to the project to overcome some of the deficiencies of the VA dataset. We have filled in several gaps with the dataset and are currently consolidating the data into RedCap.

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

- Publish Research

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. 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 dexmedetomidine administered IP. The anesthetized animal is placed in the custom rat holder also designed in Year 1 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. These eyes were later shipped to Excalibur Pathology for slicing and staining. The length of the survival period was increased to 8 weeks and the number of blast levels investigated was decreased to two. In 10 additional animals, intraocular pressure (IOP) was measured directly during the blast exposure using fiber optic pressure sensors. These data were compared to blast overpressure measured in the shock tube.

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 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. Thickness of the retina, RPE, and NFL/GC 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, inferior medial/distal, nasal medial/distal, and temporal medial/distal.

Cornea thickness measurements were initially automated, but we since switched to manual measurements of the overall, stromal, and epithelial thicknesses, each measured at the thickest region of the cornea. The switch

to manual measurements was intended to capture the most injured regions of the cornea.

A subset of control and blasted rat brains (N=16) collected during experimental studies were submitted to the University of Utah Histology Core for processing. Samples were embedded, sliced and NISSL stained. We are quantifying cell density in brain regions of the CA1, CA2, CA3, dentate gyrus, cortex, and thalamus. Densities in each region will be compared between the directly and indirectly exposed sides of the brain as well as between blast exposed and control animals. These comparisons will allow us to determine if some of the vision loss seen in our studies could be attributed to injury in the brain rather than in the eye itself.

After multiple discussions with the research team, we decided that the lack of scaling algorithms between rodents and human eyes for blast research made translating our data challenging. Therefore, we created a finite element model of an eye. The goal of this model was to investigate the effects of differences in size, geometry, and blast loading on IOP and the stresses and strains in the cornea, lens, and retina. The model includes four components of the eye: sclera, cornea, vitreous, and lens.

Six total models were created to evaluate the parametric space between the size and anatomical features of the rat and human eye (**Fig. 1A**). These models incorporated a combination of linear elastic, hyperelastic, and viscoelastic material properties (**Table 2**). The pressure-time history used to define the blast loading was taken from a single shock tube recording. A total of five different blast pressure profiles were applied to each model geometry, representing $\pm 2SD$ in pressure and duration (**Fig. 1B**). These load curves essentially stretched the baseline curve in the y- and x-axis, respectively. The rat model was validated using the intraocular pressure measurements recorded during the animal experiments.

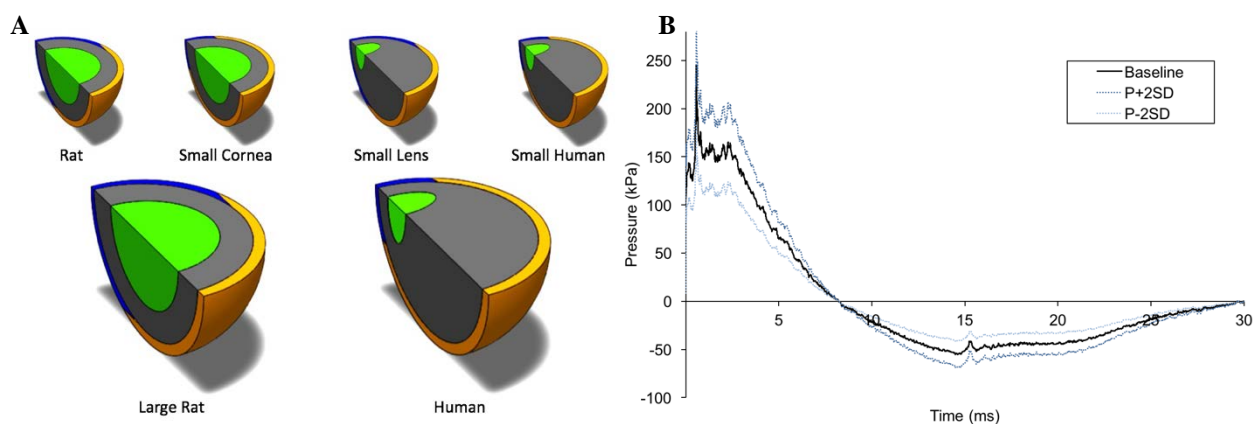


Figure 1: (A) Baseline quarter-symmetry rat model (top left) compared to the five varied model geometries. (B) Peak blast overpressure was scaled by $\pm 2SD$ relative to baseline. Positive phase duration was similarly varied (not shown).

Table 2: Constitutive models used for each model component

Model Component	Constitutive Model	Poisson's Ratio	Density (kg/m ³)
Cornea	Hyperelastic 3rd-order Ogden	0.42	1076
Lens	Isotropic, Linear Elastic, E=5MPa	0.48	1200
Vitreous	Isotropic, Bulk Modulus K=2.272 GPa	0.4999	1006
Sclera	Hyperelastic 1st-Order Ogden Viscoelastic Prony-series	0.47	1243

Results

Animal Histology. A manuscript containing data relevant to blast device development, IOP during blast, OCT anatomical changes, and visual acuity was submitted to IOVS. We addressed minor comments from the reviewers concerning images, clarifications and edits, and resubmitted to IOVS in May. A second round of reviewer feedback was received and stated that we should include ocular histology in the paper. Endothelial cell density counting was completed and histology evaluated. The manuscript will be resubmitted in two weeks. Brain histology will be reserved for a second manuscript. This histology includes H&E, NISSL, and GFAP staining. Analysis of these stains is currently underway.

Initial evaluation of the brain histology suggests increases in red neurons in the hippocampus (**Fig. 2**). The developmental timeline of this pathology is still being determined, but it may contribute to some of the visual acuity deficits found during the animal studies.

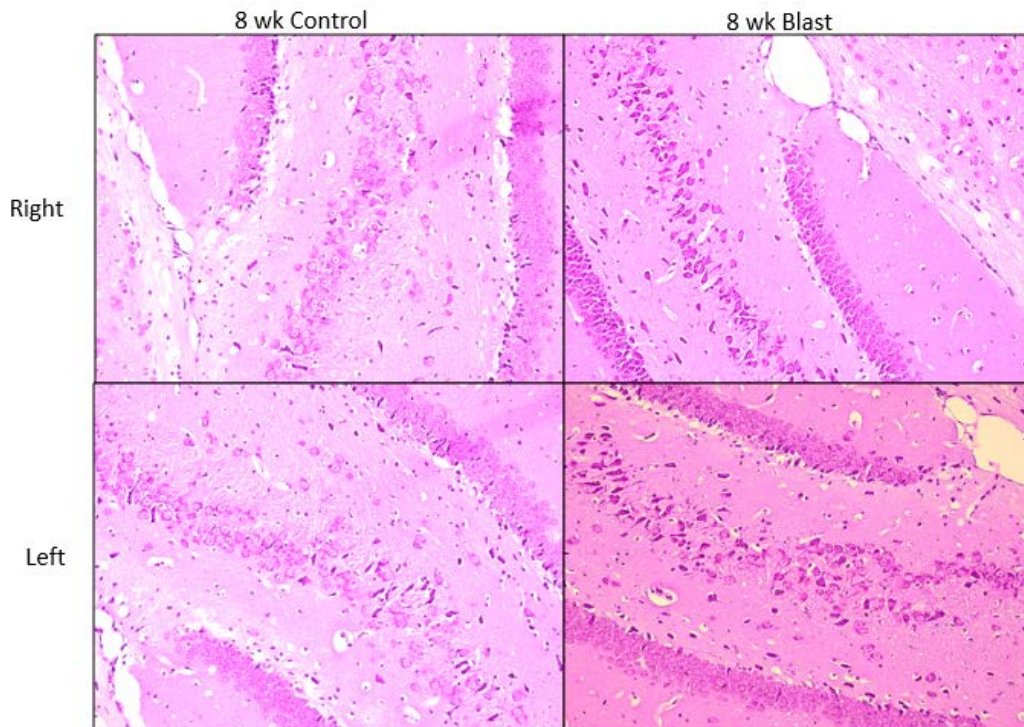


Figure 2. Preliminary assessment of brain histology shows a decrease in healthy neurons and increase in red neurons in the hippocampus. This assesment was present in both left and right eyes, but was more pronounced in the directly exposed right eye.

Computational Studies. Reducing the size of the lens or cornea to match human proportions lowered IOP by 18% and 10%, respectively. Reducing both effects simultaneously reduced IOP by 28%, the sum of the individual changes. Scaling the rat eye to the size of the human eye resulted in less than a 1% change in peak IOP. Central corneal deformation was small, but was very sensitive to global scaling (**Fig. 3**). Additionally, geometries with increased vitreous space inside the globe experienced increased corneal deformation. Tissue strains were generally small. The lens strain was more sensitive to global eye size than anatomical feature scaling, while the retinal strain was more sensitive to changes in anatomical features (**Fig. 4**). These initial findings from these models were presented at the 6th Military Vision Symposium in Boston, MA. The poster that covered the model findings is included with this report. At the same Vision Symposium, we gave a talk titled “Temporal Progression of Eye Injury following Blast Exposure.” This talk included data from our animal studies and other studies in the literature.

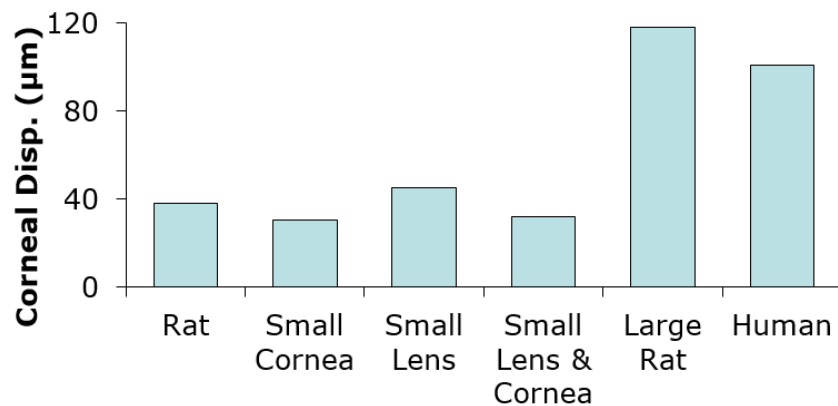


Figure 3. Globe size scaling had the largest effect on corneal displacement. The peak displacement was located at the center of the cornea in all models.

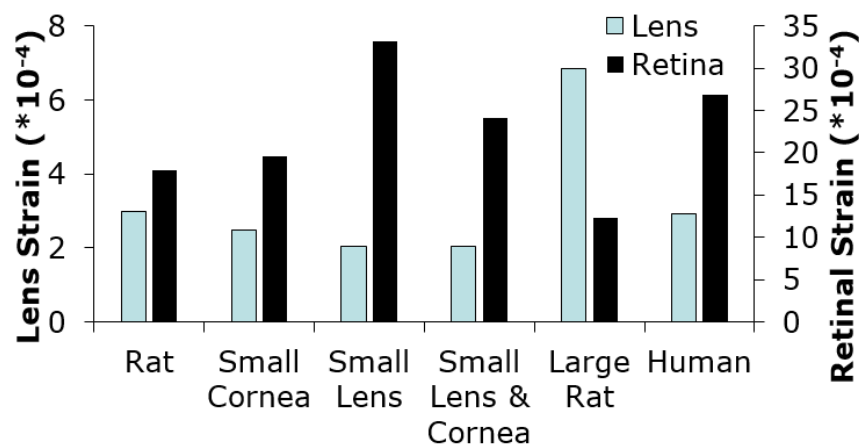


Figure 4. Lens strain increased with general scaling of the globe. The location of peak strain shifted posteriorly with increased lens size. Retinal strain was most influenced by scaling of individual anatomical components.

Findings of different ocular pressures in each geometry led to the development of a scaling equation to relate eye size, anatomy, and blast pressure to intraocular pressure. The goal of developing this equation is to allow researchers to translate loading conditions in animal models to corresponding blast conditions in humans (**Fig. 5**). The equation was computationally validated by generating two new ocular geometries (not used to generate the equation) and verifying the predictions of IOP (**Fig. 6**).

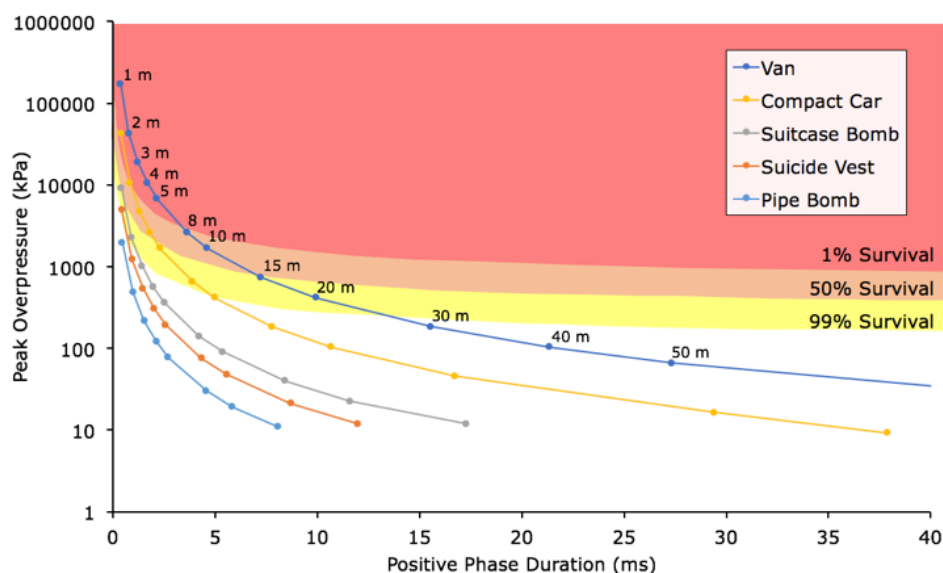


Figure 5. Survival curves for human blast exposure from various explosive sources at a range of standoff distances.

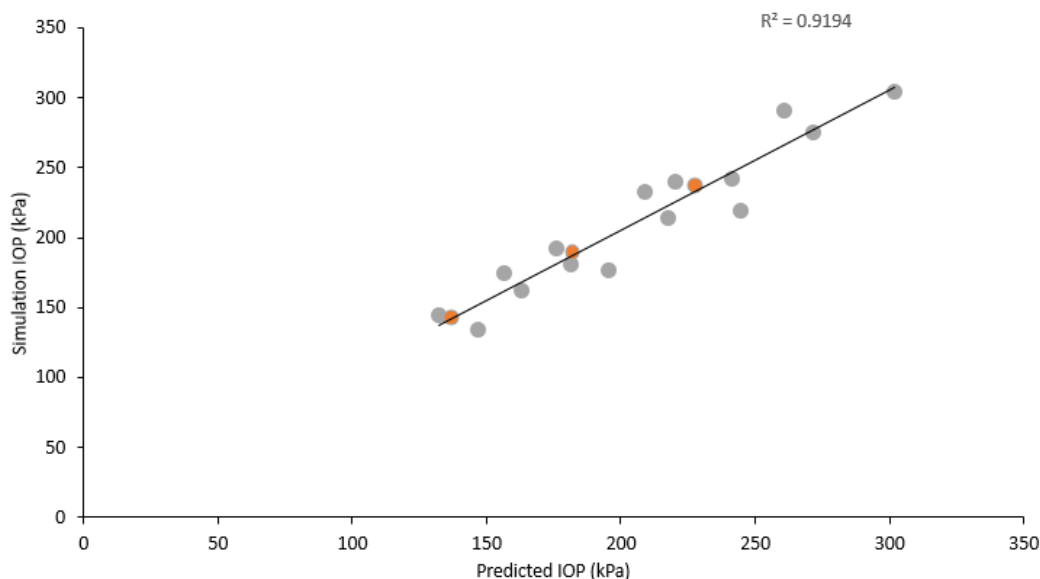


Figure 6. Comparison of novel equation predicted IOP (x-axis) with IOP predicted by FE simulations (y-axis). Prediction is based on blast overpressure, lens axial length, and globe axial length. $R^2 = 0.92$

It is difficult to evaluate whether the scaled blast accurately predicts injury severity in the models due to the lack of quantitative data, different time points used, and different assessment methodologies in previous experimental studies. However, the work of Jones et al. ("Low-Level Primary Blast Causes Acute Ocular Trauma in Rabbits", *J Neurotrauma*, 2015) quantified corneal thickness changes in rabbits 48 hours post blast. They found corneal thickening similar to the corneal thickness increases measured one day after blast in the present study. The scaled blast of 312 kPa in the present study resulted in normalized thickness changes at 24 hours post blast of 39.9% and 17.6% in directly and indirectly exposed eyes, respectively (bilateral average = 28.7%). The highest blast level in the Jones et al. study was a lower scaled blast level of 155 kPa, and resulted in a smaller normalized thickening of 11.8% at 48 hours post blast.

The more severe corneal thickening found in the present study when compared to the Jones et al. study is expected with the increased scaled blast levels. However, the unscaled blast pressures in the models (228 and 132 kPa) would also predict more severe injuries in the present study than is reported Jones et al. study. Further, it is difficult to compare between the two studies due to differences in time points (24 or 48 hours) and animal orientation (side-on or face-on). Experimental evaluations using multiple species is required to fully validate the relationship.

Progress and Accomplishments

This year was focused on histological analysis, computational simulation, and publication. All histology samples have been processed, with ocular histology analysis completed for inclusion into our upcoming journal article resubmission. This work was also included in two conference presentations and a PhD dissertation (July 27, 2017). The dissertation is under format review and will be published via ProQuest.

Computer modeling was used to understand how the findings in our experimental animal models (and those of other researchers) can be correlated to real-world human blast exposures. We developed a finite element model of the rat eye in blast, and validated the intraocular pressure recorded in the model against experimental data obtained in the previous annum. This model was then expanded to a range of geometries and anatomies representing a wide range of species. The results of these models were used to propose a scaling equation allowing intra-species comparison of ocular blast exposure levels.

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

Current Objectives

- Publish results

Key Methodology

The eyes used for vitreous protein analysis were eviscerated and the vitreous and lens removed. The vitreous was isolated by centrifuge and diluted with phosphate buffer saline (PBS) (50 μ L:100 μ L). The diluted sample was then separated into three equal tubes for quantification of Neurofilament Heavy Chain (NfH) and inflammatory cytokines.

To quantify NfH, an ELISA protocol was used according to a technique previously developed by Petzold et al.²⁴. Microtitre plates were coated overnight at 4°C with 100 μ L of capture antibody, SMI35. The plates were washed three times for 10 minutes using a barbitone buffer containing 0.1% BSA, and 0.05% Tween 20. After washing, 250 μ L 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, 50 μ L of sample, standard, or negative control was added to each well of the plate in triplicate. After one hour incubation at RT the wash processes were repeated. After washing, 100 μ L of second antibody was added to each well of the plate and incubated for 1 hour at RT. Following a third wash cycle 100 μ L HRP-labeled swine anti-rabbit antibody was added to the plates and incubated for one hour at RT. After a final wash 100 μ L TMB substrate was added and incubated for 20 minutes in a dark room, the reaction was stopped by adding 50 μ L of 1 M HCL. The absorbance was read using an ELISA plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek, Winooski, VT) at 450 nm with 750 nm reference wavelength.

Quantification of the inflammatory cytokines was performed using a commercially available array (RayBio® Rat Cytokine Antibody Array G). These arrays tested for 19 cytokines including VEGF, LIX and TNF- α Arrays were processed according to the manufacturer's instructions, and the intensities read using a GENEPIX™ 4000A microarray scanner at an excitation frequency of 532 nm. Cytokines quantification was normalized using positive controls.

Results

The left and right eyes of blast exposed animals showed an increase in NfH concentration ($p < 0.05$, **Fig. 7A**). Between four and eight weeks, NfH concentration decreased significantly but was still elevated with respect to control. In general, there appeared to be a transient increase in inflammatory cytokine expression at one day and one week after blast that resolved by the end of the study (**Fig. 7B**). VEGF appeared to increase in blast exposed eyes between 1 day and 4 weeks, but the increase was not significant (**Fig. 7C**). IL-10 intensity significantly increased in the right eye

of blast exposed animals at one day and four weeks after blast, but returned to baseline values by eight weeks (**Fig. 7D**).

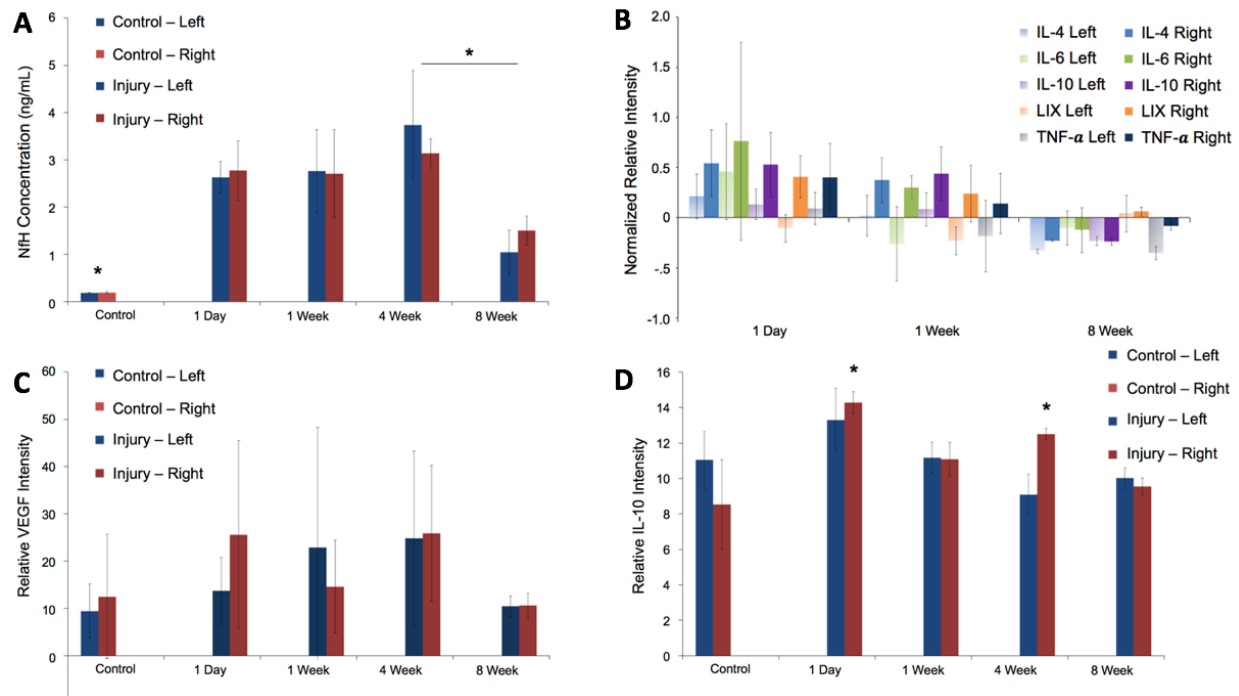


Figure 7. Results of vitreous protein quantification. (A) NfH was significantly greater in injured animals compared to controls for all time points ($p < 0.05$). At 8 weeks, NfH significantly decreased towards to control levels. (B) Selected cytokines normalized by relative intensities in control animals. Both eyes had a general increased in response 1 day after injury, but returned to control levels by 8 weeks. (C) VEGF increased from 1 day to 4 weeks and then returned to baseline, but this trend was not significant. (D) IL-10 was seen to be significantly higher in the right eye at 1 day and 4 weeks following injury compared to controls.

Progress and Accomplishments

The vitreous protein findings have been finalized. The findings from this aim are contained in a manuscript currently under review.

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- α 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.
- Initial analysis of histology shows significant damage to both the cornea and lens. Inflammation, bullae, and neovascularization were common findings in the cornea. Some of the examined lens exhibited damage indicative of the development of cataract. Analysis of this brain histology is ongoing.
- Computer models were used to develop a novel scaling equation allowing comparison of blast overpressure levels between species with different ocular anatomy and scale.

CONCLUSION:

The successful completion of the studies proposed in this 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, the bulk of animal studies were completed and resulted in some remarkable findings regarding the time course of retinal and corneal injury following a blast exposure. During Year 4, the retrospective human study advanced, and the last experimental animal studies were completed. During Year 5, data analysis from the animal studies was completed, along with investigative work using computer

modelling. 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 remainder of this project will be focused on the clinical study and publishing results.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Journal Publications

Shedd DF, Benko N, Jones J, Zaugg B, Coats B. Long Term Temporal Changes in Structure and Function of Rat Visual System after Blast Exposure. *Investigative Ophthalmology & Visual Science* (in review).

Dissertation

Shedd, DF. Ocular Injury Following Primary Blast Exposure. *University of Utah*, 2017.

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. 2016.

Conference Posters

Shedd DF and Coats B. Verification of a shock tube finite element model for blast ocular injury simulations. Proceedings of the 11th International Symposium of Computer Methods in Biomechanics and Biomedical Engineering. Salt Lake City, UT. April 2013.

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

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. Snowbird, UT. June 2015.

Coats B, Shedd DF, Jones J. Temporal Changes in Vitreous Inflammatory Cytokines and Neurofilament Heavy Chain Following Ocular Blast Trauma. Association for Research in Vision and Ophthalmology Annual Meeting 2016. Seattle, WA. May 2016.

Shedd DF and Coats B. Finite Element Investigation of Geometric Scaling Between Rat and Human Eyes During Primary Blast Exposure. Military Vision Symposium. Boston, MA. March 2017.

Conference Podium Presentations

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.

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

Benko N, Shedd DF, Coats B. Measurement of Intraocular Pressure during Blast Wave Loading. Summer Biomechanics, Bioengineering and Biotransport Conference 2017. Tuscon, AZ. June 2017.

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.
- Developed novel method for measuring intraocular pressure during blast exposure.
- Developed and validated computer model of rat eye under blast loading
- Developed scaling equation to allow comparison of blast exposure levels between species

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: 2016 ARVO Poster

Appendix I: 2017 Military Vision Symposium Poster

Appendix J: 2017 Summer Biomechanics, Bioengineering and Biotransport Conference Abstract (Podium presentation)

Appendix K: Brittany Coats (PI) CV

Appendix L: Quad Chart



VISION CHANGES FOLLOWING BLAST EXPOSURE

Participants Needed for Research Study on Vision Changes Following Blast Exposure

The Ocular Biomechanics Lab at the University of Utah, in collaboration with the Salt Lake City VA Hospital, are investigating changes in vision following direct or indirect exposure to a blast. We are looking for volunteers to participate in a 1 to 2 year study evaluating possible changes in vision following blast exposure. Participants will be interviewed, fill out questionnaires, and receive eye examinations every four months. Participants will receive a \$50 gift card for every eye examination study visit.

If you are interested in learning more and/or participating in the study, please visit the following URL:

<http://j.mp/29j7kAx>



Project funded through the generous support of
USAMRAA grant#W81XWH-12-1-0243

**PAID VOLUNTEERS
NEEDED**

FREE VISION EXAMS

NON-INVASIVE

**HELP US IDENTIFY
BLAST MECHANICS
LEADING TO AN
INCREASED RISK OF
DECREASED VISION**

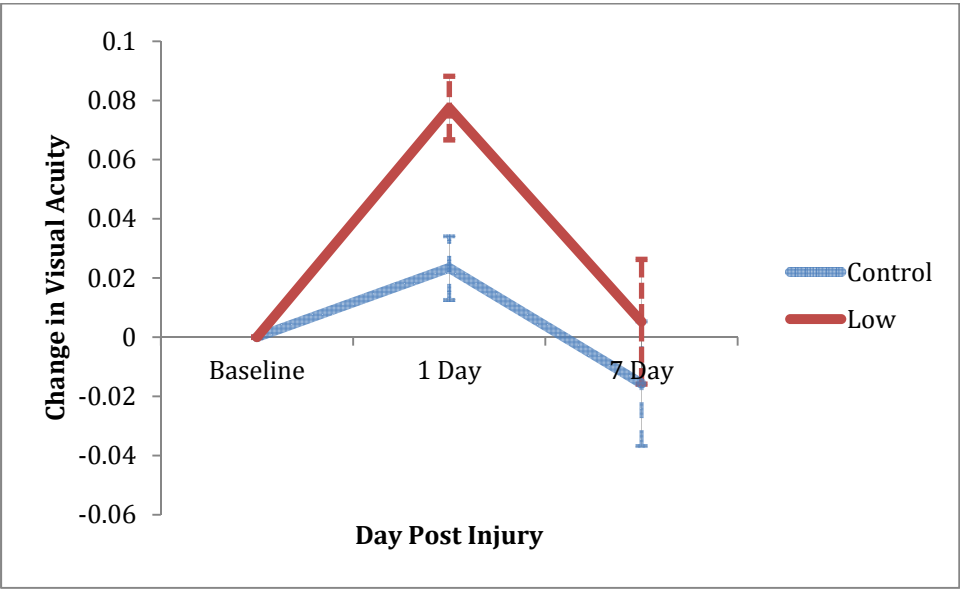
QUESTIONS?

Feel free to contact the
Principal Investigator:

Brittany Coats, PhD
Mechanical Engineering
University of Utah
brittany.coats@utah.edu
801-585-0586

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.



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.

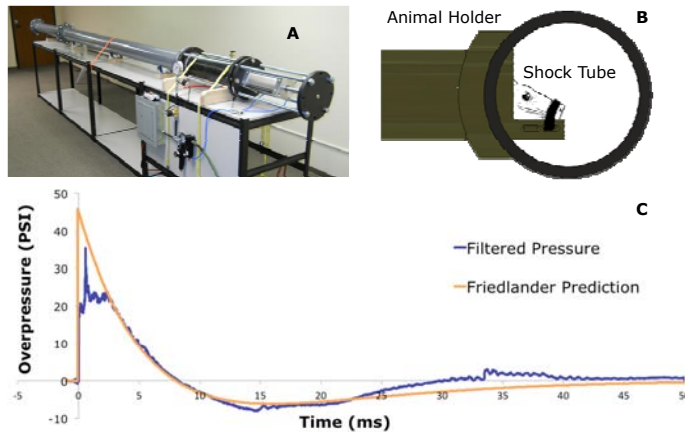


Fig. 1. (A) The 6" experimental shock tube was triggered via rupturing BoPET membranes and instrumented with 1 MS/s pressure sensors (PCB 113B26) along the length of the driven section. (B) Animal placement within shock tube. (C) Representative filtered pressure profile used to apply blast insult. Comparison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

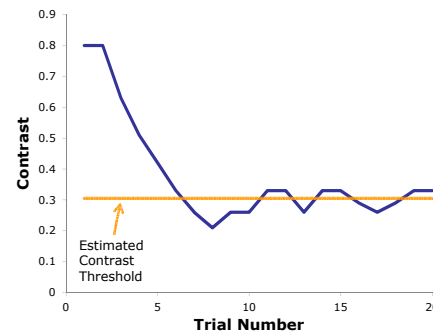


Fig. 3. Representative plot identifying visual acuity in a rat following blast exposure.

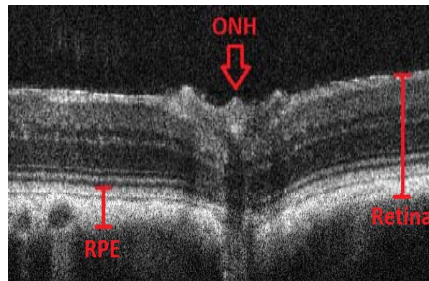


Fig. 4. Representative B-scan centered at optic nerve head (ONH). Total retinal thickness and RPE thickness were extracted across retina width and averaged. For the purposes of analysis, RPE included the photoreceptor inner/outer segments.

References

- [1] DeFraités, 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

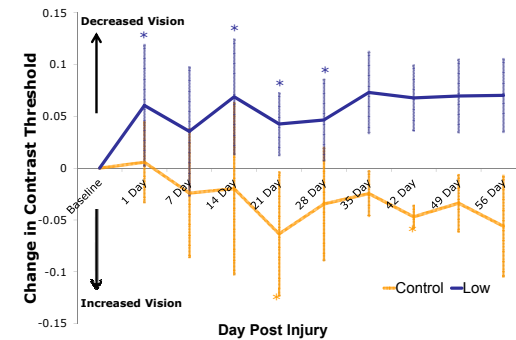


Fig. 5. Change in contrast threshold from baseline over time for control and low-level blast-exposed animals. * $p < .05$

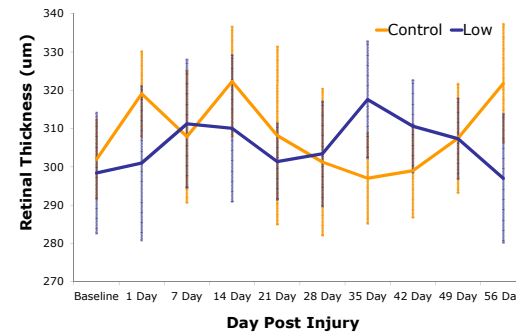


Fig. 6. Thickness of retinal layer over time, representing blast animals (n=3) and control animals (n=3). All data was gathered from eyes ipsilateral to blast insult. No significant trends were found.

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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Email: d.shedd@utah.edu Email: brittany.coats@utah.edu

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

DATA CALL SUMMARY <i>Please review and approve</i>	
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, Daniel¹; Coats, Brittany¹

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 the findings of decreased functional visual acuity.

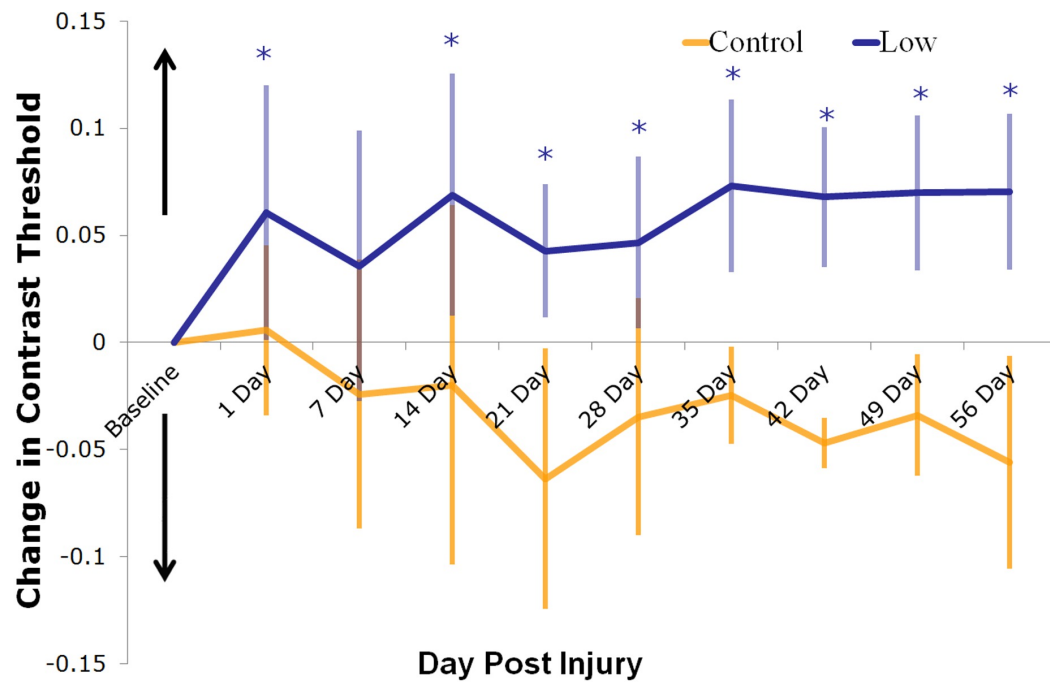


Figure 1. Change in contrast threshold from baseline over time for control and low-level blast exposed animals.
* $p < 0.05$.

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

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.

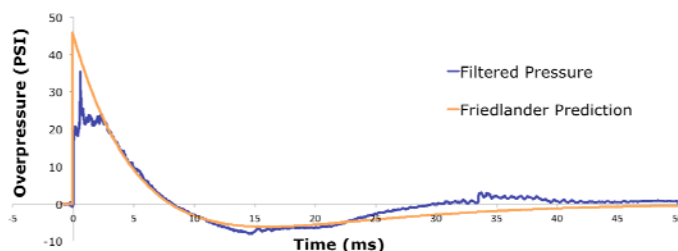


FIGURE 2. BLAST WAVE MEASURED 1-IN IN FRONT OF ANIMAL LOCATION COMPARED TO PREDICTION OF BLAST FROM FRIEDLANDER WAVE EQUATION

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).

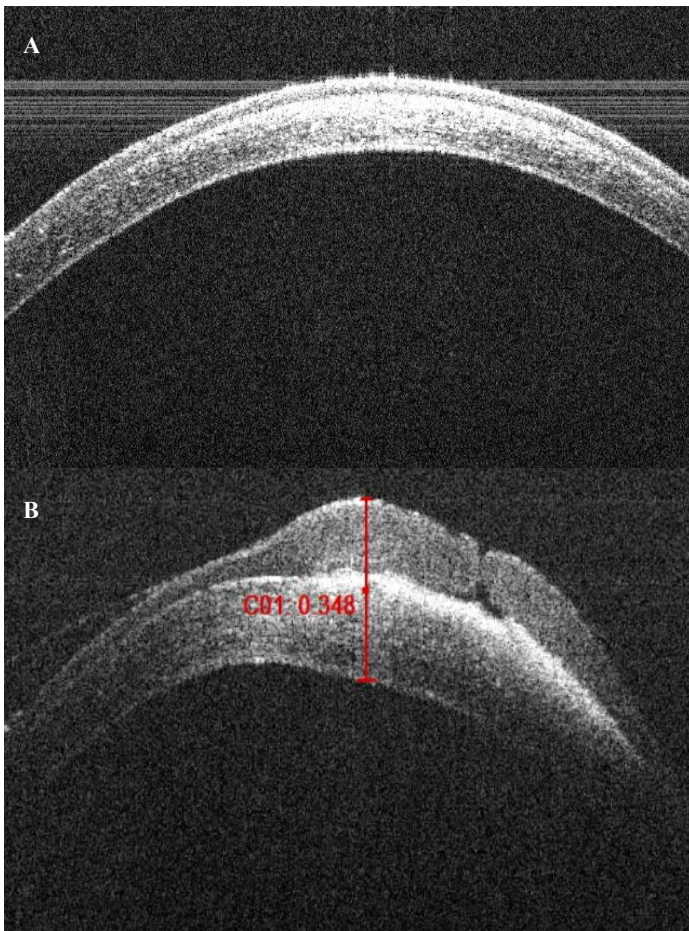


FIGURE 3. (A) HEALTHY CORNEA IMAGE. (B) CORNEA EXHIBITING THICKENING OF EPITHELIUM INDICATIVE OF INFLAMMATION. THE CORNEAL STROMA IS ALSO SLIGHTLY ENLARGED, AND THE BORDER BETWEEN LAYERS IS UNHEALTHY.

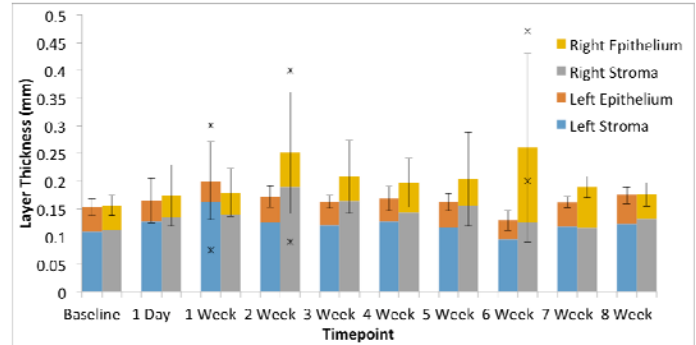


FIGURE 4. AVERAGE THICKNESS FOR TOTAL CORNEA AS WELL AS STROMA AND EPITHELIUM. STARRED COLUMNS INDICATE STATISTICAL SIGNIFICANCE COMPARED BASELINE (PRE-INJURY).

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.

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

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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 μ L) was diluted with phosphate buffer saline (PBS) until a total volume of 150 μ L was reached. The sample was then separated into three equal tubes.

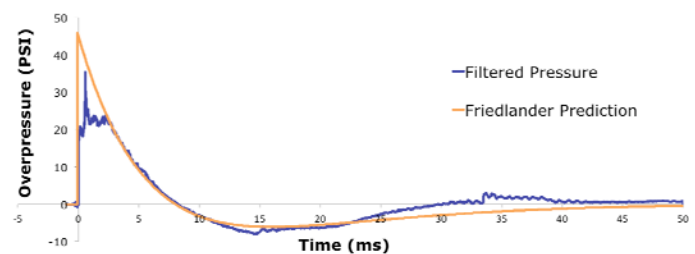


FIGURE 1. PRESSURE-TIME HISTORY AT LOCATION OF ANIMAL PLACEMENT WITHIN BLAST TUBE

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

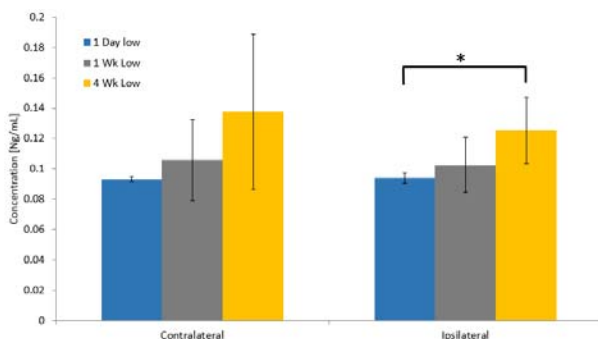


FIGURE 2. CHANGES IN NFH CONCENTRATION POST INJURY IN IPSILATERAL (RIGHT) AND CONTRALATERAL (LEFT) EYES. *p<0.05

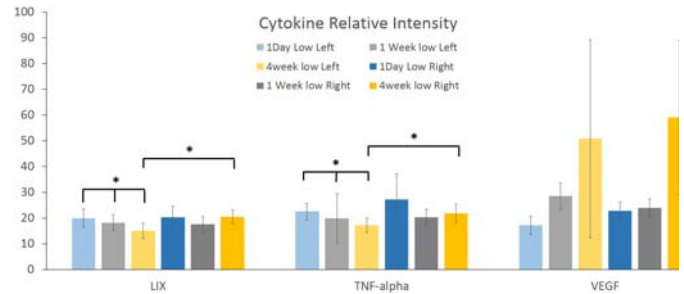


FIGURE 3. CYTOKINE RELATIVE INTENSITIES

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 to determine if they are merely a generic inflammatory response to the blast that is quickly resolved.

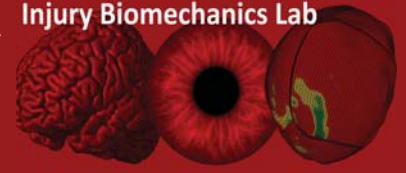
In summary, the blast pressure in this study appears to create some damage to the retina that is potentially recovered quickly in the contralateral eye, but not in the ipsilateral eye. Longer time points are currently being explored to determine the resolution of NfH to baseline levels post injury. Future 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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Poster# 6395-C0182

INTRODUCTION

The 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 due to blast exposure include corneal swelling, neovascularization, retinal degeneration, and optic neuropathy. [1] Our current blast rodent model exhibits these injuries in a temporally multiplexed manner that may be exploited for protein biomarker detection and drug delivery.

The goal of this study was to measure proteomic changes to inflammatory cytokines and neurofilament heavy chain in the vitreous over 8 weeks following blast exposure and correlate the findings with the injury pathology time-course.

METHODS

The right side of anesthetized Long-Evans rats (300-350g, n=20) were exposed to 34 psi 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.

After the respective survival time, the eyes were harvested and the vitreous humor removed. Neurofilament heavy chain (NfH), a protein resulting from degenerating retinal ganglion cells [2], was evaluated using an ELISA specifically developed for quantifying NfH concentration [3]. The absorbance was read using a Synergy HT Plate at 450 nm with a reference wavelength of 750 nm. Inflammatory cytokine changes were quantified using a commercially available kit (RayBio® Rat Cytokine Antibody Array G). A GenePix® 4000A micro array scanner and software were used to scan (532 nm) and analyze the arrays. The time-dependent response of all proteins, and comparison of experimental to control levels of concentration at each time point were evaluated with two one-way ANOVAs. A p-value < 0.05 was considered significant.

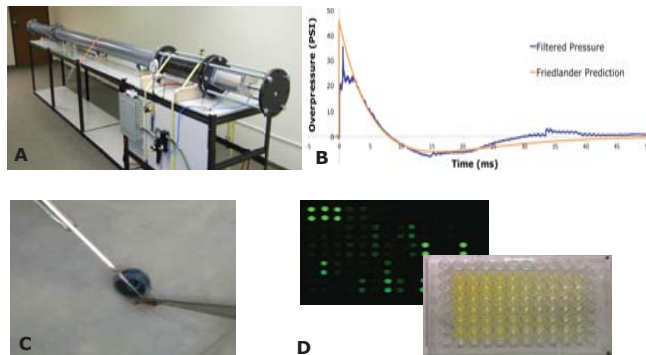


Fig. 1 (A) The 6" diameter experimental shock tube was triggered via rupturing BoPET membranes (B) Pressure of shock wave compared to Friedlander wave form. (C) Vitreous removal by evisceration. (D) Biomarker assays resulting from the experiment.

RESULTS

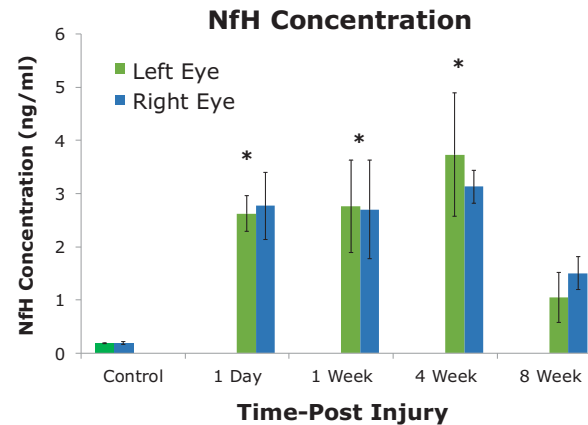


Fig. 2 NfH was significantly greater in injured animals compared to controls for all time points (p < 0.05) up to 4 weeks. At 8 weeks, NfH significantly decreased, approaching levels found in control animals.

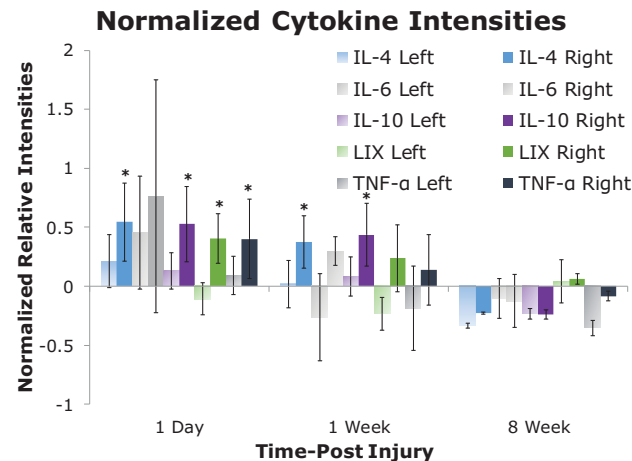


Fig. 3 Cytokines normalized by intensities in control animals. Both eyes had a general increased inflammatory response 1 day after injury, but returned to control levels by 8 weeks. Most significant changes were observed in the eye directly exposed to the blast (right eye).

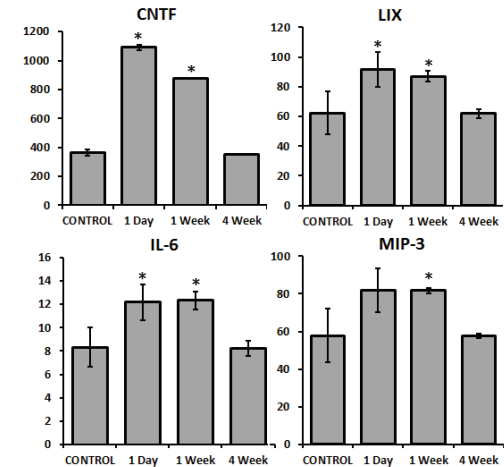


Fig. 4 Most cytokine changes returned to control values by 4 weeks, and were soon followed by structural changes observed on OCT (e.g., retinal thinning, corneal swelling/scarring).

KEY FINDINGS

- A 34 psi blast is sufficient to elicit immediate proteomic responses in both eyes that typically resolve by 4 or 8 weeks.
- NfH concentration significantly increased immediately after injury and remained elevated until at least 4 weeks.
- Most inflammatory cytokines (IL- α , IL- β , IL-10, LIX, GM-CSF, Leptin, MIP-3, β -NGF) increased immediately after injury, and resolved by 4 or 8 weeks.
- The timing of these proteomic changes correspond to decreases in visual acuity in the animal model, and supersede structural changes to the retina (thinning) and cornea (swelling).

CONCLUSION

The immediate vitreous proteomic changes prior to retinal and corneal pathology suggest that cytokines and NfH may be used as biomarkers for damage progression. Further, the profound inflammatory response identifies a potential avenue for exploring drug treatment to mitigate or reduce ocular damage from blast exposure.

ACKNOWLEDGEMENTS

We are very grateful to USAMRMC #W81XWH-12-1-0243 for their financial support of this project.

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

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Introduction

Animal models are commonly used to simulate and evaluate ocular blast trauma, but it is unclear how anatomical differences between animal and human eyes affect mechanical loading and injury outcomes. The goal of this study was to use finite element analysis to investigate the implications of cornea, lens, and globe size differences between humans and rats on intraocular pressure and deformation during primary blast exposure.

Methods

Geometry: Six simplified quarter-symmetry ocular geometries were created in SolidWorks and imported into Abaqus. The baseline geometry replicated the size and shape of a rat eye ($d = 6.26$ mm), with four major structural components: sclera, cornea, lens, and vitreous. Cornea, lens, and globe size were systematically varied in the baseline model until a proportionally accurate human eye ($d = 24.2$ mm) was replicated. Three of the model geometries are shown in Fig. 1.

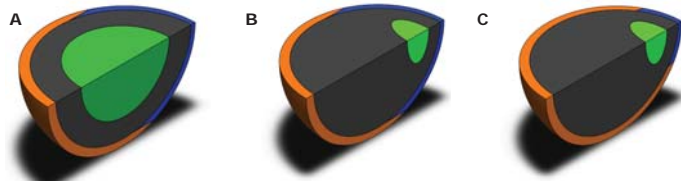


Fig. 1. Three of the six geometries: (A) baseline rat (B) rat with scaled smaller lens (C) rat with scaled smaller lens and cornea. Color Code: Orange = Sclera, Blue = Cornea, Grey = Vitreous, Green = Lens

Table 1. Material properties for major model components. Material source data taken from in house testing^a, Bhardwaj 2014[†], and Uchio 1999[‡]

Geometry Component	Material Model	Model Parameters	Density (kg/m ³)
Lens	Linear Elastic [†]	E = 500 MPa $\nu = 0.48$	1200
Vitreous	Linear Elastic [†]	E = 21 Pa K = 2.27 GPa	1006
Cornea	3 rd Order Ogden [†]	$\nu = 0.42$ Uniaxial Stretch	1076
Sclera	1 st Order Ogden [†] Prony Series Viscoelastic [*]	$\nu = 0.47$ Uniaxial Stretch Relaxation Data	1243

Mesh: Linear hexahedral elements with reduced integration were used for all structures with the exception of the interface between cornea and sclera, which was modeled with linear tetrahedral elements. The convergence study was performed on the baseline rat model. The lens mesh was converged first, followed by the cornea/sclera mesh, and then the vitreous mesh. This resulted in 80,740 elements for the baseline rat model. The number of elements was maintained for all subsequent models.

Loading & BCs: Blast loading was simulated by applying an incident pressure wave with peak overpressure 234 kPa and duration 7 ms as measured during animal experiments (Fig. 2). The rear portion of the sclera was fixed in space.

Outputs: The intraocular pressure (IOP) in the posterior vitreous of the baseline rat model was validated against IOP measured during blast experiments in rats. Output variable for all models included vitreous peak IOP; cornea, lens, and retina strain; cornea and lens displacement.

Results

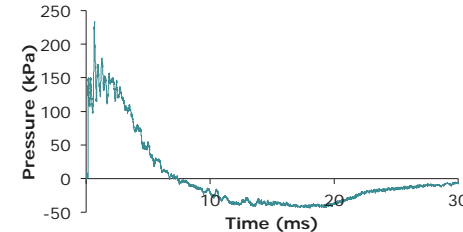


Fig. 2. Blast load applied to model. Side-on overpressure measured in shock tube during IOP experiments.

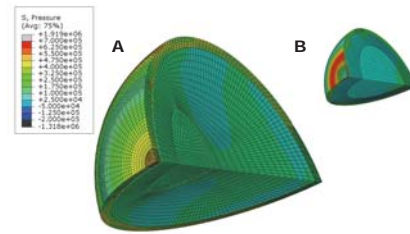


Fig. 3. Pressure during blast loading for the (A) human and (B) baseline rat models.

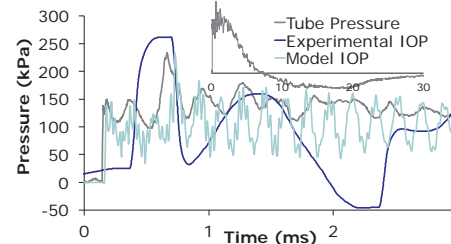


Fig. 4. IOP results compared to experimental IOP for initial peak. Tube pressure represents input to model. Peak IOP predicted by the model was within 12% of experimental data. Inset: Complete input pressure.

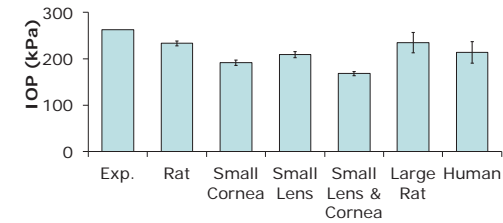


Fig. 5. Peak IOP predicted by each model compared to measured experimental IOP (Exp.).

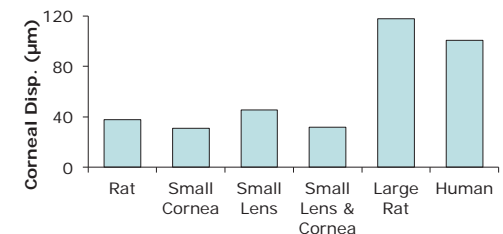


Fig. 6. Peak cornea displacement. The peak displacement was located at the center of the cornea in all models.

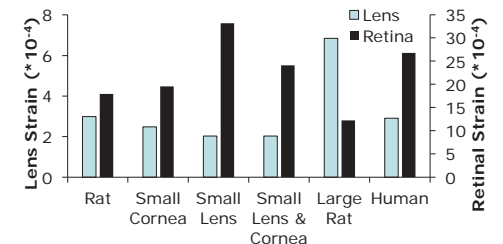


Fig. 7. Lens and retinal strain. Lens strain increased with general scaling of the globe. The location of peak strain shifted posteriorly with increased lens size. Retinal strain was influenced by scaling of anatomical features.

Conclusions

- Model peak IOP measured at sensor location recreated experimental peak IOP within 12%.
- Reducing the size of the lens or cornea to match human proportions lowered IOP by 18% and 10%, respectively. Reducing both effects simultaneously reduced IOP by 28%, the sum of the individual changes. Scaling the rat eye to the size of the human eye resulted in less than a 1% change in peak IOP.
- Central corneal deformation was small, but was very sensitive to global scaling. Additionally, geometries with increased vitreous space inside the globe experienced increased corneal deformation.
- Tissue strains were generally small. The lens strain was more sensitive to global eye size than anatomical feature scaling, while the retinal strain was more sensitive to changes in anatomical features.

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Acknowledgements

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More information about the Utah Head Trauma Injury Biomechanics Lab is available at our lab website: pedtrauma.mech.utah.edu



MEASUREMENT OF INTRAOCULAR PRESSURE DURING BLAST WAVE LOADING

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INTRODUCTION

Ocular primary blast injury has become a subject of great interest due to recent exposures in American and coalition warfighters in the Middle East [1][2]. The two most popular methods of research thus far have been injury replication with small-species animal models using mice, rats, and rabbits, and injury simulation with finite element models [3-6]. To date, there has been little work to relate the two methods of study and no experimental data exists to verify that predicted finite element model pressures are accurate. As such, there is little known about the mechanical forces inside the eye during blast exposure. As part of our lab's recent work on an animal model of ocular blast injury, we conducted the first known *in vivo* measurements of intraocular pressure (IOP) during primary blast injury.

METHODS

All animal studies were reviewed by the Institutional Animal Care and Use Committee of the University of Utah and were performed in adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Long-Evans rats, (n=10, 399±68g) were fit with fiber optic pressure transducers (FISO FOP-LS-2FR-30, Quebec, Canada), surgically placed in each eye to measure the intraocular pressures (IOP) of blast exposed eyes. Animals were anesthetized with an intraperitoneal injection of 64.0 mg/kg of ketamine and 0.25 mg/kg dexmedetomidine. The lateral commissure was clamped with a hemostat for several seconds to limit bleeding, and then cut to gain access to the posterior half of the eye. The IOP

transducers were pre-threaded through surgical tubing and placed into the midsection of an 18G hypodermic needle (Fig. 1). Tweezers were used to grasp the conjunctiva near the commissure and rotate the eye in the medial direction. The needle containing the pressure transducer was inserted through the posterior sclera and positioned into the central vitreous chamber. The conjunctiva was released and the eye allowed to gently rotate back into its natural position. Skin sutures and cyanoacrylate adhesive secured the surgical tubing to the back of the rat to limit sensor cable motion during animal positioning and blast wave exposure. The procedure was repeated for the other eye. Rats were secured in the blast tube in a 3D printed holder such that the right eye was directly exposed while the left was indirectly exposed to blast. After blast exposure, animals were euthanized and sensors removed.

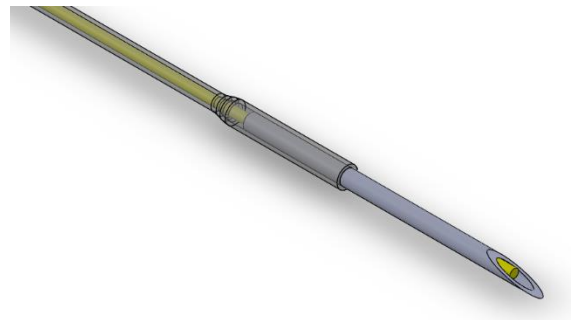


Figure 1: Computer rendering of pressure transducer situated inside an 18 gauge needle and shielded by surgical tubing.

Side-on blasts were generated using a 521 cm long shock tube. The tube has a 64 cm long driver section, 457 cm driven section, and constant internal diameter of 14 cm. The shock tube was tuned to reproduce the Friedlander waveform that is representative of an open field blast. The driver section was pressurized with compressed air until a 0.01" axially-oriented polyethylene terephthalate membrane ruptured. Pressure waveforms within the tube were recorded 2 cm upstream of the right eye for each blast-exposed animal at 1 MHz using a flush-mounted pressure sensor (PCB Piezotronics, NY). The pressure time history profiles were filtered using a custom hardware anti-aliasing filter with $f_c=180$ kHz. Fiber optic transducers were connected to a FISO signal conditioner with a sampling frequency of 15 kHz. Data from the signal conditioner and tube-mounted pressure sensors was recorded using a NI DAQ 9223 with LabView software (National Instruments, Austin, TX). No post-hoc filtering was applied. Pressure data traces were analyzed in MATLAB to record peak pressure, rise time, initial slope, positive, negative, and net impulse for each curve.

RESULTS

Intraocular pressure was successfully captured in both eyes in eight of the ten animals. During initial blast wave contact with the directly and indirectly exposed eyes, IOP closely mimicked the pressure waveform measured in the shock tube. However, after the initial insult, IOP readings became erratic (Fig. 2). This could have been caused by sensor movement within the eye, contact between the ocular lens and the sensor tip, and/or wave reflection in the eye. Based on the initial 5 ms of the pressure waveform it was observed that peak pressure in the directly exposed eye matched the tube pressure measured 2 cm in front of the animal within 0.1% and with an expected 0.6775 ms delay (Fig. 3). Peak intraocular pressure measured in the indirectly exposed eye was lower than the tube pressure by 30%, but this difference was not statistically significant. Pressurization rate decreased by 39% as the blast wave traveled from the tube into the directly exposed right eye and continued to decrease as the wave traveled through the head into the indirectly exposed left eye ($p<0.0001$). No significant difference was observed in net impulse in the tube or either eye.

DISCUSSION

Recent animal models have demonstrated the occurrence of various ocular pathologies as a result of primary blast wave exposure [2]. However, little is known about the mechanical stresses and strains within the eye during exposure. Such knowledge is key for determining injury thresholds, design of protective equipment, and translating findings from animal studies to the human eye. A technique for measuring IOP in rodents during blast exposure has been introduced, and can successfully capture the initial pressurization during blast wave exposure. After peak pressure, however, it is likely there is substantial sensor movement, so identifying reflective waves in the eye is not possible.

In vivo peak ocular pressure during the first 5 ms of blast exposure in directly exposed eyes closely matched external tube

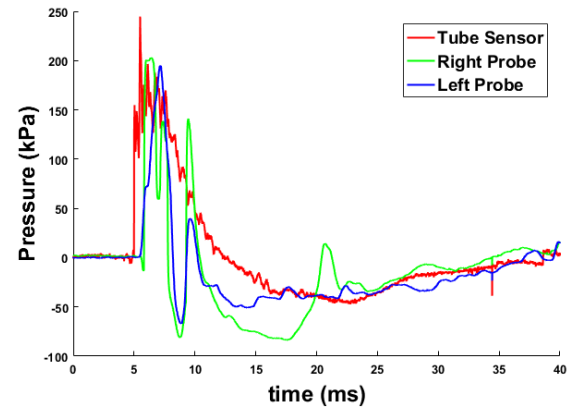


Figure 2: Pressure waveform during a side-on blast insult

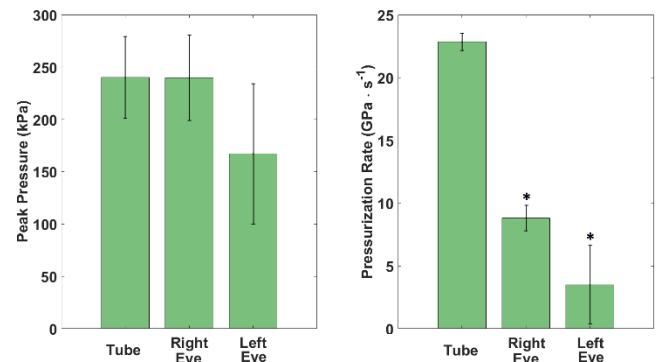


Figure 3: Peak pressure and pressurization rate in the 3 sensor locations. Bars indicate standard deviation (* $p<0.0001$)

measurements. Indirectly exposed eyes, however, experienced lower mechanical loads than those measured in the tube. Therefore, tube peak pressure measured 2 cm before the animal is a suitable surrogate for peak pressure in directly exposed eyes. Tube pressure is not a good surrogate for peak pressure in indirectly exposed eyes. Furthermore, the tube pressurization rate will likely overestimate the ocular pressurization rate. Decreases in peak pressure and pressurization rate observed in the indirectly exposed eye help account for a lower incidence of ocular injury found in our associated animal studies[7.] These data highlight the important role of facial structures and blast wave direction in determining risk of ocular trauma from blast.

ACKNOWLEDGEMENTS

We are grateful for USAMRAA award #W81XWH-12-1-0243 which financially supported this work.

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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

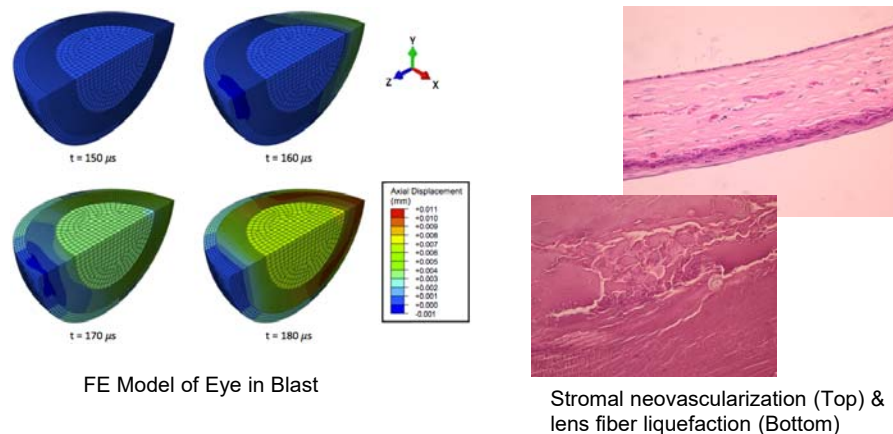


Study/Product Aim(s)

- 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: Created computational models of various eye geometries during blast exposure to developed scaling relationship between rodent and human exposure levels. Completed ocular histology which shows corneal and lens damage in blast-exposed animals.

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, 2017

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 data analysis
- ✓ Complete data analysis of protein assays

Comments/Challenges/Issues/Concerns: None at this time.

Budget Expenditure to date

Projected Expenditure: 997,528 Actual Expenditure: 946,287.59