



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Registration and Introduction (0830 – 0925)	
08:30	Registration
09:00	MITRE welcome and venue logistics Ms. Rachel W. Spencer, <i>MITRE Health Transformation Tech Center</i>
09:05	MITRE Leadership Welcome and Introduction Dr. Ronald T. Williams, <i>MITRE National Security Engineering Center</i>
09:15	Program Chair and Co-Chair Overview Program Chair: Dr. Raj Gupta (<i>US Army Medical Research and Materiel Command (USAMRMC), Department of Defense Blast Injury Research Program Coordinating Office (PCO) (USA)</i>) Program Co-Chair: Prof. Shunichi Sato (<i>National Defense Medical College (NDMC) (Japan)</i>)
09:25	General Chair and Co-Chair Overview General Chair: Mr. Michael Leggieri (<i>USAMRMC, PCO) (USA)</i>) General Co-Chair: Prof. Daizoh Saitoh (<i>NDMC) (Japan)</i>)
Session 1: Injury (0940 – 1345) Co-Chairs: Dr. R. Gupta and Dr. S. Kawachi	
09:40	Tutorial: Synergistic interaction of oxidative stress, blood-brain barrier breakage and neuroinflammation in the pathogenesis of blast TBI Namas Chandra, <i>New Jersey Institute of Technology</i>
10:10	Pathophysiological differences of hearing impairment caused by different conduction pathways of shock wave Eiko Kimura, Kunio Mizutari, Katsuki Niwa, Yasushi Satoh, Shun-ichi Sato, and Akihiro Shiotani <i>National Defense Medical College, Japan</i>
10:30	Blast exposure leads to accelerated aging in rat brain Peethambaran Arun, Franco Rossetti, Donna M. Wilder, Sujith Sajja, Steve VanAlbert, Ying Wang, Irene D. Gist, Joseph B. Long, <i>Walter Reed Army Institute of Research</i>
10:50	MORNING BREAK
11:05	Investigation of Blast Effects on Wound Infection development and Antibiotic Disposition Vlado Antonic et al., <i>Walter Reed Army Institute of Research</i>
11:25	Potential cause of primary, blast-induced brain injury: direct vs. indirect mechanism J. Rubio ^{1,2} , G. Unnikrishnan ^{1,2} , V. Sajja ³ , S. van Albert ³ , J. Long ³ , M. Skotak ⁴ , E. Alay ⁴ , N. Chandra ⁴ , A. Sundaramurthy ^{1,2} , D. Subramaniam ^{1,2} , and J. Reifman ¹ , ¹ <i>US Army Medical Research and Materiel Command</i> , ² <i>Henry M. Jackson Foundation for the Advancement of Military Medicine</i> , ³ <i>Walter Reed Army Institute of Research</i> , ⁴ <i>New Jersey Institute of Technology</i>
11:45	Animal Model of Multiple Low-Level Blast Injury Displays a Spectrum of Neuropathological and Neurobehavioral Changes Venkata Kakulavarapu Rama Rao, Arunreddy Ravula, Namas Chandra, <i>New Jersey Institute of Technology</i>
12:05	LUNCH (catered lunch requires prepaid voucher)
13:05	Effect of Blast Overpressure on 3D Neuronal Cell Cultures Thomas J. O'Shaughnessy, K.M. Gilpin, Y. Chen, and A. Bagchi, <i>U.S. Naval Research Laboratory</i>
13:25	Developing Correspondence Rules for Traumatic Brain Injury in Different Species Robert Saunders, X. Gary Tan, Amit Bagchi, <i>U.S. Naval Research Laboratory</i>
13:45	Dietary Zinc Modulates Matrix Metalloproteinases in Traumatic Brain Injury Angus G. Scrimgeour ¹ , Michelle L. Conklin ¹ and Marloes J.A. Joosen ² , ¹ <i>US Army Research Institute of Environmental Medicine</i> , ² <i>TNO Defence, Netherlands</i>
14:05	Role of resident microglia and circulating monocytes in chronic neuroinflammation and behavioral outcomes in a mouse model of blast-induced traumatic brain injury Madhuvika Murugan, N. Chandra, <i>New Jersey Institute of Technology</i>



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Session 2: Experimental blast injury methodology (14:05 – 17:10)	
Co-Chairs: Dr. N. Chandra and Dr. N. Shinomiya	
14:25	Tutorial: Neural basis for chronic headache and photophobia after mild traumatic brain injury Akimasa Tashiro, <i>National Defense Medical College, Japan – 30 mins</i>
14:55	Experimental focal blast vs non-blast traumatic brain injury research: a comparison between laser-induced shock wave model and CCI model Satoshi Tomura, Satoko Kawauchi, Shunichi Sato, Daizoh Saitoh, <i>National Defense Medical College, Japan</i>
15:05	AFTERNOON BREAK
15:25	Roles of nitric oxide in shock wave-caused cerebral hemodynamic abnormalities in rats: laser-induced shock wave study S. Kawauchi ¹ , M. Inaba ² , Y. Muramatsu ¹ , A. Kono ¹ , Y. Komuta ¹ , I. Nishidate ² , K. Kaida ¹ , T. Adachi ¹ and S. Sato ¹ <i>¹National Defense Medical College, Japan, ²Tokyo University of Agriculture and Technology</i>
15:45	Meningeal damage may be associated with spreading depolarization and glial scar formation in the cortex: laser-induced shock wave study S. Sato ¹ , S. Kawauchi ¹ , Y. Komuta ¹ , A. Kono ¹ , Y. Muramatsu ¹ , T. Osawa ² , M. Inaba ² , I. Nishidate ² and K. Kaida ¹ <i>¹National Defense Medical College, Japan, ²Tokyo University of Agriculture and Technology</i>
16:05	5-cm-by-5-cm Detonation-Driven Blast Simulator for Fluid Dynamic Research on Blast Injury Toshiharu Mizukaki ¹ , A. Kato ¹ , M. Mori ¹ , Y. Sekine ² , and D. Saitoh ² <i>¹Tokai University, ²National Defense Medical College, Japan</i>
16:25	Effects of animal orientation on brain responses to primary blast Ginu Unnikrishnan ^{1,2} , H. Mao ^{1,2} , A. Sundaramurthy ^{1,2} , V. Sajja ³ , S. van Albert ³ , J. Long ³ , J. Rubio ^{1,2} , D. Subramaniam ^{1,2} , and J. Reifman ¹ <i>¹U.S. Army Medical Research and Materiel Command, ²Henry M. Jackson Foundation for the Advancement of Military Medicine, ³Walter Reed Army Institute of Research</i>
16:45	Multiple Degree of Freedom Blast Effects Simulator Robert Kargus, <i>U.S. Army Research Laboratory</i>
17:05	Fluid Dynamical Evaluation of Pressure History of Blast Simulator at National Defense Medical College Toshiharu Mizukaki ¹ , A. Kato ¹ , M. Mori ¹ , Y. Sekine ² , and D. Saitoh ² <i>¹Tokai University, ²National Defense Medical College, Japan</i>
17:25	WIAMan Underbody Blast ATD demonstration Jacques Eckles
17:40	Closing remarks Dr. R. Gupta
17:45	Optional meet-and-greet with heavy hors d'oeuvres and cash bar until 7:30 pm EST. (NOTE: Hors d'oeuvres are prepaid during online registration and require a voucher).



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Registration and Introduction (08:30 – 09:15)	
08:30	Registration
09:00	MITRE welcome and venue logistics Ms. Rachel W. Spencer, <i>MITRE Health Transformation Tech Center</i>
Session 3: Broad surveys and intergovernmental collaborative efforts (09:15 – 10:25) Co-Chairs: Dr. R. Gupta and Dr. S. Sato	
09:15	Keynote Address: Sizing Up Blast Injury Research: A Trans-Disciplinary Evidence-Base To Maximise Impact & Relevance J. Denny, R. Brown, A. Dickinson and J. Batchelor*, <i>University of Southampton, United Kingdom</i>
09:45	Findings from the U.S. Military Health System Blast Injury Prevention Standards Recommendation (BIPSR) Process Jeffrey B. Colombe ¹ , Anthony Santiago II ¹ , Elizabeth Brokaw ¹ , Brian Colder ¹ , Rachel W. Spencer ¹ , Lisa Lalis ¹ , Raj Gupta ² , Michael Leggieri ² ¹ <i>The MITRE Corporation</i> , ² <i>US Army Medical Research and Materiel Command</i>
10:05	DoD Working Group on Computational Modeling of Human Lethality, Injury, and Impairment from Blast-related Threats Anthony Santiago II ¹ , Matthew Downs ¹ , Lisa Lalis ¹ , Rachel Spencer ¹ , Raj Gupta ² , Michael Leggieri ² ¹ <i>The MITRE Corporation</i> , ² <i>US Army Medical Research and Materiel Command</i>
10:25	MORNING BREAK
Session 4: Computational modeling (10:40 – 14:20) Co-Chairs: Dr. A. Bagchi and Dr. T. Mizukaki	
10:40	Mechanism-based brain models to study primary blast loading effects on axonal deformation: the past, present and future Reuben H. Kraft ¹ , R. Menghani ¹ , H. T. Garimella ³ , A. Eidsmore ² , M. Kleinberger ² , A. J. Przekwas ³ ¹ <i>The Pennsylvania State University</i> , ² <i>U.S. Army Research Laboratory</i> , ³ <i>CFD Research Corporation</i>
11:00	Investigation of blast-induced traumatic brain injury thresholds and mechanisms using a rodent finite element model Molly Townsend and N. Chandra, <i>New Jersey Institute of Technology</i>
11:20	Arteriole inflation as an injury mechanism following blast TBI Amy Dagro ¹ , K.T. Ramesh ² , ¹ <i>US Army Research Laboratory</i> , ² <i>Johns Hopkins University</i>
11:40	Hierarchical Validation of the WIAMan LS-Dyna FEM for Application in Underbody Blast Nicholas A. Vavalle ¹ , Christian W. Lomicka ¹ , Connor O. Pyles ¹ , Matthew T. Shanaman ¹ , and Randolph S. Coates ² ¹ <i>The Johns Hopkins University Applied Physics Laboratory</i> , ² <i>U.S. Army Futures Command, Data and Analysis Center</i>
12:00	LUNCH (catered lunch requires prepaid voucher)
13:00	Computational model development of blast-induced pressure wave impact on a surrogate head model Rohan Banton ¹ , Thuvan Piehler ¹ , Nicole Zander ¹ , Richard Benjamin ¹ , Oren Petel ² , Josh Duchworth ³ , ¹ <i>U.S. Army Research Laboratory</i> , ² <i>Carleton University</i> , ³ <i>The Uniformed Services University of the Health Sciences</i>
13:20	Experimental Study on Effect of Porosity of Porous Media on Pressure Behind Simulated Head Model with Helmet Subjected to Simulated Blast Wave Taketoshi Koita, Kou Miyashita, Hiromitsu Hisano, Shouta Kajii, Kazuki Ueguri, and Susumu Kobayashi, <i>Saitama Institute of Technology</i>
13:40	Numerical Simulation of Traumatic Brain Injury from Primary Blast Effects and Protection of Combat Helmet X. Gary Tan and A. Bagchi, <i>U.S. Naval Research Laboratory</i>
14:00	Fast Running Tools for Sensor Based Reconstruction of Warfighter Blast Exposure in Training and Operations , Andrzej J. Przekwas ¹ , Z.J. Chen ¹ , Harsha T. Garimella ¹ , Tim Zehnbaauer ¹ , Gary H. Kamimori ³ , Raj K. Gupta ² , ¹ <i>CFD Research Corporation</i> , ² <i>US DoD Blast Injury Research Program Coordinating Office</i> , ³ <i>Walter Reed Army Institute of Research</i>



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Session 5: Field sensors (14:20 – 1700)	
Co-Chairs: Dr. J. Colombe and Dr. I. Nishidate	
14:20	Force Preservation Through Individual Blast Exposure Surveillance D. Borkholder, S. Featherman, and K. Sims, <i>BlackBox Biometrics Inc.</i>
14:40	Ship Crew Injury Risk and Survivability—An Assessment of Injury Mechanics Weapons Effects Testing as Part of RIMPAC J. Mackiewicz ¹ , A. Olson ¹ , M. Clouser ^{1,2} , M. Galarneau ¹ , ¹ <i>Naval Health Research Center</i> , ² <i>Leidos</i>
15:00	Field Evaluation of the Blast Gauge Sensor (BGS) Compared to the Pencil Probe in an Open Blast Environment Thanyani Pandelani, Z. Schutte, S. Hamilton, D. Reinecke and R. Ahmed, <i>CSIR Defence, Peace, Safety and Security, South Africa</i>
15:20	AFTERNOON BREAK
15:35	Monitoring Occupational Exposures to Blast – What Have We Learned? What Are We Doing? Walter Carr, <i>Oak Ridge Institute for Science and Education, Walter Reed Army Institute of Research</i>
15:55	Interpretation of Body-Worn Sensor Data Tim Walilko ¹ , Mark Tommerdahl ² , Suthee Wiri ¹ , Ted Argo ¹ , Christina Wagner ¹ , Tim Bentley ⁴ , Laila Zai ³ ¹ <i>Applied Research Associates</i> , ² <i>Cortical Metrics</i> , ³ <i>Lucent Research</i> , ⁴ <i>Office of Naval Research</i>
16:15	Pressure Profile Distribution Across Varying Levels of Protection Adam Fournier and A. Farkas, <i>U.S. Army PEO Soldier</i>
16:35	Neurocognitive Effects of Blast Overpressure from Breaching Courses: Using DANA To Assess Performance Related To Immediate And Cumulative OP Exposure C.H. LaValle, Michael J. Egnoto, W. Carr, G.H. Kamimori, <i>Walter Reed Army Institute of Research</i>
16:55	Debate: Are field blast/IMU sensors of sufficient fidelity for their intended uses? (medevac decision, informing treatment, predicting injury and incapacity, improving design of safe systems)
17:25	Closing remarks Dr. R. Gupta
17:40	Banquet Dinner: Cash bar on first floor, banquet dinner upstairs from 6:30 pm until 8:30 pm EST <i>(NOTE: Dinner is prepaid during online registration and requires a voucher)</i>



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Registration and Introduction (08:30 – 09:15)	
08:30	Registration
09:00	MITRE Welcome and Venue Logistics Ms. Rachel W. Spencer, <i>MITRE Health Transformation Tech Center</i>
Session 6: Human Injury Assessments (09:15 - 14:00)	
Co-Chairs: Dr. S. Krna and Dr. D. Saitoh	
09:15	Tutorial: Leveraging Epidemiological Research to Identify Service Members at Risk for Deleterious Overpressure-Induced Outcomes Jennifer N. Belding ^{1,2} , Shannon Fitzmaurice ^{1,2} , Hannah Kwiatkowski ^{1,2} , Robyn Englert ^{1,2} , Jourdan Jackson ^{1,2} , Cynthia J. Thomsen ² , and LT Uade Olaghere da Silva ² , ¹ <i>Leidos</i> , ² <i>Naval Health Research Center</i> – 30 mins
09:45	Persistence of Concussion-Related Symptomology: An Examination of Major Blast Exposure and Chronic, Low-Level Overpressure Exposure Using Post-Deployment Health Reassessment Records Jennifer N. Belding ^{1,2} , Shannon Fitzmaurice ^{1,2} , Hannah Kwiatkowski ^{1,2} , Robyn Englert ^{1,2} , Jourdan Jackson ^{1,2} , Cynthia J. Thomsen ² , and LT Uade Olaghere da Silva ² , ¹ <i>Leidos</i> , ² <i>Naval Health Research Center</i>
10:05	The NICoE TBI Injury History Assessment: An adaptation of the Ohio State University TBI Identification Method for use in a military population Theresa Teslovich Woo et al., <i>The National Intrepid Center of Excellence (NICoE)</i> , <i>Walter Reed National Military Medical Center</i>
10:25	MORNING BREAK
10:40	A Comprehensive Blast-Related Auditory Injury Database (BRAID) of Injured US Military Personnel A. Joseph ^{1,2} , M. Clouser ^{1,2} , A. MacGregor ² , M. Galarneau ² , ¹ <i>Leidos</i> , ² <i>U.S. Naval Health Research Center</i>
11:00	Laboratory and Field Studies of Middle Ear Muscle Contractions as Protection Against Impulsive Noise Gregory Flamme ¹ , S. Tasko ^{1,2} , K. Deiters ¹ , M. Smith ¹ , H. Jones ³ , W. Murphy ⁴ , W. Ahroon ³ , N. Greene ³ ¹ <i>Stephenson and Stephenson Research and Consulting</i> , ² <i>Western Michigan University</i> , ³ <i>U.S. Army Aeromedical Research Laboratory</i> , ⁴ <i>National Institute for Occupational Safety and Health</i>
11:20	Assessment of chronic mTBI status in veterans Elizabeth B. Brokaw ¹ , Michael S. Fine ¹ , Anthony Santago II ¹ , Stacey Harris-Carriman ² ¹ <i>MITRE</i> , ² <i>New Mexico VA Health Care System</i>
11:40	Neuroimaging Biomarkers for TBI Detection and Monitoring Matthew Downs ¹ , K. Keenan ² , S. Russek ² , J. Dagher ¹ ¹ <i>MITRE</i> , ² <i>National Institute of Standards and Technology</i>
12:00	LUNCH (catered lunch requires prepaid voucher)
13:00	Blast-Induced Neurotrauma Results in Spatially Distinct Gray Matter Alteration Alongside Hormonal Alterations: Evidence from a Canadian Military and Veteran Cohort S.C. Hellewell ¹ , Ibolja Cernak ² ¹ <i>University of Sydney, Australia</i> , ² <i>STARR-C (Stress, Trauma, and Resilience Research Consulting) LLC</i>
13:20	Neuropathologic, Neuroimaging, and Biomarker Findings in Veterans with Blast-Related mTBI: Correspondences with Neurovascular Dysfunction and Immune Cell Infiltration in Blast Exposed Animals David Cook et al., <i>Veterans Affairs Puget Sound Health Care System, University of Washington (see abstract for all authors and affiliations)</i>
13:40	Is there an indication for pre-hospital trepanation in patients having a traumatic brain injury and a blast injury? Gregor Freude ¹ , R. Dulou ² , J.M. Delmas ² , C. Schulz ¹ , U.M. Mauer ¹ ¹ <i>Bundeswehrkrankenhaus, Germany</i> , ² <i>Hôpital d'Instruction des Armées Clermont-Tonnerre, France</i> , ³ <i>Hôpital d'Instruction des Armées Percy, France</i>



4th International Forum on Blast Injury Countermeasures 2019

8-10 May 2019

The MITRE Corporation, McLean, VA

Program Day 1 (8 May 2019)

Session 7: Injury and TBI biomarkers (14:00 - 16:15)	
Co-Chairs: Mr. M. Leggieri and Dr. S. Tomura	
14:00	<p>Hippocampus-Cerebellum Axis Specific Transcriptomic Stratification to Discriminate Mild from Moderate TBI Nabarun Chakraborty¹, Rasha Hammamieh¹, Aarti Gautam¹, Stacy-Ann Miller¹, Michelle L. Conclin², Marti Jett¹, Angus G. Scrimgeour² ¹US Army Center for Environmental Health Research, ²US Army Research Institute of Environmental Medicine</p>
14:20	<p>Microbiome signatures associated with rodent model of traumatic brain injury vs. psychological stress Aarti Gautam et al., US Army Center for Environmental Health and Research (see abstract for all authors and affiliations)</p>
14:40	<p>Visualization of neurovascular functions in in vivo rat brain using RGB camera-based diffuse reflectance imaging Izumi Nishidate¹, T. Osawa¹, M. Inaba¹, S. Kawauchi² and S. Sato³ ¹Tokyo University of Agriculture and Technology, ²National Defense Medical College, Japan</p>
15:00	AFTERNOON BREAK
15:15	<p>In Situ Synthesis and application of fluorescent metal nanoclusters to understand the effects of blast overpressure at the cellular level Karima Jeneh Perry¹, P. Aggarwal² R. Kumar² S.P. Karna¹ and R.K. Gupta³ ¹U.S. Army Research Laboratory, ²Institute of Nuclear Medicine & Allied Sciences, India, ³U.S. Army Medical Research and Materiel Command</p>
15:35	<p>Primary blast-induced mild traumatic brain injury shows changes in MRI and immunohistology in a rat model of blast-induced behavioral abnormality Hiroshi Matsuura¹, Mitsuo Ohnishi¹, Yoshichika Yoshioka², Sanae Hosomi¹, Kentaro Shimizu¹, Hiroshi Ogura¹, Takeshi Shimazu¹ ¹Osaka University Graduate School of Medicine, ²National Institute of Information and Communications Technology (NICT) and Osaka University</p>
15:55	<p>Vascular and Autoimmune Pathologies in Training-Associated Repeated Exposures to Subconcussive Blasts; Relevance for Force Readiness D.V. Agoston¹, A. Kamnaksh¹, J. McCullough¹, R. Aniceto¹, W.M. Graves III¹, L.S. Russeth² and J.L. Duckworth^{1,2} ¹Uniformed Services University of the Health Sciences, ²Marine Corps Base Camp Pendleton</p>
16:15	<p>Glial and vascular response after exposure to blast-associated shock wave K. Nishii¹, Y. Satoh¹, T. Higashi¹, T. Matsui¹, M. Kashitani², D. Saitoh¹ and Y. Kobayashi¹ ¹National Defense Medical College, ²Department of Aerospace Engineering, National Defense Academy</p>
16:35	<i>Debate: Does blast TBI share mechanisms and consequences with impact TBI?</i>
17:05	Closing remarks and final meeting adjournment, Dr. R. Gupta

Presentation abstracts

Vascular and Autoimmune Pathologies in Training-Associated Repeated Exposures to Subconcussive Blasts; Relevance for Force Readiness

D.V. Agoston¹, A. Kamnaksh¹, J. McCullough¹, R. Aniceto¹, W.M. Graves III³, L.S. Russeth³ and J.L. Duckworth^{2,3}

Departments of ¹Anatomy, Physiology & Genetics and ²Neurology, Uniformed Services University, Bethesda, MD, U.S.A.
³NeuroTactical Research Team, Marine Corps Base Camp Pendleton, Camp Pendleton, CA, USA

Background: Explosive blast creates a unique form of injurious environment [1]. The energy of the shock wave dissipates at the boundaries of biological structures with different acoustic impedances such as the vasculature and brain tissue and results in a unique form of brain injury [2]. During heavy weapons training, Students and Instructors are subject to multiple exposures to low level of explosive blasts. Many of them experience wide ranging symptoms including headaches, disorientation, and memory disturbances that can negatively affect force readiness short term. If there are lasting molecular level changes from these repeated sub-concussive blast exposures (RSCBE), long-term force-readiness will also be negatively impacted. The molecular level changes induced by RSCBE, the potential long-term consequences of prolonged exposures are not known. Our current study, “INvestigating the neurologic effects of Training Associated Blast (I-TAB)”, is an objective evaluation of the clinical and physiologic responses to RSCBE. In order to improve training protocols and to develop countermeasures aimed to mitigate the consequences of repeated exposures to military occupational blast, we have collected blood before and after the heavy weapons training period and analyzed the samples by proteomics. Based on the unique physical properties of explosive blast, we focused on biomarkers that are indicators of vascular and neuronal injury and whether RSCBE can trigger an autoimmune response. These biomarkers, including circulating autoantibodies can be indicators of long-term effects of RSCBE that can have significant implications for the mental health of service members, the military health care system, and importantly for force readiness. *The objective of our study and the long-term goal of I-TAB is to optimize training protocols and to develop countermeasures to mitigate potential consequences of RSCBE in order to maintain and improve force readiness.*

Methods: The subjects for the I-TAB study were service members undergoing heavy weapons training (HWT). The I-TAB protocol was approved by the Institutional Review Boards of the Uniformed Services University of the Health Sciences and Naval Medical Center San Diego. After subjects provided informed consent, clinical and TBI histories were taken, blast overpressure gauges were issued, and subjects were evaluated for neurocognitive, neurobehavioral, neuromotor, autonomic nervous system, vision, and hearing measures and blood samples were collected for proteomics/biomarker analyses. Time points for testing were baseline [2 weeks prior to heavy weapons training (HWT)], and at 6 hrs, 24 hrs, 72 hrs, 2 weeks, and 3 months after HWT. Blood samples were collected from Group I (Students n=6) and Group II (Instructors n=10). Sera were isolated, snap frozen, and shipped frozen for proteomics analysis using the reverse phase protein microarray (RPPM) platform [3]. We evaluated the sera for levels of the following markers: ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), a neuron damage marker and glial fibrillary acidic protein (GFAP), an astroglia damage marker; neuronal acetylcholine receptor subunit alpha-7 (CHRNA7), a putative marker of altered cholinergic transmission; integral membrane proteins Claudin-5 (CLDN5) and Occludin (OCLN), components of tight junctions between endothelial cells their elevated serum levels indicate vascular damage/leakage, blood-brain barrier dysfunction. Serum samples were subjected to 8-fold dilution using a Janus liquid handling robot and diluted samples were printed in triplicate on Avid slides using an Aushon 2470 microarrayer [4]. Slides were incubated with primary antibodies specific to UCH-L1, GFAP, CHRNA7, CLDN5, and OCLN. All primary antibodies were quality tested for specificity prior to their use in RPPM assay, as described previously. Slides were washed and incubated with secondary antibodies conjugated with near infrared dye (excitation wavelength 795 nm and emission wavelength of 805 nm). After washing and drying, the slides were scanned using an Innopsys InnoScan 710IRAL Microarray Scanner. Individual spot intensities were quantified and data analyzed as follows: 1) two-way ANOVAs were performed for each of the 5 markers (UCH-L1, GFAP, CHRNA7, CLDN5 and OCLN) to determine the main effects in *Group*, *Time*, and any Interactions; 2) ANOVAs of each marker at each time point were performed to determine the main effect in *Group*; 3) ANCOVAs were performed at each time point to identify the main effects in *Group* accounting for baseline differences; 4) and independent t-tests were performed at each time point to compare the two *Groups* (Students vs. Instructors). For autoantibody screening, a protein microarray platform was used [5]. Protein microarrays representing the human proteome were screened for autoantibodies with the sera obtained before HWT and 3 months after the training from a subset of students.

Results: 1) Serum levels of all the measured biomarkers were elevated following HWT compared to the baseline levels; 2) Serum biomarker levels were significantly higher in Instructors than in Students probably reflecting their overall higher level of exposures; 3) the effect of HWT on the serum levels of these protein biomarkers lasted for at least 3 month 4) and all but OCLN showed the highest serum levels at the 3 month time point -long after the end of the training period for Students; 5) autoantibody titers of UCH-L1, GFAP, CHRNA7, CLDN5 and OCLN were significantly elevated in the Student cohort at 3 month after HWT, indicating an autoimmune response to the vascular

and neuroglia injury.

Conclusions: Our pilot study indicates that: a) HWT involving RSCBE in its current configuration may result in vascular and neuroglia damage as reflected in the elevated serum levels of the relevant protein biomarkers; b) the damage appears to last for at least 3 months following exposures which may suggest that the initial exposures can initiate a long-lasting/chronic pathobiological response that keeps attacking the vasculature, neurons and glia long after cessation of HWT and RSCBE; c) the presence of autoantibodies against vascular and neuroglia specific proteins may be part of this chronic pathobiological process. Analyzing sera collected at later post-exposure time points, 6 and 12 months in a larger cohort will help to better understand the potential long-term effects of RSCBE as well as will allow us to elucidate the potential mechanisms of direct and indirect responses to RSCBE in service members undergoing heavy weapons training. *These studies will help to assess current safety protocols, develop countermeasures aimed to safeguard and improve short- and long -term force readiness.*

References:

1. Nakagawa, A., et al., *Mechanisms of primary blast-induced traumatic brain injury: insights from shock-wave research.* J Neurotrauma, 2011. **28**(6): p. 1101-19.
2. Agoston, D., et al., *Military Blast Injury and Chronic Neurodegeneration: Research Presentations from the 2015 International State-of-the-Science Meeting.* J Neurotrauma, 2017. **34**(S1): p. S6-s17.
3. Agoston, D.V., et al., *Proteomic biomarkers for blast neurotrauma: targeting cerebral edema, inflammation, and neuronal death cascades.* J Neurotrauma, 2009. **26**(6): p. 901-11.
4. Kamnaksh, A., et al., *Factors affecting blast traumatic brain injury.* J Neurotrauma, 2011. **28**(10): p. 2145-53.
5. Ayoglu, B., J.M. Schwenk, and P. Nilsson, *Antigen arrays for profiling autoantibody repertoires.* Bioanalysis, 2016. **8**(10): p. 1105-26.

Investigation of Blast Effects on Wound Infection development and Antibiotic Disposition

Vlado Antonic, PhD¹, V. Sujith Sajja, PhD², Jason Sousa, PhD³, MAJ Ken Nguyen, PhD³, Yonas Alamneh, MS¹, Brittany Garry¹, Maria Medina-Rojas MS¹, Donna Wilder², Chau Vuong MS³, Brittney Potter MS³, Daniel Zurawski, PhD¹, MAJ Jonathan Shearer, DVM, PhD¹, LTC Chad Black, DVM, PhD³, MAJ Samandra Demons, PhD¹, Joseph Long, PhD², LTC Stuart Tyner, PhD¹

- 1- Wound Infections Department, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD, 20910-7500, USA
- 2- Blast Induced Neurotrauma Branch, Center for Military Psychiatry and Neurosciences, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD, 20910-7500, USA
- 3- Experimental Therapeutics Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD, 20910-7500, USA

Objective:

One of the key characteristics of the current conflict is that the majority of wounded soldiers suffer injuries as a result of blast exposure. Explosion-related injuries are complex in nature, characterized by large tissue defects and deep embedding of bacterial contaminants within soft tissues [1, 2]. Furthermore, extremity injuries accounted for 65% of service member wounds in Operation Iraqi Freedom and Operation Enduring Freedom [3]. Improvements in protective equipment (mainly body armor and helmets) resulted in a shift of the anatomical location of the wounds to the extremities and an increase in survival. However, the increase in initial survival and the penetrating nature of injuries result in delayed pathologies and morbidities. Wound infections occur in approximately 25% of wounded soldiers. These wound infections present an enormous burden on soldiers, their families and the military healthcare system as a whole and can result in delayed return to duty time, in non-healing wounds, amputations and in the most severe cases sepsis and death. There is very limited data available on the effects of blast exposure on wound healing/infection development. This significantly hampers our ability to care for blast-exposed wounded service members. Our lack of understanding of these wounds and wound infections results in less-than-optimal care for blast related injuries. This has been identified as important readiness gap by the DoD. Despite the lack of research on the effect of blast on wound healing and infection, recent epidemiologic studies on military populations show a significant rise in the incidence of fungal infections in these wounds. This demonstrates that these effects are real and grant further studying [4, 5]. We leveraged WRAIR's unique blast, infection, and pharmacology research capabilities to address this knowledge gap in the pathophysiology of blast-related wound infections, namely: 1) long term effects of Blast Overpressure (BOP) on the immune system; 2) BOP effects on infection development; and 3) BOP effects on antibiotic pharmacokinetics and dynamics. We performed a series of three experiments to gain a better understanding of these effects.

Material/Methods:

All experiments are performed in male BALB/c mice. Cyclophosphamide (CP, immunosuppressing agent) pre-treatment at days - 4 and -1 was given to positive controls. An advanced blast simulator (ABS®, ORA Inc., Fredericksburg, VA) located at WRAIR with a 0.5 ft long compression chamber that is separated from a 21 ft long transition/expansion test section by rupturable VALMEX® membranes (Mehler technologies, VA) was used for blast exposure. The anesthetized mice are secured in a transverse side-on orientation in the test section and were exposed to a single 19psi BOP using the high-fidelity ABS that mimics “free-field” blast.

Experiment 1. Long term effects of blast overpressure on blood cell populations. Following blast exposure on day 0 (n=80) and CP treatment (n=80, non-blast exposed), animals were euthanized at days 1, 2, 3, 4, 5, 6, 7, and 14, blood was collected using EDTA tubes, and blood cell numbers were determined using HemaVet 950FS.

Experiment 2. Effect of blast overpressure on infection. The animals (n=60) were subdivided to receive CP, blast or Sham. Each group was subdivided to receive an incisional wound or an infected incisional wound. Infection was established using bioluminescent *A. banumantii* 5075 in a dose of 5×10^4 CFU/wound. Animals were followed for 15 days. At days 1, 3, 5, 7, 9, 11, 13 and 15 we collected photographs of the wounds and determined rates of wound healing. On the same days, we determined bacterial burden *in situ* using IVIS *in vivo* imaging system.

Experiment 3. Effect of blast overpressure on antibiotic pharmacokinetics. A total of 160 animals were divided to sham and blast. At 1h post-exposure, all the animals received an i.v. injection of cefazolin. Animals were euthanized at 3 min, 10 min, 15 min, 30 min, 1h, 3h, 6h or 10h after the injection. Plasma and liver were analyzed for concentration of cefazolin using mass-spectrometry.

Results:

Experiment 1. We observed significant a decrease in the number of immune cells in the blood of animals exposed to blast when compared to sham controls. The effects of blast were comparable to CP treatment Figure 1.

Experiment 2. We observed trend increases in the number of bacteria in wounds of blast exposed animals when compared to Sham at early time points. Blast exposure did not result in delay in wound healing (Figure 2).

Experiment 3. We observed increases in the concentration of cefazolin in the plasma and liver of blast exposed animals at later time points and increases in the elimination of half-life (Figure 3).

Conclusion:

Our results suggest that blast induces a wide spectrum of immunological, physiological and pharmacokinetic effects that may cumulatively promote infection development and hamper antibiotic therapeutic efficacy for combat wounds.

Figure 1. Single blast exposure results in decrease in number of circulating immune cells

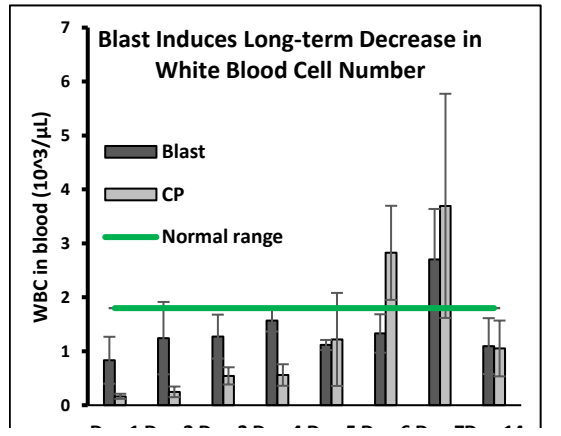


Figure 1. BOP contributes to increased number of bacteria in wound bed.

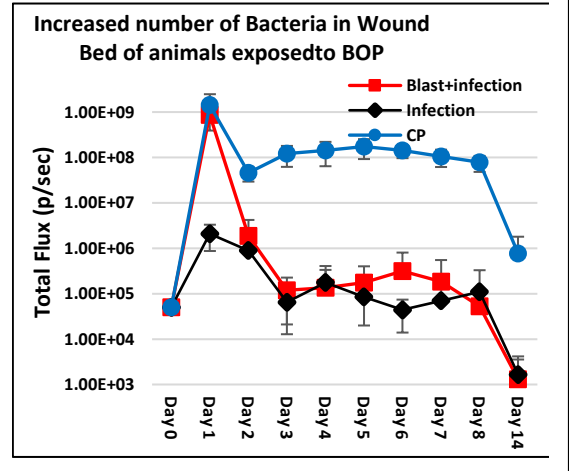
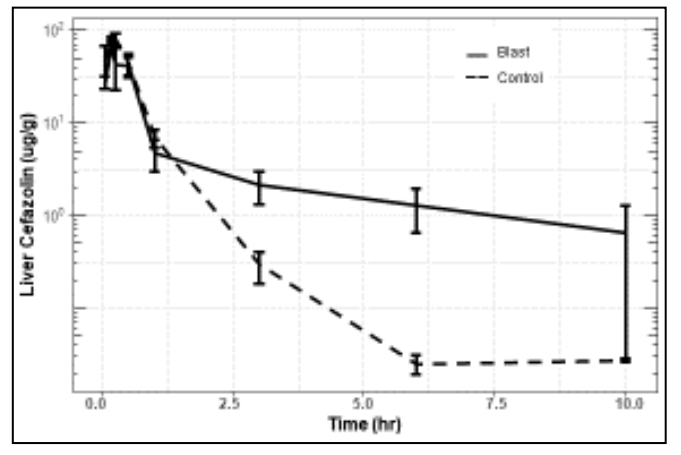


Figure 3. BOP results in change of cefazolin PK. Change in concentration of cefazolin in liver.



REFERENCES:

- Melvin, J.S., et al., *Open tibial shaft fractures: I. Evaluation and initial wound management.* J Am Acad Orthop Surg, 2010. **18**(1): p. 10-9.
- Rozen, P.a.D., I., *Wound Ballistic and Tissue Damage, in Armed Conflict Injuries to the Extremities,* A.a.S. Lerner, M., Editor. 2011, Springer Heidelberg Dordrecht. p. 21-33.
- Murray, C.K., *Infectious disease complications of combat-related injuries.* Crit Care Med, 2008. **36**(7 Suppl): p. S358-64.
- Tribble, D.R. and C.J. Rodriguez, *Combat-Related Invasive Fungal Wound Infections.* Curr Fungal Infect Rep, 2014. **8**(4): p. 277-286.
- Heaton, S.M., et al., *Histopathological techniques for the diagnosis of combat-related invasive fungal wound infections.* BMC Clin Pathol, 2016. **16**: p. 11.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation/publication.

Blast exposure leads to accelerated aging in rat brain

Peethambaran Arun, Franco Rossetti, Donna M. Wilder, Sujith Sajja, Steve VanAlbert, Ying Wang, Irene D. Gist, Joseph B. Long

Blast-Induced Neurotrauma Branch, Center for Military Psychiatry and Neuroscience,
Walter Reed Army Institute of Research, Silver Spring, MD 20910

Blast-induced traumatic brain injury (bTBI) is one of the major causes of persistent disabilities in Service Members, and a history of bTBI has been identified as a primary risk factor for developing age-associated neurodegenerative diseases. Initial clinical observations using diffusion tensor imaging to evaluate military victims exposed to blast revealed that blast exposure causes a rapid age-related loss of white matter integrity in the brain. In the present study, using an advanced blast simulator (ABS) we have tested the effect of single and tightly coupled repeated blast exposures on aging of the rat brain. Anesthetized rats were exposed to either a single or 2 closely coupled blasts (19 psi peak total pressure, 4-5 msec duration) in the ABS. Rats were euthanized and brains were collected at 24 hr, 1 month and 1 year post-blast to determine the changes in molecular and neuropathological markers of aging. Brain sections were prepared with senescence marker stain which measures senescence-associated- β -galactosidase activity in the cells. Real-time quantitative RT-PCR (qRT-PCR) was carried out to determine the differential expression of other protein markers of aging. Single and closely coupled repeated blast exposures resulted in significantly increased senescence marker staining in several neuroanatomical structures, including the cortex, hippocampus, auditory cortex, optical layer of the superior colliculus, thalamus and geniculate. The increases in senescence-associated- β -galactosidase activity were more pronounced at 1 month than at 24hr or 1 year post-blast and were also greater after repeated than after single blast exposures. In addition, qRT-PCR analysis of brain homogenates indicated decreased mRNA levels of senescence marker protein 30 (SMP30) and increased mRNA levels of p21 (cyclin dependent kinase inhibitor 1A, CDKN1A). These findings are consistent with the earlier observations in veterans exposed to military blast and reveal that exposure to blast triggers accelerated aging of cells in the brain. It is noteworthy that the rapidly aging cells were not uniformly observed in the brain after blast exposure and were restricted to specific brain regions. The increased senescence observed in some of these affected brain structures may be implicated in several long-term sequelae after exposure to blast, including memory disruptions and impairments in auditory and ocular functions.

Computational model development of blast-induced pressure wave impact on a surrogate head model

Rohan Banton¹, Thuvan Piehler¹, Nicole Zander¹, Richard Benjamin¹, Oren Petel², Josh Duckworth³

¹ U.S. Army Research Laboratory, Aberdeen Proving Ground, Maryland

² Carleton University, Ottawa, Ontario, Canada

³ The Uniformed Services University of the Health Sciences, USA

Blast related traumatic brain injuries experienced by the warfighter continue to be of major concern. Specifically, there is an urgent need to unravel the mechanisms leading to mild traumatic brain injuries from blast. In recent years, numerical modeling and simulation have emerged as viable tools for assessing blast wave interaction with the head. To aid in this effort, the current research has adopted a systematic numerical approach to investigate the intracranial pressure loading on a surrogate head impacted by pressure waves from a RDX explosive. A simplified finite element head model of a skull/brain assembly was first constructed and then inserted in an Eulerian air domain in a shock physics code. The surrogate head was subjected to blast wave impact of 300 kPa peak overpressure generated from the RDX charge placed at 180 mm standoff distance in the anterior direction. The qualitative comparisons of the simulated wave propagation and flow about the surrogate head were in good agreement with high speed shadow graph images generated from experiments. The calculations also agreed with pressure data obtained from blast experiments performed on a physical surrogate head model. In particular, at a depth of 5 mm below the kocher point the pressure data and calculated results revealed peak overpressure values of 95 and 85 kPa respectively. Pressure history calculated results also showed oscillatory behavior with strong compressive and tensile peak values. These results were also in agreement with experimental pressure probe data. This oscillatory behavior between compressive and tensile forces could give rise to tearing and shearing in brain tissue. To further examine the shear stress distribution within the surrogate brain we also imposed a time-dependent shear relaxation modulus with our model. Further results from this work will be shared at the upcoming IFBIC-2019 conference.

Persistence of Concussion-Related Symptomology: An Examination of Major Blast Exposure and Chronic, Low-Level Overpressure Exposure Using Post-Deployment Health Reassessment Records

Jennifer N. Belding^{1,2}, Shannon Fitzmaurice^{1,2}, Hannah Kwiatkowski^{1,2}, Robyn Englert^{1,2}, Jourdan Jackson^{1,2}, Cynthia J. Thomsen², and LT Uade Olaghere da Silva²

¹Leidos, San Diego, CA

²Naval Health Research Center, San Diego, CA

Introduction: Concussions and more severe neurological injuries, most often caused by improvised explosive devices have contributed substantially to service member morbidity and mortality in recent conflicts. However, little research has examined factors that place service members at increased risk for sustaining a concussion following a major blast. Preliminary evidence suggests that service members working in occupations marked by repeated exposure to low-level blasts such as those associated with firing weapons—commonly referred to as overpressure exposure—are particularly susceptible to developing a concussion following a major blast during deployment. However, few studies have attempted to characterize differences in concussion severity and recovery as a function of either service members' occupational overpressure exposure or major blast exposure. In this study, we examined the independent and combined neurological impact of acute, high-level (i.e., blast) and chronic, low-level overpressure exposure over 6 months following return from deployment.

Method: We matched Post-Deployment Health Assessment (PDHA) records with Post-Deployment Health Reassessment (PDHRA) records for enlisted active duty Marines who reported exposure to a qualifying event that may cause traumatic brain injury (TBI) on the 2003 or 2008 versions of the PDHA ($N = 8,106$). Based on their Military Occupational Specialty, we categorized Marines into groups with relatively high versus low risk for occupational overpressure exposure on the basis of judgments by 10 Marine Corps experts. We used a mixed model analysis of variance to examine the number of symptoms Marines reported experiencing during deployment as a function of probable TBI (yes/no), major blast exposure (yes/no), occupational risk (high/low), type of symptom (neurological/musculoskeletal/immunological), and time of measurement (PDHA/PDHRA).

Results: In general, both those with blast exposure and those with low-level overpressure exposure reported significantly more neurological symptoms, as did concussed Marines. Neurological symptoms improved over time. However, the recovery process was slower for those with blast (vs. physical impact to the head) exposure, and neurological symptoms did not fully dissipate in concussed Marines by 6 months postdeployment. Additionally, while blast induced (vs. impact induced) concussions were associated with significantly more neurological symptoms at return from deployment, rate of recovery was statistically equivalent for both mechanisms of injury. Recovery from concussion was also slower for Marines with (vs. without) chronic, low-level overpressure exposure, and the neurological impact of overpressure exposure did not fully dissipate by 6 months postdeployment.

Conclusion: These findings suggest two key points: (1) While blast-induced concussions are associated with significantly more neurological symptoms than impact-induced concussions, these symptoms dissipate at equal rates. As a result, the neurological impact of blast-induced concussions exceeds that of impact-induced TBIs both at return from deployment and 6 months later. (2) Chronic, low-level overpressure exposure is associated with significant neurological impact. Marines in high (vs. low) risk occupations reported elevated levels of neurological symptoms even in the absence of concussion. Furthermore, Marines working in high (vs. low) risk occupations were more susceptible to concussion following acute blast and recovered more slowly. This suggests that Marines working in high- (vs. low-) risk occupations may be more susceptible to post-concussive syndrome. Suggestions for policy and future research will be discussed.

Disclaimer: I am a military service member or employee of the U.S. Government. This work was prepared as part of my official duties. Title 17, U.S.C. §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C. §101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

This work was supported by U.S. Navy Bureau of Medicine and Surgery under work unit no. N1518. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

The study protocol was approved by the Naval Health Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Research data were derived from an approved Naval Health Research Center Institutional Review Board protocol number NHRC.2016.0024.

Tutorial: Leveraging Epidemiological Research to Identify Service Members at Risk for Deleterious Overpressure-Induced Outcomes

Jennifer N. Belding^{1,2}, Shannon Fitzmaurice^{1,2}, Hannah Kwiatkowski^{1,2}, Robyn Englert^{1,2}, Jourdan Jackson^{1,2}, Cynthia J. Thomsen², and LT Uade Olaghere da Silva²

¹Leidos, San Diego, CA

²Naval Health Research Center, San Diego, CA

Injuries resulting from overpressure exposure have increased dramatically in recent military conflicts. However, not all service members exposed to overpressure have clinically diagnosable injuries. This tutorial will achieve two objectives: (1) present a theoretical framework that articulates the relationship between overpressure exposure and injury, along with several findings from epidemiological investigations of overpressure exposure; and (2) summarize populations who are at risk of overpressure-induced injury for assessment in future research.

We will first present our theoretical perspective and explain that overpressure exposure can push people along a continuum from noninjury to injury. After defining two forms of overpressure exposure (i.e., acute, high-level blast exposure and chronic, low-level overpressure exposure), we will discuss how these exposures may both independently and jointly represent a threat to force wellness even in the absence of clinically diagnosable injury. In particular, we will argue that chronic, low-level overpressure exposure moves people along this continuum, and thus, increases susceptibility to injury following acute blast. In support of this theoretical perspective, we will present findings from several ongoing large-scale epidemiological investigations of the Post-Deployment Health Assessment (PDHA; $N = 181,423$) and Post-Deployment Health Reassessment ($N = 102,075$), in which we assessed concussion screens and self-reported symptomology as a function of acute blast exposure and chronic low-level overpressure exposure using Military Occupational Specialty (MOS) as a proxy. We will demonstrate that acute, high-level blast exposure is associated with probable concussion during deployment, but Marines who work in occupations with more (vs. less) repetitive low-level overpressure exposure are 1.45 times more likely to screen positive for concussion following blast. Additionally, concussed Marines who work in these high-risk occupations reported experiencing significantly more neurological symptoms upon return from deployment than those with less repetitive overpressure exposure, suggesting that chronic, low-level overpressure exposure can be harmful, even in the absence of an acute blast. Examination of the persistence of these self-reported symptoms approximately 6 months later showed that Marines working in high-risk occupations reported significantly more neurological symptoms than those in low-risk occupations, even in the absence of a concussion, and Marines in these high-risk occupations recovered more slowly from concussion than those in low-risk occupations. Furthermore, Marines in high-risk occupations without acute blast reported as many neurological symptoms as those in low-risk occupations with acute blast, suggesting that both forms of overpressure can be associated with indications of injury, even if that injury does not reach the threshold of a clinical diagnosis.

Next, we will briefly summarize a forthcoming technical report that provides descriptive data on the number of acute blast exposures, positive concussion screens (for both blast-induced concussions and impact-induced concussions), and self-reported symptomology at the level of broad MOS categories for active duty enlisted Marines who completed the PDHA between 2003 and 2012. For example, we will show that more than 15% of deployed Marines in the following occupational categories reported exposure to acute blast during deployment: Combat Camera (26.09%), Ammunition and Explosive Ordnance Disposal (20.70%), Infantry (20.48%), Military Police (18.08%), and Engineering, Construction, Facilities and Equipment (17.20%). Taken together, these data will provide concrete suggestions for populations who are at risk for deleterious overpressure-induced injuries that should be studied, assessed, and treated. Recommendations for future research and policy will also be discussed.

Disclaimer

I am a military service member (or employee of the U.S. Government). This work was prepared as part of my official duties. Title 17, U.S.C. §105 provides the “Copyright protection under this title is not available for any work of the United States Government.” Title 17, U.S.C. §101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person’s official duties.

This research was supported by the U.S. Navy Bureau of Medicine and Surgery (BUMED) under work unit no. N1518. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of the Army, Department of the Air Force, Department of Veterans Affairs, Department of Defense, or the U.S. Government. Approved for public release; distribution unlimited.

Human subjects participated in this study after giving their free and informed consent. This research has been conducted in compliance with all applicable federal regulations governing the protection of human subjects in research (NHRC.2016.0024).

Force Preservation Through Individual Blast Exposure Surveillance

D. Borkholder¹, S. Featherman², and K. Sims³

^{1 2 3} BlackBox Biometrics, Inc.

Background: Blast overpressure (BOP) is a serious environmental hazard unique to military operations. While there are some rudimentary standards in place to protect servicemembers, no safety policy exists to monitor repeat exposure or look at its long-term effects. Current standards are based on likelihood of overt injury and don't account for the long-term morbidity associated with environmental exposures. This lack of a clearly defined safety program incentivizes commanders to avoid exposure monitoring and only act if the injury is acutely severe enough to incapacitate a servicemember. Decades of data suggest that repetitive exposure to the rapid pressure changes associated with blasts decreases the threshold for future injury. Further, it's well documented that routine training firing current munitions exposes servicemembers to levels of BOP that are unsafe; it would be unethical to knowingly subject human subjects to these overpressures for purposes of a prospective trial. Yet no record of exposures is kept, and no mitigation strategy exists to minimize exposure. It's imperative that the US Department of Defense and militaries around the world implement a safety program mirroring those used for radiation safety to ensure that proper steps are taken to mitigate hazards from BOP.

Methods: A review of environmental exposure programs provides a template for effective blast exposure surveillance, with four critical elements to protect the servicemember from the occupational hazard. At-risk personnel are equipped with sensors to provide objective and quantitative blast exposure data in both training and operations.

- (1) **TRIAGE** - Individuals exposed to dangerous levels of BOP are identified allowing for targeted medical assessment.
- (2) **TACTICS** - Real-time and post-training analysis drives refinement and reinforcement of the tactics, techniques, and procedures (TTPs) based on individual and unit exposures.
- (3) **TRACE** - Individual exposure history is correlated to emergent mild traumatic brain injury (mTBI) symptoms.
- (4) **TRACK** - Personnel are enrolled in longitudinal health surveillance to ensure they are tracked over time for progressive disease and provided the appropriate care.

Results: A proven environmental blast sensor technology (NSN 6665-01-632-3508) exists to monitor individual dosing of blast exposure in both training and operations. The three sensors worn on the helmet, chest, and shoulder accurately document and quantify individualized blast exposure. This individual monitoring provides data that allows unit medics and commanders to keep the number of exposures as low as reasonably achievable. Reduced exposures coupled with targeted medical assessment based on exposure history supports maintenance of units at peak performance and operational readiness, aiding force preservation. The collected individualized exposure history and associated acute symptoms and long-term injury informs injury algorithms, establishes safe dose limits, and supports advances in exposure mitigation approaches.

Conclusions: Formal blast exposure surveillance for military personnel is critical to understand the dose-response relationship between BOP and acute and chronic injury. It's the only way to gather data on enough individuals to unravel the complex relationship between exposure history and progressive neurodegenerative disease. The required environmental blast sensors are available, proven, and ready to meet this critical capability gap.

Assessment of chronic mTBI status in veterans

Elizabeth B. Brokaw¹, Michael S. Fine¹, Anthony Santago II¹, Stacey Harris-Carriman²

¹The MITRE Corporation, McLean, VA

²New Mexico VA Health Care System, Albuquerque, NM

Background: Approximately 308,853 military personnel were diagnosed with mild traumatic brain injury (mTBI) between 2000 and 2017 (Defense and Veterans Brain Injury Center, 2017). The VA has screened all Veterans from Operation Enduring Freedom and Operation Iraqi Freedom for possible mTBI since 2007. However, clear identification of mTBI is difficult to attain due to the diffuse nature of injury and the heterogeneity of clinical presentation. Using conventional assessment questionnaires and medical imaging techniques, clinicians often have difficulty clearly identifying mild brain injury (Shenton et al., 2012). Clear identification of mTBI is critical to informing clinicians' allocation of care decisions and would result in improved health outcomes for Veterans.

Methods: This cross-sectional study is investigating the inclusion of a 3-minute visuomotor tracking (VMT) test for mTBI screening at the VA. During the test, individuals are asked to modulate their grip force, as measured by a hand dynamometer, to match a variable target force that is displayed visually on an iPad. Previous research suggests that test performance is sensitive to subacute mTBI (Fine et al., 2016) and repeated subconcussive head impacts (Brokaw et al., 2017). Individuals referred for a Second-Level Polytrauma Assessment at the Albuquerque VA were recruited. Study participants completed the Polytrauma Assessment, which includes the Neurobehavioral Symptom Inventory (NSI-22), as well as conventional assessments such as Trail Making B (TMB) and the Standardized Assessment of Concussion (SAC), in addition to the VMT test. Study participants also completed the PTSD Checklist and provided information about the suspected injury mechanism (e.g., blast or impact) to compare VMT performance (as quantified by root-mean-square error, lag, and a model of feedback control, previously published [Fine, 2016]) to these secondary factors.

Results: All study participants were more than 6 months post suspected brain injury when they were referred for the Polytrauma Assessment at the Albuquerque VA. Participants reported disruptive levels of mTBI symptoms on the NSI-22, including headaches, depression, sleep difficulty, and hearing difficulty. Participants generally presented with unimpaired scores on TMB and SAC, but performance on the VMT test was higher than previously seen in non-Veteran controls. PTSD, as indicated by a score greater than 36 on the PTSD checklist, was common.

Conclusions: mTBI is associated with significant medical and quality of life consequences, including an increased risk of PTSD and other health problems that continue for many months after injury, affecting home life (Hoge et al., 2008). The ability to rapidly, quantitatively, and objectively assess mTBI could be beneficial for determining interventions for Veterans. Preliminary results indicate that a VMT test could potentially provide increased resolution of mTBI relative to conventional assessment techniques. Additional research would be needed to separate out the effects of PTSD and injury mechanism (i.e., blast, impact).

© 2018 The MITRE Corporation. ALL RIGHTS RESERVED. Approved for Public Release; Distribution Unlimited. Case Number 18-0629.

Portions of this report were recently presented to the U.S. DoD Military Health System. These results are presented again here for an international community of interest.

This material is the result of work supported with resources and the use of facilities at the New Mexico VA Health Care System, Albuquerque, NM.

References

- [1] Brokaw EB, Fine MS, Kindschi KE, Santago II AC, Lum PS, Higgins M (2017) Cross-sectional Evaluation of Visuomotor Tracking Performance Following Subconcussive Head Impacts. *Technology and Health Care*.
- [2] Defense and Veterans Brain Injury Center (2017) *DoD Numbers for Traumatic Brain Injury 2000-2017*.
- [3] Fine MS, Lum P, Brokaw E, Caywood M, Metzger A, Libin A, Dromerick AW et al. (2016) Dynamic Motor Tracking is Sensitive to Subacute mTBI. *Experimental Brain Research* **234**:3173–3184. <https://doi.org/10.1007/s00221-016-4714-5>
- [4] Shenton ME, Hamoda HM, Schneiderman JS, Boulx S, Pasternak O, Rathi Y, Zafonte R et al. (2012) A Review of Magnetic Resonance Imaging and Diffusion Tensor Imaging Findings in Mild Traumatic Brain Injury. *Brain Imaging and Behavior*, **6**:137–192. <https://doi.org/10.1007/s11682-012-9156-5.A>

Monitoring Occupational Exposures to Blast – What Have We Learned? What Are We Doing?

W. Carr^{1,2}

¹ Oak Ridge Institute for Science and Education

² Walter Reed Army Institute of Research

Background.

Traumatic brain injury is widely cited as the signature injury of recent military conflicts and explosive blast as the key threat, now and going forward. A targeted capability for assessment of this threat has been measurement and quantification of blast exposures with wearable sensor technologies. Operation Enduring Freedom deployment of blast sensor technologies did not yield the hoped-for level of insight from combat exposures but did, unexpectedly, quantify relatively large magnitude exposures in training. This presentation reviews recent findings from focused studies of exposure to blast in training, ongoing research, and potential for blast surveillance.

Methods.

Studies reviewed are human subjects designs, including prospective field-based observation, longitudinal follow up, self-report survey, and archival studies of clinical records.

Results.

Prospective observational studies during routine training with explosives and weapons afford opportunity to quantify overpressure exposures with sensors worn by personnel. A key finding has been sensor-based overpressure measurements that are not in accordance with a priori algorithm predictions based on charge and distance; both underestimates and overestimates have been observed. Also, measurement of overpressure “dose” is complicated by repeated exposure factors and unmeasured chronic exposure.

Effects on personnel have been observed in symptomology, behavioral performance, hearing thresholds, diagnosed tinnitus, and subclinical biomarker changes in biofluid and neuroimaging assays.

Discussion.

Associations between exposure to blast in training and meaningful negative outcomes are not clear enough for specific intervention and are still the subject of active research. However, on prospect of risk, training units have developed and implemented local changes to reduce overpressure exposures, while preserving training effectiveness.

What is clear is that the absence of routine quantification of exposures is a gap in advancing understanding of dose-response relationships and individual differences. Congressional committees have urged sensor-based blast surveillance that can be leveraged as research findings emerge. Consideration of such programs will be discussed.

NOTE.

This material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.

This work is an integration of DoD and NIH institutional resources and multiple DoD funded work units, with key sponsorship from USAMRMC, OTSG, DVBIC, BUMED, and DARPA. Human subject research protocols have completed DoD requirements for scientific and IRB review of protocols. There are no conflicts of interest to report. Multiple prior publications and presentations will be directly referenced.

This work is an update on a parallel presentation made at the 2018 Special Operations Medicine Scientific Assembly (SOMSA), by the following contributors:

MAJ Walter Carr, Research Psychologist, Military Psychiatry, Walter Reed Army Institute of Research, Silver Spring, MD;

MAJ Charmaine M. Tate, Medical Officer, New Zealand Defence Force, Auckland, New Zealand;

Dr. Shawn G. Rhind, Defence Scientist, Defence Research and Development Canada, Toronto Research Centre, Toronto, Ottawa, Canada;

Dr. Catherine Tenn, Defence Scientist, Defence Research and Development Canada, Suffield Research Centre, Medicine Hat, Alberta, Canada;

Dr. Jason M. Bailie, Senior Clinical Research Director and Neuropsychologist, Defense and Veterans Brain Injury Center, Camp Pendleton, CA;

Dr. Jessica Gill, Lasker Clinical Research Scholar, National Institutes of Health, National Institute of Nursing Research, Bethesda, MD;

Katie Edwards, RN, Pre-doctoral Fellow, National Institutes of Health, National Institute of Nursing Research, Bethesda, MD;

Dr. Fatemeh G. Haghighi, James J. Peters VA Medical Center, Associate Professor, Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY;

Dr. Natalya Weber, Health Science Officer, Department of Epidemiology, Preventive Medicine Branch, Walter Reed Army Institute of Research, Silver Spring, MD

Dr. Stephen Recchia, PhD, Lead Mechanical Engineer, Analysis and Evaluation Technology Division, U.S. Army Armament Research, Development and Engineering Center, Picatinny Arsenal, NJ;

COL Sidney R. Hinds II, DoD Brain Health Research Program Coordinator, Blast Injury Research Program Coordinating Office, Medical Advisor to the Principal Assistant for Research and Technology United States Army Medical Research and Materiel Command, Fort Detrick, MD;

Dr. Thuvan N. Piehler, Injury Prevention Program Deputy Program Manager, Military Operational Medicine Research Program, U.S. Army Medical Research and Materiel Command, Fort Detrick, MD;

Dr. Richard Shoge, Senior Scientist, PM - Injury Prevention and Reduction Portfolio, Military Operational Medicine Research Program, U.S. Army Medical Research and Materiel Command, Fort Detrick, MD;

Theresa Lattimore, Director, Army TBI Program, Rehabilitation & Reintegration Division, U.S. Army Office of the Surgeon General, Falls Church, VA;

Dr. Gary H Kamimori, Research Physiologist, Blast-Induced NeuroTrauma, Walter Reed Army Institute of Research, Silver Spring, MD;

Hippocampus-Cerebellum Axis Specific Transcriptomic Stratification to Discriminate Mild from Moderate TBI

Nabarun Chakraborty^{1,2}, Rasha Hammamieh², Aarti Gautam², Stacy-Ann Miller^{2,3}, Michelle L. Conclin⁴, Marti Jett², Angus G. Scrimgeour⁴

¹ Geneva Foundation, US Army Center for Environmental Health Research, Fort Detrick, MD 21702

² Integrative Systems Biology, US Army Center for Environmental Health Research, Fort Detrick, MD 21702

³ ORISE, USACEHR, Fort Detrick, MD 21702

⁴ Military Nutrition Division, US Army Research Institute of Environmental Medicine, Natick, MA 01760

The variations of psycho- and physiological deficits caused by traumatic brain injury (TBI) correlate with the degree of brain injury. However, the objective stratification of TBI is yet elusive. A modified closed-head injury Marmarou model was used to induce differential closed-head injury models for mild TBI and moderate TBI, respectively. Figure 1 shows the assay design and experiment timeline. The skull was fitted with a helmet to permit a diffuse axonal injury. The frontal cortex was the focal point of injury, with the hippocampus (HC) and cerebellum (CB) susceptible to diffuse shock injury (secondary). The bodyweights of rats exposed to moderate TBI took longer time to increase in comparison to those exposed to mild TBI (Figure 2). The HC-CB axis coordinates visuomotor performance, which is known to be vulnerable to TBI. Rats exposed to moderate TBI demonstrated deficient visuomotor performance, spending significantly longer periods on the Barnes maze compared to rats exposed to mild TBI (Figure 3). Brain samples were collected 6 hours, 48 hours and 14 days post TBI. Two-color cDNA microarray was performed using RNA samples extracted from HC and CB tissues (sample size >8). Genes showing differential expression between mild and moderate TBI ($p < 0.05$ and fold change $> |1.5|$) were mined. Furthermore, genes showing temporal reduction (or increment) across the three time points were selected for functional analysis. The time resolved- and HC-CB specific transcriptomic analysis focused on genes that enabled discrimination of mild from moderate TBI at 14d post injury. Release of prostaglandin, an established marker of brain injury was suggested by both HC and CB genomic shifts post 14d post injury. The functional analysis revealed an active neurorepair mechanism in the HC in rats exposed to mild TBI. In contrast, moderate TBI caused delayed neurorepair and active cell death in the HC. The regional specific functions significantly enriched 14 days post TBI were mapped on the healing dynamics to conceptualize the temporal trend of repairing process undertaking by HC-CB axis (Figure 4). In conclusion, the graded brain injuries differentially implicated the HC-CB axis, despite the use of a helmet to minimize a focal injury. Time resolved functional dynamics informed the distinct consequences of mild vs. moderate TBI.

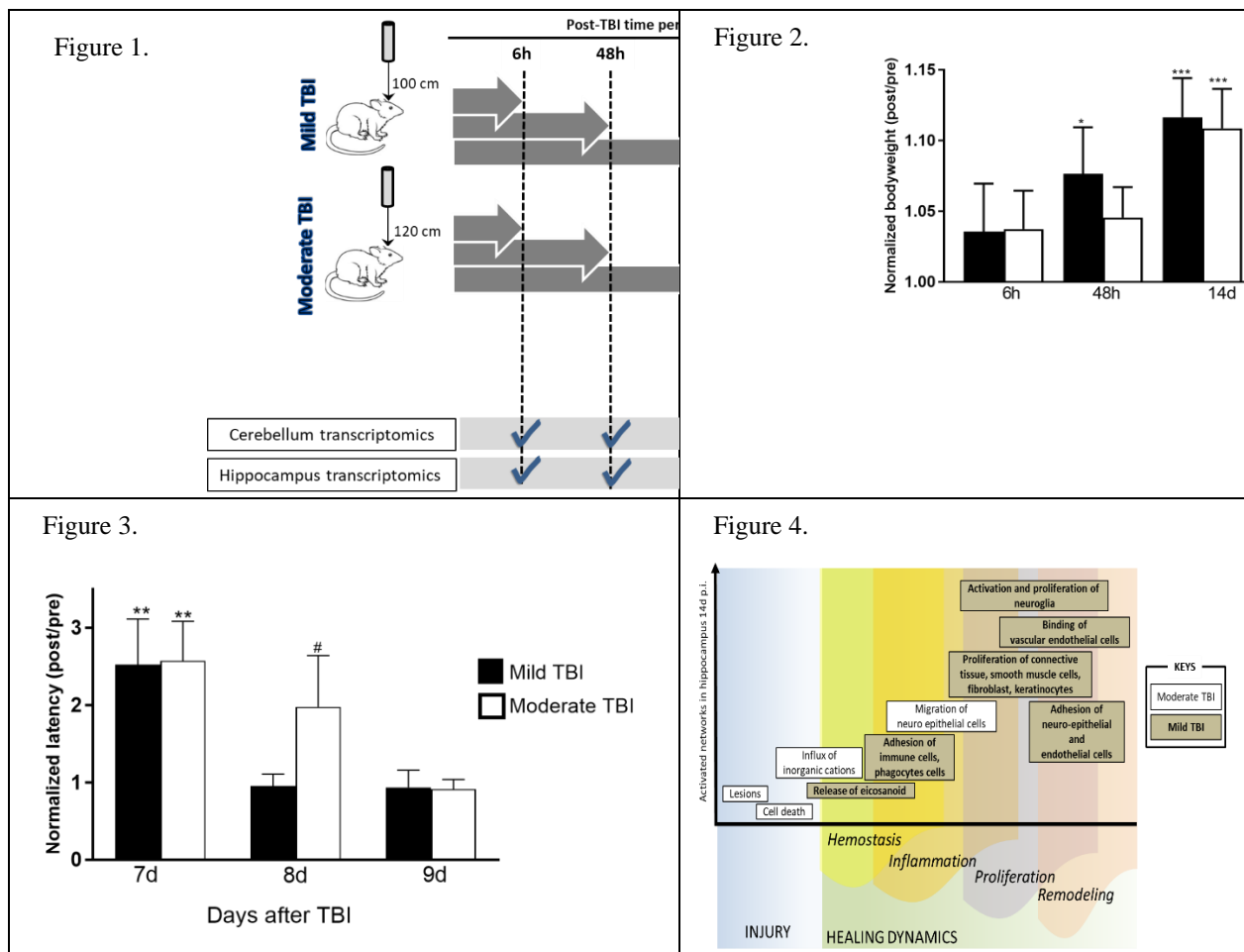


Figure 1. Study design. Rats equipped with a helmet were exposed to mild vs. moderate TBI (injury differed by the height of slug drop). Barnes maze tests were performed over 5 consecutive days pre-TBI and again over 5 consecutive days post-TBI (day 6-10 post-TBI). Post-TBI, rats were euthanized at 6h, 48h, 14d time points as marked in the diagram. Transcriptomics assays were performed post mortem at each time point.

Figure 2. Temporal shift of normalized bodyweight: Bodyweights were normalized by the average pre-TBI bodyweights ($N = 9-12$ rats). The bar plots indicate the average bodyweight normalized to the pre-TBI data \pm SEM. At 48h post-TBI, rats exposed to mild TBI were significantly heavier in body weight ($*p < 0.05$). Rats exposed to moderate TBI showed no significant gain in bodyweight at 48h post-TBI. At 14d rats exposed to both mild and moderate TBI were significantly heavier in body weight. $*p < 0.05$ and $***p < 0.001$.

Figure 3. Barnes maze result. Rats performed Barnes maze behavioral tests pre- and post-TBI for day 6-10 post-TBI ($N = 9-12$ rats). The bar plots indicate the average latencies normalized to the pre-TBI data \pm SEM. Results from the first three testing days are shown above. Latencies of subsequent days were at the baseline and thus excluded from figure. Day 1 latencies of both mild and moderate TBI were significantly elevated from the baseline. Day 2 latency in moderate TBI only, was slightly elevated ($p = 0.08$). 2-way ANOVA showed time was the most significant variable ($p = 0.002$), not severity of TBI nor their interactions: TBI \times Time.

Figure 5. The status of the hippocampal injury out to 14d post TBI. The functional networks significantly activated by either mild or moderate TBI were aligned with the different steps of the healing cascade.

Disclaimer:

Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.

Tutorial

Synergistic interaction of oxidative stress, blood-brain barrier breakage and neuroinflammation in the pathogenesis of blast TBI

N. Chandra ¹

¹Center for Injury Biomechanics, Materials, and Medicine, Department of Biomedical Engineering,
New Jersey Institute of Technology, Newark, NJ

CONTENT

In this tutorial, we will discuss the basic biochemical mechanisms that actively play a role in the acute and chronic progression of injury in blast induced traumatic brain injury. While the biomechanical mechanisms have not been clearly delineated, the loading effects at the tissue, molecular and cellular levels initiate a secondary injury cascade that is manifested as cognitive and behavioral deficits. Though there are numerous pathways identified in the degradation process, the three major mechanisms: oxidative stress, blood-brain barrier breakage, and neuroinflammation act both independently and interactively in a synergistic manner.

In this tutorial, we will first outline various causative mechanisms that have been identified to influence the TBI outcome. We will show how blast TBI is different in terms of spatial and temporal distribution compared to blunt or penetrating TBI. We will show the temporal profile of the three mechanisms and how each influences the other. We will show that low level repeated exposures can be understood from the effects of single-exposures in terms of blast intensity, number of repetitions and inter-exposure intervals.

REFERENCES

- M. Kuriakose, K.V. Rama Rao, D. Younger, N. Chandra. “*Temporal and Spatial Effects of Blast Overpressure on Blood-Brain Barrier Permeability in Traumatic Brain Injury.*” **Nature-Scientific Reports**, 8(1), 8681, (2018)
- K.V. Rama Rao, S. Iring, D. Younger, M. Kuriakose, M. Skotak, E. Alay, R. K. Gupta, N. Chandra, *A single primary blast-induced traumatic brain injury in rodent model causes cell-type dependent increase in NADPH oxidase isoforms in vulnerable brain regions*, **Journal of Neurotrauma**, , 35,1-14, (2018)
- D. Younger, M. Murugan, K.V. Rama Rao, L.J. Wu, N. Chandra, “*Microglia Receptors in Animal Models of Traumatic Brain Injury*”, **Molecular Neurobiology**, 1-27, (2018)
- M. T. Townsend, E. Alay, M. Skotak, N. Chandra, “*Effect of tissue material properties in blast loading: coupled experimentation and finite element simulation*”, **Annals of Biomedical Engineering**, ABME-D-18-0051, 1-14, (2018)
- E. Alay, M. Skotak, A. Misistia, N. Chandra, *Dynamic loads on human and animal surrogates at different locations in compressed-gas-driven shock tubes*, **Shock Waves**, 28, 1, 51-62, (2018)

Findings from the U.S. Military Health System Blast Injury Prevention Standards Recommendation (BIPSR) Process

Jeffrey B. Colombe¹, Anthony Santago II¹, Elizabeth Brokaw¹, Brian Colder¹, Rachel W. Spencer¹, Lisa Lalis¹, Raj Gupta², Michael Leggieri²

¹ The MITRE Corporation

² US Army Medical Research and Materiel Command

81% of injuries in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) were due to blasts [1], resulting from multiple physical effects of blasts, including, 1) blast pressure shockwave effects, 2) penetrating ballistic fragments, 3) accelerative loading and blunt force trauma, 4) toxic gas inhalation and dermal burns, and 5) contamination from chemical, biological, or radiological substances. The first of its kind, stakeholder-driven U.S. Military Health System (MHS) Blast Injury Prevention Standards Recommendation (BIPSR) Process seeks to identify existing knowledge to support development of MHS Blast Injury Prevention Standards.

An MHS Blast Injury Prevention Standard is a “biomedically-valid description of the physiologically- or biomechanically-based injury and performance response of a human to blast insults.” Standards may range from simple dose-response curves and injury thresholds that address single components of blast insults, such as peak force, to complex algorithms and computational models that address multiple components of blast insults, such as force-time history. Candidate standards include injury thresholds, human injury probability curves, and injury prediction tools needed to generate the information for informed trade-off and risk acceptance decisions by appropriate decision makers in the RDT&E, medical, and operational Stakeholder communities across the DoD Components. These standards support weapon system health hazard assessments, combat platform occupant survivability assessments, and protection system development and performance testing.

Designed to address the above requirement, the MHS BIPSR Process is the DoD’s first unbiased, inclusive, stakeholder-driven process designed to identify and assess the suitability and applicability of existing candidate standards and to recommend standards that meet DoD Stakeholder needs with a suitable level of validity, rigor, precision, and confidence. Fourteen BIPSR Process Blast Injury Types were identified by DoD Stakeholders (Figure 1) based on body systems that are vulnerable to blast injury, and organized in priority order of evaluation:

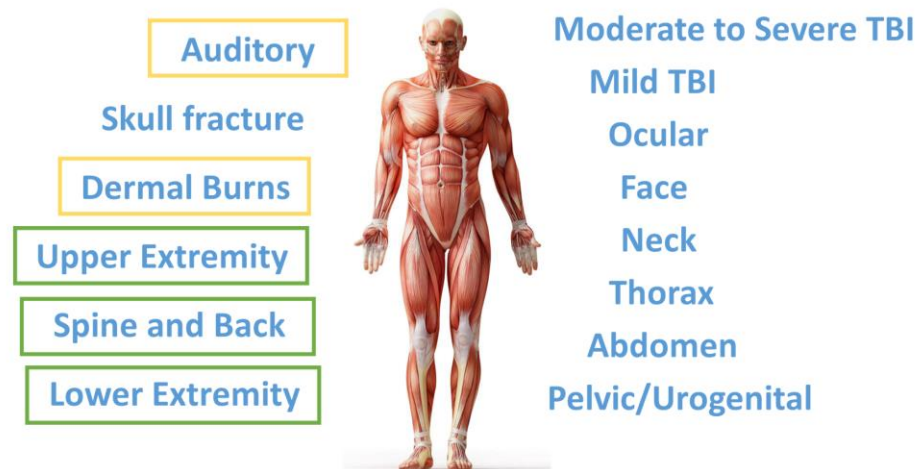


Figure 1. MHS BIPSR Process Blast Injury Types (completed, in progress)

The BIPSR Process has two major objectives: 1) identify existing biomedically-valid candidate standards for immediate use by the DoD; 2) inform the research community of knowledge gaps where no suitable candidate standards exist.

Methods

The MHS BIPSR Process involves a systematic review of biomedical literature on injury risk under conditions characteristic of blasts and interviews with subject matter experts, leading to a set of injury criteria that could be used as MHS Blast Injury Prevention Standards. Intended uses for standards were gathered from the DoD BIPSR Process Focused Stakeholder Committees and compared against currently available injury risk criteria and models, yielding science and technology (S&T) knowledge gaps that the Stakeholders recommended for targeted research.

Results and conclusions

A collection of injury risk criteria were found across the now-completed evaluations of the Lower Extremity, the Spine and Back, and the Upper Extremity Blast Injury Types, all relating to ballistic penetration and accelerative loading inclusive of blunt trauma. No injury risk criteria were found that were sufficient to recommend as MHS Blast Injury Prevention Standards. BIPSR Process Stakeholders recommended the following knowledge gaps across the above body systems to inform the DoD S&T research community:

- Test methods must more effectively replicate measured operational blast conditions
- Test subjects must span military age, sex, body size, and mounted/dismounted postures with appropriate statistical power
- Experimentally validated human surrogates, both physical and computational, are needed
- Scientific consensus is needed on sufficiency of injury risk criteria for use in MHS Blast Injury Prevention Standards
- Further study is required on vulnerability of soft tissues and substructures such as ligaments, on effects of multi-directional hazards on the risk of injury, and on effects of cumulative blast exposure on organs and tissues
- Research is needed to understand how blast injuries affect functional incapacitation, and the impact on operational readiness

Portions of this report were recently presented to the U.S. DoD Military Health System [2,3,4,5]. These results are presented again here for an international community of interest.

The opinions and assertions contained herein are the private views of the author and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense; This technical data deliverable was developed using contract funds under Basic Contract No. W15P7T-13-C-A802; Approved for Public Release; Distribution Unlimited. 18-2545

- [1] Owens BD, Kragh JF, Wenke JC, Macaitis J, Wade CE, and Holcomb JB (2008) Combat wounds in operation Iraqi Freedom and operation Enduring Freedom. *Journal of Trauma* **64**:295–299.
- [2] Colombe JB, Santago II A, Brokaw E, Colder B, Spencer R, Lalis L, Gupta R, Leggieri M, and McConnell J (2018) Science and technology knowledge gaps in blast injury risk to the lower extremity, the spine and back, and the upper extremity. *Military Health Service Research Symposium, Kissimmee, Florida, August 2018*.
- [3] Colombe JB, Spencer R, Lalis L, Gupta R, Leggieri M, Santago II A, Brokaw E, McConnell J (2016) The MHS Blast Injury Prevention Standards Recommendation (BIPSR) Process. *Military Health Service Research Symposium, Kissimmee, Florida, August 2016*.
- [4] Colombe JB, Santago II A, Brokaw E, Spencer R, Lalis L, Gupta R, Leggieri M, McConnell J (2016) Survey of blast injury risks to structures of the upper extremity and knowledge gaps. *Military Health Service Research Symposium, Kissimmee, Florida, August 2016*.
- [5] Santago II A, Colombe JB, Brokaw E, Liao Z, Colder B, Spencer R, Lalis L, Gupta R, Leggieri M, McConnell J (2016) Can existing knowledge on injuries to the spine and back be used to prevent injuries due to blasts? Literature review and knowledge gaps. *Military Health Service Research Symposium, Kissimmee, Florida, August 2016*.

Neuropathologic, Neuroimaging, and Biomarker Findings in Veterans with Blast-Related mTBI: Correspondences with Neurovascular Dysfunction and Immune Cell Infiltration in Blast Exposed Animals

David G. Cook^{1,2}, James S. Meabon^{3,4}, Aric Logsdon^{1,2}, Abigail Schindler^{1,4}, Donna Cross⁵, Garth Terry^{3,4}, Mayumi Yagi¹, William A. Banks^{1,2}, Murray Raskind^{3,4}, Daniel Perl⁶, C. Dirk Keene⁷, and Elaine Peskind^{3,4}

1. Geriatric Research, Education, and Clinical Center (GRECC), Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA
2. Department of Medicine, Division of Gerontology and Geriatric Medicine, University of Washington, Seattle, WA, USA
3. Veterans Affairs (VA) Northwest Network (VISN 20) Mental Illness, Research, Education, and Clinical Center (MIRECC), Seattle, WA, USA
4. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, USA
5. Department of Radiology, University of Utah, Salt Lake City, UT, USA
6. Uniformed Services University of the Health Sciences, Bethesda, MD, USA
7. Department of Pathology, University of Washington, Seattle, WA, USA

Background. Repetitive blast-related mild traumatic brain injury (mTBI) is associated with a range of chronic cognitive and neuropsychological symptoms. Repetitive mTBI is also associated with perivascular tau deposition characteristic of chronic traumatic encephalopathy (CTE) [1], as well as distinctive forms of chronic reactive gliosis [2]. In spite of significant clinical and preclinical progress, it remains critically important to elucidate the neuro-pathological and molecular correspondences between blast-exposed humans and animal models of blast.

We have found structural and functional brain neuroimaging abnormalities in Iraq and Afghanistan war Veterans greater than four years after their last blast-related mTBI [3]. In a mouse model of repetitive blast mTBI we have also demonstrated functional blood-brain barrier (BBB) disruption in specific brain regions that corroborate structural neurovascular neuropathology [4, 5]. Collectively, these findings raise the possibility that acute vascular disruption may initiate latent pathologic processes that give rise to chronic neuropathology. To better understand these issues we have carried out multi-modal neuroimaging, neuropathological analyses, and biomarker investigations in plasma and cerebrospinal fluid (CSF) of Veterans with blast-related mTBI. These studies have been carried out in conjunction with closely coordinated neuropathology, investigations of BBB integrity, and studies of peripheral immune cell dynamics within the central nervous system (CNS) of blast-exposed mice.

Results. Findings to be reported: (i) In both blast exposed mice and Veterans with blast-related mTBI, we found chronic dysmorphology of Purkinje cell dendritic arbors and reduced expression of the neuronal glutamate transporter, EAAT4. We also found reactive gliosis denoted by intense glial fibrillary acidic protein (GFAP) immunoreactivity, which in some instances was concentrated at the interface between the cerebellar molecular and granule cell layers; (ii) In blast exposed mice, delayed BBB disruption was blocked by inhibiting nitric oxide synthase (NOS) using N(G)-Nitro-L-arginine methyl ester (L-NAME) ($p \leq 0.05$). In addition, blast-induced delayed T-cell infiltration into the cerebellum was also blocked by NOS inhibition ($p \leq 0.05$); (iii) In blast mTBI Veterans, we have found elevated CSF levels of Monocyte Chemo-attractant Protein 1 (MCP-1) ($p \leq 0.05$) and Macrophage-Derived Chemokine (MDC) ($p \leq 0.06$), which are well-characterized chemokines that mediate trafficking of monocytes, T-cells, and other peripheral immune cells into the brain; (iv) Vascular endothelial growth factor-A (VEGF-A), a critical regulator of vascular function, was significantly elevated in the plasma of Veterans with blast-related mTBI compared to deployed Veteran controls ($p \leq 0.01$); and (v) In blast mTBI Veterans, plasma VEGF-A levels inversely correlated with fractional [¹⁸F]-fluorodeoxyglucose (FDG) uptake in the cerebellum (left, right lobule 9 and left, right Crus II; $p \leq 0.045, 0.030, 0.015, 0.004$, respectively).

Conclusions. Taken together, these findings suggest that vascular and neuroimmune disturbances may play important, interrelated roles in the pathogenic cascades that underlie chronic blast-related mTBI. Hindbrain disturbances to structures such as the cerebellum may contribute to the manifestations of chronic mTBI disorders. These data also indicate that VEGF-A levels in blood are associated with greater functional hypometabolism in cerebellum, a brain region that is vulnerable to blast-induced neurovascular and BBB dysfunction. In addition, these findings argue that further study is warranted to elucidate the mechanisms by which repetitive mTBI affects NOS signaling, which is a key regulator of VEGF-A.

References:

1. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Keene CD, Litvan I, Perl DP, Stein TD, Vonsattel JP, Stewart W, Tripodis Y, Cray JF, Bieniek KF, Dams-O'Connor K, Alvarez VE, Gordon WA, group TC, *Acta Neuropathol.*, **131** (2016) 75.
2. Shively SB, Horkayne-Szakaly I, Jones RV, Kelly JP, Armstrong RC, Perl DP, *Lancet neurology*, **15** (2016) 944.
3. Petrie EC, Cross DJ, Yarnykh VL, Richards T, Martin NM, Pagulayan K, Hoff D, Hart K, Mayer C, Tarabochia M, Raskind M, Minoshima S, Peskind E, *J. Neurotrauma*, **31** (2014) 425.

4. Meabon JS, Huber BR, Cross DJ, Richards TL, Minoshima S, Pagulayan KF, Li G, Meeker KD, Kraemer BC, Petrie EC, Raskind MA, Peskind ER, Cook DG, *Science Translational Medicine*, **8** (2016) 321.
5. Huber BR, Meabon JS, Hoffer ZS, Zhang J, Hoekstra JG, Pagulayan KF, McMillan PJ, Mayer CL, Banks WA, Kraemer BC, Raskind MA, McGavern DB, Peskind ER, Cook DG, *Neuroscience*, **319** (2016) 206.

Arteriole inflation as an injury mechanism following blast TBI

A. Dagro¹, K.T. Ramesh²

¹ US Army Research Laboratory, Aberdeen Proving Ground, MD

² Johns Hopkins University, Dept. of Mechanical Engineering

The cerebral arterioles---small vessels that carry oxygenated blood from the larger arteries to the capillary bed---can create a pressurized bottleneck following injury or disease states since arterioles are largely unbranched vessels with a high resistance to flow¹. Numerous experimental studies suggest that the blood-brain barrier (BBB), which is formed by endothelial cells of the capillary wall, becomes compromised following blast events². Although physical damage of the BBB can be observed post mortem, perivascular damage is commonly found surrounding the arterioles as well, which exist at a larger length scale than the capillaries. As shown in the post mortem pathology of blast TBI patients in Fig. 1, the cellular injury that surrounds the arterioles can take on the pattern of either "normal" perivascular damage (Fig. 1a-b), or "honeycomb" perivascular damage. Here, we address the question, *What type of mechanical loading gives rise to these two types of damage patterns?*

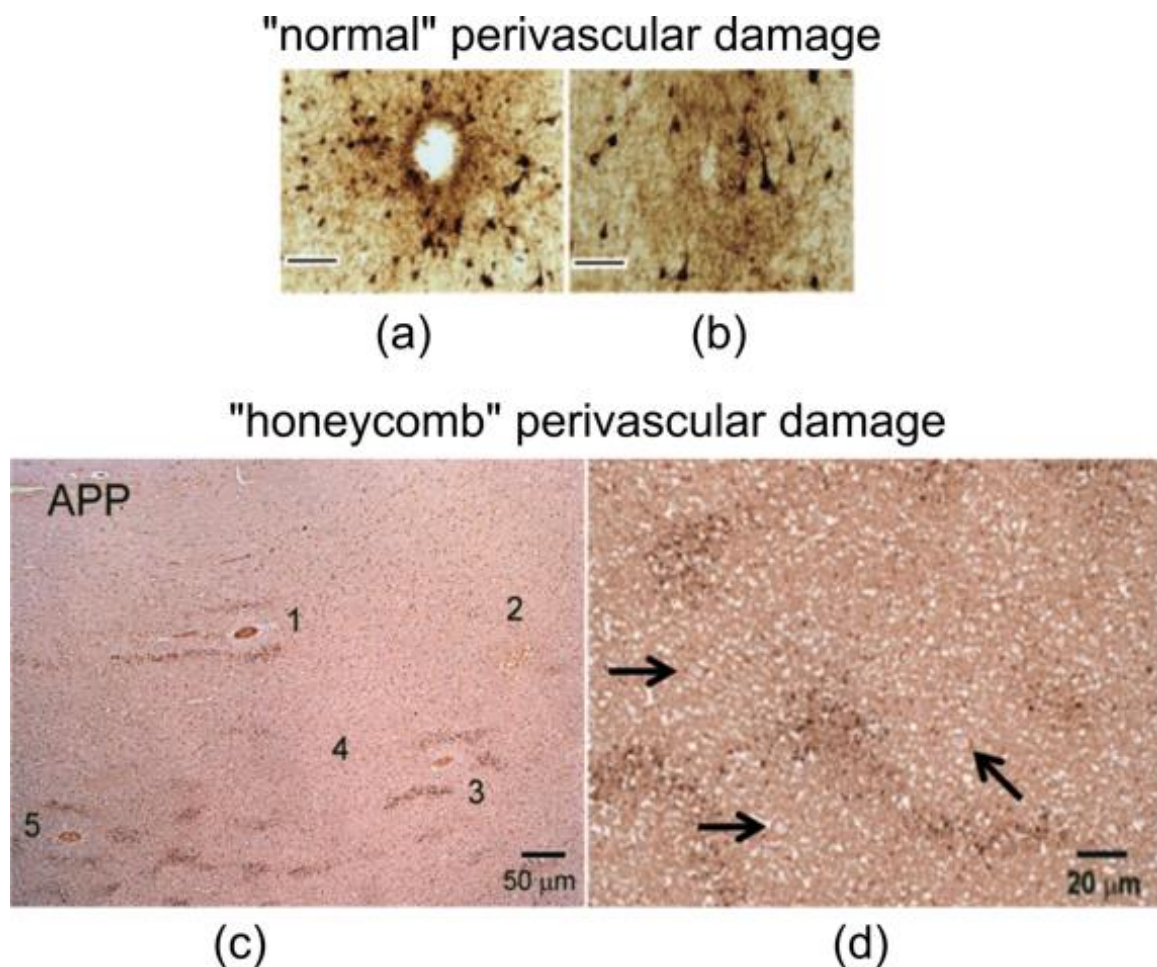


Fig. 1. Neuropathology of post-mortem blast TBI patients often reveals damage surrounding small cerebral vessels, at the length scale of arterioles. (a) Phosphorylated tau with neurofibrillary degeneration in the frontal cortex of a military veteran with exposure to a single blast event. (b) Phosphorylated tau in the frontal cortex of a military veteran with exposure to two blast events (adapted from Goldstein et al. 2012)³. Scale bars in (a) and (b) are 100 microns. (c-d) Examples of amyloid precursor protein (APP) staining of neuronal damage surrounding arterioles in military veterans subjected to blast exposure (adapted from Ryu et al. (2014)⁴).

In this work we present a computational model that captures the physical interaction of neighboring pressurized arterioles and the surrounding brain tissue. Assuming a strain-induced injury criterion, we find that the injury depends on vessel spacing, proximity to an unconfined free surface, and the relative difference in stiffness between the arterioles and the surrounding tissue. An analytical model is used to provide an approximate mathematical expression describing the interplay between internal pressure, arteriole thickness, and the variation in mechanical properties of arterioles. We find that a steeper heterogeneity (stiffer vessels surrounded by softer brain tissue) causes larger axial strains to develop at some distance from the arteriole wall. Finally, we discuss the timescales of arteriole inflation due to the "bottleneck

effect” and whether deformation on the venous compartments can create a high enough pressure to instantaneously disrupt the BBB integrity at short timescales.

- [1] Cipolla MJ (2009) The cerebral circulation. *Integr Syst Physiol Mol Funct* **1(1)**:1–59
- [2] Hue CD, Cho FS, Cao S, Nicholls RE, Vogel EW III, Sibindi C, Arancio O, Bass CR, Meaney DF, Morrison B III (2016) Time course and size of blood–brain barrier opening in a mouse model of blast-induced traumatic brain injury. *J Neurotrauma* **33(13)**:1202–1211
- [3] Goldstein LE, Fisher AM, Tagge CA, Zhang XL, Velisek L, Sullivan JA, Upreti C, Kracht JM, Ericsson M, Wojnarowicz MW, Goletiani CJ (2012) Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci Transl Med* **4(134)**:134ra60
- [4] Ryu J, Horkayne-Szakaly I, Xu L, Pletnikova O, Leri F, Eberhart C, Troncoso JC, Koliatsos VE (2014) The problem of axonal injury in the brains of veterans with histories of blast exposure. *Acta Neuropathol Commun* **2(1)**:153

Sizing Up Blast Injury Research: A Trans-Disciplinary Evidence-Base To Maximise Impact & Relevance

J. Denny¹, R. Brown², A. Dickinson¹ and J. Batchelor²

¹ Bioengineering Group, Faculty of Engineering and Physical Sciences, University of Southampton

² Clinical Informatics Research Unit, Faculty of Medicine, University of Southampton

Blast injuries caused by conflict, legacy landmines and explosive remnants of war represent a global challenge, posing a serious and ongoing threat to military forces, emergency services and civilians. The threat of blast injuries affects millions globally, particularly vulnerable populations in low-to-middle income countries. Blast injury research is an accelerating field, receiving increased interest and considerable funding in recent years. The current evidence-base to inform research methodologies, strategies, funding decisions and health policy is widely accepted to be insufficient and out of date. This makes it challenging to interpret and assess the impact of blast injury research, the effectiveness and fairness of funding and how health systems, clinicians and protection engineers can sustainably absorb findings to direct new priority research areas, utilise correct research methodologies and improve health outcomes.

Our vision is to improve the impact, effectiveness, fairness and relevance of blast injury research to address health issues caused by landmines, explosive remnants of war and conflict. We bring together expertise in blast engineering, public health, clinical informatics and research-on-research to establish an evidence-base to ensure that future blast injury research methodologies and investment reflects priority areas that best translate into improved health outcomes and population health. Through unique investment mapping and blast engineering critical reviews, we are building an evidence-base of the global blast injury research field.

The development of a unique investment map of the blast injury research funding landscape has so far indicated an estimated \$891 million invested by public and philanthropic funders into blast-related research across 1,204 individual awards between 2000 and 2018 (Figure 1). This highly comprehensive dataset is being extensively categorised to provide granular detail on the research landscape and the spatial and temporal distribution of funding for blast-related research. Further analysis of this dataset examines the distribution of blast injury types being researched, the locations and demographics concerned and the extent of translation into improved protective equipment, clinical health systems and treatments. Using blast engineering analysis, blast injury studies are being critically reviewed to determine the methodologies and blast loading parameters being adopted and how these correspond to idealised explosive scenarios. Preliminary analysis has explored the distribution of blast loading parameters, evaluated the relevance of different research methodologies and identified their respective limitations.

Continued development and analysis of this evidence-base will provide valuable evidence to inform future research priorities and strategies to maximise future research capacity and impact. This will ensure that future research and funding into blast injury reflects relevant and priority areas that best translate into improved protection, mitigation, treatment and health outcomes.

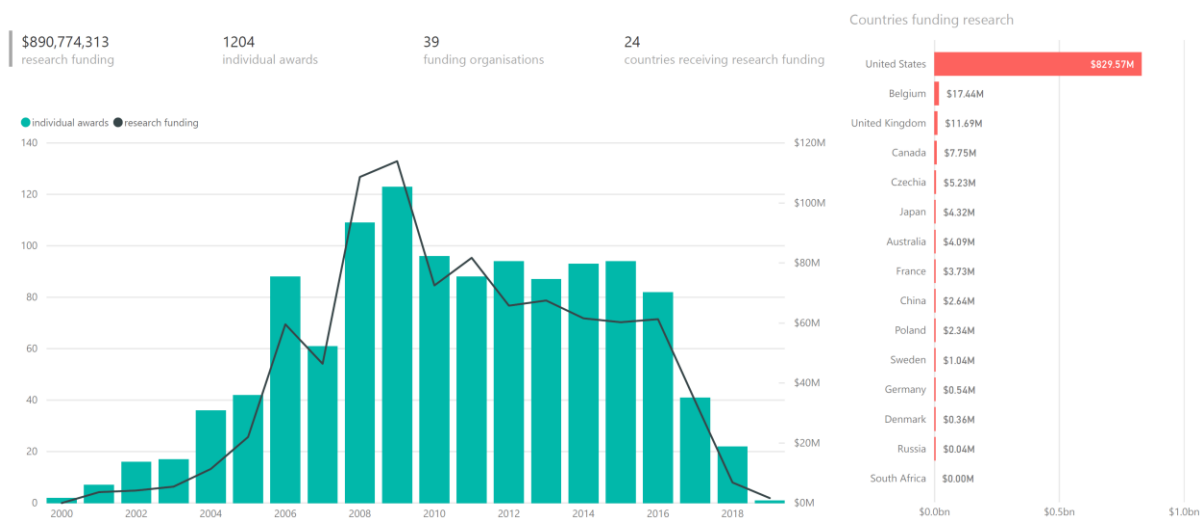


Figure 1: Preliminary investment mapping analysis – Overview of blast-related research funding 2000-2018.

Neuroimaging Biomarkers for TBI Detection and Monitoring

M. E. Downs¹, K. Keenan², S. Russek², J. Dagher¹

¹ The MITRE Corporation

² National Institute of Standards and Technology

Traumatic Brain Injury (TBI) has become known as a “Signature Wound” of Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) as incidence of this injury has been much greater than in prior conflicts. A subset of TBI, mild Traumatic Brain Injury (mTBI), accounts for 84.8% of all TBI injuries. Despite technical breakthroughs, identifying and monitoring damage caused by mTBI remain limited due to the inability to quantitatively detect and monitor damage. Biomarkers have been implemented for numerous diseases, but there currently are no known objective, *in-vivo*, quantitative measurements of biomarkers for mTBI. While there are multiple candidates that can be used as a neurological biomarker for mTBI, iron has been identified as the most promising biomarker. Based on extensive histological research, our hypothesis is that iron deposition observed in the brains of mTBI patients is directly implicated in neurodegeneration. As iron is ferromagnetic, we can detect its presence in the diseased brain using novel Magnetic Resonance Imaging (MRI) tools that are designed to measure Electro-Magnetic Tissue Properties (EMTP). Current MRI EMTP methods are limited with their accuracy and precision, which reduces the spatial resolutions at which the measurements can be performed. Here we aim to develop and verify a novel MRI EMTP technique to improve on current methods to allow for quantitative measurement of iron in the brain. With the development of this technique we then can begin to correlate observed iron concentrations in the brain of mTBI patients to injury severity, and treatment recovery to facilitate identification of optimal therapeutic strategies to help warfighters return-to-duty.

We are taking a multistep approach to understand iron accumulation in patients with neurodegenerative diseases. This includes identifying iron concentration in the diseased brain from prior histological studies for a numerical phantom, fabricating an iron MRI phantom to correlate MR EMTP signal to known iron concentrations, develop optimal MRI EMTP techniques through verification and validation with the numerical and MRI phantom, and finally employ the optimal MRI EMTP techniques and MRI phantom to assess iron concentrations in the brains clinical patients.

Preliminary work has been completed on both the numerical and physical iron phantom along with the identification of optimal MRI EMTP techniques. The numerical phantom shown in Figure 1 has both cortical and subcortical segmented brain sections along with CSF, bone, soft tissue and air sections. The MRI EMTP techniques evaluated with the numerical phantom and MRI simulator have been applied to an initial collection of clinical data from 10 patients exhibiting mTBI injury at the University of Arizona. Additional collaborations have begun for collection of additional mTBI data along with patients exhibiting other neurological diseases.

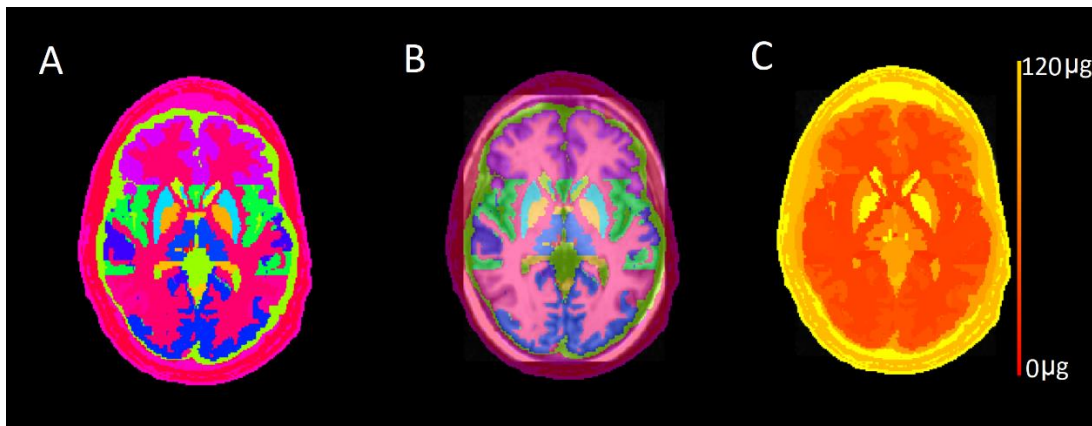


Figure 1: Numerical Phantom. A) Numerical Phantom with individual segmented brain sections. B) Numerical Phantom overlaid onto T1 structural scan. C) Iron concentration values.

Through collaborating with NIST and clinical collaborators, we are developing, as well as validating and verifying a novel MRI EMTP technique sensitive to iron accumulation in the damaged brain. This technique can be used as not only a way to monitor the damage after it has happened, but quickly detect and localize damaged area if the patient has a preliminary scan before the injury. This ability could revolutionize the ability to closely monitor the neurological health of warfighters or citizens in high mTBI risk professions.

Portions of this report were recently presented to the U.S. DoD Military Health System [1]. These results are presented again here for an international community of interest.

[1] Downs ME, Keenan K, Russek S, Dagher J. (2018) Imaging Biomarkers for TBI Detection and Monitoring. *Military Health Service Research Symposium, Kissimmee, Florida, August 2018.*

Approved for Public Release; Distribution Unlimited, Case Number 18-2532. © 2018 The MITRE Corporation. ALL RIGHTS RESERVED

Pressure Profile Distribution Across Varying Levels of Protection

A. Fournier¹ and A. Farkas¹

¹ Program Executive Office – Soldier, Project Manager - Soldier Protection and Individual Equipment, Technical Management Directorate

Questions regarding the profile distribution of loading during blast event, and the role protection systems play in affecting these, is an area which may help lead to improved medically based injury metrics, inform program managers on risk and requirements, and may influence the direction of solution designs.

The objective of this work is to provide a baseline of the pressure profile distribution for varying levels of protection against dynamics pressure threats. Shock tube and free field blast tests were conducted. Shock tube was chosen to gather pressure distribution data in a controlled and repeatable environment while free field enables gathering of pressure distribution data in a live-fire testing environment with short time durations, as compared to shock tube testing. An array of nineteen (19) pressure transducers and nineteen (19) force gauges were mounted into an anthropomorphic torso base. Instrumentation was symmetrically placed about the mid-sagittal plane of the torso. Data taken was used to assess peaks for pressure and force, impulse, and duration.

Protection configurations in shock tube testing included bare, Army Combat Uniform (ACU), ACU + Improved Outer Tactical Vest (IOTV), and ACU + IOTV + Enhanced Small Arms Protective Insert (ESAPI). Characterization testing was conducted to establish required testing configurations (number of Mylar sheets, standoff, etc) for the five (5) nominal reflected impulses chosen. Three (3) tests were conducted in each protection configuration at each reflected impulse test level.

For free field only bare and ACU + IOTV + ESAPI protection configurations were assessed. Five (5) test levels were chosen to replicate the nominal reflected impulse test levels from shock tube testing. One test was conducted at each nominal reflected impulse test level.

Shock tube results indicate the addition of the ACU and ACU + IOTV increased both peak pressure and peak force. The ACU + IOTV + ESAPI significantly reduced the peak pressure, but it is not clear how this influences the peak force. Impulse, as measured by the force gauge, indicates the addition of armor increases the impulse. In contrast, the pressure transducer did not record an increase in impulse over the bare configuration, with the exception of the addition of the ACU. The impulse, measured by the pressure gauges, describes a decrease over the base configurations with the ACU + IOTV and ACU + IOTV + ESAPI configurations. One possible explanation of the discrepancy between the pressure and force gauge impulse calculations is the total cross sectional area each type measures. The distribution for the bare configuration indicates a relatively uniform distribution across the torso, with slightly higher magnitudes at the midsagittal plane, which may be due to the location relative to the loading. The ACU and ACU + IOTV followed a similar distribution profile to that of the bare configuration. The ACU + IOTV + ESAPI moved the peak loads away from the mid-sagittal plane, towards the periphery, and may be attributed to the ESAPI presence. [1]

Free field results indicate wearing armor reduces the peak pressure and peak force measured. Of interest is the impulse felt by the torso, where the addition of armor appears to increase the duration of the blast wave (based on pressure sensor durations). The duration data from the force sensors appears unreliable, potentially because of the amount of noise created by the load cap. In terms of distribution, the bare configuration indicates a relatively uniform distribution across the torso, with slightly higher magnitudes at the midsagittal plane, which may be due to the location relative to the loading repeating what was observed in shock tube testing. The addition of armor compares well with that of the shock tube, where peaks are moved towards the periphery and may be attributed to the ESAPI presence. Using an arbitrary comparative measure of 100psi, the peak pressure drops below 100psi when greater than 13 feet away while wearing body armor and the bare torso does not reliably drop below 100psi until the torso is greater than 20 feet away. [2]

Together the results suggest the addition of armor may reduce the peak values; however the time duration, and thus the impulse, may present an issue. Increasing the armor worn does not appear to change the pressure profile distribution until a hard armor insert is added, moving the peak values from the mid-sagittal plane to the edge of the insert. More work is needed to assess how the observed changes in profile distribution might be attributed to injury and understand the mechanism for this.

The authors would like thank Ms. Katie Francia, Mr. Dan Cook, and Mr. Brandon Hepner for their work in providing test support under ATEC Project Number: 2013-DT-ATC-ARMOR-F5303.

References

- [1] K. Francia, D. Cook and B. Hepner, *Test Report for Behind Armor Pressure Measurement Study*, (2016).
- [2] A. Farkas, *Behind Armor Force & Pressure Measurement Study - Free Field Blast Test*, **TMD-16-356-F** (2017).

Laboratory and Field Studies of Middle Ear Muscle Contractions as Protection Against Impulsive Noise

G. Flamme¹, S. Tasko^{1,2}, K. Deiters¹, M. Smith¹, H. Jones³, W. Murphy⁴, W. Ahroon³, N. Greene³

¹ Stephenson and Stephenson Research and Consulting

² Western Michigan University

³ U.S. Army Aeromedical Research Laboratory

⁴ National Institute for Occupational Safety and Health

Several damage-risk criteria (DRC) for impulsive noise have presumed a protective role for middle ear muscle contractions (MEMC) [1,2]. The Auditory Hazard Assessment Algorithm for Humans (AHA AH), an electroacoustic model predicting permissible impulsive noise exposures, presumes that if an individual is warned of an impending impulse, full activation of an MEMC will occur prior to the initial impulse and until completion of any subsequent impulses. The awareness of an impending impulse has been assumed to afford the listener ten times more permissible exposures (Figure 1). There is reason to suspect that a warned /early MEMC is not reliable protection, but few studies have systematically examined reflexive and/or warned/early MEMC in response to impulsive noise. In recent years, our research group has been focused on filling this knowledge gap.

This presentation (1) reviews results from a series of laboratory studies of reflexive and anticipatory MEMC that indicate that MEMC should not be included in DRC; (2) describe s the influence and prevalence of MEMC elicited by *voluntary* or *incidental* electromyographic (EMG) head, neck,

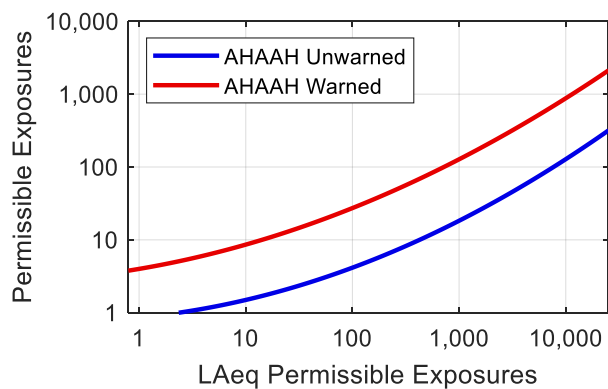


Figure 1. Comparison of permissible exposures between two DRC methods. The AHA AH Warned option affords the listener ten times greater permissible exposures than the AHA AH Unwarned option.

and/or upper extremity activity; (3) present s results of laser doppler vibrometry (LDV) measurements of anticipatory MEMCs confirming that MEMC should not be included in DRC, and; (4) describe s a large, ongoing field study designed to assess early MEMC during live-fire exercises with M4 rifles. The live-fire study examine s whether warned/early MEMC results obtained in laboratory studies generalize to an environment common to the warfighter.

Results of acoustic and non -acoustic elicitors on reflexive MEMC have demonstrated that MEMC are not pervasive and that non -acoustic elicitors are more likely to produce an MEMC th an acoustic elicitors in a group of highly screened participants (N=190) (Figure 2). To date, little evidence has been seen for a pervasive warned/early MEMC.

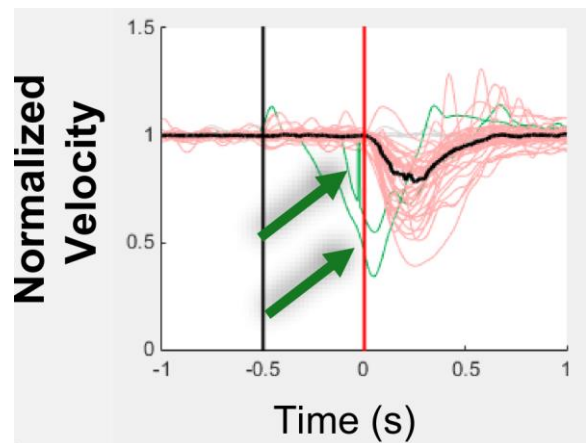


Figure 2. Laser Doppler vibrometry results indicating that warned/ea rly MEMC are not often observed. Pink traces represent results from individual participants. The black trace is the mean across the participant group. The green traces indicated by the green arrows indicate the two participants showing evidence of warne d/early MEMC.

Voluntary eye closure was one of the most reliable MEMC elicitors. Following this observation, further

analyses were conducted to determine if incidental motor activity influenced MEMC during acoustic and non-acoustic reflex conditions. Incidental motor activity was assessed via analysis of electromyographic (EMG) traces obtained during MEMC data collection. Regression analysis was used to examine the relationship between activity in the ear during silent baseline periods and during contralateral elicitor presentation periods with incidental EMG

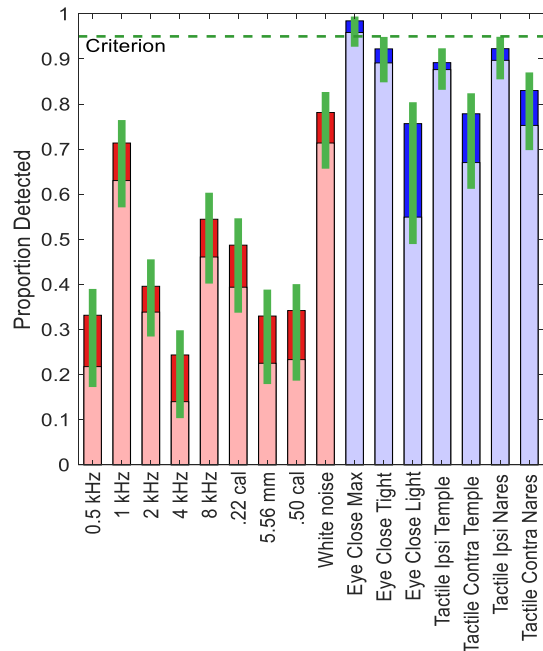


Figure 1. Proportions of participants in which an MEMC was observed for brief acoustic and non-acoustic stimuli. Lighter (consensus rater agreement) and darker (2/3 agreement) indicate non-acoustic MEMC are more likely than MEMC elicited via acoustic elicitors

activity from five head, neck and upper extremity recording sites. Findings suggest that incidental motor activity is common even in highly controlled laboratory settings (Figure 3). For a substantial minority of measurements (10%), 20-70% of the variance in MEMC probe signals is predicted by facial muscle activity. Multiple regression may be a viable tool for discriminating between MEMC elicited by incidental motor activity and MEMC that could be considered reliable protection. This is particularly important when considering MEMC in tasks involving other motor activities (e.g., firing a weapon) or tactical/operational exercises and maneuvers.

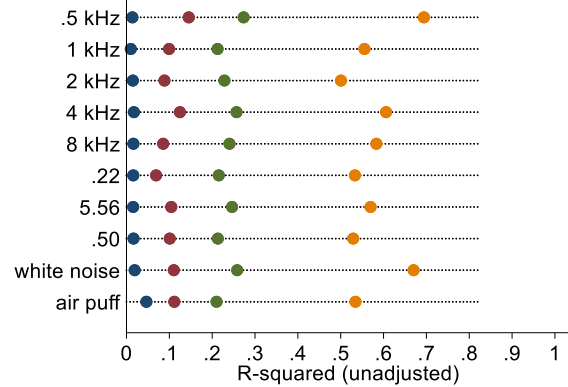


Figure 2. Proportion of variance described by EMG results during silent and elicitor periods. Results indicate median (blue), 75th percentile (red), 90th percentile (green) and maximum (yellow) results, indicating that for the upper 10th percentile, EMG results explain between 20-70% of the variance.

- [1] G. R. Price and J. Kalb, *J. Acoust. Soc. Am.*, **90**(1) (1991) 219-227.
- [2] W. Ward, *Washington DC: National Academy of Sciences, National Research Council*, (1968).

Is there an indication for pre-hospital trepanation in patients having a traumatic brain injury and a blast injury?

G. Freude¹, R. Dulou², J.M. Delmas², C. Schulz¹, U.M. Mauer¹

¹ Dep. of Neurosurgery; Bundeswehrkrankenhaus Ulm, Germany

² Dep. of Neurosurgery; Hôpital d'Instruction des Armées Clermont-Tonnerre, Brest, France

³ Dep. of Neurosurgery; Hôpital d'Instruction des Armées Percy, Clamart, France

corresponding author: Gregor Freude, M.D.

Dep. Of Neurosurgery

Armed Forces Hospital Ulm

Oberer Eselsberg 40

89081 Ulm

GERMANY

gregor.freude@uni-ulm.de

Keywords: traumatic brain injury (TBI), blast injury, imaging, emergency medical service, pre-hospital trepanation

Abstract:

Traumatic brain injury (TBI) and blast injury can be severe injuries. The combination of a tbi and other injuries (for example blast injury) increase lethality [2]. The pathophysiology of a combined tbi and polytrauma is very complex [3] and blast induced brain injury is a complex scientific problem [5].

In case of a severe traumatic brain injury the patient should arrive intubated and ventilated in a trauma center within 60 minutes [4]. A Computed tomography scan (ct scan) of the brain has to be performed quickly in order to assess the extent of intracranial injury [4]. If required (after the computed tomography) an operation should be performed. The effect of decompressive craniectomy on clinical outcomes in patients with intracranial hypertension remains unclear [1].

Sometimes there is a discussion if pre-hospital trepanation or exploratory burr hole without ct scan should be performed. The authors illustrate with clinical cases that there is no indication for pre-hospital trepanation in military missions or in the civilian emergency medical service. Trepanation without diagnostic is life-threatening for the patient.

[1] Hutchinson PJ, Koliass AG, Timofeev IS et al, *N Engl J Med*, **375** (2016) 1119

[2] Leitgeb J, Mauritz W, Brazinova A et al, *Arch Orthop Trauma Surg*, **133** (2013) 659

[3] Huber-Lang M, Lambris JD, Ward PA, *Nat Immunol*, 2018 **19** (2018) 327

[4] Firsching R, Rickels E, Mauer UM et al, *J Neurol Surg A Cent Eur Neurosurg*, **78** (2017) 478

[5] Risling M, Davidsson J, *Front Neurol*, **3** (2012) 30

Microbiome signatures associated with rodent model of traumatic brain injury vs. psychological stress

Aarti Gautam¹, James C. DeMar², Nabarun Chakraborty³, Raina Kumar⁴, Allison Hoke⁵, John G. Rosenberger², Andrew B. Batuure², David J. Bloodgood², Donna M. Wilder², Venkatasivasaisujith Sajja², Rasha Hammamieh¹, Marti Jett¹ and Joseph B. Long²

¹Integrative Systems Biology Program, US Army Center for Environmental Health and Research Frederick, MD, USA 21702-5000

²Blast-Induced Neurotrauma Branch, Center for Military Psychiatry and Neuroscience Research, WRAIR, Silver Spring, MD

³The Geneva Foundation, ISB, USACEHR, Frederick, MD

⁴Advanced Biomedical Computing Center, FNLCR, Frederick, MD

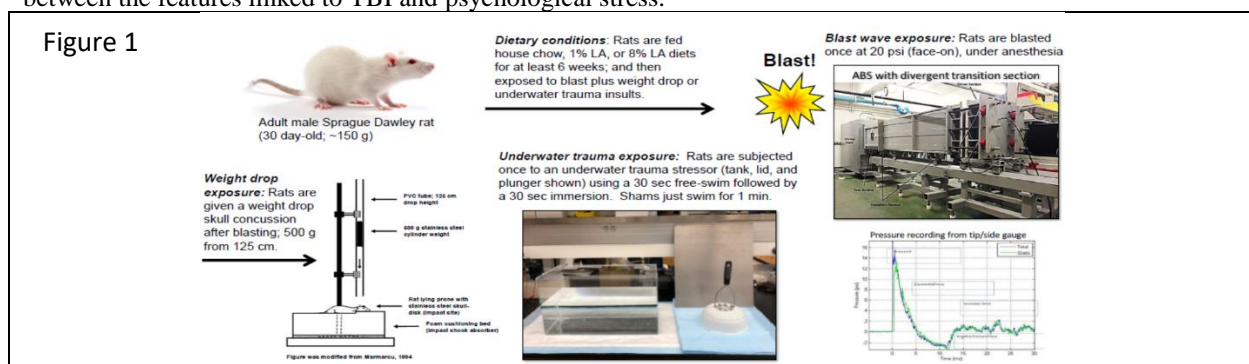
⁵ORISE, ISB, USACEHR, MD, USA 21702-5000

Traumatic brain injury (TBI) is a major source of morbidity and mortality within current war zones; and psychological disorders, such as post-traumatic stress disorder (PTSD), are the major career limiting factors for combat soldiers. Discrimination between psychological stress and TBI using a knowledge-driven unbiased panel of biomarker signatures will be essential for designing precise care management. However, the biological importance of these alterations in traumatic injury or stress is not well understood. Recent data suggest a role of alterations of the microbiome and gut architecture leading to bacterial translocation in both these conditions. To determine how the shift in the microbiome population is coordinated with the physical injury and stress responses, two animal models were chosen. The present study was conducted to identify distinct factors between psychological stress and TBI in the fecal microbiome of rats and further to study the effect of diets enriched with varied polyunsaturated fat compositions..

A closed-head TBI model consisting of blast overpressure (BOP) wave exposure coupled with a weight drop concussion (Marmarou method) was used on a group of adult male rats (N=12 each exposed and sham). The anesthetized animals were subjected once to BOP (18 psi) inside an Advanced Blast Simulator (ABS), which was then immediately followed by dropping a 500-gram metal weight from 125 cm above onto a stainless steel disc affixed to the rat's skull midway between lambda and bregma, whereas shams received anesthesia. In parallel, an independent group of rats (N=12 each exposed and sham) was subjected to an underwater trauma (UWT) stressor model that consisted of 30s of swimming and habituation, followed by 30s of forced whole body immersion. Shams received one min of free swimming. Existing literature and our data shows that UWT elicits prolonged "anxiety" behaviors. Figure 1 shows the assay design. Prior to TBI or UWT, animals were maintained for six weeks and continued thereafter on three different diets (N=4 per diet group): (i) Standard house chow; (ii) Custom chow enriched with 1% of calories as Linoleic acid (LA) and (iii) Custom chow enriched with 8% LA. Unlike the house chow, the two custom diets contained no long chain ω -3 polyunsaturated fatty acids (PUFAs) to offset their high LA (ω -6 PUFA) content. The fecal bacterial populations were characterized by identification of 16S ribosomal RNA using Illumina MiSeq. Microbiome data analysis was conducted using a standard metagenomics pipeline.

Principal coordinate analysis (PCoA) showed clear separation between taxonomic phylogenetic profiles linked to TBI and UWT, respectively from their corresponding shams (Figure 2A-B). Within each group, TBI or UWT, the taxonomic phylogenetic profile linked to rodents fed on house chow showed diversion from the other two diet groups. No significant separation was observed between rats fed on either the 1% or 8% LA enriched diets that were absent in long chain omega-3 PUFAs. *Firmicutes* and *Bacteroidetes* were the dominant phyla observed and the relative abundance of each of the phyla with respect to the type of the stress will be discussed.

This study indicates that the effects of 1% or 8% LA enriched diet compared to standard diet and its effects on the gut microbiome following exposure TBI as well as psychological stress. The present results will be extended to incorporate the genomic alterations within tissues (e.g., blood and brain) that can be attributed to the different traumatic injury and stress factors and diet compositions. Final deliverables will articulate the role of the gut-brain axis in different traumatic injury and stress conditions while also defining how fecal microbial signatures can discriminate between the features linked to TBI and psychological stress.



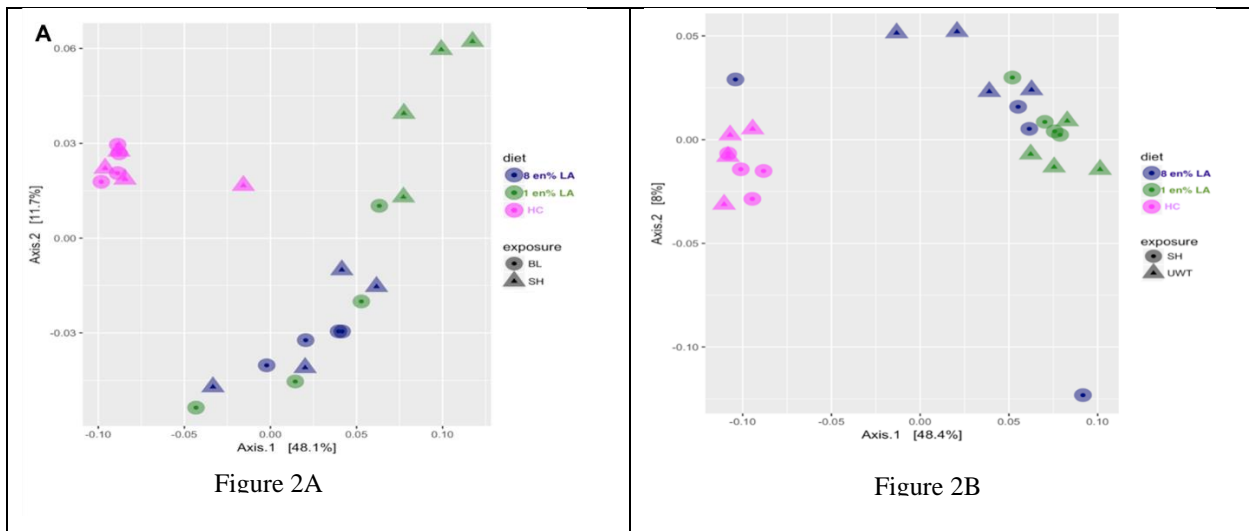


Figure 1. The study design.

Figure 2A. PCoA plot based on unweighted UniFrac-based method showing variance in microbiota composition. Hose chow (HC) were distinctly separated from the special diet groups independent of the sham (SH) and Blast/TBI (BL) group. Among the special diets, SH fed on diets enriched with 1% LA (1 en% LA) were clustered separately from others.

Figure 2B. PCoA plot based on unweighted UniFrac-based method showing variance in microbiota composition. Hose chow (HC) were distinctly separated from the special diet groups independent of the sham (SH) and underwater trauma (UWT) group. Among the special diets, certain separation between two diets (8% vs. 1% LA enrichment) dominated.

Disclaimer:

Research was conducted under an institutionally approved animal care and use protocol in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the U.S. Army.

Blast-Induced Neurotrauma Results in Spatially Distinct Gray Matter Alteration Alongside Hormonal Alterations: Evidence from a Canadian Military and Veteran Cohort

S.C. Hellewell¹ & I. Cernak²

¹ Sydney Translational Imaging Laboratory, The Heart Research Institute; Charles Perkins Centre, University of Sydney, Sydney, NSW, Australia

² STARR-C (Stress, Trauma, and Resilience Research Consulting) LLC, Philadelphia, PA, USA

Introduction: Blast-induced neurotrauma (BINT) is a growing concern in both military and civilian populations due to an increasing prevalence of explosive weaponry in combat and terrorist actions. Among military personnel, repeated mild exposures to blast occur frequently during training and deployment. BINT events are increasingly recognized to cause long-term neuropsychological and neurocognitive change, and may also result in brain structural alterations, with particular consequences for gray matter. As military occupations are linked to frequent exposures to stress, BINT might negatively influence the personnel's stress coping abilities and contribute to chronic stress-induced health impairments. In this study we examined gray matter volumetric change after BINT or chronic stress, and investigated the cooccurrence of salivary testosterone and cortisol changes to establish potential BINT-specific impairments.

Methods and Materials: Participants in the BINT group were active Canadian Armed Forces (CAF) personnel and CAF veterans [1] with self-identified exposure to BINT at least 6 months prior to examination (n = 11). The chronic stress group consisted of emergency first responders who experienced similar workplace stressors without exposure to BINT (firefighters, paramedics, corrections officers, n = 8). Saliva samples were collected *via* passive drool technique on the morning of testing, and analysed for testosterone and cortisol concentrations, with raw concentrations converted to Z scores. The testosterone to cortisol (T/C) ratio (pg/mL) was also calculated to determine their relationship. MRI data were acquired using a 3 Tesla Siemens Prisma scanner (Siemens Healthcare GmbH, Erlangen, Germany) with a 64-channel head coil. Structural T1 weighted anatomical volumes were obtained (axial orientation, TR=2080 ms, TE=4.38 ms, FOV=256 mm, slice thickness =1 mm). T1 images were preprocessed using SPM12, normalized using an affine followed by non-linear registration, corrected for bias field inhomogeneities, registered to standard MNI space and then segmented into gray matter, white matter and cerebrospinal fluid components. Whole-brain VBM was performed to determine gray matter volume using the Computational Anatomy Toolbox (CAT12).

Results: Widespread and largely symmetric loci of reduced gray matter volume specific to BINT were found when compared to the chronic stress group. Predominant clusters (Figure 1) were found bilaterally in the superior temporal gyrus, cuneus, thalamus and cerebellum, while significant hemispheric changes were noted in the left middle occipital gyrus and right middle and posterior cingulate. Examination of saliva testosterone and cortisol Z scores (Figures 2A and 2B, respectively) revealed distinct and opposing patterns in the BINT and chronic stress groups, with significantly elevated testosterone levels in the BINT group and reduced testosterone levels in the chronic stress group. The T/C ratio (Figure 2C) was also significantly increased in the BINT group compared to the chronic stress group, for which values were within the normal range.

Conclusion: This study highlights that discrete patterns of gray matter loss occur in anatomically specific regions after BINT, which are not observed after chronic stress. Distinct alterations were found in the BINT and chronic stress groups with regard to profiles of testosterone, cortisol and their T/C ratio, suggesting that BINT and chronic stress have differential consequences for HPA axis function. These findings establish that the pathophysiology of blast injury has important structural and endocrine components, and emphasize the distinction between BINT and chronic stress.

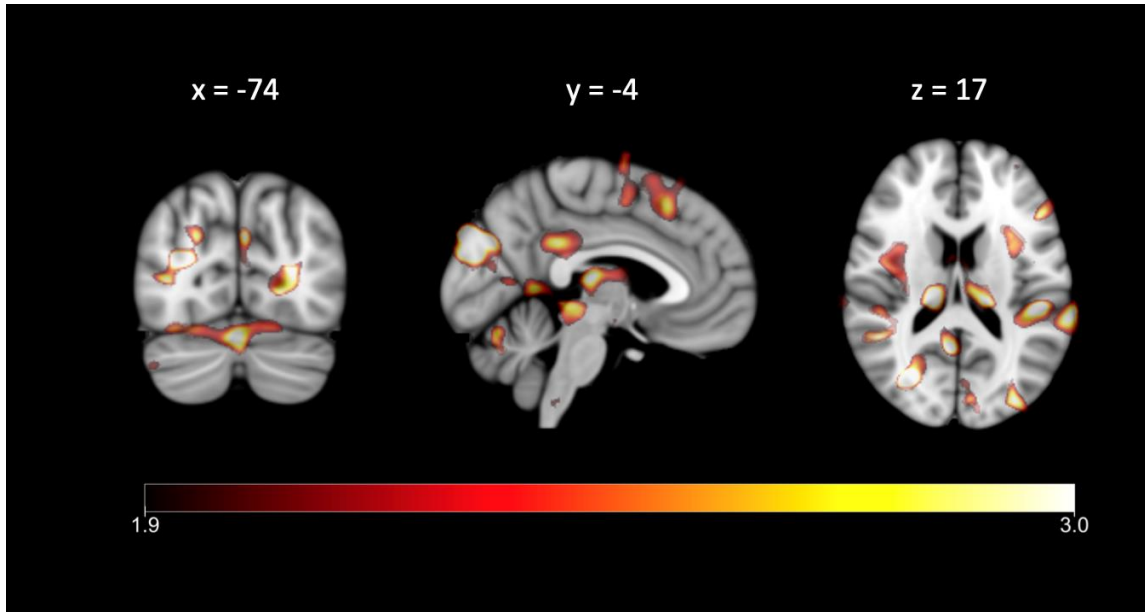


Figure 1. Significant clusters of gray matter alteration in BINT participants. Whole-brain Voxel based morphometry was performed to determine volume differences between BINT and chronic stress groups. Color map indicates scale for t-statistic.

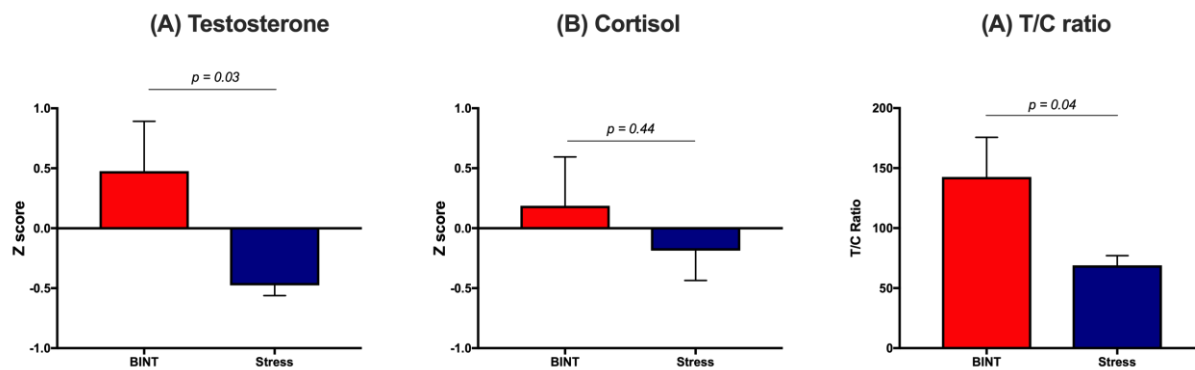


Figure 2. Testosterone, cortisol and T/C ratio in BINT vs. chronic stress groups. Testosterone and cortisol are presented as Z scores of hormone concentrations, while the T/C ratio is calculated from raw values in pg/mL.

[1] S.C. Hellewell and I. Cernak, *J Trauma Stress*, **31** (2008) 89.

A Comprehensive Blast-Related Auditory Injury Database (BRAID) of Injured US Military Personnel

A. Joseph^{1,2}, M. Clouser^{1,2}, A. MacGregor², M. Galarneau²

¹Leidos, San Diego, California

²Naval Health Research Center, San Diego, California

Hearing preservation and restoration has been identified as a top research initiative by the Navy. Blast injury to the ear has emerged as one of the most common combat-related injuries among service members deployed between 2001 and 2013 and may result in symptoms of tinnitus, hearing loss, and/or hearing-threshold shifts. It is well known that exposure to hazardous noise, such as blasts, can compromise the ability to hear and communicate, reducing situational awareness and operational readiness. Military personnel who deploy to battle zones are increasingly at risk for adverse hearing outcomes, as indicated by the burgeoning cost of hearing-related disability claims. Although several studies have demonstrated adverse auditory outcomes following blast exposure, none have followed a large number of military personnel with blast exposure through multiple monitoring audiograms.

To address these issues, Naval Health Research Center (NHRC) created a comprehensive database that combines audiometric data from the Defense Occupational and Environmental Health Readiness System-Hearing Conservation (DOEHRS-HC) module with point-of-injury data from the Expeditionary Medical Encounter Database (EMED) and additional personnel records. The objective of merging these data was to develop a long-standing capability for surveillance, assessment, and investigation of blast-related hearing outcomes—the Blast-Related Auditory Injury Database (BRAID). To date, NHRC has used the BRAID for several studies and research is ongoing. The BRAID has been used to: (1) determine if or how male Navy and Marine Corps personnel with minor to moderate blast- or nonblast-related injury (BRI vs. NBRI) differed on hearing outcomes; (2) examine hearing loss, significant threshold shifts, and pure tone averages that were calculated from audiometric data pre- and postinjury; (3) examine mean audiometric thresholds for the left and right ear at the pure-tone test frequencies of 500, 1,000, 2,000, 3,000, 4,000, and 6,000 Hz for audiograms prior to and following injury; and (4) compare low- and high-frequency pure-tone averages (LFPTA and HFPTA) by injury group.

The BRAID contains 16,525 Navy and Marine Corps members who were identified in the EMED with deployment-related injuries from 2001–2013, and had at least one audiogram recorded in DOEHRS-HC. The average age at time of injury for the entire BRAID cohort was 24 years, 97% were male, 93% enlisted, and 92% Marine Corps. Most injuries were sustained in combat (73%) and were blast-related (63%). To date, research with the BRAID has focused on comparisons between BRI and NBRI groups. Among those with at least one audiogram, 1,813 (781 BRI, 1,032 NBRI) had a recorded audiogram both within the year prior to sustaining an injury and within 1 year postinjury. Mean Injury Severity Score was higher for those with BRI than NBRI (3.4 vs. 1.7). Of those with normal hearing pre-injury, pure-tone audiometric hearing loss occurred in 23% of those who sustained blast injuries compared with 12% in the NBRI group ($P < .001$). To look more closely at the specific frequency hearing loss, the same BRAID group was used and those with evidence of prior hearing loss on their pre-injury audiogram were excluded. There were 482 service members with a BRI and 704 with a NBRI who had at least one audiogram prior to and following the date of injury who met this criteria ($N = 1,186$). Overall, mean-threshold audiograms revealed hearing within normal limits (responses ≤ 25 dB HL) at all test frequencies for both ears. However, when investigating hearing loss at individual frequencies and by ear, new-onset hearing loss primarily affected the frequency range of 4,000–6,000 Hz, and hearing shifts were greater in the left ear. Postinjury LFPTAs and HFPTAs were significantly higher in the BRI group than the NBRI group for both ears ($P < .001$ for all comparisons). On average, new-onset LFPTA loss was also accompanied by HFPTA loss. All pure-tone average distributions for the BRI group were right-skewed and included intensity levels as high as 70 dB HL, which indicates severe hearing loss.

The BRAID has far-reaching applications for future research and surveillance of short- and long-term outcomes. Findings suggest there are greater hearing shifts in those with a BRI than those with NBRI, and further scrutiny may reveal unique patterns in population subgroups. It is imperative to continually monitor and analyze the effects of blast injury on hearing sensitivity and configurations of audiometric-threshold data over time. Doing so can help identify at-risk populations for the purposes of early intervention and hearing loss prevention, develop best-practice guidelines for clinicians, and implement supportive hearing-health policies. Auditory health and readiness is critical for situational awareness and quality of life for the US military.

Disclaimer: I am a military service member or employee of the U.S. Government. This work was prepared as part of my official duties. Title 17, U.S.C. §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C. §101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. Approved for public release; distribution is unlimited.

This work was supported by the U.S. Navy Bureau of Medicine and Surgery under work unit no. 60332. The views

expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. The study protocol was approved by the Naval Health Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Research data were derived from an approved Naval Health Research Center, Institutional Review Board protocol number NHRC.2003.0025.

Multiple Degree of Freedom - Blast Effects Simulator

Robert G. Kargus

U.S. Army Research Laboratory

Surrogate devices for replicating the response of vehicles to underbody blast continue to be attractive for their repeatability in evaluating crew safety systems and hardened components. One challenge has been the replication of severe localized motion that occurs simultaneously with the global motion of the target vehicle (Fig. 1). A device called the Multiple Degree of Freedom - Blast Effects Simulator (MDOF-BES) was designed and built to replicate this type of motion (Fig. 2). Momentum exchange masses are used to represent localized inertia and provide the “spring back” effect to the test article. Building on past experience in the development of mechanical shock machines for studying vehicular blast effects, it is anticipated that the MDOF-BES will complement and significantly exceed the capability of existing vertical shock machines such as the Crew Seating Blast Effects Simulator (CSBES) [1]. Performance specifications and intended applications will be presented.

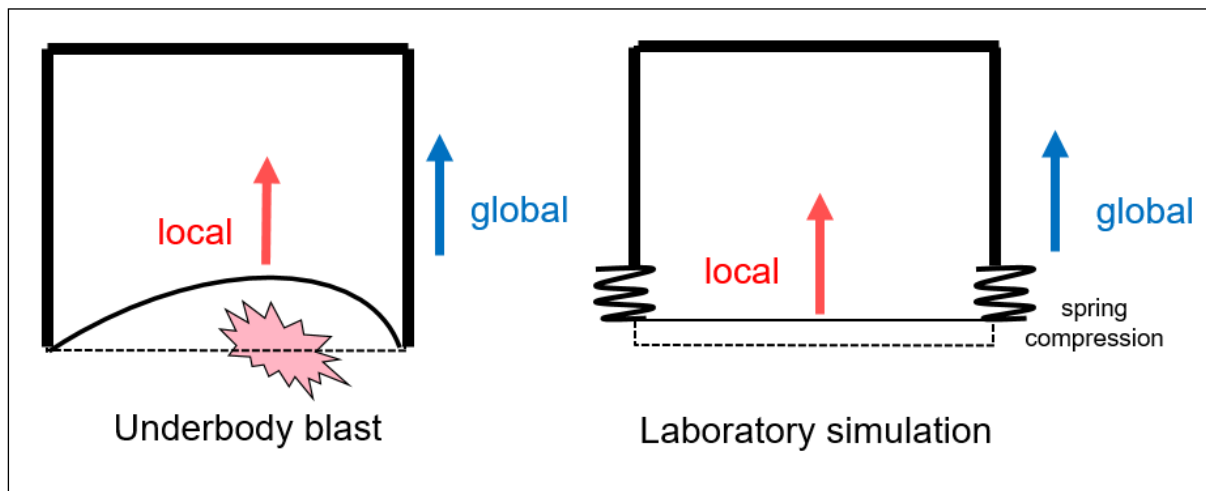


Figure 1 Local response to UBB and its laboratory analog

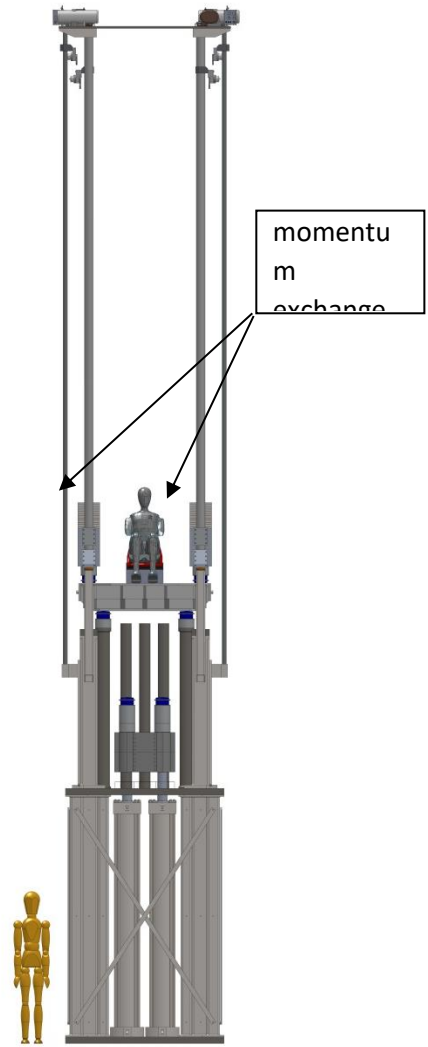


Figure 2 MDOF-BES

[2] <http://www.lansmont.com/crew-seating-blast-effects-simulator-april-18-2011>

DISTRIBUTION A. Approved for Public release.

Roles of nitric oxide in shock wave-caused cerebral hemodynamic abnormalities in rats: laser-induced shock wave study

S. Kawauchi¹, M. Inaba², Y. Muramatsu¹, A. Kono¹, Y. Komuta³, I. Nishidate²,
K. Kaida³, T. Adachi⁴ and S. Sato¹

¹ Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute
² Graduate School of Bio-Applications & Systems Engineering, Tokyo University of Agriculture and Technology
³ Division of Neurology, Department of Internal Medicine, National Defense Medical College
⁴ Division of Cardiology, Department of Internal Medicine, National Defense Medical College

Introduction: Despite extensive studies on blast-induced traumatic brain injury (bTBI), the pathophysiology and mechanisms have not been fully elucidated. This would be partly due to, in addition to the complex interactions of the brain with a shock wave, the lack of information on the physiological, structural, and functional changes in the brain during and immediately after shock wave exposure *in vivo*. Since initial events caused by a shock wave can trigger the following cascading processes leading to pathological consequences in the chronic phase, real-time observation of the cerebral responses to a shock wave is crucial for understanding the mechanisms of bTBI. We previously performed real-time observation of the rat brain exposed to a laser-induced shock wave (LISW) and observed that spreading depolarization (SD) was generated and spread over the entire ipsilateral cortex, which was followed by long-lasting hypoxemia/oligemia [1]. We hypothesized that such impaired cerebral hemodynamics were associated with the generation of nitric oxide (NO) due to a shock wave (Fig. 1). An overpressure from a shock wave can increase intracellular calcium and stimulate endothelial NO synthesis (eNOS), causing NO generation. On the other hand, SD substantially increases neuronal intracellular calcium and can generate reactive oxygen species (ROS) such as superoxide anion (O_2^-) in mitochondria. Two radical species, O_2^- and NO, can efficiently react, producing highly toxic peroxynitrite ($ONOO^-$) and hence causing alteration of vascular functions. In this study, we investigated such roles of NO in the hemodynamic changes by applying an NO synthesis inhibitor, L-NAME (*N*^G-Nitro-L-arginine methyl ester, hydrochloride) to the same rat model.

Materials and Methods: L-NAME was administered through the drinking water (100 mg/L) for 6 weeks to Sprague-Dawley male rats. On the day of LISW application, ten-weeks-old L-NAME (+) rats and L-NAME (-) rats were anesthetized with isoflurane inhalation and placed in a stereotactic frame. An LISW (diameter, 4 mm; shock wave impulse, ~30 Pa·s) was transcranially applied to the left parietal cortex, where the center of the LISW was positioned at 2.5 mm posterior and 3.0 mm lateral to the bregma. Fiber-based diffuse reflectance measurement was performed in the vicinity of the site of LISW application. From the measured reflectance spectra, we quantified tissue oxygen saturation (StO_2) in the cortex on the basis of multiple regression analysis aided by Monte Carlo simulation.

Results and Discussion: Figure 2 shows time courses of StO_2 in the cortices of the rats with and without L-NAME administration. After the occurrence of SD, L-NAME (-) rats showed sudden and large decreases in StO_2 as previously reported [1]. The maximum decrease in StO_2 reached to -20%. We defined hypoxemia as the condition showing StO_2 decrease of more than 13% [2]. The results clearly show that hypoxemia persistently occurred in the L-NAME (-) rats later than ~10 min after LISW application. The decrease in StO_2 in L-NAME (+) rats, on the other hand, was around -5% later than 10 min after LISW application and did not reach the hypoxemic level. These results suggest that the impaired cerebral hemodynamics caused by a shock wave is associated with generation of NO. Currently, we are also conducting experiments using free radical scavenger to clarify contributions of peroxynitrite to the impaired hemodynamics.

Acknowledgements

This work was supported by the Ministry of Defense Research Program for Advanced Defense Medicine.

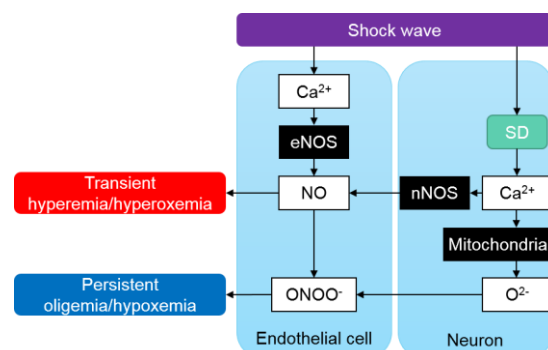


Figure 1 Hypothesis of impaired cerebral hemodynamics caused by a shock wave.

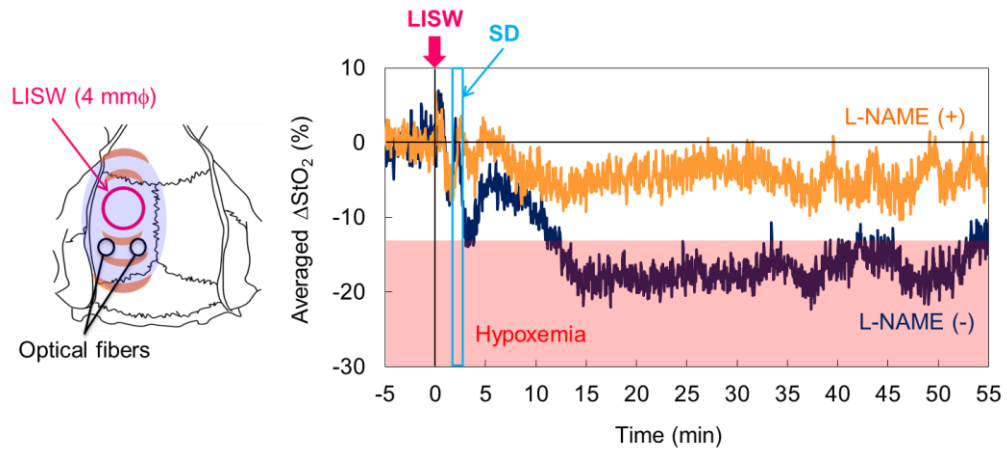


Figure 2 Averaged time courses of StO_2 in L-NAME (+) rats (n=4) and L-NAME (-) rats (n=4) after LISW application (right); the red shadow region indicates hypoxemic condition [2]. Positions of LISW application and optical fibers for diffuse reflectance measurement are shown on the left.

References:

- [1] S. Sato, S. Kawauchi, W. Okuda, I. Nishidate, H. Nawashiro and G. Tsumatori,, *PLoS ONE*, **9** (2014) e82891.
- [2] P. G. Al-Rawi and P. J. Kirkpatrick, *Stroke*, **37** (2006) 2720-2725.

Pathophysiological differences of hearing impairment caused by different conduction pathways of shock wave

Eiko Kimura¹, Kunio Mizutari¹, Katsuki Niwa¹, Yasushi Satoh², Shun-ichi Sato³, and Akihiro Shiotani¹

¹ Department of Otolaryngology, Head and Neck Surgery, National Defense Medical College, Japan

² Department of Pharmacology, National Defense Medical College, Japan

³ Division of Bioinformation and Therapeutic Systems, National Defense Medical College, Japan

Background

Blast induced hearing loss has been an important topic in military medical research, because ear is the most fragile organ for blast. In the Boston Marathon experience, sensorineural hearing loss, for example, hearing loss, tinnitus, hyperacusis, and difficulty hearing in noise is the most numerous permanently subsequent complications. [1] Although several blast models have been established, the detailed pathophysiology on blast induced hearing loss has not been revealed. The most critical etiology of blast-induced hearing loss is cochlear permanent injury. However, measurement of detailed cochlear function after blast exposure has been difficult, because conventional blast exposure models have caused tympanic membrane perforation, following conducting hearing loss. We have established two different types of blast induced hearing loss models, which have pure sensorineural hearing loss without tympanic membrane perforation. In this study, we analyzed the pathophysiology on these two blast induced hearing loss models; the first was induced by air-conducting shock wave generated from an air-compressed shock tube, the other was induced by bone-conducting percutaneous shock wave generated by laser chamber (laser-induced shock wave: LISW).

Methods

Shock tube: SUS-tubing inflated compressed nitrogen gas was used. Polyester membrane divides this shock tube to high and low pressure part (length of low-pressure part: 800mm, high pressure part: 400mm). Shock wave propagates to low-pressure part by breaking the membrane. The pressure and waveform of the shock wave were measured using pressure sensor and an oscilloscope. The peak pressure was set to 25 kPa, and irradiated to the mouse from diagonally upward. (Figure 1)

LISW: Shock wave was generated by irradiating a laser target with a 532-nm Nd: YAG laser. The laser irradiated to posterior ear of mice through the target made by a PET seat rubber. The diameter of laser spot was 3mm, and the outputs of the laser pulses were set to 2.0 J/cm². (Figure 2)

Six-week-old male CBA/J mice (BW: 17-20 g) were used, and we observed tympanic membrane using a small endoscope (AVS) immediately after irradiation. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were used to cochlear electrophysiological function measurement. To evaluate the morphological changes of cochleae, cochlear immunohistochemistry was performed 28 days after shock wave exposure, using Myosin VIIA, Cochlear hair cell marker), CtBP2 (synaptic ribbon marker), and GluR2 (synaptic receptor marker) on organs of Corti.

Results

Electrophysiological function of cochlear: In both shock tube and LISW groups, ABR thresholds, which indicate the “hearing level”, were remarkably elevated at one day after shock wave exposure, and have spontaneously recovered gradually. However, the permanent ABR threshold shifts about 10 - 20 dB were remained up to 28 days after shock wave exposure in both groups. The wave I amplitudes of ABR, which indicate the total amount of “cochlear signal output”, were also significantly reduced at one day after shock wave exposure in both groups. Interestingly, the wave I amplitude was not recovered in shock tube group, although the ABR threshold in the same group was spontaneously recovered. In contrast, in LISW group, the wave I amplitude was recovered at low frequencies, accompanied with the threshold recovery. DPOAE thresholds, which indicate the outer hair cell function, were not elevated in both shock tube and LISW group. **Pathological findings of cochlear:** Loss of the inner and outer hair cells was not observed in both shock tube and LISW groups. These results would be consistent with the results of DPOAE. Significant reduction of the number of synapses of the inner hair cells, and the orphan synapse degeneration (loss of receptor alone in the synapse) were observed in both groups. However, these changes were observed more prominent in LISW group compared to shock tube group. Moreover, tendency of severer synapse degeneration at high frequencies area in the organ of Corti was observed in LISW, whereas the consistent degenerative changes of synapse was observed in shock tube group. These results would also consistent with the patterns of synapse reduction in each group, because the wave I amplitude is thought to directly reflect the number of inner hair cell synapses.

Discussion

Recent previous reserches revealed that blast could induce hearing loss in all frequency range, while noise induced hearing loss would mainly be occurred only in higher frequencies^[2]. Both presented models could replicate these characteristics of blast induced hearing loss, thus these pathophysiology should be similar as the human physiologies in the patients with blast exposure in military field.

In both shock tube and LISW groups, the pathological findings were characteristics in reduction of synapses. However,

the patterns of synaptic degeneration were different, appeared at whole area of cochlea in shock tube group, whereas mainly damaged at high frequencies area in LISW group. It is suggested that pathophysiological differences between shock tube group and LISW group might be caused by the difference of conduction pathway of shock wave. The air-conducting shock wave generated by shock tube would be conducted via tympanic membrane to the inner ear directly. Therefore the excessive energy would directly injure all cochlear region, because the power spectrum of shock wave includes all frequency range. Alternatively, LISW conducted toward the inner ear via bone-conduction. Thus, low frequency elements of shock wave energy might be absorbed by skin and bone during transduction of shock wave vibration. This mechanism would result in the high frequency dominant damage of the synapses in the organ of Corti in LISW exposed mice. This phenomenon would represent the situation of blast exposure with ear protection, such as soldiers in the battle field with ear muff.

Conclusion

We analyzed the pathophysiology of blast induced hearing loss mouse models. These models were induced mild permanent sensorineural hearing loss, which represents blast induced hearing loss in human. The main etiologies of hearing loss of these models were degenerative changes of the inner hair cell synapses. The pattern of these degenerative changes would be varied according to the conduction pathway of shock wave energy.

[3] Remenschneider AK, Lookabaugh S and Quesnel AM, *Otol Neurotol*, **35** (2014) 1825.

[4] Joseph AR, Shaw JL, Galarneau MR, *AM J Epidemiol*, **187** (2018) 7

[5] Sato S, Kawauchi S, Okuda W, *PLOS ONE*, **9** (2014) e82891

[6] Satoh Y, Araki Y, Ishizuka T, *J Neuropathol Exp Neurol*, **77** (2018) 827

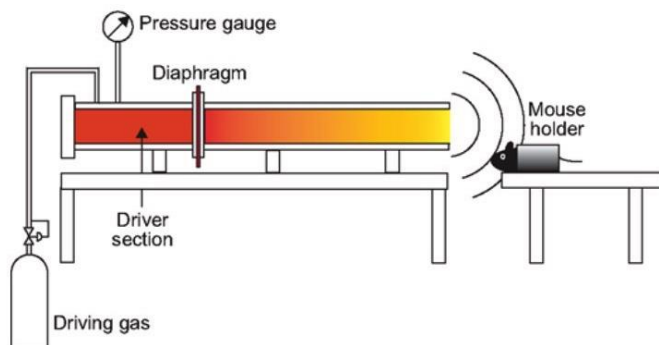


Figure 1: Setup of the shock tube system [3]

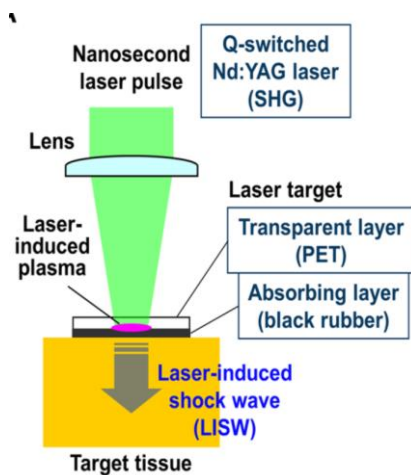


Figure 2: Setup for generating an LISW [4]

Experimental Study on Effect of Porosity of Porous Media on Pressure Behind Simulated Head Model with Helmet Subjected to Simulated Blast Wave

Taketoshi Koita¹, Kou Miyashita¹, Hiromitsu Hisano¹, Shouta Kajii¹, Kazuki Ueguri¹
and Susumu Kobayashi¹

¹ Faculty of Engineering, Saitama Institute of Technology, 1690 Fusaiji, Fukaya, Saitama, 369-0293, Japan

The mechanism of blast-induced traumatic brain injury (bTBI) has been studied medically [1,2]. The experimental and numerical studies on the interaction between the blast wave and the head model subjected to the blast wave loading were conducted to investigate the bTBI mechanism [3]. In these studies, the blast wave was generated by the explosive or the shock tube simulating the blast wave. The development of shock tube generating the simulated blast wave was also studied experimentally and theoretically in the study of bTBI [4,5]. The interaction of simulated blast wave generated by the shock tube with the simulated head model including the helmet was visualized to discuss the change of density gradient of gelatin simulating brain by the wave loading [6]. On the other hand, the buffer material inside the helmet reducing the blast wave loading to the head was not much investigated. The purpose of this study is to investigate the effect of porosity of porous media, the buffer material using in the helmet, on the attenuation of pressure behind the simulated head model with the helmet loaded by the simulated blast wave.

Figure 1 shows the schematic diagram of experimental model, the simulated head model with the helmet including the porous media. In this experiment, the head model was sandwiched by the porous media that width $w = 10$ mm and the acrylic plate of $w = 3$ mm simulating the buffer material and the helmet. The polyurethane foam was employed as the porous media. The four porosities of foam, $\phi = 0.934, 0.9603, 0.980, 0.982$ were tested by changing the foam, Foam 80, 55, 20, 13, respectively. The experiment at $\phi = 1.000$ that was the model with the air, the atmosphere, filled instead of Foam was also performed to research the effect.

We employed the averaged width and length of head for Japanese adult male [7] into the size of head model. In the head model, the gratin (20 wt%, $40 \times 100 \times 39.5$ mm) simulating brain was filled into the acrylic container ($50 \times 106 \times 49.5$ mm) simulating the averaged size skull as seen in Fig. 1. The experimental model was subjected to the simulated blast wave loading that pressure increased rapidly and dropped with the negative pressure by the shock tube developed in our previous study [6]. The model was fixed to the inner bottom wall of test section ($50 \times 200 \times 50$ mm) of the shock tube by the screws. Figure 2 shows the schematic diagram of shock tube with the abrupt expansion high pressure chamber generating the simulated blast wave. In this experimental conditions, Mach number was set to 1.48, the TNT equivalent of 0.90 kg and the distance from explosive point of 2.00 m were tested. The length x_a and the pressure p_4 of high pressure chamber in the shock tube was calculated by the theoretical method [6] in the conditions. The calculated value, $x_a = 800$ mm and $p_4 = 7.11$ atm, were set in the high pressure chamber. The diaphragm was ruptured by the firing needle. To research the effect of porosity of foam on the attenuation of over pressure behind the experimental model subject to the blast wave loading, the over pressures behind the model with the changed Foams and with the air, were measured by the pressure sensor 3 (Type603C, KISTLER) set in the test section of shock tube as seen in Fig. 2.

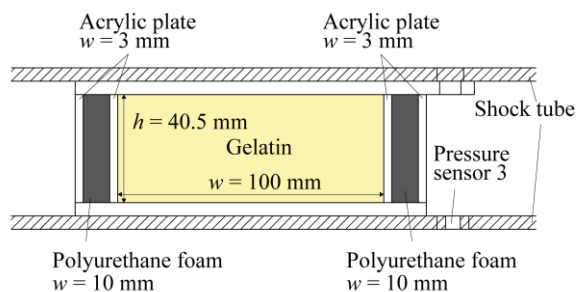


Fig. 1 Schematic diagram of simulated head model without the helmet set in shock tube.

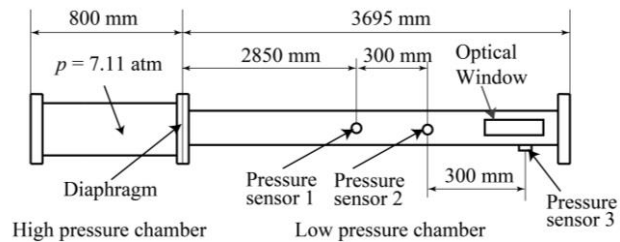


Fig. 2 Schematic diagram of simulated head model with the helmet set in shock tube.

Figure 3 indicates the time histories of over pressure of simulated blast wave measured by the pressure sensor 3 without the experimental model. The measurement was conducted three times to investigate the repeatability of generation of the blast wave. The over pressure profiles for three shots were almost same each other in Fig. 3. This meant the simulated blast wave was repeatably generated by the shock tube with high repeatability. The peak over pressure of shock wave reached about 185.5 kPa and the negative pressure of wave appeared about -30 kPa in Fig. 3. Figure 4 presents the time histories of over pressure behind the simulated head model mounting the helmet with the changed Foams and the air subjected to the pressure loading of simulated blast wave as shown in Fig. 3. The peak over pressures Δp_{\max} at all Foams and the air were largely reduced compared to the peak over pressure of the blast wave. These pressures were decreased by repeating the sudden rise and fall fluctuations in Fig. 4. Figure 5 and 6 show the peak over pressure Δp_{\max} and the peak negative pressure Δp_{\min} behind the model for the porosities. The order of peak

over pressures for all Foams were similar with that for the air in Fig. 5. It seems that Δp_{\max} is decreased and Δp_{\min} is increased as ϕ increases in Fig. 5 and 6. These results suggest two conclusions. One conclusion is that the helmet with the polyurethane foam decreases the peak over pressure behind the simulated head model with the foam loaded by the blast wave as well as that with air instead of foam. The other is the increase of porosity in the Foam decreases the peak over pressure and increases the peak negative pressure behind the simulated head model mounting the Foam.

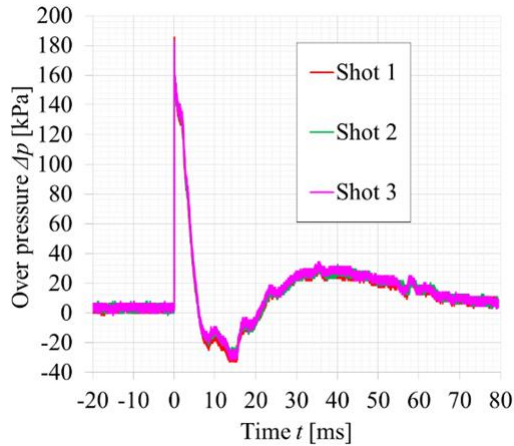


Fig. 4 Time histories of over pressure of simulated blast wave measured by the pressure sensor 3.

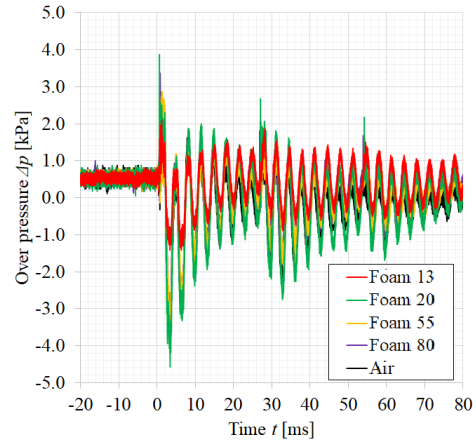


Fig. 5 Time histories of over pressure behind the simulated head model mounting the helmet with changed porosity of foam and the air.

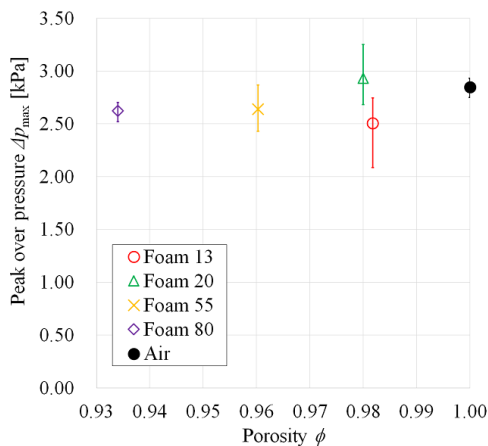


Fig. 6 Relationship between the porosity and the peak over pressure behind the experimental model.

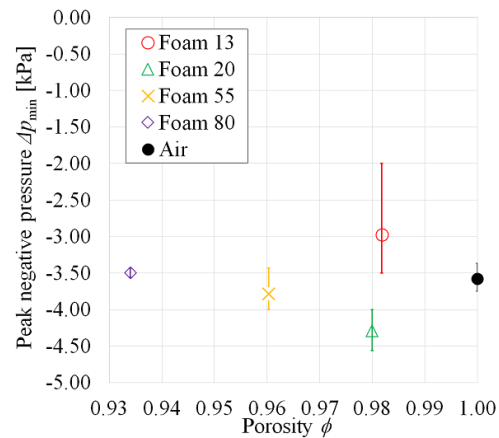


Fig. 7 Relationship between the porosity and the peak negative pressure behind the experimental model.

References

- [7] S. Sato, S. Kawauchi, W. Okuda, I. Nishidate, et. al., *PLoS One*, **9** (2014) 1.
- [8] A. Nakagawa, G. T. Manley, A. D. Gean, K. Ohtani, R. Armonda, et. al., *J. neurotrauma*, **28** (2011) 6.
- [9] K. Ohtani, A. Nakagawa, et. al., *Proc. of the Symp. on Shock Waves in Japan FY2013*, **1B3-3** (2014).
- [10] T. Mizukaki, Y. Yabe, T. Nagao, et. al., *Proc. of the Symp. on Shock Waves in Japan FY2016*, **1C1-2** (2017).
- [11] T. Koita, S. Kobayashi, *Proc. of the Fall Japan conference of Japan Explosive Society*, **38** (2018).
- [12] T. Koita, S. Kobayashi, *Proc. of the 3rd Japan Japan-US Technical Information Exchange Forum on BLAST INJURY*, (2018).
- [13] National Institute of Advanced Industrial Science and Technology, Data base of basic human body measurements1991-92, **A1, A2** (1992).

Mechanism-based brain models to study primary blast loading effects on axonal deformation: the past, present and future

R. H. Kraft¹, R. Menghani¹, H. T. Garimella³, A. Eidsmore², M. Kleinberger², A. J. Przekwas³

¹ The Pennsylvania State University

² U.S. Army Research Laboratory

³ CFD Research Corporation

Subject-specific computer models (male and female) of the human head were used to investigate the possible axonal deformation resulting from the primary phase blast-induced skull flexures [1]. The corresponding axonal tractography was explicitly incorporated into these finite element models using a recently developed technique based on the embedded finite element method [2]. These models were subjected to extensive verification against experimental studies which examined their pressure and displacement response under a wide range of loading conditions. Once verified, a parametric study was developed to investigate the axonal deformation for a wide range of loading overpressures and directions as well as varying cerebrospinal fluid (CSF) material models. This study focuses on early times during a blast event, just as the shock transverses the skull (< 5 milliseconds). Corresponding boundary conditions were applied to eliminate the rotation effects and the corresponding axonal deformation. A total of 138 simulations were developed - 128 simulations for studying the different loading scenarios and 10 simulations for studying the effects of CSF material model variance - leading to a total of 10,702 simulation core hours. Extreme strains and strain rates along each of the fiber tracts in each of these scenarios were documented and presented here. The results suggest that the blast-induced skull flexures result in strain rates as high as 150-378 s⁻¹. This rapid deformation of the axonal fiber tracts, caused by flexural displacement of the skull, suggests the possibility of rate dependent micro-structural axonal damage according to the published experimental studies. Ongoing work is focused on quantifying helmeted versus un-helmeted effects. In the second part of the presentation we will discuss the development of history-dependent tissue models that further enable mechanism-based modeling and ideas on how computational brain simulations can be used more broadly. This topic touches on the emerging field of computational brain medicine [3].

Conflict of Interest: Dr. Kraft has a financial interest in Digital Brain Technologies, LLC, a company which could potentially benefit from the results of this research. This interest has been reviewed by Penn State University in accordance with its Individual Conflict of Interest policy for the purpose of maintaining the objectivity and integrity in research and is being managed.

Funding: The authors acknowledge support provided by the U.S. Army Research Laboratory under contracts W15P7T-10-D-D416 and DOTC-17-01-INIT0086, and CFD Research Corporation under a subcontract funded by the Department of Defense, Department of Health Program through contract W81XWH-17-C-0216 and NSF CAREER Award # 1846059 (PI Kraft).

[1] H. T. Garimella, A. Przekwas, and R. H. Kraft, "Do blast-induced skull flexures result in axonal deformation?," *PloS ONE*, vol. 13, no. 3, p. e0190881, 2018.

[2] H. T. Garimella and R. H. Kraft, "Modeling the mechanics of axonal fiber tracts using the embedded finite element method," *Int. J. Numer. Methods Biomed. Eng.*, vol. 33, no. 5, 2017.

[3] H. T. Garimella and R. H. Kraft, "A new computational approach for modeling diffusion tractography in the brain," *Neural Regen. Res.*, vol. 12, no. 1, p. 23, 2017.

Neurocognitive Effects of Blast Overpressure from Breaching Courses: Using DANA To Assess Performance Related To Immediate And Cumulative OP Exposure

C.H. LaValle¹, M.J. Egnoto¹, W. Carr¹, G.H. Kamimori¹

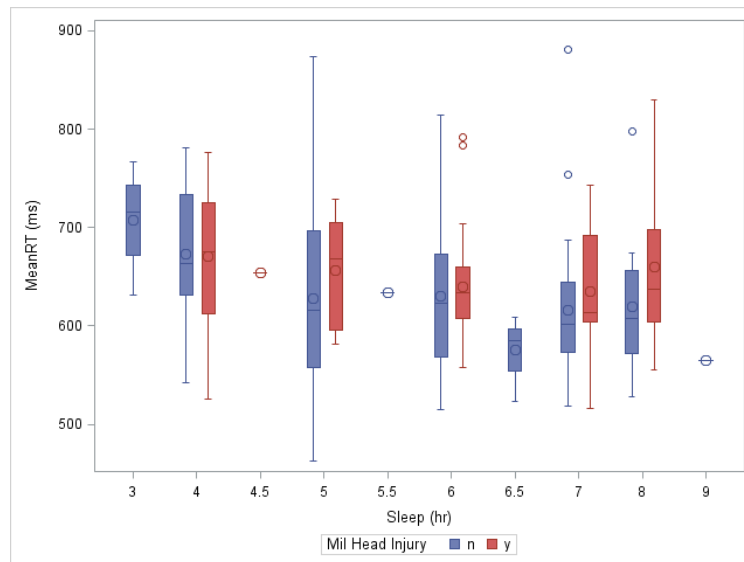
¹ Walter Reed Army Institute of Research

Background: Blast overpressure (OP) consequences from low to moderate pressure exposures have received increasing military/research attention. Previous investigations on OP have found effects from exposure, upon anecdotal reports of ‘breachers brain’, which create issues with sleep disturbance, mild cognitive impairment, and ringing of the ears [1,2,3]. Other works have investigated the lasting effects of OP exposure by looking at variation in baseline scores of military personnel with different OP experiences [4]. This work evaluates a cohort of 117 students over four breacher training courses conducted with the 35th Engineer Battalion located at Ft. Leonard Wood Missouri from 2017-2018 on neurocognitive function with use of the DANA Rapid [5]. Specifically, this work evaluates how prior experiences (like military-based head injuries) impact baseline performance on the DANA Rapid, how environmental variables like sleep duration influence DANA Rapid scores, and how blast exposure impacts both sleep quality and DANA performance.

Methods: From 2017 to 2018 a total of four breacher training courses were evaluated by a team of researchers investigating the effects of blast OP exposure across student subjects. After analysis for outliers, 117 of the original 136 subjects remained. Primary reasons for removal were status (instructors were eliminated from analyses, n = 8), or baseline data that was greater than 3 mean absolute deviations (n = 11). All four courses evaluated had students experience two major blast exposures during training (concrete wall blasts: NEW 10lbs). Students were equipped with three B3 gen 6 blast gauges (Rochester, NY) located on the helmet, and on the outside of both shoulders (near the flag patch on a modern military combat shirt). OP exposure ranged from 2.57 to 9.17 psi as measured from the shoulder of the participant (best incident measurement) with an average OP of 4.86 psi (SD = 0.13). Impulse ranged from 3.93 to 18.8 psi*ms with an average impulse of 11.87 psi*ms (SD = 5.34). DANA Rapids were administered at baseline (prior to the first training event on day 1) and immediately following back-to-back breaching charges. The DANA Rapid consists of three tests, evaluating Simple Reaction Time (SRT) Procedural Reaction Time (PRT) and Go/No-Go (GNG). As tests move from SRT to GNG they increase in cognitive complexity, testing reaction time along with decision-making. Additionally, an occupational history questionnaire was administered asking about duration of military service, prior head injury, general health (like average sleep duration) and general demographics.

Results: Baseline – To evaluate how subject history affected reaction time, open-ended responses on head injury prior to enrollment in the course were evaluated and cross coded by two-researchers ($r_s = .87$) to determine if head injuries described were incurred in the military. Of the 4 total disagreements in the dataset, two were coding error and the remaining two were based on vocabulary. The most common head injury found in the sample was IED/VBIED exposure. Results indicated a NS difference in SRT, PRT, and GNG, though in each instance prior head injury scores were slower reaction times – the difference did not meet statistical significance thresholds. Additional testing years of military service, hours of sleep prior to the training course, and SRT, PRT, and GNG were conducted. Years of military service had similar findings, such that NS trends existed (as years of experience increase, reaction time gets slower), but failed to hit criteria of research or clinical significance. Sleep duration, however, was statistically significant for SRT and PRT resulting in a 5 ms and 11 ms reaction time delay for each hour of sleep loss respectively. PRT yielded the most intriguing results (see figure 1) that suggested a trend whereby those with prior military head injuries while sleep deprived had slower reaction times. Overall, subjects with more sleep performed better on DANA responses. The rate of improvement did diverge such that on average, each hour of sleep would decrease performance (increase time for tests) on DANA testing for PRT and GNG by 13 seconds for those without prior head injury, but only improved on average by 6 seconds for those with prior military head injuries. These findings too however, failed to meet thresholds of significance.

Figure 1 – PRT Mean Reaction Scores and Amount of Sleep Between Those With/without Prior Military Head Injuries



Post-Blast Effects - A series of General Linear Models (GLM) were conducted to evaluate how blast experience from the course (above or below 4psi peak OP), years of military experience, sleep duration, and history of self-reported head-injury impacted DANA results as compared to baseline; analyses were conducted on percent change from baseline. Trends were consistent such that more experience, less sleep, and higher overpressure exposures resulted in worse DANA performance, but findings were sporadic in terms of meeting statistical thresholds (see table 1 below).

Ship Crew Injury Risk and Survivability—An Assessment of Injury Mechanics Weapons Effects Testing as Part of RIMPAC

J. Mackiewicz¹, A. Olson¹, M. Clouser^{1,2}, M. Galarneau¹

¹Naval Health Research Center, San Diego, California

²Leidos, San Diego, California

Navy knowledge gaps exist regarding quantification of the real-world weapon effects loading environment and crew casualties resulting from attacks by real-world, above-water weapons. Key risks posed to crew include blast overpressure, penetrating shrapnel and debris, and physical impacts against ship infrastructure or equipment. The lack of this understanding impedes preparation for injury mitigation and treatment strategies. The Navy has validated computational models to predict ship survivability and the dynamic loading environments resulting from a range of threats and threat engagement scenarios due to AIREX (missiles) and UNDEX (underwater detonations). However, lacking in this capability is the technical understanding of the forces, loading states, and subsequent injury levels for ship crews due to a threat engagement scenario. This understanding and the ability to correlate forces/loads to injuries resulting from a threat engagement are essential to predicting injury outcomes and mitigation strategies and informing models. Ultimately, a software toolkit is needed to practically link the dynamic loadings potentially experienced by ship occupants with likely injury outcomes that will effectively evaluate both ship and crew survivability.

Recently, the Navy (Naval Health Research Center and Naval Surface Warfare Center) has been conducting ship impact testing during AIREX and UNDEX threat engagements, which includes instrumented human manikin systems to collect data that may be relevant for injury prediction methods. To date, data on the weapon effects loading environment experienced by a ship's crew under real-world attack by an above-water weapon have been collected during live-weapons firing events at RIMPAC 2018 Fleet Exercise. Data collections are being planned for future live weapons firing events with more sophisticated manikins and instrumentation. Having data from these live-fire events is invaluable; however, this collected data must be translated into injury profiles to be useful in modeling.

The Navy Survivability Assessment Model (NavSAM) is a demonstrated, fast-running computational platform that is well positioned to provide a foundation that bridges the gap between loads and injury levels, once the correlations to injury have been developed. The current focus is to develop the understanding and relationships between forces and loads generated by a threat to the subsequent injury levels that crew may experience, accounting for relative locations to the threat detonation center and shipboard confinement using current data and future collections. The key outcomes of this effort will be experimental data, insights to injury mechanics, and computational models that address injury risk prediction for exposures relevant for Surface Combatant engagements. This will ultimately enable fast-running, statistically-based assessments of ship crew survivability for penetrating, blast, and blunt-impact trauma. Ultimately, NavSAM will be used to make injury risk predictions and survivability assessments for ship occupants positioned in multiple ship locations (e.g., bridge, command centers, engine rooms, sleeping compartments, magazines, etc.), generating survivability and functionality assessments to enhance the Navy's ability to address escalating threat engagements and provide response strategies to protect the ship and crew.

Disclaimer: I am a military service member or employee of the U.S. Government. This work was prepared as part of my official duties. Title 17, U.S.C. §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C. §101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. Approved for public release; distribution is unlimited.

This work was supported by the U.S. Navy Bureau of Medicine and Surgery under work unit no. N1215. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

Primary blast-induced mild traumatic brain injury shows changes in MRI and immunohistology in a rat model of blast-induced behavioral abnormality

Hiroshi Matsuura¹, Mitsuo Ohnishi¹, Yoshichika Yoshioka², Sanae Hosomi¹, Kentaro Shimizu¹, Hiroshi Ogura¹, Takeshi Shimazu¹,

¹Department of Traumatology and Acute Critical Medicine, Osaka University Graduate School of Medicine,

²Center for Information and Neural Networks (CiNet), National Institute of Information and Communications Technology (NICT) and Osaka University,

Introduction: Victims of blast-induced mild traumatic brain injury (mTBI) are increasing worldwide due to terrorism, and many suffer from after-effects of physical or mental impairment. Diagnosing primary blast-induced mTBI is difficult due to few findings on imaging. This study aimed to detect new findings for therapeutic intervention in a rat model of behavioral abnormality after blast-induced mTBI.

Methods: We used a bench-top blast wave generator with the blast wave exiting through a 20-mm I.D. nozzle aimed at the focused target. The blast wave was directed at the head of male SLC:Wistar rats weighing 247 ± 3.9 g under general anesthesia positioned prone 2.5 cm below the nozzle. Peak shock wave pressure at this point was 646.2 ± 70.3 kPa. Our previous study showed post-blast behavioral abnormality in this rat model in a forced swim test and Y-maze test. We assess this rat model with specialized magnetic resonance imaging (MRI) modalities (11.7-T scanner) and immunohistochemically at day 3, 2 weeks and 6 weeks after blast injury.

Results: The blast-induced mTBI model showed no macroscopic findings of brain hemorrhage or contusion. However, food intake decreased significantly in the blast group rats, and they lost weight compared to control rats (-18 vs. +10 g on day 3; $P=0.001$) in the early post-injury phase. Behavioral analysis in the blast group showed increased immobility time in the forced swim test at 2 (165 vs. 125 s; $P=0.006$) and 6 weeks (199 vs. 162 s; $P=0.01$), and the percentage of spontaneous alternation in the Y-maze test was significantly smaller than that of the control group (82% vs. 60%; $P=0.03$) at 2 weeks. Specialized MRI showed bilateral inflammation of the oriented layers of the hippocampus at 3 days and 2 weeks. Immunohistochemical analysis by Iba1 showed microglial accumulation in the same region, and detected activated microglia at 3 days and 2 weeks. Anti-neuronal nuclei (NeuN) antibody immunostaining showed a gradual decrease in NeuN-positive neurons over time in the pyramidal cell layer of the hippocampus.

Conclusions: Specialized MRI and immunohistochemical analysis enabled visualization of new abnormal findings of depressive-like behavior and short-term memory disturbance in a rat model of blast-induced behavioral abnormality.

This abstract have submitted to the 78th Annual Meeting of The American Association for the Surgery of Trauma.

5-cm-by-5-cm Detonation-Driven Blast Simulator for Fluid Dynamic Research on Blast Injury

T. Mizukaki¹, A. Kato², M. Mori², Y. Sekine³, and D. Saitoh³

¹ Dept. of Aeronautics and Astronautics, Tokai University, Japan

² School of Engineering, Graduate School, Tokai University, Japan

³ National Defense Medical College, Ministry of Defense, Japan

Typically, experimental blast wave studies require costly, large scale, free field testing. Shock waves can be generated on the laboratory scale with the use of shock tubes. However, the gas dynamic evolution of the flow generated by many shock tubes is not representative of free field blast waves, decreasing their usefulness in such studies. The focus of this work is the design and implementation of a shock tube facility capable of producing realistic blast wave profiles on a laboratory scale with a high degree of reproducibility.

Compare to plane shock waves generated inside a shock tube, blast waves have three characteristics. Firstly, blast wave has a sharp peak overpressure following decayed pressure profile. Secondary, at the latter part of the decayed pressure profile, blast wave often has “negative pressure” that is lower than the initial pressure at quiet region. Finally, blast wave has the secondary shock wave that is the reflected shock wave of the implosion shock wave generated by overexpansion of the combustion gas. In order to simulate blast wave for blast injury research, the flow field generated must have the characteristics described above.

As the first step of this study, to generate blast-like-planer shock waves, modifications of a diaphragm-type-shock tube both on volume and shape of the high-pressure chamber, method of high-pressure release, and the cross-section of the low-pressure channel, have been investigated. The shock tube which has cross section of 50-by-50-mm², a high-pressure chamber with 500-mm in length, a low-pressure channel with 3040-mm in length, and a middle-pressure chamber with 20-mm in length, has been used (Fig. 1). Negative pressure was observed inside the shock tube with an expanded channel which was attached between the high-pressure chamber and the low-pressure channel. Finally, detonation driver has been connected to produce strong shock wave into the low-pressure channel. Both experimental and numerical investigation has been examined to obtain blast-like-planer shock waves inside the shock tube (Fig. 2).

As the second step, the high-pressure chamber was replaced by the detonation tube which is shown in Figure 3. The detonation tube is 58 mm in inner diameter, 1 m in length. Ethylene as fuel and Oxygen as oxidizer are mixed under stoichiometric condition in the high-pressure chamber with total pressure 1 atm. and separated with a diaphragm (PET) from low-pressure channel. The mixture gas was ignited with spark-plugs SP connected to a high-voltage source HV. Traveling Combustion wave was obtained with ion probes IP with power supply PS and pressure history was with PZT-type pressure transducer PT through signal conditioner SC. Both signals were recorded with digital storage oscilloscope DSO.

Figure 4 shows the signal history of pressure transducers and ion-probes. The combustion wave which were detected with ion-probes and discontinuously pressure rise which were detected with pressure transducers simultaneously arrived at the measurement points. This simultaneous arrival of the both waves indicates that detonation was successfully generated.

The final step of the present study is to connect the detonation-driver to the low-pressure channel which had been optimized for obtaining blast-like pressure wave at the test section. The pressure wave obtained at the test section is shown in Fig.5. The obtained history indicates that overpressure of 700 kPa with appx. 1.5 ms and positive pressure which was longer than 8.0 ms was produced at the test section.

As the summary of the present study, 5-cm-by-5cm detonation driven shock tube with long low-pressure channel is produced blast-like pressure profile at the test section and useful for fluid dynamic study on blast injury mechanism.

[1] Chandra, N., Ganpule, S., Kleinschmit, N.N., Feng, R., Holmberg, A.D., Sundaramurthy, A., Selvan, V., and Alai, A., Shock Waves, 2012. 22: p. 403-415.

[2] Ritzel, D.V., Parks, S.A., Roseveare, J., Rude, G., and Sawyer, T.W., The Research and Technology Organization of NATO Halifax, Canada.

[3] Schraml, S.J. and Pearson, R.J., Computer Program for LB/TS Test Design: Technical Description, Usage Instructions and Source Code Listings. 1995, Army Research Laboratory.

[4] Schraml, S.J., 1991, Ballistic Research Laboratory.

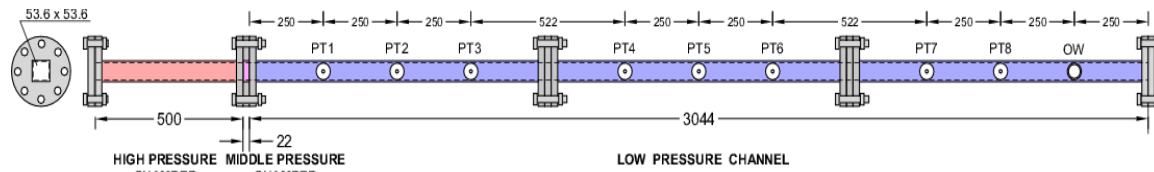


Figure 1. Schematic diagram of the shock tube for blast-like-pressure study.

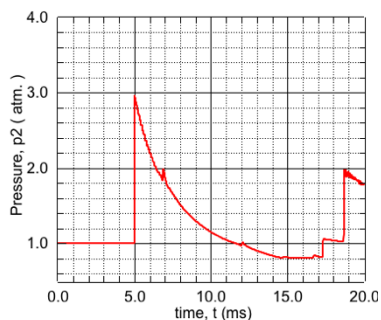


Figure 2. Pressure history inside the test section.

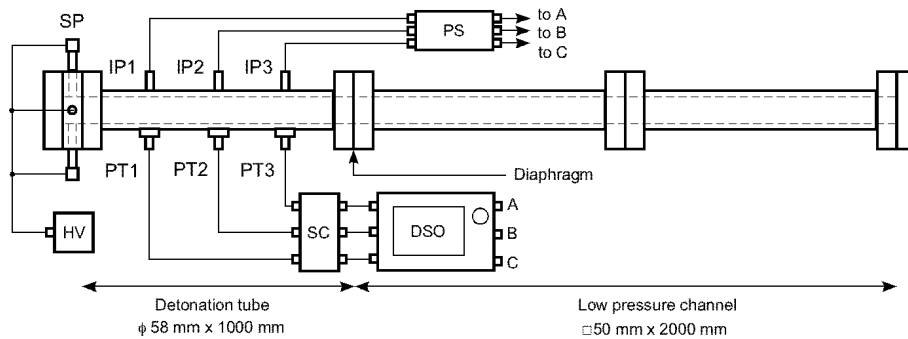


Figure 3. Schematic diagram of the detonation driver and low-pressure channel.

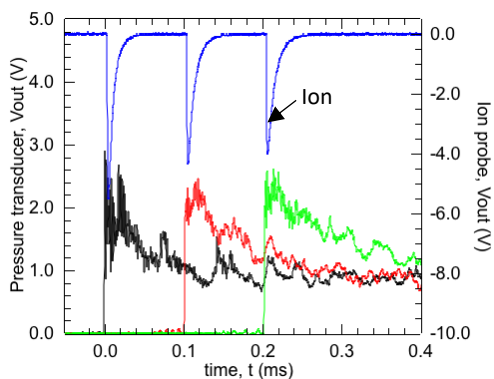


Figure 4. Pressure and ion-probe signal inside the detonation driver.

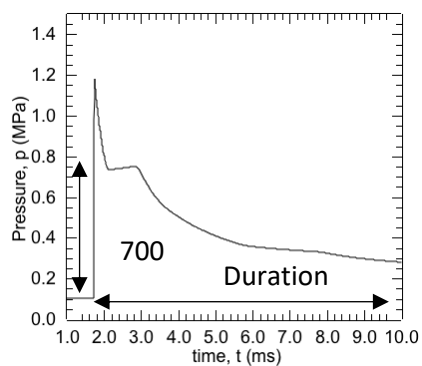


Figure 5. Pressure history of detonation driven blast wave at the test section.

Fluid Dynamical Evaluation of Pressure History of Blast Simulator at National Defense Medical College

T. Mizukaki¹, A. Kato², M. Mori², Y. Sekine³, and D. Saitoh³

¹ Dept. of Aeronautics and Astronautics, Tokai University, Japan

² School of Engineering, Graduate School, Tokai University, Japan

³ National Defense Medical College, Ministry of Defense, Japan

Pressure history inside the blast simulator which has been installed at National Defense Medical College, Japan., is experimentally and numerically evaluated from fluid dynamical point of view. Both the pressure history inside shock tube and the test room of the blast simulator have been obtained and compared with computational result. Pressure history was recorded with PZT-type pressure transducers. Statistic and dynamic pressure transducers were installed just in front of the examined animal with careful alignment toward the center point of discharge surface of the blast simulator. High-pressure air was charged up to 5 MPaG and released by rupturing of double-type diaphragm which is made of stainless steel. Speed and Mach number of the incident shock wave was determined by time-difference between the pressure transducers. Finally, the incident shock wave was discharged from the open-end of the blast simulator and propagated toward the examined animal. Interaction between the incident shock and body surface of the examined animal is computed with commercially available hydro-code, AUTODYN.

[1] Chandra, N., Ganpule, S., Kleinschmit, N.N., Feng, R., Holmberg, A.D., Sundaramurthy, A., Selvan, V., and Alai, A., Shock Waves, 2012. 22: p. 403-415.

[2] Ritzel, D.V., Parks, S.A., Roseveare, J., Rude, G., and Sawyer, T.W., The Research and Technology Organization of NATO Halifax, Canada.

[3] Schraml, S.J. and Pearson, R.J., Computer Program for LB/TS Test Design: Technical Description, Usage Instructions and Source Code Listings. 1995, Army Research Laboratory.

[4] Schraml, S.J., 1991, Ballistic Research Laboratory.

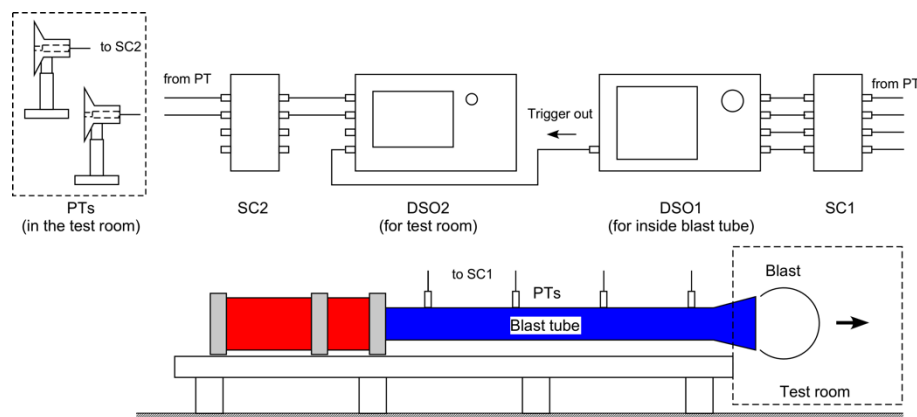


Figure 1. Schematic diagram of experimental setup of the blast simulator installed NDMC.

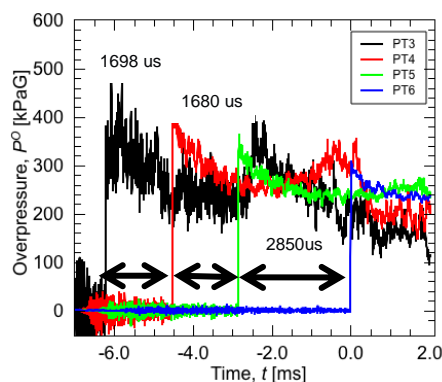


Figure 2. Pressure history inside the blast simulator.

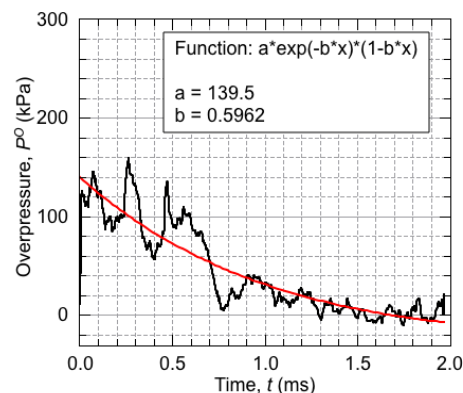


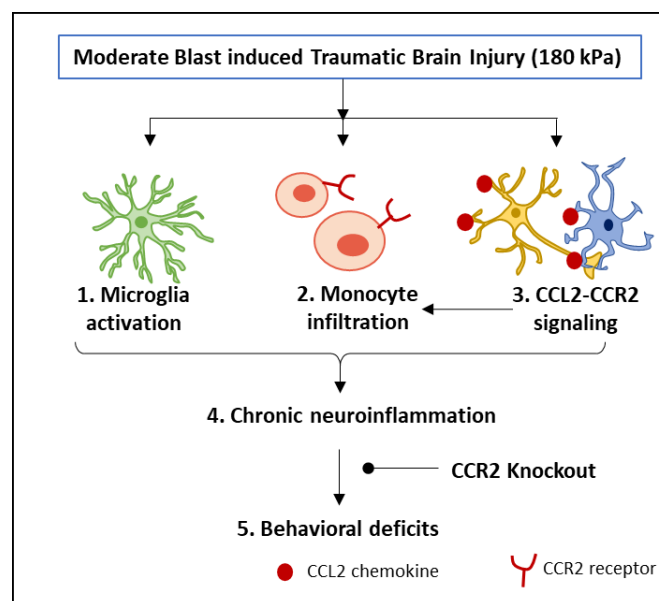
Figure 3. Statistic pressure history in front of examined animal.

Role of resident microglia and circulating monocytes in chronic neuroinflammation and behavioral outcomes in a mouse model of blast-induced traumatic brain injury

M. Murugan^{1*}, N. Chandra¹

¹ Department of Biomedical Engineering, Center for Injury Biomechanics materials and medicine, New Jersey Institute of Technology, Newark, NJ 07102

Moderate blast-induced traumatic brain injury (bTBI) is associated with microglia/macrophage accumulation, chronic neuroinflammation and neurobehavioral deficits [1-3]. However, the origin of macrophages, whether it is sourced by local microgliosis alone, or supplemented by infiltration of peripheral monocytes remains to be investigated [4]. In this talk, we discuss the possibility of circulating blood borne monocytes entering the brain following bTBI. This requires the use of transgenic mice to tease out the differences between resident microglia and infiltrating monocytes. Using state of the art tools such as time lapse two-photon imaging and double transgenic mice- CCR2RFP/+; CX3CR1GFP/+, in which CX3CR1-positive resident microglia are tagged with GFP and CCR2-positive infiltrating monocytes are tagged with RFP, we were able to identify a spatial and temporally distinct pattern of microglia activation and monocyte infiltration following moderate bTBI (180 kPa). The mechanism underlying monocyte infiltration in bTBI, including mechanical disruption of the blood brain barrier and chemokine attraction of circulating monocytes will be elucidated. Results will be presented that demonstrate how activation of resident microglia and infiltration of monocytes contribute to chronic increase in production of proinflammatory cytokine IL-1 β that further leads to neurobehavioral consequences such as increased anxiety and decline in short-term memory. While neuroinflammation is a common target for treating bTBI, the talk will attempt to illustrate how infiltrated monocytes might be an ideal target for ameliorating bTBI-induced behavioral alterations or any pathology in which monocyte infiltration is implicated.



[1] B.R. Huber, et al., *Neuroscience*, **319** (2016): p. 206-20.

[2] E.C. Petrie, et al., *J Neurotrauma*, **31**(2014): p. 425-36.

[3] A.F. Ramlackhansingh, et al., *Ann Neurol*, **70** (2011): p. 374-83.

[4] D.M Trahanas, et al., *Shock*, **43** (2015): p. 255-60.

Visualization of neurovascular functions in *in vivo* rat brain using RGB camera-based diffuse reflectance imaging

I. Nishidate¹, T. Osawa¹, M. Inaba¹, S. Kawauchi² and S. Sato³

¹ Graduate School of Bio-applications and Systems Engineering, Tokyo University of Agriculture and Technology

² Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute

Changes in optical properties of the brain have been used to deduce spatial and/or temporal changes of neuronal activity and tissue viability in the brain as intrinsic optical signal (IOSs). It is believed that IOSs in the brain are mainly caused by the following three processes: changes in absorption and scattering originating from cerebral hemodynamics, changes in absorption due to redox states of cytochromes in mitochondria, and changes in scattering generated by cell swelling or shrinkage caused by water movement between intracellular and extracellular compartments [1]. Cerebral hemodynamics are important for evaluating brain function and tissue viability. IOSs also have potential for evaluating pathophysiology of brain, such as cortical spreading depolarization (CSD). CSD is a wave of neuronal and glial depolarization propagating at 2 to 4 mm/min over cerebral cortex followed by the temporal changes in cerebral blood flow [2]. CSD is an important disease model for migraine and is related to other neurological disorders, such as neurotrauma, seizure, ischemia and traumatic brain injury. We have developed a simple and rapid imaging method with a digital red-green-blue (RGB) camera to quantify optical properties of *in vivo* brain tissue and demonstrated the feasibility of the method to evaluate physiology and functions in *in vivo* rat brain [3].

In the developed method [3], the RGB values were converted into tristimulus values in the CIE (Commission Internationale de l'Éclairage) XYZ color space, which is compatible with the common RGB working spaces. Monte Carlo simulation for light transport in tissue was then used to specify the relationship among the tristimulus XYZ values and the concentrations of oxygenated hemoglobin (C_{HbO}), deoxygenated hemoglobin (C_{HbR}), and the scattering power b in the expression of $\mu_s' = a\lambda^{-b}$ as a scattering parameter. Total hemoglobin concentration is simply calculated as $C_{HbT} = C_{HbO} + C_{HbR}$, whereas tissue oxygen saturation of blood is determined as $StO_2\% = [C_{HbO}/(C_{HbO} + C_{HbR})] \times 100$. In the animal experiments, A white-light emitting diode (LED) illuminated the surface of the exposed cortex via a light guide and a ring-shaped illuminator with a polarizer. The light source covered a visible wavelength range from 400 to 700 nm. Diffusely reflected light was received by an RGB CCD camera via an analyzer and a camera lens to acquire an RGB image. The primary polarization plate (ring-shaped polarizer) and the secondary polarization plate (analyzer) were placed in a crossed Nicols alignment in order to reduce specular reflection from the sample surface. A standard white diffuser was used to regulate the white balance of the camera. The field of view of the system was 9.31×6.98 mm² with 1024×768 pixels. The lateral resolution of the images was estimated to be 9.1 μ m. The RGB images were then used to estimate the images of C_{HbO} , C_{HbR} , C_{HbT} , StO_2 and scattering power b according to the process described above.

Figure 1 shows typical *in vivo* resultant images for C_{HbO} , C_{HbR} , C_{HbT} , StO_2 , and b obtained from an exposed rat brain while varying fraction of inspired oxygen FiO_2 . Values of C_{HbO} and C_{HbR} decreased and increased, gradually, as FiO_2 decreased, which caused decreases in StO_2 . The value of C_{HbT} gradually increased after the onset of severe hypoxia ($FiO_2=6\%$), implying an increase in blood flow compensating for a lack of oxygen to the brain. Time courses of C_{HbO} , C_{HbR} , C_{HbT} , and StO_2 while changing FiO_2 were consistent with well-known physiological responses to changes in FiO_2 . The value of b began to increase before the onset of anoxia ($FiO_2=0\%$), and then decreased rapidly immediately after respiratory arrest (RA). The change in light scattering after respiratory arrest therefore implies morphological changes in brain tissue induced by anoxic depolarization (AD) [4,5] caused by osmotic cell swelling due to failure of the Na^+K^+ ATPase pump [4,5]. The results indicate the potential of the method to evaluate tissue viability.

Figure 2 (a) shows typical sequential images of measured RGB color, change in total hemoglobin ΔC_{HbT} , and change in scattering power Δb obtained from a rat brain that was exposed to a laser-induced shock wave LISW. The wave propagations of changes in both ΔC_{HbT} and Δb spreading over the cerebral cortex can be observed in Fig. 2(a). The wave of decreased Δb preceded the wave of increased ΔC_{HbT} . Figure 2 (b) shows the typical time courses of C_{HbT} and b averaged over the region of interest (ROI) on parenchyma area. Two distinct hemodynamic changes can be observed in Fig. 2(b) as responses to LISW. The first is hypoperfusion due to vasoconstriction that synchronizes with the decrease in scattering power b . The second change is profound hyperemia that is observed at or soon after the onset of decrease in scattering power b . It is said that the direct current shift of extracellular local field potential is coincident with a rise in extracellular potassium and can evoke cell deformation generated by water movement between intracellular and extracellular compartments, and hence the light scattering by tissue [5]. Therefore, the decrease in b before the profound increases in C_{HbT} is indicative of the change in light scattering related to morphological alteration due to CSD induced by LISW. Figure 2 (c) shows long-term observation of StO_2 before and after application of LISW. The value of StO_2 significantly dropped to at most 33% of the level before LISW application, which indicates long-lasting hypoxemia.

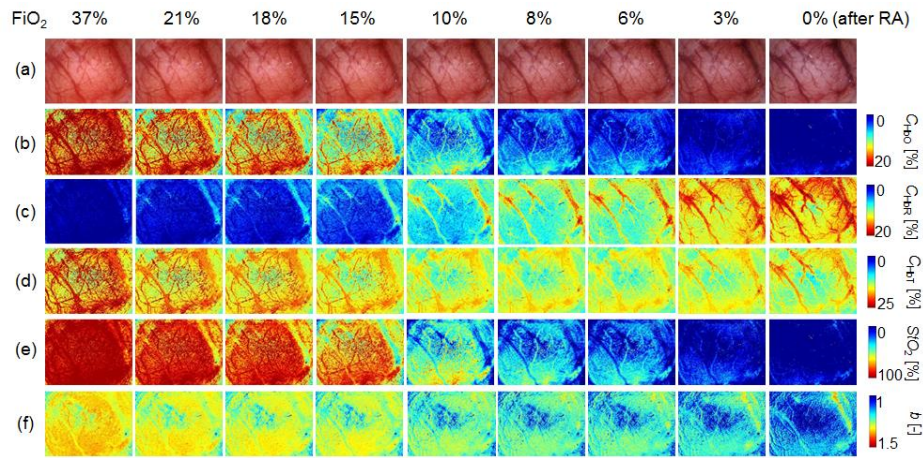


Fig. 1 Typical images of exposed rat brain obtained while changing FiO_2 for (a) original RGB image, (b) oxygenated hemoglobin C_{HbO} , (c) deoxygenated hemoglobin C_{HbR} , (d) total hemoglobin C_{HbT} , (e) tissue oxygen saturation StO_2 , and (f) scattering power b for scattering power.

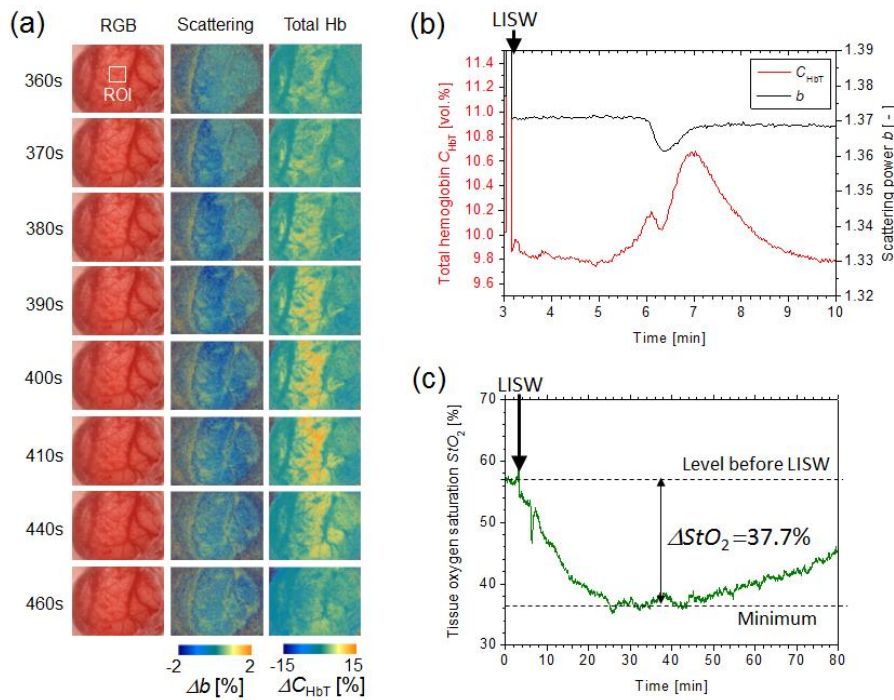


Fig. 2 (a) Typical sequential images obtained from the rat brain that was exposed to LISW for the RGB color, ΔC_{HbT} and Δb . (b) Time courses of C_{HbT} and b averaged over the area for the region of interest (ROI) on the parenchyma region (corresponding to the white square on the RGB image in Fig.2 (a)) immediately after application of LISW. (c) Long-term observation of StO_2 averaged over the area for the ROI on the parenchyma region before and after application of LISW.

Acknowledgement

Part of this work was supported by the US -ARMY ITC-PAC Research and Development Project (FA5209-15-P-0175, FA5209-16-P-0132).

- [1] T. Bonhoeffer and A. Grinvald, [Brain mapping: The Methods], Academic Press, San Diego, 1996.
- [2] C. Ayata, H. K. Shin, S. Salomone, Y. Ozdemir-Gursoy, D. A. Boas, A. K. Dunn, and M. A. Moskowitz, *J. Cereb. Blood Flow Metab.*, **24** (2004) 1172.
- [3] A. Mustari, T. Kanie, S. Kawauchi, S. Sato, M. Sato, Y. Kokubo, and I. Nishidate, *Int. J. Mol. Sci.*, **19** (2018) 491.
- [4] A. J. Hansen, *Physiol. Rev.* **65** (1985) 101.
- [5] C. R. Jarvis, T. R. Anderson, and R. D. Andrew, *Cereb. Cortex*, **11**(3), (2001) 249.

Glial and vascular response after exposure to blast-associated shock wave

K. Nishii¹, Y. Satoh², T. Higashi¹, T. Matsui¹, M. Kashitani³, D. Saitoh⁴ and Y. Kobayashi¹

¹ Department of Anatomy and Neurobiology, National Defense Medical College

² Department of Pharmacology, National Defense Medical College

³ Department of Aerospace Engineering, National Defense Academy

⁴ Division of Traumatology, Research Institute, National Defense Medical College

Background

Pathophysiology of mild traumatic brain injury (mTBI) resulting from exposure to blast-associated shock wave (BSW) includes vascular leakage due to disruption of blood-brain barrier (BBB) integrity [1]. BBB is a multicellular vascular structure that separates the central nervous system from the peripheral blood circulation [2]. Precise sequence of BBB disruption and how it affects glial response are not well-understood.

Methods

Single BSW with peak pressure of 25 kPa was loaded on male C57BL6/J mice at the age of 8 weeks as described elsewhere [3]. An Evans Blue dye solution (4% w/v in saline) was injected intravenously (2.5 μ l/g) through the tail vein 0.5-1 h prior to or 4 h, 1 d, 3 d, and 7 d after BSW exposure. After labeling for 2-3 h, the mice were transcardially perfused for 2 min with heparinized phosphate buffered saline including 40 kDa FITC-dextran (3 mg/ml), and then with 10% formalin neutral buffer solution. Fluorescence images of dye extravasation were scanned using an Olympus IX83 inverted microscope equipped with a cellSens imaging software. The slices were then cryosectioned and immunostained for GFAP, CNPase, Iba1, and iNOS.

Results

We determined the precise sequence of fluorescent dye extravasation using Evans Blue and FITC-dextran. Dye extravasation did not occur immediately after exposure to BSW, but was prominent as early as in 4 h. Its extent showed a significant alteration between 4 and 6 h. Then, it declined gradually from 1 to 3 d, and ceased in 7 d. We found microglia changed its form immediately after exposure to BSW. Astrocyte activation as indicated by GFAP overexpression was observed in regions where dye extravasation occurred (Figure 1). In contrast, we found no difference in intensity and distribution of CNPase immunoreactivity within the time-window of this study.

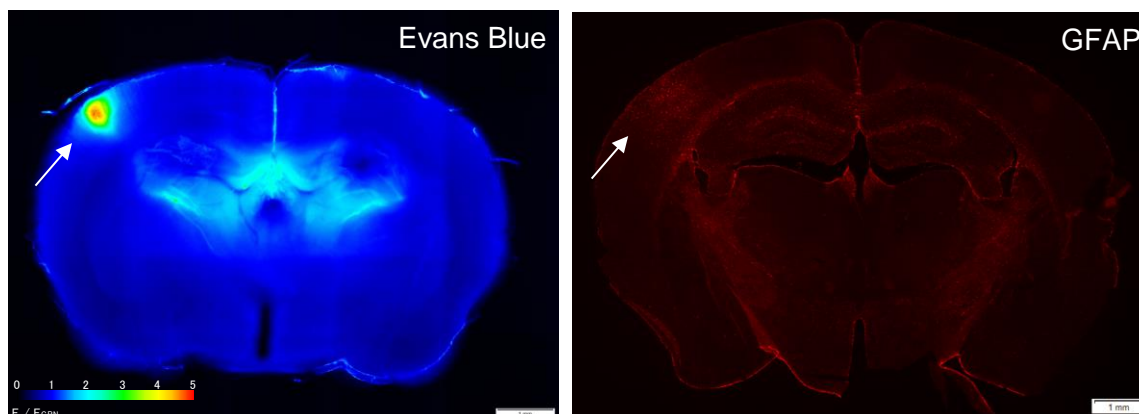


Figure 1. GFAP overexpression in a region where Evans Blue dye extravasation was observed at 3 d after exposure to BSW (arrows).

Discussion

Using the mTBI model in mice, we determined the precise sequence of fluorescent dye extravasation. Transient disruption of BBB integrity should be one of the causes of the following chronic neurological disorders because the same region showed both dye extravasation and GFAP overexpression (Figure 1). On the other hand, microglia was an early responder in our mTBI model. These changes may play important roles in the etiology and pathophysiology of mTBI.

[1] M. Kuriakose, K.V. Rama Rao, D. Younger and N. Chandra, *Sci Rep*, **8** (2018) 8681.

[2] B. Obermeier, R. Daneman and R.M. Ransohoff, *Nature Medicine*, **19** (2013) 1584-1596.

[3] Y. Satoh, Y. Araki, M. Kashitani, K. Nishii, Y. Kobayashi, M. Fujita, S. Suzuki, Y. Morimoto, S. Tokuno, G. Tsumatori, T. Yamamoto, D. Saitoh and T. Ishizuka, *J Neuropathol Exp Neurol*, **77** (2018) 827-836.

Effect of Blast Overpressure on 3D Neuronal Cell Cultures

T.J. O'Shaughnessy¹, K.M. Gilpin², Y. Chen¹, and A. Bagchi¹

¹Materials Science and Technology Division, U.S. Naval Research Laboratory, Washington, DC, USA

²ASEE Post-Doctoral Fellow at U.S. Naval Research Laboratory, Washington, DC, USA

Blast-induced traumatic brain injury is a major topic of concern for the military. Recent conflicts have resulted in a large number of warfighters being diagnosed with mild-to-moderate traumatic brain injuries (mTBIs), characterized by an array of behavioral and physical symptoms, but with no detectable lesion in the brain. Also of concern are repeated exposures to low-level blast overpressures and how multiple exposures might sum to result in an increased risk of neurodegenerative disease later in life. The injury mechanism behind blast-induced mTBI is unclear, especially at the cellular level. However, the nature of cell culture makes realistic exposure of neuronal cells to blast events difficult. Here we present 1) a system capable of maintaining three-dimensional (3D) neuronal cell cultures in a field environment, 2) a method for exposing the cells to live blast events in a surrogate head system that can accommodate protective head gear, and 3) results of several bioassays of blast-exposed neurons.

For this work, a surrogate head system has been paired with a small, sealed device (the cell pack) for the maintenance of cells outside the laboratory. The device consists of a plastic body with wells for four independent cell cultures. The cell packs are sealed on each end by silicone gaskets that permit the cells to breathe, while allowing the blast pressure waves to pass through with minimum impedance (Figure 1A). For the experiments described here, the cell packs contained primary murine cortical neurons in 3D collagen hydrogels.



Figure 1. A) The cell pack is a 5 cm square device with four independent cell culture wells capable of supporting 3D neuronal cultures for up to 2 weeks. B) The cell pack in a gel-based brain (rear half) in a surrogate headform. Not shown are the front gel-brain half that fits tightly against the cell pack and the skull cap form the full head.

The cell packs can be placed in either a “flat panel” system or an anthropomorphic head model that contains a ‘brain’ simulant material. The flat panel system can be used for simple shock tube exposure testing in the laboratory, while the full head allows for either shock tube or live blast testing (Figure 1B), with protective headgear if desired. Once the cells have been exposed to a blast, they can be tested with standard biological assays. The primary assay used in our work is a metabolic assay (MTT assay) that quantitates overall cell culture metabolism via a simple colorimetric dye. We have demonstrated, using the flat panel system, a dose-response relationship between peak overpressure exposure from a shock

tube and a reduction in cell culture metabolism (Figure 2).

We have additionally looked at the effect of multiple overpressure insults on cell culture metabolism. Results showed that when the overpressure insults were less than 15 minutes apart, there was no significant difference between one or two insults relative to controls. However, when the two insults were delivered at an interval of 20 to 60 minutes, the two insult condition had a much greater decrease in metabolism, relative to controls, than the single insult condition.

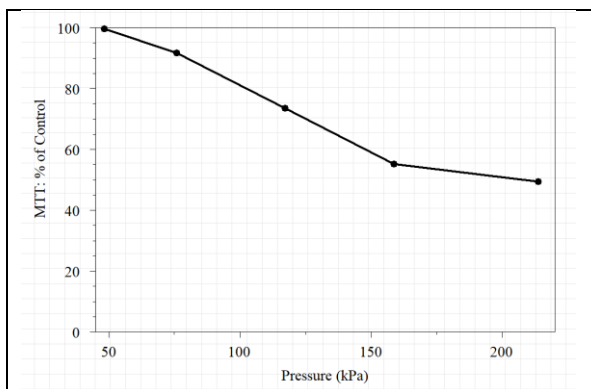


Figure 2. Dose response relationship between overpressure insult (x-axis) and change in metabolism relative to control cultures (y-axis).

Efforts are now underway to determine pathways that might be involved in the above phenomena. To this end, quantitative western blots are being used to look at changes in protein expression levels in response to overpressure exposures. The first proteins tested were caspase-3 (apoptotic pathway) and tom-20 (outer mitochondrial membrane protein). Small increases in caspase-3 levels were detected one hour post overpressure insult, but these changes were absent by 48 hours post insult. In contrast, no changes were detected in tom-20 expression levels in response to overpressure insults. Initial testing of neurofilament-M (a cytoskeletal protein) and chondroitin sulfate (an extracellular matrix protein) suggest that expression levels of both may be affected by neuronal exposure to overpressure insults.

The comments expressed here are those of the authors and do not represent the opinion of the US Naval Research Laboratory or the US Department of Defense.

Field Evaluation of the Blast Gauge Sensor (BGS) Compared to the Pencil Probe in an Open Blast Environment

T. Pandelani¹, Z. Schutte¹, S. Hamilton¹, D. Reinecke¹ and R. Ahmed¹

¹ CSIR Defence, Peace, Safety and Security, 1 Meiring Naude Road, Pretoria, 0001, South Africa

Abstract

last Traumatic Brain Injury (tTBI) has become the “signature wound” of conflicts in Iraq and Afghanistan. In 2018 the Defense and Veterans Brain Injury Center (DVBIC) worldwide numbers for service men, diagnosed with TBI from 2000-2018, stood on 383,947, giving reason why TBI is known as the signature injury of modern war [1,2].

59% of soldiers exposed to blast, suffer from TBI. Of those, 56% of the injuries are being classified as severe. However, actual figures may be higher, due to difficulties associated with diagnosis of closed TBI (cTBI). This has grave implications, as soldiers reporting some degree of tTBI have been shown to be five times more likely to report a major decline in health in the six months following injury. A Full 41.3% reports a severe decline in health, and quality of living, in the five years following deployment. Classifying the TBI correctly can ensure correct treatment and prevent decline of health on the long term following tTBI [3,4].

Battlefield medical personnel rely on the visual signs and personal accounts of patients, to alert them of the possibility of TBI. The identification of both the magnitude, as well as the characteristics of clinically relevant blast waves, which caused the brain damage, is of paramount importance for correct diagnosis. Measuring an individual’s exposure level becomes key to provide a good understanding of the mechanisms of blast TBI, and can provide insight for the choices about treatment and therapies [4].

The Blast Gauge Systems (BGS) provides a quantitative means for measuring blast related exposure, thus providing a mechanism for medical personnel to better identify those at risk for TBI. The BGS collects quantitative data to provide medics with a screening tool and data for uncovering the mechanisms of TBI.

A series of laboratory and field tests were conducted using the BGS with industry standard pencil probes (PP) as controls. The Pencil Probe (PP) is designed to measure shock waves, caused from explosions in air, and has been widely used since the 1960’s. The tests were conducted with PE4 detonated at different Height of Burst (HOBs) with a mass 300 g and 1 kg.

The HOBs were selected to reproduce scenarios typically incurred on the battlefield. These HOBs represented a charge positioned close to the ground, a charge positioned at mid-sternum human height and a charge positioned at an intermediate height, halfway between the two previously mentioned locations. These charge heights thus produce intermediate, simple and Mach regimes respectively.

The experimental results were compared with respect to incident pressures, positive phase duration of the blast wave, impulse and relevant injury criteria. The pencil probe measured higher peak pressure as compared to the BGS. The time to peak for the BGS was slower than that of the pencil probe. These differences peak peak pressure and time to peak are likely due to the slower sampling rate of the BGS and piezoresistive properties of the sensing element. The BGS data was repeatable and reliable, which supports the importance of the BGS for the measurement of both incident overpressures in the field environment.

References

- [1] Lindquist LK, Love HC, Elbogen EB. Traumatic Brain Injury in Iraq and Afghanistan Veterans: New Results From a National Random Sample Study. *J Neuropsychiatry Clin Neurosci*. 2017;29(3):254-259.
- [2] DoD Worldwide Numbers for Traumatic Brain Injury. Number of Service Members Diagnosed with Traumatic Brain Injury 2018 ,<https://dvbic.dcoe.mil/dod-worldwide-numbers-tbi>
- [3] Armonda RA, Bell RS, Vo AH, Ling G, DeGraba TJ, Crandall B, Ecklund J, Campbell WW. Wartime traumatic cerebral vasospasm: recent review of combat casualties. *Neurosurgery*, 2006;59(6):1215–25; discussion 1225.
- [4] Heltemes KJ, Holbrook TL, Macgregor AJ, Galarneau MR. Blast-related mild traumatic brain injury is associated with a decline in self-rated health amongst US military personnel. *Injury*, 2012; 43(12):1990–5.

In Situ Synthesis and application of fluorescent metal nanoclusters to understand the effects of blast overpressure at the cellular level

K.J.Perry¹, P. Aggarwal², R. Kumar², S.P. Karna¹ and R.K. Gupta³

¹ US Army Research Laboratory Weapons and Materials Research Directorate, RDRL-WM, Aberdeen Proving Ground, MD, USA 21005

² Radiation Biotechnology Laboratory, Institute of Nuclear Medicine & Allied Sciences, Delhi 110054 India

³ DoD Blast Injury Research Program Coordinating Office, USMRMC, Ft. Detrick, MD, USA 21702

Blast-induced traumatic brain injury (BI-TBI) has been a subject of intense focus in recent years because of its debilitating effects on the health and quality of life of Soldiers exposed to blast conditions. Major studies have been devoted to understand the biological effects of blast overpressure in animal models. The focus of these activities have been to understand, quantify and blast-induced TBIs. Detection and prognosis of injuries are best defined by Computed Tomography (CT) and Magnetic resonance (MR) imaging techniques. However, these techniques do not offer the fundamental details of TBIs which are complex in terms of cellular and molecular modulation. One approach to develop a fundamental understanding of BI-TBI is to embed imaging and pressure sensing agents at cellular levels. Recent studies have shown that bio-synthesized metal nanoclusters exhibit intense fluorescence that can be used for high resolution imaging of the host matrix^{1,2}. Additionally, the intensity of the fluorescence emission from bio-synthesized nanoclusters show sensitivity to external pressure³, that can be used to quantify local pressure at the site of injury. Such novel properties of bio-synthesized metal nanoclusters offer a unique opportunity to establish pressure-biomechanical injury threshold criteria as well as real time imaging at cellular levels.^{1,2} In order to achieve pressure-injury relationship and real-time imaging, a major first step requires embedding fluorescent metal nanoclusters at cellular levels and the ability to establish pressure-fluorescence base-line. To date, numerous studies have shown protein and DNA-synthesized metal nanoclusters to exhibit intense fluorescence in the visible spectrum. However, there has been little effort in the synthesis of fluorescent metal nanoclusters in cellular medium.

Recently, in situ synthesis of gold (Au) NCs in tumorigenic (cancerous) rat cells has been reported⁴, suggesting the involvement of reactive oxygen and nitrogen species in the underlying chemistry. We have successfully synthesized fluorescent Au NCs in situ in non-tumorigenic Human Embryonic Kidney (HEK) cells, expanding the application space of fluorescent AuNCs to probing/imaging healthy cells. Figure 1 shows the HEK cells (a) without Au NCs and (b) with *in situ* synthesized AuNCs. We also discovered that HEK cells can uptake protein-mediated pre-synthesized Au NCs. This is significant because it suggests the ability to image TBI at the cellular level. Our studies also show that AuNCs do not affect the bio-availability of the cells, which is extremely important for further diagnostic and mechanical studies on healthy cells.

Ultimately, these NCs could drive TBI research forward by helping scientists understand the relationship between blast over pressure and mechanical properties of the cells, which is essential in determining and predicting injury criteria. The details of the in situ synthesis and characterization of the HEK-embedded fluorescent AuNCs, including the spectroscopic properties will be presented at the meeting.

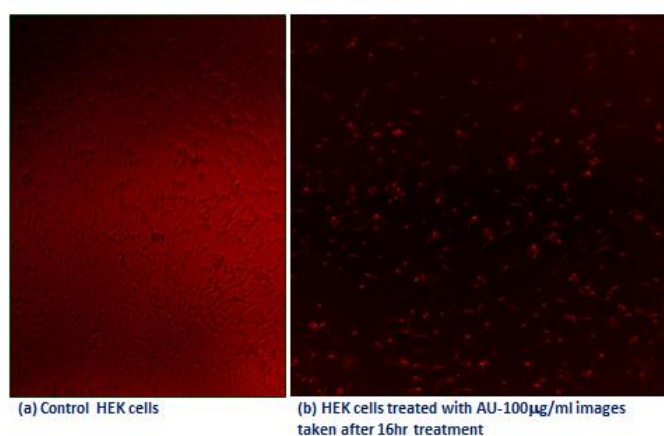


Fig.1 In situ synthesis of fAuNCs in HEK 293 cells (a) control without AuNCs (b) with AuNCs

[1] J. Xie, Y. Zheng, and JY. Ying, *J Am Chem Soc.* **131** (2009) 888-889.

[2] A. West, M. Griep, D. Cole, and S. P. Karna, *Anal Chem*, **86** (2014) 7377-7382.

[3] M. Zhang, et al., *J. Phys. Chem.*, **117** (2013) 639-647.

[4] D. Chen, C. Zhao, J. Ye, Q. Li, X. Liu, M. Su, H. Jaing, C. Amatore, M. Selke, X. Wang, *ACS Appl. Mater Interfaces*, **7** (2015) 18163-18169.

Fast Running Tools for Sensor Based Reconstruction of Warfighter Blast Exposure in Training and Operations

Andrzej J. Przekwas¹, Z.J. Chen¹, Harsha T. Garimella¹, Tim Zehnbauer¹,
Gary H. Kamimori³, Raj K. Gupta²

¹ CFD Research Corporation, 701 McMillian Way NW, Huntsville, AL 35806

² US DoD Blast Injury Research Program Coordinating Office, 810 Schreider Street, Fort Detrick, MD 21702-5000

³ Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910

During training and operations military personnel may be repetitively exposed to low-level blast (LLB) while using explosives to gain entry, firing the heavy weapon systems such as recoilless weapons or high caliber sniper rifles. This repeated exposure, even within allowable limits, has been associated with cognitive deficits similar to that of accidental and sports concussion such as delayed verbal memory, visual-spatial memory, and executive function. Following recommendations from US Congress the DoD is taking steps to understand and mitigate any potentially harmful effects from blast exposure. An integrated experiment and computational framework is needed for better understanding of physics of blast exposure and neurophysiological responses and to establish safety measures during training and combat operations.

One way to quantify the blast exposure, in these scenarios, is through the use of pressure traces from body-worn sensors on military personnel exposed to blast waves. However, this data comprises only of pressure loading at discrete points on the body and may not accurately capture the loading distribution profile over the entire body. Novel computational tools are needed to predict the blast exposure on anatomically critical regions such as the brain, neck, and thorax from the few discrete pressure traces. Here, we present a novel framework for automated reconstruction of the weapon blast “signature” and overpressure loading on humans using wearable pressure sensor data. This framework utilizes data collected by pressure sensors on human subjects or equipment as inputs to an inverse problem solver to calculate the location and charge mass of an explosive device and detailed pressure loads on human bodies exposed to the blast wave. The results could be used for calculation of blast wave loads on the whole human body and on specific organs vulnerable to blast loads, such a head and torso.

An integrated experimental-computational framework comprising of fast running blast dynamics model, human body anatomy models, human body biodynamics and inverse problem solver tools. The main components of the framework can be summarized as follows:

(1) Human body models: A human body anatomical geometry model generator was developed to account for the anthropometric/postural variations and protective equipment among the military personnel involved in training. This tool has been validated on military population anthropometric data available in ANSUR II database. The model can generate postural and operational scenarios involving multiple human subjects, e.g., gunner team, artillery squad or breachers stack.

(2) Fast-running blast dynamics and human body loading tools: CoBi blast loading modeling tools, similar to ConWep, account for ground/wall reflection and calculate blast loads with minimal computational cost and loss of accuracy. The accuracy of these tools was evaluated by comparison to experimental data and high-fidelity CFD simulations.

(3) Inverse problem solver: CoBi inverse blast wave dynamics tools are used to characterize the loading on the human body models using multiple pressure sensor data as inputs. Fast execution of these inverse tools is made possible by the use of fast-running forward blast loading tools.

(4) Model Validation: The predictive capabilities of CoBi inverse blast tools are being validated against filed data collected during military training of breachers, gunners and snipers. The model uses recorded information on weapon firing scene, positions and postures of operators and instructors, protective armor, wearable and free field sensor locations and position and orientation of weapons.

Results of parametric studies will be presented to analyze and optimize military training protocols. Predicted blast loading on specific organs, brain in particular, are being used to calculate injury criteria that could be used for diagnostics, protection and treatment. In the future this framework could be extended to correlate blast loads with chronic neuro-physiological responses including biomarker kinetics.

Disclaimer: Material has been reviewed by the USAMRMC. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25. Funding for the data was received from USAMRMC, RAD III and the Office of the Assistant Secretary of Defense for Health Affairs, Broad Agency Announcement Award No. W81XWH-16-2-0001.

Keywords: training, fast-running, inverse-blast-tools, human body modeling, traumatic brain injury

Animal Model of Multiple Low-Level Blast Injury Displays a Spectrum of Neuropathological and Neurobehavioral Changes

Kakulavarapu V. Rama Rao¹, Arunreddy Ravula¹, Namas Chandra¹

¹Center for Injury Biomechanics, Materials, and Medicine, Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in individuals due to variety of head injuries resulting from blast waves generated by detonation of explosives in combat zones, automobile accidents as well as injuries caused by a variety of sports events. Among these, blast-induced neurotrauma (BINT) is becoming a leading cause of morbidity and mortality in soldiers in the battle field as well as training sites. Among different modes of BINT, severe BINT are always associated with primary, secondary and tertiary injuries and display visible neuropathological changes. In contrast, mild BINT, particularly resulting from repeated low-level (rLLB) blasts usually experienced by Service Members (SMs) in training for select occupations including artillery, mortars, heavy weapons, explosive breaching does not display an overt phenotype but does have a significant bearing on the long-term neuropsychiatric changes. Available evidence reports an association between repeated mild blast and neurologic dysfunction¹, but reliable and effective means to objectively measure such effects remain elusive². A comprehensive survey on SMs exposed to rLLB in various training facilities reported a spectrum of symptoms ranging from headache, fatigue, and an increase in memory deficits,³ while an anonymous survey of self-reported symptomatology in SMs with rLLB also reported difficulty sleeping, irritability, cognitive impairment. MRI studies with susceptibility weighted images (SWI) performed in SMs with rLLB also display significant microbleeds consistent with blood brain barrier compromise in select areas of brain⁴ which we also recently identified in animal models of moderate blast injury in addition to oxidative stress. While a variety of neuropsychological assessments were performed to SMs across the training period, including computer-based neurocognitive assessments and fMRI during memory and word retrieval tasks, the current lack of clearly documented neurologic effects and effective measurement methods for those affected hinders the evaluation and mitigation of neurotrauma risk from occupational exposure to rLLB². These constraints preclude implementation of standard protocols to limit the number of blast exposures received by SMs at a given time. Further, lack of well-established animal model to precisely represent field validated multiple exposures of blasts at low blast overpressure (BOP) by far is another constraint. The existing animal models of multiple blast at low BOP are thus far limited to 3 exposures, which is by far less compared to SMs concurrently experience during training. Our group has developed a state-of-the-art blast tube and able to measure accurate dynamic and incident pressures and optimized the exposure conditions without artifacts⁵. More recently, we have optimized our shock tube allowing the constant flow of isoflurane to perform multiple exposures to animals under anesthesia within a short duration (5-8 concurrent exposures in ~ <5 min) at low BOPs (2-10 psi), which allowed us to *hypothesize* that injury severity (pathophysiological and neurobehavioral changes) in rLLB is directly proportional to the extent of BOP and number of successive exposures. Accordingly, changes in blood brain barrier permeability, microglia activation were evaluated as pathological outcomes. Since both events are known to contribute to neurobehavioral deficits, we also performed a battery of neurobehavioral tests in these animals.

Methods

Blast Exposures: Sprague-Dawley rats (n=28 for neurobehavioral studies, n=12 each for BBB extravasation and immunofluorescence studies), male, 350 ± 50g) were allocated into three groups (control, single blast, repeated blast) and exposed low BOP of 10 psi (70 kPa) one time or 5-successive times within a short duration in the helium-driven shock tube housed in CIBM3 at NJIT. Before the exposure to blast, both control and experimental groups of animals were anesthetized by 5% isoflurane in a chamber. For multiple blast exposures, shock tube has been continuously supplied with isoflurane by a connecting tube attached to the isoflurane chamber to ensure that the animal is under anesthesia during multiple exposures (which lasted ~ total 5 min).

BBB Extravasation: BBB disruption in both control and bTBI animals was evaluated using brain extravasation of sodium fluorescein (NaFl, 360Da), Evan's Blue (EB, 66kDa) which were introduced to rats intravenously through the tail vein. Twenty-four hours after blast exposure, rats were transcardially perfused with PBS followed by 4% PFA and 100 µm sections from different brain regions were prepared and fluorescent intensities of EB and NaFl.

Immunofluorescence: To evaluate the activation of microglia near blood vessels and the following blast injury, double immunofluorescence studies were conducted for Iba-1 (Marker of microglia) and RECA-1 (marker of brain endothelial cells) in the frontal cortex hippocampus, thalamus in 20 µm brain sections. Stained sections were containing different brain regions were digitized (20x magnification) using Leica Aperio Versa 200 fluorescent microscope and slide scanner. Morphological changes and migration of microglia cells were analyzed manually by calculating soma size, processes length and number of cells near the compromised BBB.

Neurobehavioral assessment: A battery of neurobehavioral tests was conducted in animals exposed to single and multiple low-level blast (10 psi) and animals were examined for signs of anxiety-depression (elevated plus maze), motor coordination (accelerated rotarod), short-term memory (Novel object recognition test) and sleep patterns (Sleep Recognition Apparatus, Signal Solutions).

Results

BBB permeability: We found a correlation between the extent of BBB permeability and the intensity of the injury: while no measurable extravasation was apparent in animals exposed to single blast at 10 psi, the leakage was obvious in animals exposed to multiple (5x) blasts at 5 psi, which strongly suggesting a cumulative effect of repeated blast exposures causing BBB permeability changes (Figure 1).

Microglial Activation (Neuroinflammation) in rLLB: Double immunofluorescence staining of tissue sections from hippocampus with microglial marker Iba1 with endothelial marker RECA-1 in animals exposed to rLLB (10 psi 5X) showed sustained microglial activation until 30 days post injury suggesting that neuroinflammatory mechanisms are chronic pathological events in low-level repeated diffuse brain injuries (Figure 2).

Neurobehavioral deficits: Assessment of motor coordination using accelerated rotarod showed a significant motor deficit in animals exposed to rLLB at different time points after blast, whereas single blast exposure at 10 psi did not show any motor incoordination again suggesting a cumulative effect of repeated blast exposures causing motor deficits. Likewise, assessment of anxiety using elevated plus maze also showed a significant level of anxiety-like symptoms in animals exposed to rLLB while no such symptoms were observed in animals with single blast at 10 psi (Figure 3). We also observed short-term memory deficits in animals exposed to rLLB as assessed by novel object recognition tests.

Sleep studies were performed using PiezoSleep apparatus (Signal Solutions Inc, Lexington, KY), the activity of control and animals exposed to repeated low-level blast (10 psi 5 times) were recorded continuously in a 24 hrs. cycles. Analysis of data showed that repeated blast exposure to rats disturbed the sleep wake cycle 4 days post-injury and such pattern continued for up to 8 days' post-injury as indicated by increased amount of sleep in both light cycle (day time) and dark cycle.

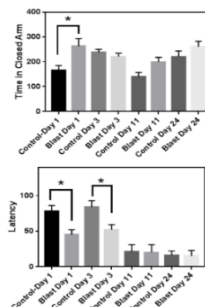


Figure 3: Motor performance and anxiety-depression assessments in animals exposed to repeated LLB (10 psi 5x). Note a significantly reduced motor coordination in rats 24h and 3 days post injury, while anxiety-depression symptoms were increased 24 h post injury.

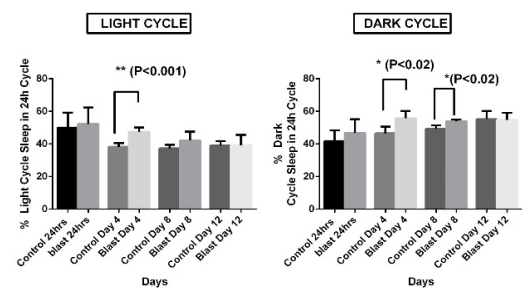


Figure 4: Sleep-Wake cycle alterations in animals exposed to repeated low-level blast. Note that both light and dark cycle of sleep were significantly increased in animals 4 days post-injury.

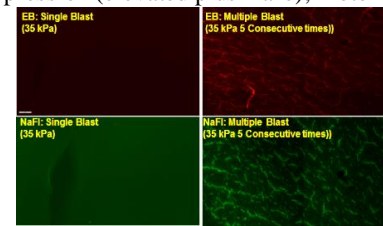


Figure 1: Fluorescent images of sodium fluorescein (NaFl) and Evans blue (EBB) extravasation in animals exposed to single low level blast (5 psi) vs. repeated (5 times) blast at the similar BOP. Note a robust increase in the extravasation of NaFl and EB in animals

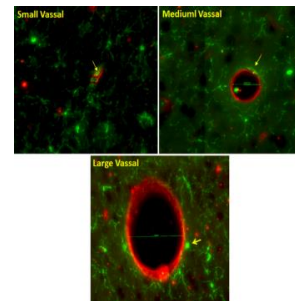


Figure 2: Immunofluorescence staining of activation of Iba1 (microglial marker) showing amoeboid morphology in 28 days after animals exposed to repeated mild blast injury consistent with chronic activation of microglia in repeated LLB.

Conclusion: We have developed an animal model for rLLB which reproduces the neurobehavioral and neuropsychiatric symptoms associated with human mild rLLB and there are a number of neurobehavioral deficits observed in the animal model of rLLB, which are believed to be resulting from upstream pathological events such as BBB disruption and neuroinflammation observed in our animal model of rLLB.

- 1 Elder, G. A., Stone, J. R. & Ahlers, S. T. Effects of low-level blast exposure on the nervous system: is there really a controversy? *Front Neurol* **5**, 269, doi:10.3389/fneur.2014.00269 (2014).
- 2 Carr, W., Dell, K. C., Yanagi, M. A., Hassan, D. M. & LoPresti, M. L. Perspectives on repeated low-level blast and the measurement of neurotrauma in humans as an occupational exposure risk. *Shock Waves* **27**, 829-836, doi:10.1007/s00193-017-0766-0 (2017).
- 3 Tate, C. M. *et al.* Serum brain biomarker level, neurocognitive performance, and self-reported symptom changes in soldiers repeatedly exposed to low-level blast: a breacher pilot study. *J Neurotrauma* **30**, 1620-1630, doi:10.1089/neu.2012.2683 (2013).
- 4 Liu, W. *et al.* Imaging Cerebral Microhemorrhages in Military Service Members with Chronic Traumatic Brain Injury. *Radiology* **278**, 536-545, doi:10.1148/radiol.2015150160 (2016).
- 5 Kuriakose, M. *et al.* Tailoring the Blast Exposure Conditions in the Shock Tube for Generating Pure, Primary Shock Waves: The End Plate Facilitates Elimination of Secondary Loading of the Specimen. *PloS one* **11**, e0161597 (2016).

Potential cause of primary, blast-induced brain injury: direct vs. indirect mechanism

J. Rubio^{1,2}, G. Unnikrishnan^{1,2}, V. Sajja³, S. van Albert³, J. Long³, M. Skotak⁴, E. Alay⁴,
N. Chandra⁴, A. Sundaramurthy^{1,2}, D. Subramaniam^{1,2}, and J. Reifman¹

¹Department of Defense Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, United States Army Medical Research and Materiel Command, Fort Detrick, MD 21702, USA

²The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., 6720A Rockledge Drive, Bethesda, MD 20817, USA

³Blast Induced Neurotrauma Division, Center for Military Psychiatry and Neurosciences, Walter Reed Army Institute of Research, 503 Robert Grant Drive, Silver Spring, MD 20910, USA

⁴Department of Biomedical Engineering, Center for Injury Biomechanics, Materials, and Medicine, New Jersey Institute of Technology, 111 Lock Street, Newark, NJ 07103, USA

Background:

Among United States Soldiers deployed to Iraq and Afghanistan, exposure to explosive devices is the main cause of traumatic brain injury. In an Army brigade team, the incidence of blast-induced brain injury, caused by the primary (due to blast overpressure), secondary (due to fragments), or tertiary (due to body translation) mode of blast injury, was reported to be as high as 88% of all clinically reported traumatic brain injuries [1]. Yet, our understanding of the primary mode of injury remains extremely limited compared to that of the secondary and tertiary modes. Despite years of research, it is not known whether or how blast overpressure causes brain injury. Several competing hypotheses, which can broadly be grouped into *direct cranial mechanism* and *indirect thoracic mechanism*, have been proposed to explain the cause of this injury. The direct mechanism assumes that injury to the brain occurs from the interaction of the blast wave with the head [2]. In contrast, the indirect mechanism assumes that injury to the brain occurs from the interaction of the blast wave with the body [3]. In this study, we aimed to quantify the nature of the changes in brain tissue due to the direct and indirect mechanisms. By quantifying the changes, we can characterize the risk of brain injury associated with each of these two mechanisms.

Methods:

We performed shock-tube experiments on 10-week old male Sprague-Dawley rats at an incident blast overpressure of 130 kPa, with the animal placed in a vertical orientation facing the blast wave. We performed histopathology and immunohistochemistry (IHC) of the serially cut rat brains (1 to 12 mm posterior to bregma) to identify changes in the brain tissue due to blast exposure. For histopathology, we used Silver staining to evaluate axonal degeneration. For IHC analyses, we assessed astrogliosis with glial fibrillary acidic protein (GFAP), microgliosis with ionized calcium-binding adapter molecule 1 protein (Iba-1), and neuron population with neuron-specific nuclear protein (NeuN). We performed separate experiments to quantify the changes in the brain tissues caused by the direct and indirect mechanisms, as detailed below.

Direct mechanism: We randomly assigned rats into sham, whole-body condition, or head-only condition groups. The rats in the sham group did not receive a blast exposure. The animals in the whole-body and head-only conditions received a single blast exposure in a vertical position. In the whole-body condition, the head and torso of the rat were exposed to the blast wave, whereas in the head-only condition, only the head protruded into the shock tube through an opening on the bottom wall of the shock tube. For histopathology and IHC analyses, we obtained perfused brains from sham rats and rats sacrificed 24 hours or 14 days after blast-wave exposure. For each brain, we performed Silver and NeuN staining in five coronal slices ($n = 5$ animals per group), as well as GFAP and Iba-1 staining in 12 coronal slices ($n = 8$ animals per group). All tests were performed at the Walter Reed Army Institute of Research.

Indirect mechanism: We randomly assigned rats into sham, whole-body condition, or torso-only condition groups, where animals in the whole-body and torso-only conditions received a single blast exposure in a vertical position. In the whole-body condition, the rat was placed in the test section of the shock tube, whereas in the torso-only condition, it was placed so that the torso was inside the shock tube and the head protruded from the shock tube through a small opening on the top wall. For histopathology and IHC analyses, we obtained perfused brains from sham rats and rats sacrificed 24 hours after blast-wave exposure ($n = 4$ animals per group). For each brain, we performed histopathology and IHC (GFAP, Iba-1, and NeuN) analyses on 12 coronal slices. All tests were performed at the New Jersey Institute of Technology.

Histopathology and IHC quantification: For each coronal slice, we assessed the extent of axonal degeneration with Silver staining (histopathology) and the fluorescence intensity for GFAP, Iba-1, and NeuN due to blast exposure. In addition, we estimated the histopathology and IHC data for the entire brain by summing the corresponding values from each slice.

Statistical analyses: For data from the entire brain, we compared the histopathology and IHC data for the three groups of rats corresponding to the individual mechanisms using linear mixed-model analysis. In addition, we performed statistical analyses for the data from each region (i.e., each coronal slice) using Kruskal-Wallis test. We tested for differences between the groups, using Dunnett's post-hoc test with the Holm-Bonferroni correction. The significance criterion was set to $p < 0.05$.

Results:

Direct mechanism: GFAP positive staining increased in blast-exposed rats relative to shams ($p < 0.05$), regardless of the time of sacrifice (i.e., 24 hours or 14 days) or exposure condition (whole-body or head-only) for the entire-brain analyses. The regional analyses (i.e., for comparison across each coronal slice) revealed an increase in GFAP positive staining in the posterior region of the brain (10 to 12 mm posterior to bregma; $p < 0.05$), regardless of the time of sacrifice or exposure condition. However, for both entire-brain and regional analyses, blast-exposed and sham rats did not differ in terms of histopathology or Iba-1 and NeuN positive staining, regardless of the exposure condition and time of sacrifice.

Indirect mechanism: For the entire-brain analyses, histopathological analyses revealed significantly more positively stained cellular fragments in whole-body exposed rats than in shams. Furthermore, GFAP positive staining decreased in whole-body exposed rats relative to shams ($p < 0.05$) but not in torso-only exposed rats. Blast-exposed and sham rats did not differ in terms of Iba-1 and NeuN positive staining. For regional analyses, Silver staining revealed an increase of cellular fragments in the anterior region of the brain (2 mm posterior to bregma; $p < 0.05$) in whole-body exposed rats relative to shams. In addition, GFAP positive staining decreased in the posterior region of the brain (9 mm posterior to bregma; $p < 0.05$) in whole-body blast exposure. Blast-exposed (whole-body and torso-only) and sham rats did not differ in terms of Iba-1 and NeuN positive staining.

Conclusion:

We characterized the changes in the rat brain due to the direct and indirect mechanisms of primary blast-induced injury for 130 kPa exposure. GFAP positive staining in both whole-body and head-only exposed rats changed relative to shams. However, there were no changes in the brain of torso-only exposed rats. These results indicate that the indirect mechanism does not lead to any changes that are identifiable through histopathological (using silver staining) or IHC analyses (via detection of GFAP, Iba-1, and NeuN). In summary, our work suggests that direct cranial transmission, rather than an indirect thoracic transmission, is the major contributor of brain changes due to primary blast exposure.

References:

- [1] H. Terrio, L.A. Brenner, B.J. Ivins, J.M. Cho, K. Helmick, K. Schwab, K. Scally, R. Bretthauer, and D. Warden, *J. Head Trauma Rehabil.*, **24** (2009) 14.
- [2] R. Bolander, B. Mathie, C. Bir, D. Ritzel, and P. VandeVord, *Ann. Biomed. Eng.*, **39** (2011) 2550.
- [3] I. Cernak, *Front. Neurol.*, **1** (2010) 151.

Disclaimer:

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the United States (U.S.) Army or the Department of Defense (DoD) or The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF). Any citations of commercial organizations and trade names in this report do not constitute an official U.S. Army, DoD, or HJF endorsement of approval of the products or services of these organizations. This abstract has been approved for public release with unlimited distribution.

DoD Working Group on Computational Modeling of Human Lethality, Injury, and Impairment from Blast-related Threats

A. Santago¹, M. Downs¹, R. Spencer¹, L. Lalis¹, R. Gupta², and M. Leggieri²

¹ The MITRE Corporation, McLean Virginia

² DoD Blast Injury Research Program Coordinating Office

Introduction: With the increase in computational power allowing increased fidelity in models of the human body, modeling and simulation (M&S) is poised to play a significant role in the Department of Defense's (DoD's) future efforts to mitigate Warfighter lethality, injury, and impairment from exposure to blast-related threats. A DoD Computational Human Body Modeling Capability (Modeling Capability) will allow development of personal protective equipment and combat platform occupant protection concepts in a virtual environment, accelerate development of effective treatment strategies, predict health outcomes and disabilities, and improve Warfighter lethality. Valid predictive computational models aim to reduce the number of expensive and time-consuming dynamic tests by identifying only the specific cases that require additional experimental verification. As our Nation's Warfighters encounter novel threats in future combat scenarios, this capability will allow the DoD to quickly and adeptly address those threats.

The Modeling Capability will require extensive, coordinated integration of many kinds of human body computational models. Available human body computational models are designed to represent one or more aspect(s) of the human condition (paradigms) such as movement, musculoskeletal and soft tissue injuries, cardiovascular system, physiological responses to drug delivery, healing, and disease progression. Each modeling paradigm requires mathematical formulations (e.g., rigid body dynamics, finite element, finite difference, lumped parameter, etc.) and underlying assumptions that may or may not be compatible with other modeling paradigms or with other models addressing the same paradigm. Furthermore, many models may exist within a paradigm that may offer competing solutions. Developing an all-encompassing Modeling Capability will require a DoD Computational Human Body Modeling Framework (Framework) supporting model selection for scenario development, execution of the scenario, guidelines and best practices for inter-model communication, guidelines and best practices for inputs and outputs, and analyses of results.

Methods: Developing the Modeling Capability and associated Framework will require a coordinated effort across the DoD and other federally agencies. In 2017, the DoD Blast Injury Research Program Coordinating Office (PCO) established the DoD Working Group on Computational Modeling of Human Lethality, Injury, and Impairment from Blast-related Threats (Working Group) to shape, focus, and coordinate the DoD's computational modeling efforts to enable a new capability for computational modeling and simulation (M&S) of human lethality, injury, and impairment resulting from the entire spectrum of blast-related threats and environments. The Working Group was tasked with developing a Strategic Plan for the development of the Modeling Capability and associate Framework. The Working Group currently consists of members from 26 DoD and 7 other federal agencies.

Results and Discussion: To date, the Working Group has held four meetings discussing specific actions related to the development of the Strategic Plan including establishing the scope for the Modeling Capability (outlined below), developing a Computational Modeling Questionnaire and a Computational Modeling Registry to collect and house information about available computational models, hosting presentations from model developers and users to understand the state-of-the-science, and participating in numerous brain storming activities to inform specific sections of the Strategic Plan.

Modeling Capability: The Modeling Capability, as envisioned by the Working Group Members, would allow computational M&S from the time of exposure to the blast hazard to return to routine, and is shown in Figure 1.

Computational models of the blast threat will interact with computational models of any protective system, such as a vehicle or body armor. The output of the protective system model will then interact with the Modeling Capability, which is represented by everything within the dotted lines of Figure 1. Additionally, the Modeling Capability might incorporate a loading scenario representing the output from the protective system or blast threat without requiring a direct output from a model of the blast threat or protective system.

Once the loading scenario enters the Modeling Capability, a feedforward loop would then persist until homeostasis is reached, which would occur when models used to predict and understand injury interact with those used to predict and understand intrinsic function (such as blood loss, oxygen deprivation, etc.) and extrinsic function (move, shoot, communicate, etc.). During this initial phase, the Modeling Capability will continually interact with the protective systems as loading conditions change due to additional hazards and/or the additional restrictions protective systems impose on the Warfighter's functional ability. Computational models of acute care treatment strategies such as injections, bandages, tourniquets, splints, etc., on both injury and function would interact with the Modeling Capability

during this feedforward loop. This would allow the Modeling Capability to generate predictions on how these acute care treatment strategies affect injury and function.

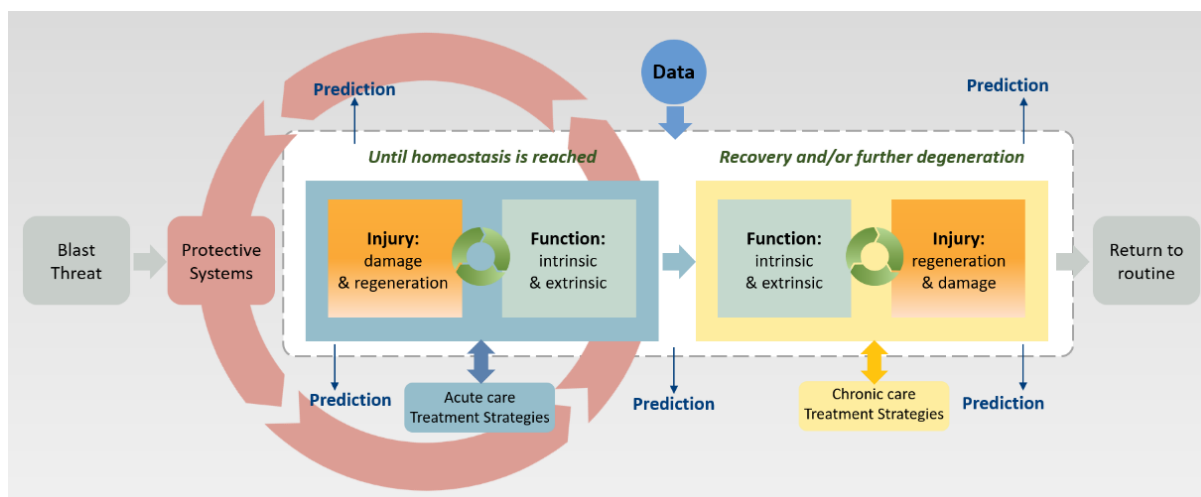


Figure 1: Scope of the DoD Modeling Capability from blast threat to return to routine. The Modeling Capability is represented by everything within the dotted lines and has two modules 1) Until homeostasis is reached and 2) Recovery and/or further degeneration.

The second phase of the Modeling Capability can be used to predict and understand both functional and physical recovery until the Warfighter can return to routine. This would involve a similar feedforward loop to that in the initial phase, where outputs from computational models that describe rehabilitative exercises interact with computational models that predict recovery based on the functional loading. Information on recovery would then feed into the functional computational models and would allow an end user of the Modeling Capability to assess functional ability and prescribe additional rehabilitation as necessary. Computational models of chronic care treatment strategies on injury and function such as the use of exercise equipment, antibiotics, etc., would interact with the Modeling Capability at this stage.

As shown in the Figure 1, the Modeling Capability will respond to user queries for information and generate predictions to assist decision makers at any point. Additionally, authorized users will be able to insert data (e.g., experimental and epidemiological) into the Modeling Capability at any point, which will allow modifications to an end user scenario of interest and/or improve prediction accuracy.

Conclusion: The DoD Working Group on Computational Modeling of Human Lethality, Injury, and Impairment from Blast-related Threats has planned an additional two meetings to finalize the Strategic Plan that will deliver the Modeling Capability and determine the next steps needed to implement the Strategic Plan. Beyond Working Group meetings, many of the members have initiated collaborative research efforts to avoid unnecessary duplication of effort and share data and models. It is expected that the Strategic Plan, information generated during the meetings, the Modeling Registry, performer presentations, and collaborations established by members of the Working Group will inform the development of future human body computational models and provide the DoD with a cohesive Modeling Capability.

The opinions and assertions contained herein are the private views of the author and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense; This technical data deliverable was developed using contract funds under Basic Contract No. W56KGU-18-C-0010; Approved for Public Release; Distribution Unlimited. Public Release Case Number 19-0953

Meningeal damage may be associated with spreading depolarization and glial scar formation in the cortex: laser-induced shock wave study

S. Sato¹, S. Kawauchi¹, Y. Komuta², A. Kono¹, Y. Muramatsu¹, T. Osawa³, M. Inaba⁴,
I. Nishidate⁴ and K. Kaida²

¹Division of Bioinformation and Therapeutic Systems, National Defense Medical College Research Institute

²Division of Neurology, Department of Internal Medicine, National Defense Medical College

³Department of Industrial Technology and Innovation, Graduate School of Engineering,
Tokyo University of Agriculture and Technology

⁴Graduate School of Bio-Applications & Systems Engineering, Tokyo University of Agriculture and Technology

Laser-induced shock wave (LISW) is a unique shock wave source to investigate the mechanisms of primary blast TBI (bTBI) due to the high controllability of shock wave energy, capability of targeted application and lack of dynamic pressure. The spatially confined nature of LISW also enables real-time diagnosis of the brain that is interacting with a shock wave, since sensors can safely be placed in the vicinity of the shock wave source. In our previous study, we performed real-time diagnosis of the rat brain exposed to an LISW and observed the occurrence of spreading depolarization (SD) in the cortex. The cerebral blood vessels showed transient hyperemia/hyperoxemia during SD propagation, but it turned to long-lasting (> 1h) oligemia/hypoxemia.^{1,2}

In the same model, we recently found that the meninges were sensitive and vulnerable to a shock wave. The transcranial application of a low-impulse LISW to the rat brain caused increased permeability of meningeal blood vessels, and in the very acute phase, microhemorrhages were often observed selectively in the meninges under the site of LISW application when SD was observed to occur in the cortex (Fig. 1). This suggests an association between the meningeal microhemorrhages and the SD occurrence, which can be interpreted as being due to the effects of potassium ions released from erythrocytes. In many such cases, however, meningeal microhemorrhages disappeared at one week after shock wave exposure, and in some cases, glial scar formation (gliosis) was observed in the cortex under the site of LISW application (Fig. 2). Gliosis has been reported to be a clinical neuropathology in bTBI patients.³ Damage to the meninges might be associated with glial scar formation.

Recently, it has been reported that meningeal lymphatics have a role to remove waste products from the brain.⁴ Thus, meningeal damage can impede this function, possibly leading to long-term brain tissue alteration. Effects of shock wave-induced meningeal damage will be discussed.



Figure 1 Photographs of the rat brain a few hours after LISW application (~25 Pa•s). With meninges (top) and without meninges (bottom).

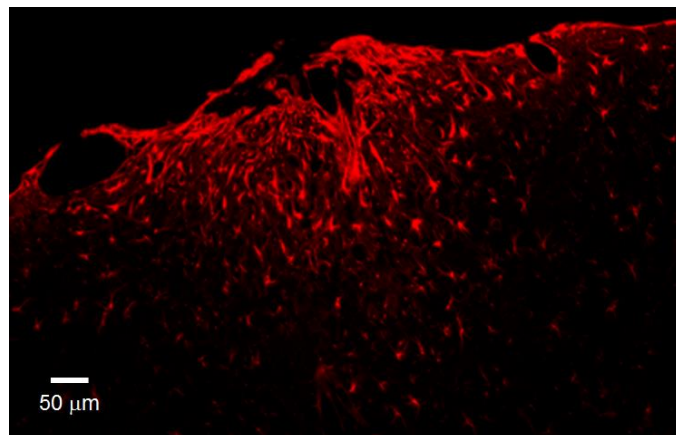


Figure 2 The result of GFAP immunostaining in the cortex at 7 days after LISW application (~25 Pa•s).

References:

- [1] S. Sato, S. Kawauchi, W. Okuda, I. Nishidate, H. Nawashiro, and G. Tsumatori, *PLoS ONE* 9, e82891 (2014).
- [2] S. Kawauchi, W. Okuda, H. Nawashiro, S. Sato and I. Nishidate, *J. Biomed. Opt.* (in press)
- [3] S. B. Shively, I. Horkayne-Szakaly, R. V. Jones, J. P. Kelly, R. C. Armstrong and D. P. Perl, *Lancet Neurol.* 15, 944-953 (2016).
- [4] A. Louveau, I. Smirnov, T. J. Keyes, J. D. Eccles, S. J. Rouhani, J. D. Peske, N. C. Derecki, D. Castle, J. W. Mandell, K. S. Lee, T. H. Harris and J. Kipnis, *Nature* 523, 337-341 (2015).

Developing Correspondence Rules for Traumatic Brain Injury in Different Species

Robert Saunders¹, X. Gary Tan¹, Amit Bagchi¹

¹U.S. Naval Research Laboratory, Washington, DC 20375

Traumatic brain injury (TBI) analysis in humans is exceedingly difficult due to the intrusive methods by which data can be collected; thus, many researchers commonly implement animal surrogates. But tests on animals are restricted by ethical concerns, cost, test logistics, and insufficient data granularity. The availability of better computational facilities and material properties at higher strain rates now allow the use of high-fidelity computational models with a high-resolution brain structure as a viable alternative to predict TBI. Computational models are not constrained by the same concerns as animal testing and are able to generate significant amounts of data in relatively short time.

In this work, TBI prediction data from validated human and pig head computational models [1-2] is generated under a range of blast conditions, such as varying charge weights, varying standoff distances, and varying subject orientation relative to the blast. The TBI prediction outputs are based on TBI thresholds available from the literature and is quantified in the model by the volume of brain exceeding the given threshold criteria at any point in the simulation. The predicted TBI data are compared and contrasted between the species to determine existence of any injury patterns. Present results indicate that equivalent blasts at a fixed standoff distance in the human and pig produce significantly different injuries, and when equating total injured brain volume, the locations of injury in the brain vary between the species (Figure 1). Further results suggest that linear regression can be used to relate injured volume to a given blast for each species, then using simple algebra, used to relate injury in the pig to injury in the human.

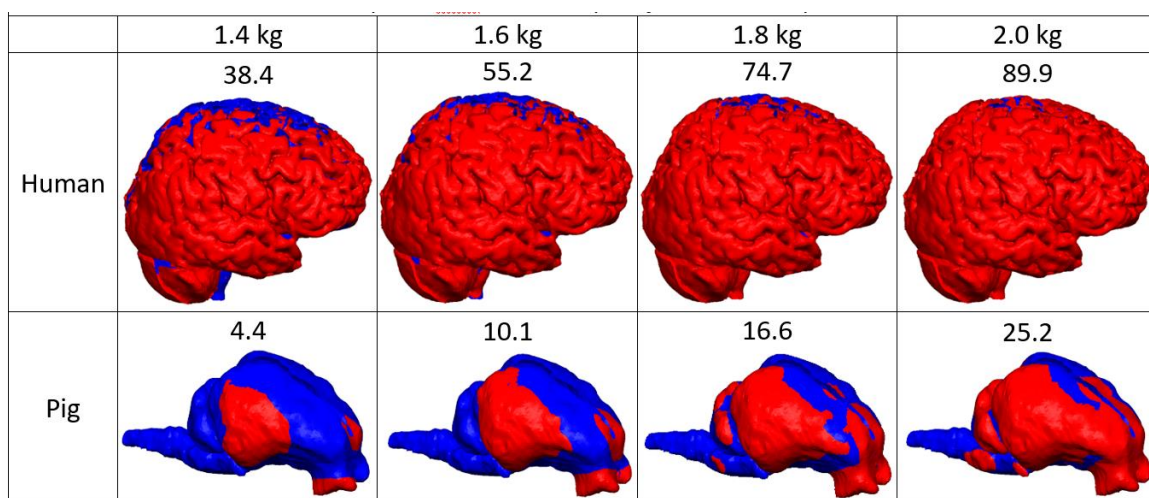


Figure 1. Spatial injury results for the for equal charge weight study for 235 kPa injury criterion (brain oriented to show coup injury). The red region represent an injured region.

References:

- [14] R.N. Saunders, N. Kota, A. Bagchi, S. Qidwai, *Def. Tech. Inf. Cent.* (2018) NRL/MR/6350-18-9807.
- [15] X.G. Tan, R.N. Saunders, A. Bagchi, *ASME-IMECE* (2017) doi:10.1115/IMECE2017-70611.

Dietary Zinc Modulates Matrix Metalloproteinases in Traumatic Brain Injury

Angus G. Scrimgeour¹, Michelle L. Conclin¹ and Marloes J.A. Joosen²

¹ Military Nutrition Division, US Army Research Institute of Environmental Medicine, Natick MA 01760

² TNO Defence, Security and Safety, 2280 AA Rijswijk, The Netherlands.

Animal models of mild traumatic brain injury (mTBI) provide opportunity to examine the extent to which dietary interventions can be used to improve recovery after injury. Animal studies also suggest that matrix metalloproteinases (MMPs) play a role in tissue remodeling post-TBI.

OBJECTIVES/METHODS: Because dietary zinc (Zn) improved recovery in nonblast mTBI models [1,2], and the MMPs are Zn-requiring enzymes, we evaluated the effects of low- (LoZn, 5ppm) and adequate-Zn (AdZn, 30ppm) diets on MMP expression and behavioral responses, following exposure to a single blast (Semtex, 130 kPa incident pressure). MMP messenger RNA expression in soleus muscle and frontal cortex tissues were quantified at 48 h and 14 days post-blast.

RESULTS: In muscle, blast resulted in significant upregulation of membrane-type (MT)-MMP, MMP-2, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 at 48 h post-injury in rats consuming AdZn. At 14 days post-blast, there were no blast or dietary effects observed on MMP levels in muscle, supporting the existence of a Zn-responsive, functional repair and remodeling mechanism. In contrast, blast resulted in a significant downregulation of MT-MMP, TIMP-1, and TIMP-2 and a significant upregulation of MMP-3 levels at 48 h post-injury in cortex tissue, whereas at 14 days post-blast, MT-MMP, MMP-2, and TIMP-2 were all downregulated in response to blast, independent of diet, and TIMP-1 were significantly increased in rats fed AdZn diets despite the absence of elevated MMPs. Because the blast injuries occurred while animals were under general anesthesia, the increased immobility observed post-injury in rats consuming LoZn diets suggest that blast mTBI can, in the absence of any psychological stressor, induce post-traumatic stress disorder-related traits that are chronic, but responsive to diet.

CONCLUSIONS: Taken together, our results support a relationship between marginally Zn-deficient status and a compromised regenerative response post-injury in muscle, likely through the MMP pathway. However, in neuronal tissue, changes in MMP/TIMP levels after blast indicate a variable response to marginally Zn-deficient diets that may help explain compromised repair mechanism(s) previously associated with the systemic hypozincemia that develops in patients with TBI.

[1] EC. Cope, DR. Morris, AG. Scrimgeour and CW. Levenson. Use of zinc as a treatment for TBI in the rat: effects on cognitive and behavioral outcomes. *Neurorehabil Neural Repair*. **26** (2012) 907-13.

[2] EC. Cope, DR. Morris, AG. Scrimgeour, JW. VanLandingham and CW. Levenson. Zinc supplementation provides behavioral resiliency in a rat model of TBI. *Physiol Behav*. **104** (2011) 942-7.

[3] AG. Scrimgeour, CT. Carrigan, ML. Conclin, RM. van den Berg, HPM. van Helden and MJA. Joosen. Dietary zinc modulates matrix metalloproteinases in TBI. *J. Neurotrauma*. **35** (2018) 2495-2506.

Disclaimer: Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. *Funded by U.S. Army MRMC and U.S Air Force Medical Support Agency (AFMSA).*

Numerical Simulation of Traumatic Brain Injury from Primary Blast Effects and Protection of Combat Helmet

X. G. Tan¹ and A. Bagchi¹

¹U.S. Naval Research Laboratory, Washington, DC 20375

The blast-induced traumatic brain injury (TBI) has become a signature wound of recent military activities and terrorist acts. Current understanding of blast wave transmission and mechanism of primary blast effects to cause traumatic brain injury while wearing a helmet is incomplete and thus limits the development of protection and therapeutic measures.

We have developed high fidelity computational tools, image-based human head model that could help in the design of next generation combat helmets with improved blast, blunt and ballistic protection. We have investigated the blast induced brain biomechanics and assessed the role of helmet by utilizing an integrated experimental and computational method. Experimental shock tube tests of head surrogate provide benchmark quality data and are used for the model validation [1]. The finite element (FE) head model is able to accurately reproduce the complex structures of the head. The tissue material parameters have been calibrated based on the available fast dynamic loading data. The head model with a combat helmet produces biomechanical quantities thus provides a more complete understanding of the conditions that may contribute to TBI. Different blast wave loadings on the helmet and head were obtained and compared by simulating both free-field explosion and shock tube blast test, via an efficient, staged computational fluid dynamics (CFD) approach [2]. The two-phase flow model with a robust and accurate shock capturing method was utilized to simulate the shock wave interaction with the head with helmet in the helium-air shock tube [3]. For the simulation of shock wave propagation, the Eulerian finite volume method was compared with Arbitrary Lagrangian-Eulerian (ALE) finite element method with respect to the cost and accuracy.

By employing the coupled Eulerian-Lagrangian fluid structure interaction (FSI) approach, we solved the dynamic problem of helmet and head under the blast exposure. We discussed possible pathways of blast energy transmission to the brain and the effectiveness of helmet systems. The model simulation showed the influence of helmet configuration, suspension pads, and shell material stiffness on the biomechanical response of brain. Response metrics including head acceleration and intracranial pressure (ICP) were used to assess and compare the protection performance of helmet configurations. The main contribution was the elucidation of blast wave brain injury pathways, including wave focusing in ocular cavities and the back of head under the helmet, the effect of neck, and the frequency spectrum entering the brain through the helmet and head. The suspension material was seen to significantly affect the ICP results and energy transmission. These findings can be used to design next generation helmets including helmet shape, suspension system, and eye protection.

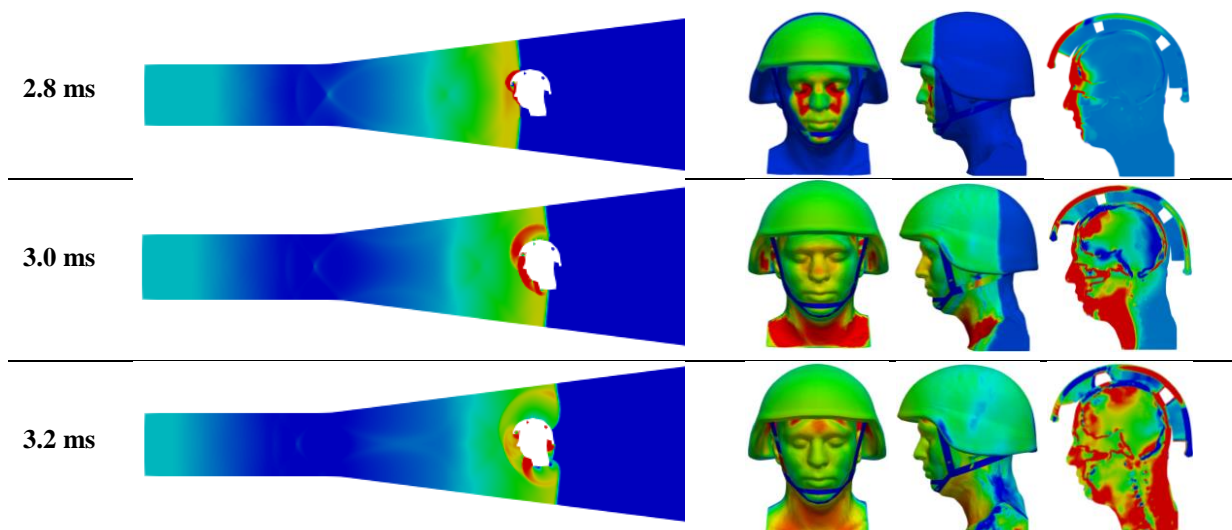


Figure 1. Shock wave interaction with the head with helmet at different times in the helium-air shock tube [3]. The diaphragm between driver and driven sections in the shock tube is ruptured at time = 0.

References

- [16] X.G. Tan, A.J. Przekwas and R.K. Gupta, *Shock Waves*, **27** (2017) 889.
- [17] X.G. Tan, R.N. Saunders and A. Bagchi, *ASME-IMECE* (2017) doi:10.1115/IMECE2017-70611.
- [18] X.G. Tan and A. Bagchi, *ASME-IMECE* (2018) doi:10.1115/IMECE2018-87689.

The comments expressed here are those of the authors and do not represent the opinion of the US Naval Research Laboratory or the US Department of Defense.

Tutorial: Neural basis for chronic headache and photophobia after mild traumatic brain injury

Akimasa Tashiro

Department of Physiology, NDMC

Mild traumatic brain injury (mTBI) accounts for the majority of head injuries, and posttraumatic headache (PTH) is the most common adverse effect. The most PTH has migraine-like phenotype (Hyperalgesia, Photophobia, Throbbing pain) and is difficult to resolve. It is well known that shock-wave induced traumatic brain injury. Recent studies show that laser-induced shock wave (LISW) lead to mTBI. The goal of this study is to establish a new model for PTH using LISW and to reveal the neural basis for PTH. The parietal region of male rats was irradiated with laser-induced shock wave (diameter 3mm, 4J/cm²) under barbiturate anesthesia. In awake male rats, ocular surface application of hypertonic saline (2.5 M; 40μl) evoked eye wipe behavior that was enhanced after mTBI. And also, in TBI rats, light-aversion behavior was enhanced compared to naïve rats. In separate rats, under isoflurane anesthesia, single cornea/dura responsive neurons were recorded at the trigeminal subnucleus caudalis (Vc). Hypertonic saline (2.5M) and blight light (irradiance=50, 300, 500 W/m²) selectively activated ocular surface and intraocular (neurovascular system), respectively. In TBI rats, Vc units had enhanced responses to hypertonic saline and blight light compared to naïve rats. To determine if mTBI caused bright light evoked intracranial blood flow increases, blood flow was monitored in arteries of the exposed cranial dura mater and the parietal cortex in naive and TBI rats. In TBI rats, bright light enhanced the magnitude of blood flow but not naive rats, and this blood flow increases evoked dura-responsive Vc units activities. Additionally, in TBI rats, bright light-increased dural blood flow was markedly inhibited by 10min after CoCl₂ injection into the subnucleus interpolaris/caudalis (Vi/Vc) transition, whereas blockade at the caudalis/upper cervical junction (Vc/C1) regions had no effect. It is concluded that mTBI produces a chronic state of hyperalgesia and light evoked vessels dilation that is reflected in the sensitization of trigeminal -parasympathetic circuit. This model may be suitable for future studies of migraine.

Experimental focal blast vs non-blast traumatic brain injury research: A comparison between Laser-induced shock wave model and CCI model

Satoshi Tomura¹, Satoko Kawauchi², Shunichi Sato², Daizoh Saitoh¹

¹ Division of Traumatology, Research Institute, National Defense Medical College

² Division of Bioinformation and Therapeutic Systems, Research Institute, National Defense Medical College

Blast induced traumatic brain injury (bTBI) caused by improvised explosive devices (IEDs) is especially frequent in recent conflicts. Mild bTBI can result in chronic neurobehavioral changes such as increased anxiety and memory disturbance. Animal models of blast injury are currently under investigation in order to understand the molecular and cellular mechanisms of mild bTBI leading to cognitive impairment. Although many approaches have been applied to elucidate bTBI, detailed mechanisms and prognosis of injury are still unclear. One of this reason is that the effects of the blast is very complex, with secondary (penetrating fragments) and tertiary (impact / acceleration) blast potentially masking any deleterious effects of primary blast on the brain. We have proposed the use of laser-induced shock waves (LISWs) to mimic primary blast-induced injury in animal models [1, 2, 3]. The characteristics of LISWs, such as safety, versatility and highly controllable shock wave energy, enable many experiments that cannot be performed with other shock wave sources. The most useful characteristic of LISWs is that can produce the focal primary blast injury whereas no other device could make that. We herein examined how to make the very mild bTBI mice model using LISWs that induces behavioral dysfunction, cognitive impairment and depression-like behavior, and also investigated the histopathological finding of this model. Furthermore, we made the very mild non-blast TBI mice model using CCI (controlled cortical impact) that is one of the most widely used models of experimental TBI, and compare the two models to investigate the difference between bTBI and non-blast TBI. All animal experiments were conducted according to the guidelines of the Institutional Review Board for the Care of Animal Subjects at the National Defense Medical College, Japan. Male C57BL/6 mice were anesthetized and LISWs were transcutaneously applied to the brain of the left parietal region. The LISWs were generated by irradiating an elastic laser target with a Q-switched ruby laser (wavelength; 694-nm, pulse width; 20 ns). The laser pulse was focused with a plano-convex lens to a 5-mm diameter spot on the target. The peak pressure of the LISWs under our conditions were approximately 1.30 MPa corresponded to the laser fluence of 0.8 J/cm², while the durations were approximately 0.02 ms. At the condition of 0.8 J/cm² and less level of LISWs, no obvious traumatic change was visible on the brain surface although some of the animals suffered from 0.9 J/cm² level of LISWs showed conspicuous hemorrhage on the brain surface. We determined that 0.8 J/cm² level of LISWs is the threshold to make the very mild bTBI mice model with no obvious traumatic change on the brain surface. Non-blast TBI model was made by using CCI device. The mice were transcutaneously injured over the left parietal region (the same point as the LISW models) at an impact depth 1 mm with a 5-mm diameter round impact metal tip (speed; 5.0 m/s, dwell time; 100ms). In this condition, no animal showed obvious hemorrhage on the brain surface after the insult although faster than 5.0 m/s impact induced some visible traumatic change on the head of mice. We assessed the behavior test and histopathological examination on day 7 and day 28 on both groups. We will report the findings and the difference between experimental focal bTBI and non-blast TBI.

References

- [1] Satoh Y, Sato S, Saitoh D, et al., *Laser Surg Med*, **42** (2010) 313-318.
- [2] Sato S, Kawauchi S, Okuda W, et al., *PLoS One*, **9** (2014) e82891.
- [3] Miyazaki H, Miyawaki H, Satoh Y, et al., *Acta Neurochir*, **157** (2015) 2111-2120.

Investigation of blast-induced traumatic brain injury thresholds and mechanisms using a rodent finite element model

M. Townsend¹ and N. Chandra¹

¹ Center for Injury Biomechanics, Materials and Medicine, Biomedical Engineering Department, New Jersey Institute of Technology, Newark, NJ, USA

The wide use of animal models of blast -induced traumatic brain injury (TBI) enables the investigation of injury biomechanics, injury mechanisms, prevention methods, and diagnostics in a way that is beyond the scope of human testing. However, anatomical differences between animal models and human create additional complexities in applying the extensive findings of animal models towards warfighter combat readiness. Computational models offer a unique opportunity to tie the injury mechanisms and responses of these species together, bridging that gap. This work seeks to understand existing mechanical injury thresholds, proposed for TBI in humans, using a computational animal model. A validated rodent head finite element model was used to simulate a wide variety of loading conditions corresponding with mild to moderate shock exposures (peak overpressure of 130, 180, and 230 kPa). Rodent simulation results were compared to human TBI mechanical injury thresholds developed. These mechanical injury thresholds were developed based upon human blunt injuries through the correlation of computer simulations with clinical injury definitions and accident reconstructions (Table 1). Simulation results were investigated to identify areas of the brain which exceeded each threshold value, indicating that a theoretical injury takes place. It was found that injury thresholds were largely non-discriminatory and without a neuropathological basis in the animal model, it offers little to no insight into the severity of injury observed in shock exposure. Of the fifteen injury criteria investigated, seven reported an injured brain volume of over 99% in the lowest severity blast investigated, 130 kPa (Figure 1A). Seven of the remaining eight reported an injured volume of under 1%. The five most severe strain-based injury criteria (15-26% strain) were not triggered for the highest severity shock simulated, 230 kPa (Figure 1B). This highlights the lack of sensitivity that existing mechanical injury thresholds have in predicting the subtle nature of blast injury in a rodent model. Although blast is known as a diffuse injury, a mechanical injury threshold should identify some spatial variability, as seen in the neuropathology of blast injuries at varying injury severity [1]. Therefore, a more sensitive injury marker is proposed which offers higher specificity for blast-induced TBI in a rodent model. This work marks an important step towards the development of mechanically-based, inter-species injury levels.

Table 1. A list of the injury criteria investigated in this work. The list and analysis techniques were based adapted from the work of Ref. [2] and expanded upon. σ_p – pressure stress, σ_{vm} – von Mises stress, τ – Tresca or maximum shear stress, ϵ – strain.

Injury Criteria	Injury Type	Reference
<i>Pressure-Based Injury Criteria</i>		
65.8 kPa max. σ_p	Concussion	Kleiven, <i>IBIA Congress</i> , (2008).
173 kPa max. σ_p	Mild TBI	Zhang et al., <i>J. Biomech. Eng.</i> , 126 (2004).
235 kPa max. σ_p	Severe TBI	Zhang et al., <i>J. Biomech. Eng.</i> , 126 (2004).
-100 kPa min. σ_p	Concussion	Takhounts et al., <i>Stapp Car Crash J.</i> , 47 (2003).
<i>Stress-Based Injury Criteria</i>		
8.4 kPa max. σ_{vm}	Concussion	Kleiven, <i>IBIA Congress</i> , (2008).
11 kPa max. σ_{vm}	Severe TBI	Kang et al. <i>SAE Techn. Pap.</i> , 41 (1997).
26 kPa max. σ_{vm}	Mild DAI	Deck and Willinger, <i>Int. J. Crashworthiness</i> , 13 (2008).
33 kPa max. σ_{vm}	Severe DAI	Deck and Willinger, <i>Int. J. Crashworthiness</i> , 13 (2008).
7.8 kPa max. τ	Mild TBI	Zhang et al., <i>J. Biomech. Eng.</i> , 126 (2004).
<i>Strain-Based Injury Criteria</i>		
5% max. principal ϵ	Moderate DAI	Marguiles and Thibault, <i>J. Biomech.</i> , 25 (1992).
15% max. principal ϵ	DAI	Takhounts et al., <i>Stapp Car Crash J.</i> , 47 (2003).
18% max. principal ϵ	DAI	Wright and Ramesh, <i>Biomech. Model. Mechanobio.</i> , 11 (2012).
20% max. principal ϵ	Mild TBI	Zhang et al., <i>J. Biomech. Eng.</i> , 126 (2004).
21% max. principal ϵ	Mild DAI	Kleiven, <i>Stapp Car Crash J.</i> , 51 (2007).
26% max. principal ϵ	Mild DAI	Kleiven, <i>Stapp Car Crash J.</i> , 51 (2007).

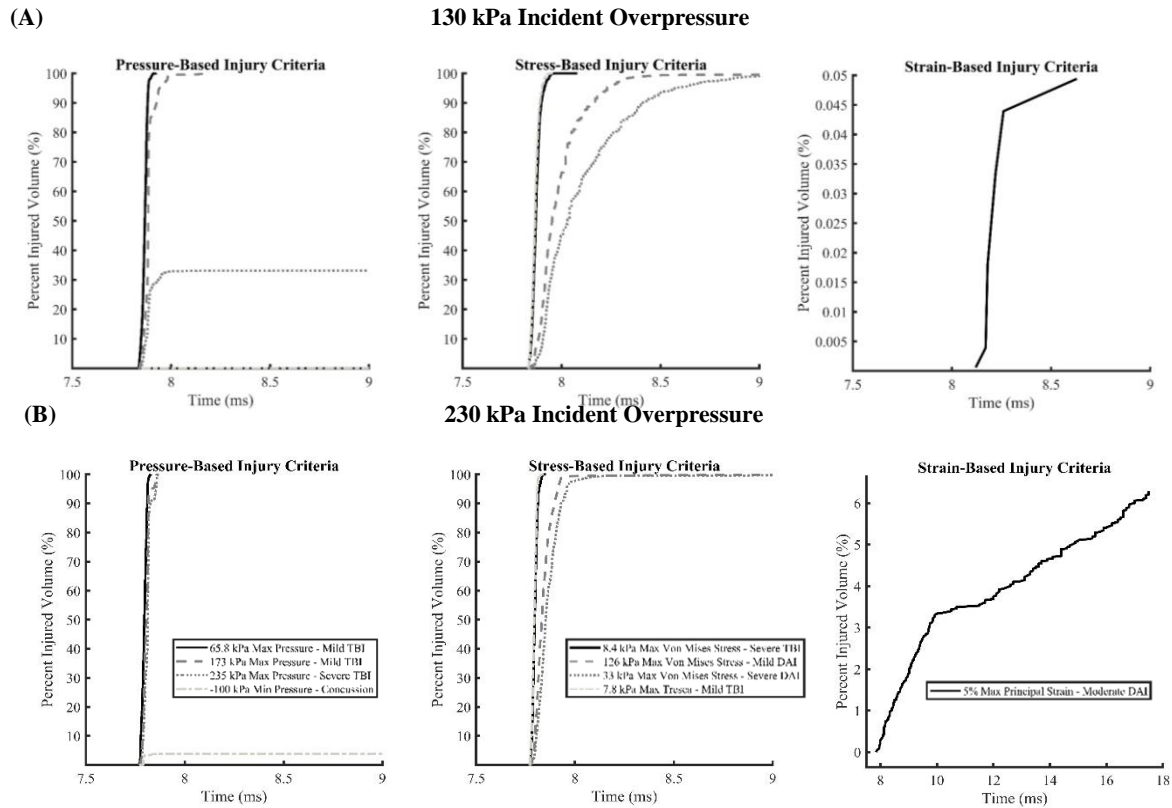


Figure 3. The percent injured volume of the brain for (left) four pressure-based injury criteria, (middle) four stress-based injury criteria, and (right) one strain-based injury criteria in (A) a 130 kPa shock exposure and (B) a 230 kPa exposure. The 11 kPa maximum von Mises stress injury criteria was excluded from the figure for clarity, as it exhibited a very similar response to the 8.4 kPa maximum von Mises stress criterion. Untriggered injury criteria were not included.

[1] M. Kuriakose, K. V. Rama Rao, D. Younger, and N. Chandra. *Sci. Rep.*, **8** (2018) 1.
 [2] R. Saunders, X. G. Tan, S. Qidwai, and A. Bagchi, *Ann. Biomed. Eng.*, (2018).

Effects of animal orientation on brain responses to primary blast

G. Unnikrishnan^{1,2}, H. Mao^{1,2}, A. Sundaramurthy^{1,2}, V. Sajja³, S. van Albert³, J. Long³, J. Rubio^{1,2},
D. Subramaniam^{1,2}, and J. Reifman¹

¹Department of Defense Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, United States Army Medical Research and Materiel Command, Fort Detrick, MD 21702, USA

²The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., 6720A Rockledge Drive, Bethesda, MD 20817, USA

³Blast Induced Neurotrauma Division, Center for Military Psychiatry and Neurosciences, Walter Reed Army Institute of Research, 503 Robert Grant Drive, Silver Spring, MD 20910, USA

Background:

Exposure to explosive devices is the leading cause of traumatic brain injury in United States Army Soldiers deployed to Iraq and Afghanistan [1]. The lack of clinical data for human exposure to primary blast waves has led to the development of experimental and computational animal-model studies to detect and quantify the potential brain injury associated with such exposure. Although there have been several animal studies to date, a consensus on the type or mechanism of this injury is lacking, in part due to differences in the type of shock tube, species of animal, and orientation of the animal in the shock tube. In recent experiments performed with rats in the vertical orientation [2, 3], brain-injury measures were compared to those from animals tested in the conventional, prone orientation. While these studies have shown that experimental measurements of intracranial pressure (ICP) are similar for certain brain locations, it is not known whether such similarity occurs throughout the brain and whether other biomechanical responses, such as shear stress and strain, are influenced by animal orientation. Because these responses may determine the intensity of brain injury, it is necessary to determine how animal orientation influences these biomechanical responses. In this study, we used a computational finite-element (FE) model to quantify the differences in the brain responses of rats when exposed to a blast wave in the prone and vertical orientations.

Methods:

We developed a high-fidelity three-dimensional (3-D) FE model of a rat, which consisted of the muscles of the rat head and torso, skull, brain, and cerebral vasculature. First, we obtained the geometry of the rat from magnetic resonance imaging scans and computed tomography images. Next, we obtained the material properties of the muscles and skull from the literature. We derived the material properties of the rat brain and cerebral vasculature from high-strain rate tests performed on these tissues [4, 5]. Finally, we assigned material properties to each component of the rat and developed the 3-D FE model. We coupled the FE model of the rat to a FE model of a shock tube and simulated a single blast exposure at blast overpressures (BOPs) of 110 and 130 kPa, for the prone orientation (i.e., the anterior-posterior axis of the animal aligned in the direction of blast-wave propagation with the head facing the blast wave) and vertical orientation (i.e., the anterior-posterior axis perpendicular to the direction of blast-wave propagation with the ventral side facing the blast wave). For both orientations, we computed the ICP and maximum principal strain at five points—anterior, central, posterior, top, and bottom—in the mid-sagittal plane of the brain.

Results:

We validated the 3-D FE model by comparing the peak ICP predicted by the model with experimental data. For the prone orientation, at an incident BOP of 110 kPa, the difference in peak ICP between the model prediction (113 kPa) and experimental measurement (104 kPa) was 8%. For the vertical orientation, at an incident BOP of 130 kPa, the difference between the predicted (125 kPa) and measured (114 kPa) peak ICP was 9%. In both orientations, the temporal profile of the ICP matched the incident blast profile.

Our results showed that the orientation of the animal in relation to the blast wave influenced the ICP predictions at the anterior and top points in the brain, but not at the central (where experimental measurements are often made), posterior, and bottom points. At 130 kPa, the difference in peak ICP for the animal in the vertical and prone orientations was as high as 30% at the top and anterior points, while it was as high as 6% at the bottom, central, and posterior points. In contrast, the maximum principal strain in the vertical orientation was nearly three times greater than that in the prone orientation at all points in the mid-sagittal plane of the brain. However, the spatial distributions of the strain predicted for the two orientations were similar. For both orientations, high strains were mostly concentrated in the peripheral cortices.

Conclusion:

In this study, we evaluated the biomechanical responses of the rat brain when exposed to a blast wave with the animal in a prone orientation and a vertical orientation. Our simulations showed that the animal orientation does not influence the pressure at the center of the brain, where measurements of brain pressure are often performed. Surprisingly, at locations away from the center, pressure predictions for the prone and vertical orientations differed by as much as 30%. In contrast to the pressure, the strain prediction in the vertical orientation was nearly three times greater than that in the

prone orientation. As the intensity of brain injuries are dependent on the biomechanical responses, we believe that the prone and vertical orientations will lead to considerably different injury patterns in the rat brain for an identical blast-wave exposure. Our high-fidelity FE model will aid in identifying correlates between the predicted biomechanical responses and observed changes in the brain tissue due to blast exposure, leading to an improved understanding of blast wave/rat-brain interactions.

Disclaimer:

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the United States (U.S.) Army, the Department of Defense (DoD), or The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF). Any citations of commercial organizations and trade names in this report do not constitute an official U.S. Army, DoD, or HJF endorsement of approval of the products or services of these organizations. This abstract has been approved for public release with unlimited distribution.

References:

- [1] B.E. Wojcik, C.R. Stein, K. Bagg, R.J. Humphrey, and J. Orosco, *American Journal of Preventive Medicine*, **38** (2010) S108.
- [2] I. Cernak, *Frontiers in Neurology*, **1** (2010) 151.
- [3] T. Sawyer, W. Yushan, R.D. V., J. Tyson, V. Mercy, S. Yimin, N. Peggy, H. Grant, W. Tracy, V. Cory, F. Changyang, and B. Julia, *Journal of Neurotrauma*, **33** (2016) 1181.
- [4] H.W. Haslach, J.M. Gipple, and L.N. Leahy, *Journal of the Mechanical Behavior of Biomedical Materials*, **68** (2017) 88.
- [5] D. Bell, M. Converse, H. Mao, G. Unnikrishnan, J. Reifman, and K. Monson, *Journal of Biomechanical Engineering*, **140** (2018) 071004.

Hierarchical Validation of the WIAMan LS-Dyna FEM for Application in Underbody Blast

Nicholas A. Vavalle¹, Christian W. Lomicka¹, Connor O. Pyles¹, Matthew T. Shanaman¹, and Randolph S. Coates²

¹The Johns Hopkins University Applied Physics Laboratory

²U.S. Army Futures Command, Data and Analysis Center

Under-body blast (UBB) has been a significant cause of injuries to the US Warfighter in recent conflicts [1]. Although testing is conducted to understand the likelihood of injury in the event of a UBB on a vehicle, the current tool used to assess Warfighter injury, the Hybrid III, is inadequate [2]. While the Hybrid III has served well to provide a cursory understanding of injury in UBB events, the Army required a more precise tool that would be able to take measurements and assess injuries relevant to UBB. In typical vehicle conditions during a UBB event, the extremities, pelvis, and lumbar spine are at particular risk for injury. Therefore, the Warrior Injury Assessment Manikin (WIAMan) ATD was developed to be a purpose-built tool to assess injury in vertical loading conditions, such as UBB.

The use of computational modeling enabled an accelerated ATD design process. Specifically, a finite element model (FEM) of the ATD (*Figure 4*) was developed to aid ATD design and serve as an injury assessment tool for the live fire community. The WIAMan FEM was able to produce fast and effective design studies to improve the biofidelity, or human-like behavior, of the ATD. Dozens of simulations were run at less cost to the project than the equivalent physical experiments would have required [3]. Further, the FEM has recently been used for ATD test planning and survivability assessment in severe conditions, which is another capability that will be valuable for the community.

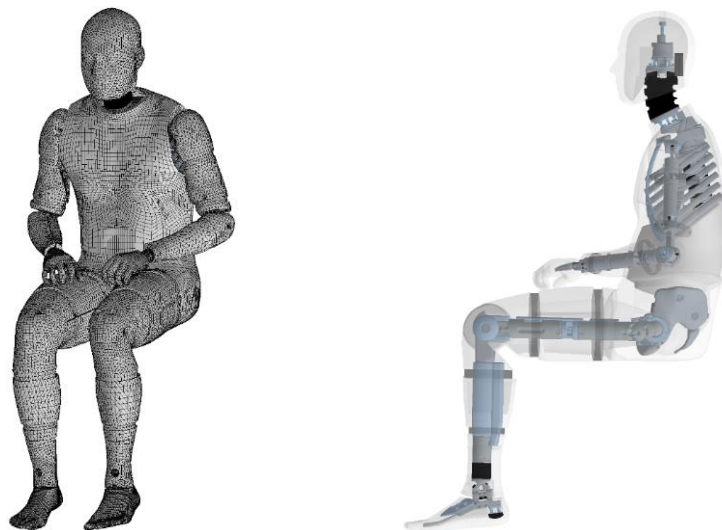


Figure 4. The WIAMan Finite Element Model will be used by the Army for injury predictions in simulated vertical loading environments. HoloLens apps were developed to showcase the results and impact of the model.

Validation was a necessary step to build trust in the model for predictive studies. The WIAMan FEM was developed and validated using a hierarchical approach. Component models were developed and validated individually and incorporated into the full system-level model. The test articles for the component simulations were the lower leg, pelvis, and lumbar spine. Validation of the component models occurred in two sets of experiments, nominally non-injurious and injurious loading conditions, with the exception of the pelvis that did not have injurious loading conditions available at the time of this study. The whole body model contains ~1.4 million nodes and was simulated in three loading conditions. Two whole body conditions were simulated from the Vertically Accelerated Load Transfer System (VALTS) test series, an upright posture (Condition 1) and a reclined-seatback posture with the legs extended (Condition 2). A unique model positioning framework was developed that leveraged Coordinate Measurement Machine (CMM) data collected during WIAMan ATD testing on the VALTS. Nodes ($n=25$) corresponding to the CMM points were prescribed to the precise experimental location, relative to the seat, using a short simulation that allowed the rest of the ATD to move naturally. This ensured maximum fidelity to the experimental boundary conditions, accounting for small asymmetries that arise during testing of the physical ATD. The third whole body simulation was of a test series run at the University of Michigan with the legs at an acute angle under the body (Condition 3). Correlation and Analysis (CORA) [4] was used to objectively evaluate the model signal traces against a reference (in this case the physical ATD). CORA rates signals from 0 to 1, with 1 being a perfect match, and has been shown to provide 4 independent evaluations of phase, magnitude, shape, and corridor (which accounts for variability in the reference) [5]. In this case only the phase, magnitude, and shape correlations were made because the physical ATD proved highly repeatable so a corridor score was unnecessary.

The typical simulation time for the whole body WIAMan FEM is approximately 13 hours for a 100 ms simulation on 100 processors. Results of the component simulations show good agreement with the experimental response. Average CORA scores for the components range from 0.64 to 0.89 across all input conditions (Figure 5). In whole body simulations, 57 signals were evaluated for each of the three whole body simulations including accelerations, rotations, forces, and moments. Results indicate a strong match to the experimental response with a CORA score of 0.77 in the nominal posture VALTS condition (Condition 1), 0.75 in the reclined posture VALTS condition (Condition 2), and 0.71 in the acute posture UMTRI condition (Condition 3).

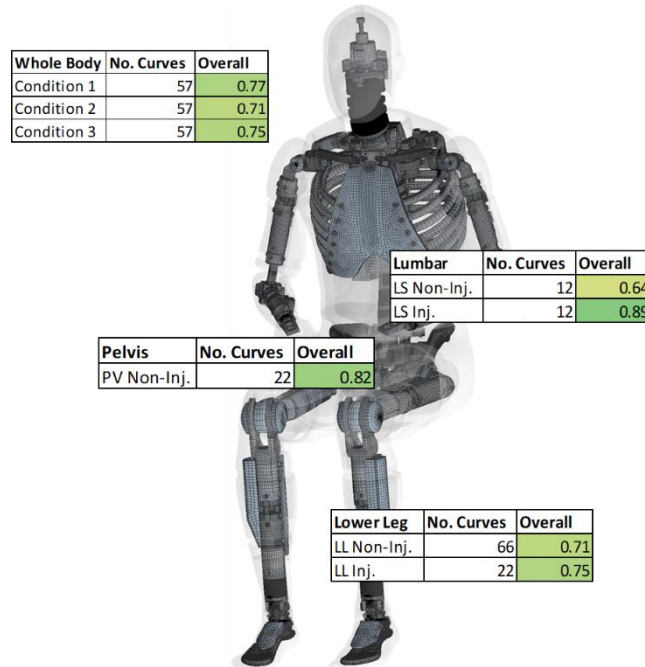


Figure 5. Overall average CORA scores for the 5 component test series and the 3 whole body conditions, with the number of curves from which the scores were calculated.

The WIAMan FEM will be used to reduce the vulnerability of service members subjected to extreme vertical accelerative loading conditions. These validation results, with over 300 signals compared using objective ratings, give confidence in the FEM’s ability to predict the ATD response to a variety of loading conditions. The WIAMan FEM will serve as a long-term complement to the physical ATD for live-fire test and evaluation. Future work includes the development of injury prediction capabilities. This study was part of the WIAMan Research sponsored by the U.S. Army Research Laboratory. The content included in this work does not necessarily reflect the position or policy of the U.S. government.

[1] B. D. Owens, et al., *J. Trauma - Inj. Infect. Crit. Care*, **64** (2008) 295.
 [2] K. A. Danelson et al., *Stapp Car Crash J.*, **59** (2015) 445.
 [3] M. P. Boyle, et al., *15th International LS-DYNA Users Conference* (2018).
 [4] C. Gehre, H. Gades, and P. Wernicke, *The 21st ESV Conference* (2009).
 [5] N. A. Vavalle, et al., *Traffic Inj. Prev.*, **14** (2013) S87.

DISTRIBUTION STATEMENT A - APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.

Interpretation of Body-Worn Sensor Data:

Tim Walilko PhD¹, Mark Tommerdahl PhD², Suthee Wiri PhD¹,
Ted Argo PhD¹, Christina Wagner PhD¹, Tim Bentley PhD⁴, Laila Zai PhD³

¹Applied Research Associates

²Cortical Metrics

³Lucent Research

⁴Office of Naval Research

Background

To mitigate the risk of TBI from repeated blast exposure, we must know the cumulative threshold of safe exposures in a given span of time, then apply that threshold to stand-down times and return-to-duty decisions. The Office of Naval Research's (ONR) Blast Load Assessment - Sense and Test (BLAST) program has produced estimated safe threshold blast overpressure limits which consider repetitions over a number of days as part of the calculations. The results have been incorporated into a probabilistic algorithm that predicts an individual's likelihood of significant physiological and functional change for the given blast profile. The BLAST Algorithm is being implemented into software that interfaces with operational blast gauges, such that each individual's cumulative exposures can be quantified and evaluated after each training day. If an individual's exposure history is at or beyond the programmed safety levels, he/she is sent for further neurofunctional assessments.

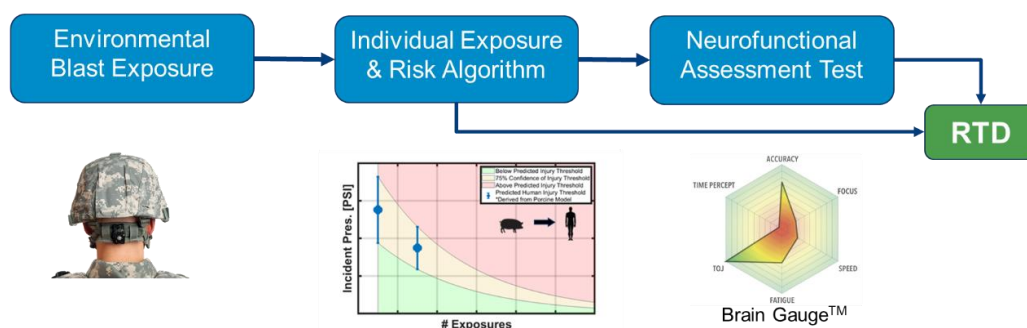


Figure 6: The BLAST technologies work in concert to track and evaluate blast exposure and provide science-driven stand-down decisions

Methods

The BLAST system of technologies has been derived using a physics-based approach by: 1) Analyzing available blast sensor data to compute each individual's unique exposure history to blast; 2) Leveraging the findings from research using a large animal blast model combined with the existing body of human clinical data to quantify biological response to blast; and 3) Applying advanced neurofunctional assessment tools to evaluate individuals who are over the threshold to assist in return-to-duty decision making. Together, when fully validated, the technologies of the BLAST program will predict the risk and severity of injury for operationally relevant blast loads.

Results

The BLAST Algorithm is being integrated into a fieldable software package that can operate on Android devices in austere environments to identify individuals of concern using data from their blast sensors. Individuals who have been identified then take the Brain Gauge™ battery of tests to determine if their nervous system has been potentially affected and whether they should seek further neurological assessment by a clinician.

Conclusion

The BLAST system of technologies is an alternative to the twenty-four hour stand-down after exposure to blast overpressure, as currently mandated by the Department of Defense. This system provides an automated methodology to interpret wearable blast sensor data in a consistent manner, regardless of operational conditions. It is believed that the rapid identification of individual's susceptibility to blast-generated injury will reduce the long term affects from repeated exposure to blast overpressure. The limitation of this approach is the current version of the technology cannot account for the synergistic effect of combined blast and blunt impact observed in the military environment.

The NiCoE TBI Injury History Assessment: An adaptation of the Ohio State University TBI Identification Method for use in a military population

T.T. Woo¹, A. Conrad², C. Davis², L.M. French¹, A. Gano², S.E. Kruger¹, W. Pettit¹, T.C. Pickett¹, C. Rhodes¹, K. Rosen², R. Sandlain², T. Staver¹, C. Tuman¹, H. Wu², K. Kenney¹ and J.M. Ollinger¹

¹ The National Intrepid Center of Excellence (NiCoE), Walter Reed National Military Medical Center, Bethesda, MD 20889

²Contractor, *in support of* the National Intrepid Center of Excellence (NiCoE), Walter Reed National Military Medical Center, Bethesda, MD 20889

Introduction. The following abstract describes the implementation of the NiCoE Injury History Assessment, a comprehensive assessment of head injuries and sub-concussive exposures based on the Ohio State University TBI Identification Method (OSU TBI-ID) in an intensive outpatient clinic serving active military [1-2]. Throughout the development of this interdisciplinary initiative, insights gleaned speak to the need for more structured and standardized assessment of head injuries sustained during the complex training and operational exposures common in active duty military personnel. Implications of and justification for the development of a blast ordnance and occupational hazards inventory to more uniformly and consistently capture recurring sub-concussive exposures, especially in the Special Operations Forces (SOF), are also provided.

The Intensive Outpatient Program at NiCoE. The National Intrepid Center of Excellence (NiCoE) is a state-of-the-art clinical and research facility, stood up by the Department of Defense in 2010 in order to advance our nation's understanding of traumatic brain injury (TBI) and associated psychological health (PH) conditions. The NiCoE is best known for its interdisciplinary four-week Intensive Outpatient Program (IOP) that integrates complementary and alternative medicine techniques with conventional neurological and behavioral health treatments. In addition to receiving clinical care, each service member (SM) is provided the opportunity to participate in various research ranging from observational to treatment studies. SMs attending the IOP often present with complex histories including 20+ years in service, multiple deployments, numerous head injuries, and frequent exposures to blast overpressures making them extremely challenging cases from both a clinical and research perspective.

The NiCoE Injury History Working Group. Prior to 2018, TBI injury histories were assessed via a self-report questionnaire included in a pre-admission packet for each SM entering in the IOP and augmented by individual clinic assessments conducted during their four-week stay. However, due to the vast number of events that many SMs have experienced prior to and during service, as well as the need for a standardized method to comprehensively collect injury history to better inform both clinicians and researchers, the NiCoE established an Injury History Working Group to begin administering a structured clinical interview based on the OSU TBI-ID. The narrative from this interview which summarizes personal histories of head or neck injuries and general blast exposure, is included as a note within the patient's medical records. Each narrative is then presented at a weekly interdisciplinary meeting, comprised of both research personnel and clinicians, where research determinations of TBI (mild, moderate, or severe based on VA/DoD guidelines) are discerned for each event, via group consensus. Events that fail to meet criteria for TBI, but are clinically suspect may be classified as potential concussive events (PCE). TBI determinations are based on the presence or absence of a witnessed loss of consciousness, alteration of consciousness, and/or post-traumatic amnesia. In the event that medical records are available from the time of injury (i.e., clinical notes, Glasgow Coma Scale (GCS) scores, radiological reports), they may be referenced to support the final research determination. In addition to classifying each qualifying injury as a TBI or PCE, the consensus group determines TBI severity, mechanism of injury (i.e., "blow", "blast", "blast+", "fall", etc.) and designation of the index (most severe) injury, where multiple qualifying events are identified within a single patient.

Beyond the Standard Interview. The Injury History Assessment itself is conducted within the first week of the IOP and consists of a 90-minute interview, to which two members of the NiCoE Injury History Working Group are assigned, one a credentialed clinician and the other a research staff member. After a brief introduction to the process, the patients are asked whether they have ever sustained injuries to their head or neck and provide as much detail as memory and/or medical records allow. In following with the OSU TBI-ID, patients are presented with five structured questions, prompting specific mechanisms of injury and situations prone to head injury, to help elicit recall and facilitate the conversation.

As the Injury History Interview protocol evolved, however, it became clear that additional service-related prompts and follow-up questions were needed to fully capture potentially injurious exposures that are commonplace within the military population, including, but not limited to: capturing training in addition to operational events, explicitly including military vehicles when inquiring about motor vehicle accidents and the review of parachute jumps and fast-roping in the context of fall injuries. Perhaps the greatest challenge in using the OSU TBI-ID in a military population is the lack of instruction and detail for capturing blast-related injuries. To address this limitation, a series of follow-up questions and qualifiers were incorporated into the Injury History Assessment. Specifically, upon disclosure of a potential blast-related head injury, a series of follow-up inquiries is initiated, including, but not limited to: the SM's

position with regard to the blast, the use or absence of personal protective equipment, the composition and size of the explosive device or ordnance, physical displacement and/or injury, and time between exposures in the case of serial or recurring events. Finally, in addition to those elements added to ensure the inclusion of common military-related events, several prompts were added to encapsulate training and operational exposures unique to SOF. Due to the multifaceted nature of these elite forces, SOF receive frequent and extensive training. To address this, exposures to high-speed watercraft, high-volume-high-caliber munitions, advanced breaching and combative training are explicitly queried and recorded.

Developing a Blast Exposure Inventory. While the development of thoughtfully-structured clinical interviews has greatly enhanced our ability to classify and characterize head injuries in the military population, each remains subject to the inherent challenges of self-reports. Namely, the loss of fidelity due to the passage of time and memory degradation, confounds of psychological and emotional distress, as well as the influence of pain and/or medication on memory consolidation at the time of injury. The intrinsically subjective nature of these assessments highlights the importance of and need for more objective measures of exposure. While blast gauges have greatly contributed to our understanding of the immediate physical effects of blast and allowed us to refine computational models of brain injury, they remain in the developmental stages and far from ubiquitous. In the absence of real-time blast gauge data, carefully-crafted inventories designed to catalogue and weight potentially injurious exposures may be vital in predicting susceptibility to TBI and severity of resulting symptomology. In reviewing the 200+ Injury History Assessments collected at NICoE over the past year, it is clear that the potential for and benefits of developing such an inventory are substantial. Consequently, the Injury History Working Group has begun the process of aggregating lists of blast exposures and occupational hazards commonly reported by SMs, including activities unique to SOF. Once these common elements have been identified, the next step will be to strategically partner with experts in the blast community to assist in the characterization and weighting of each exposure based on blast gauge data and computational models of brain injury.

DISCLAIMER: The identification of specific products or scientific instrumentation is considered an integral part of the scientific endeavor and does not constitute endorsement or implied endorsement on the part of the author, DoD, or any component agency. The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or U.S. Government.

[1] J.D. Corrigan and J.A. Bogner, *Journal of Head Trauma Rehabilitation*, **22** (2007) 318-329.

[2] J.A. Bogner and J.D. Corrigan, *Journal of Head Trauma Rehabilitation*, **24** (2009) 279-291.