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14. ABSTRACT

This is a multi-site observational study of military and law enforcement personnel who are exposed to overpressure (OP) where data is collected in conjunction with occupational training exercises. The study's primary purpose is to characterize a variety of weapon systems/environments (OP, impulse, acoustics) and measure effects (neurocognitive, biomarkers, symptom reporting, etc.) immediately after OP exposure (< 5 min)and/or at end of training day (i.e., acute effects). 6 data collections took place (n_1 = 11, n_2 = 26, n_3 = 16, n_4 = 26, n_5 = 36, n_6 = 23) at 3 different sites. Individual overpressure exposures recorded during training frequently exceeded the 4 psi incident safe threshold prescribed by US Army doctrine. Greater overpressure exposures were associated with neurocognitive deficits measured immediately after blast exposure and at the end of the training day. Greater acoustic exposure levels measured in a low overpressure blast environment were also associated with neurocognitive deficits. Changes were identified in blood-based biomarkers (targeted proteins, DNA methylation)after repeated blast overpressure exposure. Surrogate head and brain models were used by the Naval Research Laboratory (NRL) to collect data during wall breaching events at one training site. Three datasets, two blast events each, were collected by exposing neuronal cell cultures to wall breaching events during training exercises. Surrogate head and cell culture data have been analyzed.

15. SUBJECT TERMS
mTBI, neurotrauma, blast, blast exposure, immediate effects, military, breacher, explosive entry, blast injury, brain injury, blood based biomarker, injury threshold

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Military and law enforcement personnel are repeatedly exposed to overpressure (OP) during training and operations. This multi-site observational study will collect individual OP exposure data (including impulse and acoustics data when possible) and measure neurocognitive and physiological (blood based biomarkers, genetic markers, eye-tracking, tactile sensory perception, balance, symptom reporting) effects immediately after OP exposure and/or at end of day. The study will occur in conjunction with training exercises from a variety of weapon systems/environments where operators are exposed to OP ranging from safe (< 4 psi) to high exposure (> 8 psi) levels at local and national military and law enforcement training facilities. A parallel effort will collect biomechanical data using surrogate head forms that are embedded with sensors for obtaining pressures and accelerations. The surrogate head forms will be developed and customized for this project as a collaborative effort by the Naval Research Laboratory (NRL). Data collection efforts from this study will be used to quantify blast and its effects (neurocognitive and physiological) on the operator and identify variable(s) and/or test(s) which will serve to inform medical research with regards to increased risk and/or the effects of OP exposure. In addition, data derived from this study will be available for pertinent medical research in terms of OP exposure and neurotrauma modeling efforts. The Initiating Principal Investigator (PI) for the overall award and effort is Dr. Gary Kamimori at the Walter Reed Army Institute of Research (WRAIR); the Collaborating/Partnering PI for the sub-award to develop surrogate head models is Dr. Amit Bagchi at the Naval Research Laboratory (NRL).

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

mTBI, neurotrauma, blast, blast exposure, immediate effects, military, breacher, explosive entry, blast injury, brain injury, blood based biomarker, injury threshold

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Goal 1) Collect high overpressure exposure (8-12 psi) data on human operators during operational training and secondly to collect possible injury data using extensively instrumented human surrogates.

Goal 2) Identify and quantify acute, transient decrements in cognitive performance resulting from overpressure exposure within a variety of environments. This portion of the project will encompass as many different exposure environments as possible in order to characterize overpressure within these specific environments as well as individual operator exposure.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major Activities
   a. (WRAIR) Protocol was amended four times which involved increasing sample size to 800, adding audiometric testing, and adding 4 new collaborators.
   b. (WRAIR) Six human subjects data collections took place at 3 different sites, 3 x site #2 (Ft Leonard Wood, MO, Mar 2019, Aug 2019, Sept 2019); 2 x site #6 (Ft Benning, GA, Feb 2019, May 2019); 1 x site #7 (Camp Hansen, Okinawan, Japan, Oct-Dec 2019).
   c. (WRAIR) Four environmental characterization data collections took place at 2 x Camp Lejeune, NC (Mar 2019, Jul 2019); 1 x annual breaching circle (Phoenix, AZ; Apr 2019); and 1 x site #8 (Tier 1 Group, Aug 2019).
   d. (WRAIR) Blood based biomarker analyses and biological specimens transfer continues with collaborating investigators for blood based protein quantification and genetic analyses.
   e. (WRAIR) Five peer reviewed manuscripts were published; two additional publications are currently under review.
   f. (WRAIR) Two abstracts were submitted to the International Neurotrauma Symposium (29 Mar -2 Apr 2020, Melbourne, AU) on 12 Nov 2019; two abstracts were submitted to the National Capital Area Traumatic Brain Injury Research Symposium (5-6 Mar 2020) on 2 Dec 2019.
   g. (WRAIR) Symptom, demographics, and neurocognitive data from 6 data collections were submitted to the Federal Interagency Traumatic Brain Injury Research (FITBIR) Informatics System 26 Sept 2019. Blast exposure data (psi/impulse) submission was attempted, but there were issues with the data structure/submission; FITBIR is still consulting with their data curator to resolve the issue.
   h. (NRL) Completed testing with neuronal cell cultures exposed to blast. Three data collection events at Quantico (Apr 2019, May 2019, and June 2019) provided replicates for the data set. Cell culture data was processed and analyzed and a dose-response relationship developed relating cell culture metabolism to incident blast overpressure.
   i. (NRL) Fabricated replacement NRL Head-Neck Brain Models (NHBMs) for those damaged in previous experimental trials.
   j. (NRL) Using data collected at the WRAIR shock tube with a specially designed NHBM with surface mounted pressure sensors, it was determined that anomalous skull surface pressure data recorded at FLW was likely a sensor artifact due to the method of mounting the surface sensors. This data has been excluded from further analysis.

k. (NRL) Submitted one article to Frontiers in Neurology: Neurotrauma. Paper is under review.
l. (NRL) Published one poster in National Neurotrauma Society Symposium (NNS) 2019 in Pittsburgh, PA (June 2019).
m. (NRL) Published one poster in 2019 Military Health Sciences Research Symposium in Orlando, FL (August 2019).

2) Specific Objectives
   a. (WRAIR) Site #6, CY19 data collection #1, Ft. Benning, GA, Maneuver Center of Excellence
      i. Date(s): Feb 2019
      ii. Environment: Hand grenades
      iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, stress, demographics/occupational history, cheek swab, blood sampling
      iv. Number of subjects (complete data collection): 11
   b. (WRAIR) Site #2, CY19 data collection #2, Ft. Leonard Wood, MO, 35th Engineers
      i. Date(s): Mar 2019
      ii. Environment: Heavy wall breaching
      iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, demographics/occupational history, cheek swab, blood sampling
      iv. Number of subjects (complete data collection): 26
   c. (WRAIR) Site #6, CY19 data collection #3, Ft. Benning, GA, Maneuver Center of Excellence
      i. Date(s): May 2019
      ii. Environment: Hand grenades
      iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, stress, demographics/occupational history, cheek swab, blood sampling
      iv. Number of subjects (complete data collection): 16
   d. (WRAIR) Site #2, CY19 data collection #4, Ft. Leonard Wood, MO, 35th Engineers
      i. Date(s): Aug 2019
      ii. Environment: Heavy wall breaching
      iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, demographics/occupational history, cheek swab, blood sampling
      iv. Number of subjects (complete data collection): 26
   e. (WRAIR) Site #2, CY19 data collection #5, Ft. Leonard Wood, MO, 35th Engineers
      i. Date(s): Sept 2019
      ii. Environment: Heavy wall breaching
      iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, demographics/occupational history, cheek swab, blood sampling
      iv. Number of subjects (complete data collection): 36
   f. (WRAIR) Site #7, CY18 data collection #6, Camp Hansen, Okinawa Japan, Marine Expeditionary Force
      i. Date(s): Oct-Dec 2019
      ii. Environment: Close quarters training

iii. Data measures: overpressure exposure, acoustics, neurocognitive performance, symptoms, stress, demographics/occupational history, cheek swab, blood sampling
iv. Number of subjects (complete data collection): 23

g. (WRAIR) Environmental characterization data collection (overpressure, impulse, and acoustics) occurred at
i. Camp Lejeune, NC, Mar 2019
ii. Annual breaching circle, Phoenix, AZ, Apr 2019
iii. Camp Lejeune, NC, Jul 2019
iv. Site #8 Tier 1 Group (T1G), Aug 2019

h. (WRAIR) Data analysis in progress.
i. Analyses of cognitive performance, symptoms, exposure, demographics continues.
ii. Analyses of blood samples continues.
a. Continued quantitation of a seven biomarker panel (GFAP, UCH-L1, NF-L, Tau, Aβ-40 and Aβ-42) in association with symptomology before and after exposure to blast overpressure.
b. Continued proteomics analysis of serum samples (time-points tested in triplicate) collected from breacher trainees before and after acute blast overpressure exposure. Preliminary data indicate that a subset of proteins (18.5%) within the serum proteome were specific to post-overpressure exposure compared to pre-exposure and to non-breacher controls. Further, the majority of these proteins are classified as "brain-enriched". These data indicate that a select group of brain proteins are in peripheral blood as a consequence of blast overpressure.
c. Continued testing of a control dataset (non-overpressure exposed persons, commercially purchased) to build a protein data repository for biomarker comparisons. Notably, GFAP, UCH-L1, NF-L, Tau, Aβ-40 and Aβ-42 levels are significantly lower within this control group compared to participants who are sampled before and after overpressure exposure.
d. Initiated measurement of select protein biomarkers in blood samples for retrospective analysis of self-reported symptoms associated with long term breaching. This study seeks to focus on determining potential co-variates that influence biomarker levels associated with medical history, inclusive of prior brain injury or concussion status defined in subject reporting.
e. Processed and sequenced DNA methylation data from subset of explosive breaching sites samples (collected from two training courses) collected before and after exposure. Increased methylation was observed among genes as a consequence of overpressure exposure. These genes were found to be associated with neurodegenerative disease, anxiety, depression, disrupted sleep and circadian rhythms, and hearing loss.
f. Compared baseline DNA samples and genome-scale DNA methylation assays (Infinium MethylationEPIC chip) between "high" and "low" career blast exposed groups from a subset of explosive breaching participants; "high" and "low" groups were categorized using each participant's self-reported number of explosive breaching events (ranged from 0 to 400+) experienced throughout their career (Appendix A, Figure 1). Differentially methylated regions (DMRs)

of the genome were produced related to “high” vs “low” career blast exposed groups.

i. (NRL) Project is complete.
   a. Sensor based data from Quantico and Fort Leonard Wood breacher training classes using NHBMs have been compiled, and the data has been analyzed.
   b. Cell culture response changes data has been analyzed and will be developed into a publication in a peer reviewed journal.

3) Key outcomes

a. (WRAIR) Environmental characterizations of multiple weapon systems/configurations/materials were conducted and outcomes were shared with operators and command units to better understand occupational blast exposure and aid in decision making.

b. (WRAIR) Neurocognitive impairments are prevalent in the DANA Procedural Reaction Time (PRT) subtask which is a higher memory demand task. PRT places additional burden on working memory and maintenance of cognitive sets whereas the other DANA subtasks, simple reaction time (SRT) and Go/No-Go (GNG), require a single motor response to a visual target.

c. (WRAIR) Greater peak overpressure was associated with performance deficits measured immediately after blast exposure and at the end of the training day compared to baseline; as cumulative blast exposure increases over the training or on high operation days, performance defects also increase. This outcome may impact decision-making considerations on sequencing training events and cycling through personnel when tempo allows. Additionally, this information contributes to better assessment of risks against mission success and determining best courses of action.

d. (WRAIR) Concussive symptom reporting increased after training in an environment where overpressure measurements were very low [less than 0.5 pounds per square inch (psi)] and acoustic measurements (decibels; dB) were very high (>150 dB).

e. (WRAIR) Increased acoustic exposure levels measured in a low overpressure blast environment are associated with performance deficits. This outcome suggests that looking at other characteristics of blast, specifically sound pressure, in addition to blast overpressure (psi) is needed to improve our understanding and to mitigate risk in military training environments.

f. (WRAIR) GFAP suppression and amyloid beta elevation occur after blast overpressure exposure in blood samples collected from a subset of participants after explosive breaching or large caliber rifle trainings. Biomarker levels were associated with dizziness reported by participants among breachers.

g. (WRAIR) Six differentially methylated regions (DMRs) were identified in the “high” relative to “low” career blast exposed groups at baseline from a subset of explosive breaching participants; DMRs related to neuroinflammation and circadian rhythm regulation (Appendix A, Figure 2). Genome-wide methylation data were also analyzed relative to self-reported symptoms, such that these data were compared against highest reported symptoms of tinnitus (ringing in the ear), sleep disturbance, forgetfulness, and headache. DMRs were identified in regions related to tinnitus, sleep disturbance and headache.

h. (WRAIR) Symptoms reporting from a subset of explosive breaching participants were elevated after blast exposure related to headache, concentration, dizziness,

longer thinking times, and slowed thinking (Appendix A, Figure 3, pink bars). Headache was the most widely reported symptom pre vs post training; transcriptional analyses was performed focusing on gene expression changes associated with the headache symptom. There were 16 robustly differentially expressed genes (with fold change FC≥1.5) that tracked with headache (Appendix A, Figure 4). Of note, the gene growth arrest and DNA damage inducible gamma (GADD45G) shows an increase in gene expression following blast, and has previously been implicated in nervous system injury, where it has also been shown to be upregulated in animal models of peripheral nerve injury. We also identified KCNQ4 as tracking with the symptom of headache and it was significantly downregulated (Figure 4) in our expression analyses. This is particularly noteworthy because KCNQ4 is a potassium channel gene implicated in an autosomal dominant form of hearing loss, and mouse models have functionally localized KCNQ4 expression to outer hair cells of the cochlea.

i. (NRL) Neuronal cell metabolism changes to blast overpressure exposure in breaching exercises were demonstrated using an in vitro cell culture system and a biofidelic surrogate. Peak overpressure exposures of 4 to 5 psi showed initial cell response. The changes in metabolism of the neuronal cultures correlate well with peak overpressure in blast, and no correlation was noted with impulse. The dose response analysis with cell cultures thus suggests that peak pressure, not cumulative impulse, may be the more important parameter for predicting injury.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

(WRAIR/NRL) Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

(WRAIR) Dr. Kamimori (WRAIR PI) discussed/presented study progress and/or results at the following meetings and conferences,
8th Annual Breaching Circle, 22-26 April 2019, Phoenix, AZ
4th International Forum on Blast Injury Countermeasures (IFBIC), 8-10 May 2019, McLean, VA
National Neurotrauma Society Symposium (NNS), 29 Jun-3 July 2019, Pittsburgh, PA
Military Health Systems Research Symposium (MHSRS), 19-22 Aug 2019, Kissimmee, FL
In-progress review to CDMPR, 28-29 Aug 2019, Ft Detrick, MD
19th International Breachers Symposium, 27-30 October 2019, Lakeland, FL

(WRAIR) Preliminary brief back on site-specific blast exposure data (overpressure, impulse, acoustics) were presented to the following sites,
-Ft Leonard Wood, MO
-Ft Benning, GA
-Camp Hansen & Command, Okinawa, Japan
-Camp Lejeune, NC

(WRAIR) Study information and preliminary results were passed to US military and law enforcement agencies (30+ agencies); subsequently, information was provided to select NATO allies (including Netherlands, Belgium, Finland, Canada, Australia, New Zealand, Singapore).

(WRAIR) Study collaborators:
Dr. Angela Boutte (WRAIR, Brain Trauma, Neuroprotection, and Neurorestoration Branch Collaborating Investigator) presented findings at Annual Arrowhead TBI Conference on 15-16 May 2019, Arlington, VA.
Dr. Haghighi (James J Peters VA Medical Center Collaborating Investigator) presented findings at NNS 29 Jun-3 July 2019, Pittsburgh, PA; Sleep 9-12 Jun 2019, San Antonio, TX; MHSRS 19-22 Aug 2019, Kissimmee, FL.

(NRL) Regular meetings occurred between the NRL and the WRAIR research teams.

(NRL) Dr. O'Shaughnessy (NRL Co-PI) discussed/presented study progress and/or results at National Neurotrauma Society Symposium (NNS), 29 Jun-3 July 2019, Pittsburgh, PA.

(NRL) Dr. Bagchi (NRL PI) discussed/presented study progress and/or results at Military Health Systems Research Symposium (MHSRS), 19-22 Aug 2019, Kissimmee, FL

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

(WRAIR) Data collection will continue/finalize; identified sites include,
-Tacflow Academy
-Camp Pendleton, CA, I EOTG (IMEF)

-Ft Benning, GA

(WRAIR) Data analysis will continue. Statistical analyses using individual exposure data (e.g., additional cumulative overpressure exposure variables, acoustics etc.) with data collected pre/post overpressure exposure will continue. This includes correlating various demographic, behavioral, cognitive, and physical outcome metrics.

(WRAIR) Blood sample testing and comparisons using the seven-biomarker panel in serum samples from multiple sites will continue. Development and testing of the blood proteome, genome, transcriptome, bioinformatics will continue. Quantitation and analysis of biomarker levels in the context of medical history, inclusive of prior brain injury or concussion status defined in subject reporting will be completed. Testing/evaluation of a novel biomarker (undisclosed) and its ability to classify the effects of blast exposure will be initiated. Genome scale DNA methylation profiling on blood samples will continue and analyses that will incorporate demographics/cognitive response/behavioral/physical outcomes data collected during training will be initiated as funding supports.

(WRAIR) Manuscript preparation is planned for: DNA methylation data for publication in Frontiers in Neurology for the special issue on "Neurosensory Alterations from Blast Exposure and Blunt Impact"; baseline biomarker levels relevant to self-reported exposure and health history; biomarker levels as an effect of long-term blast exposure and self-reported TBI history; acoustic exposure effects on neurocognitive performance in a low blast overpressure environment; symptomology effects during breaching over multiple cohorts; neurosensory effects associated with high environmental sound pressure exposure; environmental characterization and data modeling involving overpressure, impulse, and acoustic data.

(WRAIR) Increased involvement/collaboration with Georgia Tech Research (GTRI) will occur to advance efforts related to collecting acoustic data.

(WRAIR) Data from sites #2 (Ft Leonard Wood, MO), #6 (Ft Benning, GA), #7 (Camp Hansen, Okinawa, JP and Camp Pendleton, CA), and others collected in 2020 will be submitted to FITBIR on an annual basis (submission deadline as discussed with Science Officer is 30 September). In addition, exposure data from all data collections sites will be formatted according to the FITBIR data from structure and submitted.

(WRAIR) Submit abstracts and present at the following conferences,
-National Capital Area TBI Research Symposium, (abstract accepted) 5-6 March 2020, Bethesda, MD
-9th Annual Breaching Circle, Los Angeles, CA, 23-27 Mar 2020
-International Neurotrauma Symposium, (abstract submitted) 29 Mar – 2 Apr 2020, Melbourne, AU
-National Neurotrauma Symposium, (abstracts due 1 April 2019) 27 June – 1 July 2020, Atlanta, GA
-Military Aspects of Shock and Blast (abstracts due 8 Feb 2020) 15-20 Nov 2020, Wollongong, AU
-Military Health Systems Research Symposium (abstract deadline, location, & dates: TBD)

-19th International Breachers Symposium, (location & dates: TBD)

(NRL) Nothing to report; project complete.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

(WRAIR) Characterizing environmental exposure, identifying inadequacies with current stand-off equations, expanding the variety of training environments, and including acoustic data to further environmental characterization will inform safety and occupational health groups on possible occupational training hazards.

(WRAIR) Data on the immediate and acute neurocognitive decrements associated with high blast exposure or higher acoustic levels in low overpressure environments will be used by the operational community in their risk/benefit assessment.

(WRAIR) These studies provide further evidence that proteins, peptides, and genes are biomarkers "responders" of blast overpressure exposure in a variety of training paradigms. Additionally, this work has begun to indicate that biomarker levels among military personnel is variable from non-exposed age-matched controls. Results from the past year indicate that a select group a proteins, typically associated with diagnosed TBI, are acute responders of exposure associated with operational decrement among explosive breacher trainees and asymptomatic large caliber rifle trainees in a manner that is applicable to rapid, same-day diagnostics. Proteomics and genomics have begun to further define the biological responses of overpressure exposure among study subjects compared to non-exposed controls, further expanding the repertoire of exposure related biomarkers as reflection of impairment vs. resilience, or symptomatic vs asymptomatic subjects.

(WRAIR) This is the first study to delineate the effects of blast exposure in military personnel on molecular and biological signatures in humans. Specifically, this work highlights how DNA methylation and gene expression patterns change through both acute blast exposure (before and after blast) and in cumulative (lifetime) exposure to blast throughout a career in the military. These projects will make an impact on the field of blast exposure research, mild TBI research, and research conducted in the context of the military.

(NRL) A dose-response relationship was developed relating neuronal cell metabolism changes to incident blast overpressure exposure using an in vitro cell culture system and biofidelic surrogate. Initial cell response was found to be at overpressure exposures between 4 and 5 psi peak pressure. Additionally, it was determined that the changes in metabolism of the neuronal cultures exposed to blast correlate well with peak pressure; no correlation was noted with impulse. This suggests that peak pressure, not impulse, may be the more important parameter for predicting injury.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

(WRAIR) Data for this reporting period indicates that proteins and / or genes derived from either targeted analysis or prior association with TBI, or from omics-based discovery methods are applicable to detection of overpressure effects in the context of subclinical injury. Therefore, biomarkers herein may have utility for early, or low-level, detection of nervous system injuries or diseases.

(WRAIR) This work is likely to impact policies, research protocols, and laws related to military personnel, the Department of Defense, and blast exposure. Notably, a recent 2020 National Defense Authorization Act (NDAA) ordered the history of blast exposure and blast duration from both combat exposure and trainings to be included in medical histories of troops. This decision by Congress is highly relevant to this research and may expand to include additional research outcomes related to effects of acute and chronic blast exposure on molecular signatures and blast-related symptoms.

(NRL) The finding that peak incident pressure is correlated with changes in cellular function may affect the direction of future shockwave bioeffects research. It may also help to determine an injury mechanism of shockwave damage. The dose-response curve is one of the first to relate changes in cell behavior to blast overpressure exposure, and could serve as a basis for setting exposure thresholds. Additional testing with the cell/surrogate system with helmets could lead to a better understanding of helmet effects on the brain during blast and improved helmet designs.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

(WRAIR/NRL) Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

(WRAIR) Results from environmental characterization continues to impact military and law enforcement operators by increasing knowledge of blast pressure, modifying training practices, aiding in mission planning, and improving safety.

(WRAIR) This work may impact Department of Defense protocols and practices. Congressional acts including the John S. McCain National Defense Authorization Act for Fiscal Year 2019 have called for "review of guidance on blast exposure during training" (Section 253), which emphasize the importance of understanding and mitigating the effects of blast after successive days of training and over the course of a military career. Thus, this work is relevant to delineate the biological responses to blast exposure, a primary goal of congressional acts to date, and has the potential to impact blast-related policies and training/exposure protocols.

(NRL) Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

(WRAIR/NRL) Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

(WRAIR) Processing/assaying/analyzing biospecimens has been moderately delayed due to acquisition of biochemistry, supplies, and analytical software. The collaborating investigators tasked with biospecimen analyses are coordinating with institutional internal processes and external vendors to facilitate supply pipelines. Investigators are keeping pace by prioritizing samples to process as directed by the PI.

(NRL) Nothing to report.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

(WRAIR) No cost extension has been requested to finish data collection and complete reporting/disseminating study outcomes. The remaining senior study staff person has resigned; efforts are underway to find a replacement for the position.

(NRL) Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

(WRAIR) Core protocol Amendment #9 was submitted to WRAIR HSPB/IRB on 27 August 2019, approved by WRAIR IRB on 06 September 2019, and received Commander Authorization on 13 September 2019; HRPO will be notified during annual reporting. The amendment updated site specific blood draw volume details applicable to Addenda B & G (sites #2 and #6 respectively).

(WRAIR) Core protocol Amendment #10 was submitted to WRAIR HSPB/IRB on 16 September 2019, approved by WRAIR IRB on 24 September 2019, and received Commander Authorization on 26 September 2019; HRPO will be notified during annual reporting. The amendment updated the Addendum G (site #6) informed consent form.

(WRAIR) Core protocol Amendment #11 was submitted to WRAIR HSPB/IRB on 19 September 2019, approved by WRAIR IRB on 10 October 2019, and received Commander Authorization on 1 November 2019; HRPO will be notified during annual reporting. The amendment increased the overall sample size as stated in the core protocol from 500 to 800.

(WRAIR) Core protocol Amendment #12 was submitted to WRAIR HSPB/IRB on 1 November 2019, approved by WRAIR IRB on 4 December 2019, and received limited Commander Authorization on 12 December 2019; HRPO acknowledgement is pending receipt of external collaborator's research determination letter. The amendment added audiometric testing, 4 collaborating/associate investigators, and data sharing language with data repositories. The amendment is limited by receipt of external collaborator's research determination letter and finalizing reliance agreement between US Naval Hospital Okinawa and WRAIR.

(NRL) Nothing to report.

Significant changes in use or care of vertebrate animals.

(WRAIR/NRL) Nothing to Report, not applicable

Significant changes in use of biohazards and/or select agents

(WRAIR/NRL) Nothing to Report, not applicable

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

(WRAIR)
Boutte, A. M., Thangavelu, B., LaValle, C. R., Nemes, J., Gilsdorf, J., Shear, D. A., & Kamimori, G. H. Brain-related proteins as serum biomarkers of acute, subconcussive blast overpressure exposure: A cohort study of military personnel, PLoS One, 14 no. 8: 2019; e0221036.
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0221036; published; yes.

LaValle, C.R., Carr, W., Egnoto, M.J., Misistia, A.C., Salib, J., & Ramos, A.N., & Kamimori, G.H. Neurocognitive Performance Deficits Related to Immediate and Acute Blast Overpressure Exposure. Frontiers in Neurology; 10: 2019; p.949.
https://www.frontiersin.org/articles/10.3389/fneur.2019.00949/full; published; yes.

Sajja, S.V., Kamimori, G.H., LaValle, C.R., Salib, J.E., Misistia, A.C., Ghebremedhin, M.Y., Ramos, A.N., Egnoto, M.J. and Long, J. The Role of Very Low Level Blast Overpressure in Symptomatology. Frontiers in Neurology; 10: 2019; p.891.
https://www.frontiersin.org/articles/10.3389/fneur.2019.00891/full; published; yes.

Skotak, M., LaValle, C. R., Misistia, A.C., Egnoto M. J., Chandra, N., & Kamimori, G.H. Occupational Blast Wave Exposure During Multiday .50 Caliber Rifle Course. Frontiers in Neurology; 10: 2019; p. 797.
https://www.frontiersin.org/articles/10.3389/fneur.2019.00797/full; published; yes.

Chatterton, Z., Mendelev, N., Chen, S., Raj, T., Walker, R., Carr, W., Kamimori, G., Beeri, M., Ge, Y., Dwork, A. and Haghighi, F., (2019). Brain-derived circulating cell-free DNA defines the brain region and cell specific origins associated with neuronal atrophy. bioRxiv, p.538827. https://www.biorxiv.org/content/biorxiv/early/2019/02/02/538827.full.pdf; published; yes.

Thangavelu, B., LaValle, C.R., Egnoto, M.J., Nemes, J., Boutté, A.M., Kamimori, G.H. Overpressure Exposure from .50 Caliber Rifle Training is associated with Increased Amyloid Beta Peptides in Serum. Frontiers in Neurology; under review; yes.

Khokhar B, LaValle CR, Bailie J, Carr W, Malik S, Martin EM, & Kamimori GH; Health Symptomology following environmental blast exposure during heavy wall breaching in a military cohort; Military Medicine; manuscript revisions; yes.

Chen, Y.C., O'Shaughnessy, T.J., Kamimori, G.H., Horner, D.M., Egnoto, M.J. and Bagchi, A., Role of Interfacial Conditions on Blast Overpressure Propagation into the Brain, Frontiers in Neurology; under review; yes.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

(WRAIR)
Misistia, A. C., Salib, J. E., Kamimori, G. H., LaValle, C. R., Ramos, A. N., Carr, W., & Egnoto, M. J. Acoustic Exposure Comparison on Instructors that are Experienced During Heavy Wall Explosive Breaching, Ballistic Breaching, and Grenades Training. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Salib, J. E., Misistia, A. C., Kamimori, G. H., LaValle, C. R., Ramos, A. N., Carr, W., & Egnoto, M. J. The Characterization of Flashbangs used by Law Enforcement and Military Personnel. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Wilson, C., Mendelev, N., Wang, Z., Choi, B., Ge, Y., Carr, W., Weber, N., Kelley, A. L., LaValle, C., Boutte, A., Kamimori, G. H., & Haghighi F. Biosignatures of Accumulated Blast Exposure and Associated Symptoms of Tinnitus and Hearing Loss in Exposure to Explosive Breaching. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Haghighi, F., Wang, Z., Mendelev, N., Choi, B., Wilson, C., Nemes, J., LaValle, C., Ge, Y., Kamimori, G., Shear, D., Boutte, A., & Carr, W. Peripheral Markers of Operational Blast Exposure. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Medda, A., Funk, R., Ahuja, K., & Kamimori, G. Measurements of Infrasound Signatures from Grenade Blast During Training Presentation. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; no.

Sajja, V. S., LaValle, C. R., Salib, J. E., Misistia, A. C., Ghebremedhin, M. Y., Carr, W., Egnoto, M. J., Long, J. B., & Kamimori, G. H. The Role of Acoustic Signatures in Overpressure Symptomatology. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Skotak, M., LaValle, C., Misistia, A., Egnoto, M. J., Chandra, N., & Kamimori, G. Occupational Blast Wave Exposure during Multiday .50 Caliber Rifle Course Presentation. Military Health System Research Symposium; Kissimmee, FL; 2019; conference poster; accepted; yes.

Kamimori, G.H., Salib, J.E., Misistia, A.C., Egnoto, M.J., Sajja, V.E. (2019) Acoustic and blast overpressure exposure in a military training environment. Journal of Neurotrauma. 36(13). A05-21. doi: 10.1089/neu.2019.29100.abstracts. 2019; abstract & conference poster; published; yes.

Khokhar, B., LaValle, C.R., Bailie, J., Malik, S., Martin, E., Carr, W., Kamimori, G.H. (2019) Change in health related symptoms following environmental blast exposure during heavy wall breaching training in a military cohort. Journal of Neurotrauma. 36(13). A05-02. doi: 10.1089/neu.2019.29100.abstracts. 2019; abstract & conference poster; published; yes.

Carr, W., LaValle, C.R., Egnoto M.J., Polejaeva, E., Kamimori, G.H. (2019) Neurocognitive performance deficits related to immediate and acute blast overpressure exposure. Journal of Neurotrauma. 36(13). A05-18. doi: 10.1089/neu.2019.29100.abstracts. 2019; abstract & conference poster; published; yes.

Haghighi, F., Ge, Y., Wang, Z., Mendelev, N., Choi, I., Vitale, A., Wilson, C., Nemes, J., LaValle, C., Kamimori, G., Shear, D., Boutte, A., Carr, W. (2019). Molecular correlates of occupational blast and associated sleep disturbances. Sleep. 42(abstract supplement), 0012. https://sleepmeeting.org/wp-content/uploads/2019/04/SLEEP_42_S1-Website-Final.pdf. 2019; abstract & conference poster; published; no.

(NRL)
Nothing to report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

(WRAIR)
*LaValle, C. R., Egnoto, M. J., Carr, W., Misistia, A. C., Salib, J., Ramos, A. N., & Kamimori, G. H. Neurocognitive Effects from Immediate and Acute Blast Overpressure Exposure: Insight on Using DANA to Assess Performance Change during Explosive Breaching. Military Health System Research Symposium; Kissimmee, FL; 2019; conference presentation; yes.

Kamimori, G. H., LaValle, C. R., Ramos, A. N., Misistia, A. C., Salib, J. E., Carr, W., & Egnoto, M. J. Acoustic Characterization Of Military And Law Enforcement Personnel. Military Health System Research Symposium; Kissimmee, FL; 2019; conference presentation; yes.

Khokhar, B., LaValle, C., Bailie, J., Malik, S., Martin, E. M., Carr, W., & Kamimori, G. H. Change in Health Related Symptoms Following Environmental Blast Exposure During Heavy Wall Breaching Training in a Military Cohort. Military Health System Research Symposium; Kissimmee, FL; 2019; conference presentation; yes.

Przekwas, A., Garimella,H., Chen, Z.J., Harrand, V., Kamimori, G.H., Gupta, R. A computational Framework for Personalized Monitoring of Blast Exposure in Military Training and Operations. Military Health System Research Symposium; Kissimmee, FL; 2019; conference presentation; yes.

Haghighi, F. Acute and Chronic Biomarkers of Blast. National Neurotrauma Society Annual Symposium; Pittsburgh, PA; 2019; conference presentation; no.

Boutte, A.M. Serum Biomarkers of Overpressure Exposure among Military Personnel. Arrowhead TBI Conference; Arlington, VA; 2019; conference presentation, yes.

Kamimori, G.H. & Carr, W. Evaluation of the Effects of High Level Overpressure (8+ psi) on Cognitive Performance, Brain Blood Biomarkers and Symptom Reporting. Military Operational Medicine Research Program In-progress Review; Ft Detrick, MD; 2019; presentation; yes.

Boutte, A.M. Biomarker Responses Methods & Results supplement to Evaluation of the Effects of High Level Overpressure (8+ psi) on Cognitive Performance, Brain Blood Biomarkers and Symptom Reporting. Military Operational Medicine Research Program In-progress Review; Ft Detrick, MD; 2019; presentation; yes.

LaValle, C.R., Egnoto, M.J., Carr, W., & Kamimori, G.H. Neurocognitive Effects of Blast Overpressure from Breaching Courses: Using DANA To Assess Performance

Related To Immediate And Cumulative OP Exposure. International Forum on Blast Injury Countermeasures, McLean, VA; 2019, presentation, yes.

(NRL)
O'Shaughnessy, T.J., Chen, Y., Gilpin, K.M., Kamimori, G.H. and Amit Bagchi, A. Mechanical and Cellular Response to Low Level Blast. National Neurotrauma Society Symposium (NNS), 29 Jun-3 July 2019, Pittsburgh, PA. Poster.

Chen, Y.C., Bagchi, A., Kamimori, G.H., Horner, D.M. and O'Shaughnessy, T.J., Comparison of In-Brain Pressure in Blast for Surrogate Headforms with Gel-Fluid Interface and Gel-Skull Interfaces, Proceedings of Military Health Sciences Research Symposium 2019, Orlando, FL; published: yes.

Website(s) or other Internet site(s)
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

(WRAIR/NRL) Nothing to Report

Technologies or techniques
Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

(WRAIR/NRL) Nothing to Report

Inventions, patent applications, and/or licenses
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

(WRAIR/NRL) Nothing to Report

Other Products
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*

- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

- (WRAIR) Data: Overpressure, acoustics, neurocognitive, symptom, demographic/occupational history data were collected at Ft. Leonard Wood, MO; Ft. Benning, GA; and Camp Hansen, Okinawa, JP (sites #2, #6, and #7, respectively); in addition, stress inventory data were collected at sites #6 and #7.
- (WRAIR) Biospecimen collections: cheek swab and blood samples were collected at sites #2, #6, and #7.
- (WRAIR) Symptom, neurocognitive, demographics data from sites #1 (Washington County, OR), #2, #6, #7 were submitted to FITBIR Informatics System on 26 September 2019.
- (WRAIR) Biospecimens were transferred to James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai (New York, NY) for additional biomarker (genetic) analyses.
- (NRL) Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name: Gary Kamimori
Project Role: WRAIR PI
Research Identifier (ORCID ID): 0000-0001-7899-9553
Nearest person month worked: 9
Contribution to project: no change.

Name: Angela Boutté
Project Role: WRAIR Laboratory Investigator
Research Identifier (ORCID ID): 0000-0003-3542-8122
Nearest person month worked: 6
Contribution to project: no change.
Funding Support: The Combat Casualty Care Research Program

Name: Bharani Thangavelu
Project Role: WRAIR Research Fellow; National Research Council
Nearest person month worked: 6
Contribution to project: Conducted blood based biomarker assays and analytics.

Name: Fatemeh Haghighi
Project Role: WRAIR Collaborating Laboratory Investigator, James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai
Research Identifier (ORCID ID): 0000-0003-0920-6956
Nearest person month worked: 1
Contribution to project: no change.
Funding Support: Research Career Scientist Award (#1IK6CX002074) from the Department of Veterans Affairs. RR&D: RX001705

Name: Caroline Wilson
Project Role: Research Associate, James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai
Research Identifier (ORCID ID): 0000-0002-2553-4151
Nearest person month worked: 6
Contribution to project: DNA sample processing and preparation for sequencing. Conducted blood based biomarker assays involving ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) analyses.
Funding Support: RR&D: RX001705

Name: Zhaoyu Wang
Project Role: Data Scientist, James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai
Nearest person month worked: 6
Contribution to project: Quality control and data analysis of methylation and sequencing data. Creating and running pipelines for analyzing data; figure generation.
Funding Support: RR&D: RX001705

Name: Yongchao Ge

Project Role: Senior Biostatistician, James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai
Nearest person month worked: 1
Contribution to project: Senior statistician on DNA methylation pipelines and analyses.
Funding Support: RR&D: RX001705

Name: Amit Bagchi
Project Role: NRL PI
Nearest person month worked: 1
Contribution to project: no change.
Funding Support: MIPR 11274678

Name: Thomas O'Shaughnessy
Project Role: NRL PI
Nearest person month worked: 2
Contribution to project: no change.
Funding Support: MIPR 11274678

Name: YungChia Chen
Project Role: NRL Biomedical/Biomechanical Engineer
Nearest person month worked: 2
Contribution to project: no change.
Funding Support: MIPR 11274678

Name: David M Horner
Project Role: NRL Test Engineer
Nearest person month worked: 1
Contribution to project: no change.
Funding Support: MIPR 11274678

Name: Christina LaValle
Project Role: WRAIR Data Manager/Statistician, Study Coordinator
Research Identifier (ORCID ID): 0000-0003-1529-4761
Nearest person month worked: 12
Contribution to project: no change

Name: Mike Egnoto
Project Role: WRAIR Clinical Informaticist
Nearest person month worked: 9
Contribution to project: no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

(WRAIR/NRL) Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Military
Organization Name: U.S. Army 35th Engineers of Ft Leonard Wood
Location of Organization: Ft Leonard Wood, MO
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization permitted subject recruitment to enroll study volunteers

Organization Name: U.S. Marine Corps Methods of Entry School
Location of Organization: Quantico, VA
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: 194th Armor & 198th Infantry
Location of Organization: Ft Benning, GA
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization permitted subject recruitment to enroll study volunteers

Organization Name: I & III Marine Expeditionary Force (MEF)
Location of Organization: Camp Pendleton, CA (I MEF) & Camp Hansen, Okinawa, Japan (III MEF)
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization permitted subject recruitment to enroll study volunteers

Organization Name: 171st Infantry
Location of Organization: Ft Jackson, SC
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: US Army Research, Development Engineering Command (RDECOM)
Location of Organization: Aberdeen Proving Ground, Aberdeen, MD and Picatinny Arsenal, Wharton, NJ
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: US Army Research Laboratory (ARL)
Location of Organization: Adelphi, MD
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: US Army Asymmetric Warfare Group (AWG)
Location of Organization: Ft A.P. Hill, VA
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: Engineer Research and Development Center
Location of Organization: Champaign, IL
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: US Army Training and Doctrine Command (TRADOC) Maneuver Center of Excellence (MCoE)
Location of Organization: Ft Benning, GA
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: US Army Aeromedical Research Laboratory (USAARL)
Location of Organization: Ft Rucker, AL
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: Department of Energy
Location of Organization: Oakridge, TN/Albuquerque, NM
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization permitted subject recruitment to enroll study volunteers

Organization Name: Las Vegas Special Weapons and Tactics (SWAT)
Location of Organization: Las Vegas, NV
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization will permit subject recruitment to enroll study volunteers

Organization Name: Washington County Sheriff's Office
Location of Organization: Beaverton, OR
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization will permit subject recruitment to enroll study volunteers

Organization Name: Portland Special Emergency Reaction Team (SERT)
Location of Organization: Portland, OR
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: Dallas Special Weapons and Tactics (SWAT)
Location of Organization: Dallas, TX
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities

- Collaboration – technical discussions between partner and project staff

Organization Name: Maricopa County Sheriff's Office
Location of Organization: Maricopa, AZ
Partner's contribution to the project
- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Organization Name: National Capital Region Breachers Consortium (28 team)
Location of Organization: Washington, DC
Partner's contribution to the project
- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff

Private Tactical Training Groups
Organization Name: Forced Entry Tactical Training (FETT)/Global Assets Integrated
Location of Organization: multiple sites/locations
Partner's contribution to the project
- Facilities – project staff use partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Organization Name: Tacflow Academy
Location of Organization: multiple sites/locations
Partner's contribution to the project
- Facilities – project staff used partner's facilities for project activities
- Collaboration – technical discussions between partner and project staff
- Other – partner organization will permit subject recruitment to enroll study volunteers

Organization Name: Tactical Energetic Entry Systems (TEES)
Location of Organization: Southaven, MS
Partner's contribution to the project
- Facilities – project staff use partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Academic
Organization Name: Defense and Veterans Brain Injury Center
Location of Organization: Silver Spring, MD; Colorado Springs, CO; Camp Pendleton, CA
Partner's contribution to the project
- Collaboration – technical discussions between partner and project staff

Organization Name: Georgia Tech Research Institute (GTRI)
Location of Organization: Atlanta, GA
Partner's contribution to the project
- Facilities – project staff use partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Organization Name: Johns Hopkins University Applied Physics Laboratory
Location of Organization: Laurel, MD
Partner's contribution to the project

- Facilities – project staff use partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Organization Name: New Jersey Institute of Technology
Location of Organization: Newark, NJ
Partner's contribution to the project

- Facilities – project staff used partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Organization Name: James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai
Location of Organization: New York, NY
Partner's contribution to the project

- Facilities – project staff use partner's facilities for project activities
- Collaboration – partner staff work with project staff on project

Organization Name: Penn State University
Location of Organization: State College, PA
Partner's contribution to the project

- Collaboration – technical discussions between partner and project staff

Organization Name: New York Genome Center (NYGC)
Location of Organization: New York, NY
Partner's contribution to the project:

- In-kind support - NYGC completed DNA methylation assays on Infinium MethylationEPIC chips as a service to James J. Peters VA Medical Center, Icahn School of Medicine at Mount Sinai.

Private Technology
Organization Name: Applied Research Associates
Location of Organization: San Antonio, TX
Partner's contribution to the project

- Collaboration – partner staff work with project staff on project

Organization Name: CFD Research Corporation
Location of Organization: Huntsville, AL
Partner's contribution to the project

- Collaboration – technical discussions between partner and project staff

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

a. Blood-based biomarker Figures 1 – 4
b. NRL Neurotrauma poster
c. NRL MHSRS poster

Appendix A – blood based biomarkers

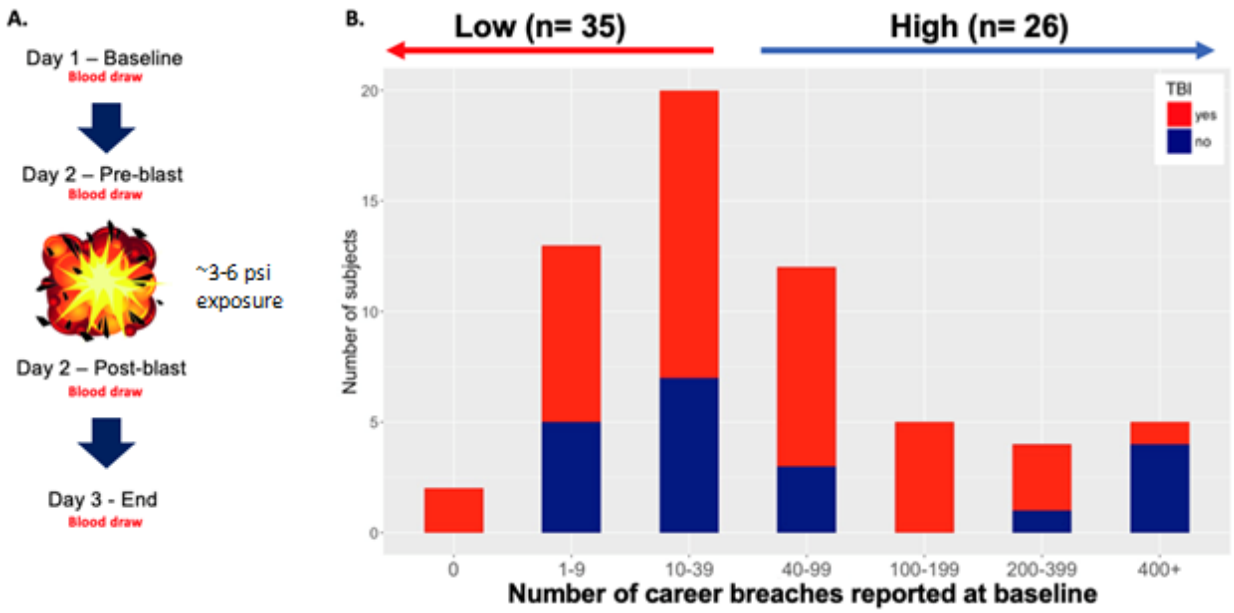

Figure 1. Study design and participants. (A) 3-day protocol schedule showing blast exposure and blood draws throughout the study. (B) Number of career breaches reported at baseline (61 participants) with and without history of traumatic brain injury (TBI) (red and blue, respectively), with ≤ 39 blasts empirically defined as low blast, and 40≤ career breaches considered high exposure to blast.

Appendix A – blood based biomarkers

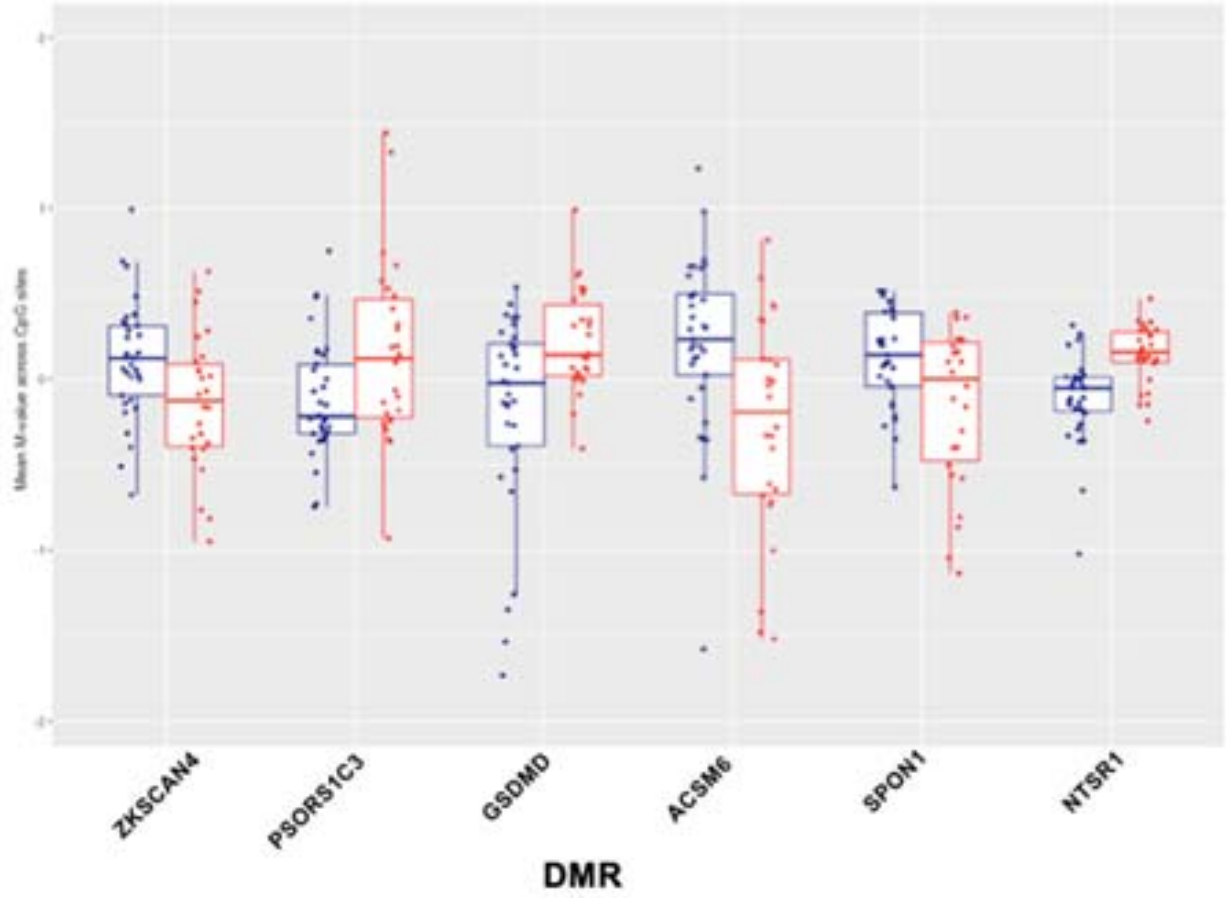

Figure 2. Six differentially methylated regions (DMRs) in high relative to low-blast exposed groups, showing low blast exposed groups in blue and high blast exposed groups in red, with mean methylation M-values across CpG sites on the y-axis.

Appendix A – blood based biomarkers

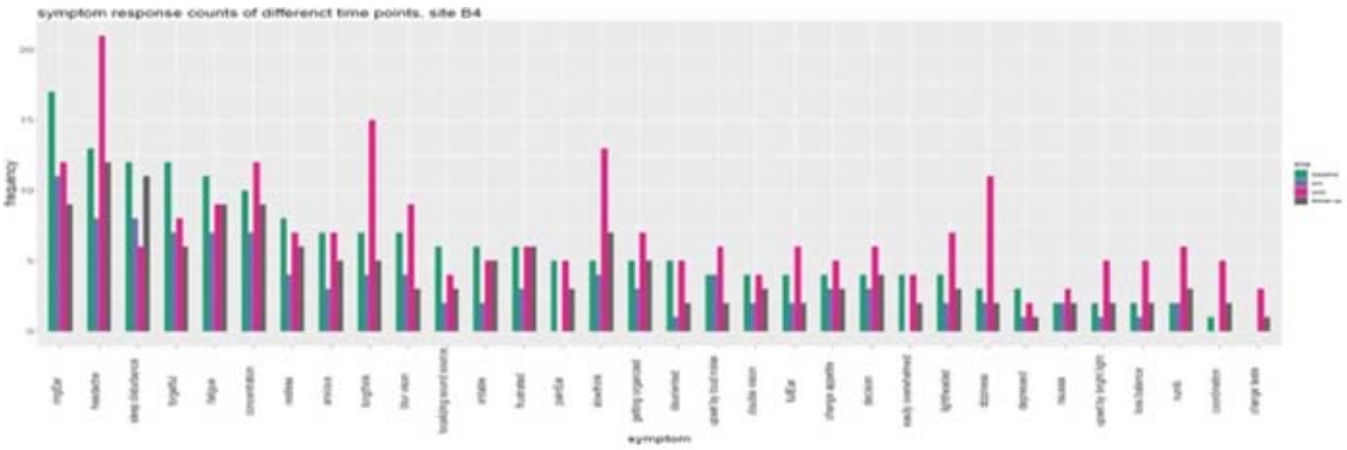

Figure 3: Symptom response frequencies from self-report surveys at each timepoint throughout the training (baseline, pre-, post-blast, and follow up), showing elevated reporting of symptoms related to headache, concentration, dizziness, long think, and slowed thinking directly following blast (pink).

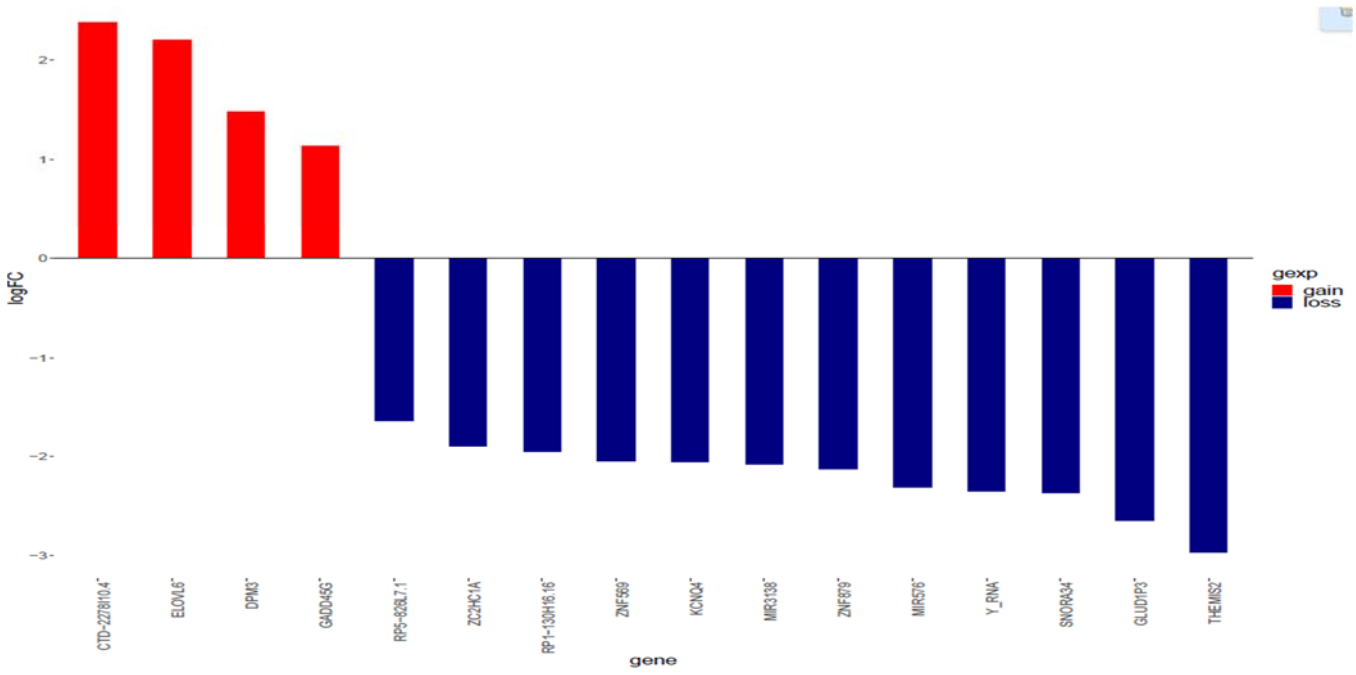

Figure 4: 16 robustly differentially expressed genes (FC≥1.5) that tracked with the most frequently reported symptom of headache, showing gain (red) and loss (blue) of expression.

Appendix B: 2019 National Neurotrauma Society Symposium (NNS) Poster

Mechanical and Cellular Response to Low Level Blast

Thomas J. O'Shaughnessy[1], YungChia Chen[1], Kathleen M. Gilpin[2], Gary H. Kamimori[3], and Amit Bagchi[1]

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U.S. NAVAL RESEARCH LABORATORY

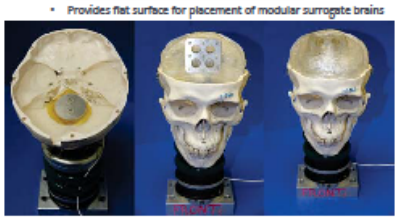
WRAIR Walter Reed Army Institute of Research

OBJECTIVE

- Exposure to low level blast overpressure is a common occurrence during military training exercises
- This effort is determining cellular response thresholds to blast
  - Using primary murine neuronal cell cultures in a surrogate head/brain
  - Using cell culture metabolism as a measure of cell response

NRL HEAD-BRAIN MODELS (NHBMs)

- **Modular skull system**
  - Pressure sensor in left eye socket (reflected pressure)
  - Custom neck mount for attachment to Hybrid-III neck
  - Bottom of skull filled with Sylgard 184
    - Covers neck mount hardware and skull sensors
    - Provides flat surface for placement of modular surrogate brains



- **Brain surrogates for live cells (via NRL cell Packs)**
  - Brain is space filling, split into front/back pieces
  - Back piece contains pocket for cell pack, made from Sylgard 184 (37:1)
  - Front piece is Sylgard 6366 (1:1.5)
  - Each cell pack contains 4 independent cell cultures
  - Cell cultures are primary murine cortical neurons growing in a 3D collagen lattice

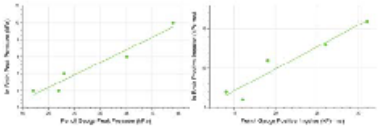
BLAST EXPOSURE OF CELL CULTURES

- All exposures occurred during Marine Corps Methods of Entry school breacher training courses at Quantico, VA
- For door breach events:
  - Single NHBM on aluminum stand with vest, no helmet
- For wall breach events:
  - Three NHBMs on aluminum stand, each with vest, no helmets
- Each stand had one or more pencil gauges attached to record incident pressure at the test fixture

Acknowledgements: The authors thank US Marine Corps Methods of Entry School for the opportunity to collect data. This work was conducted under CDMRP award W81XWH-16-2-0001.

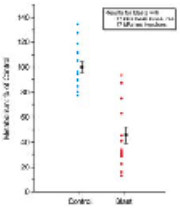
INCIDENT VS. IN BRAIN PRESSURES

- In order to correlate the incident pressures with an in brain (cell exposure) pressure level:
  - A cell pack with a pressure sensor was placed in the system
  - Multiple blast events were recorded
- The relationship between incident pressure and in brain pressure are shown for peak pressure and positive impulse in the graphs below



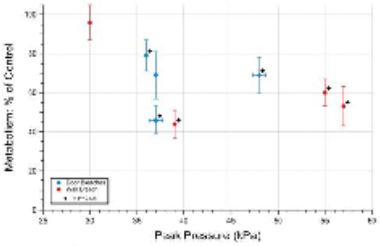
NEURONAL CELL CULTURE RESPONSE TO BLAST

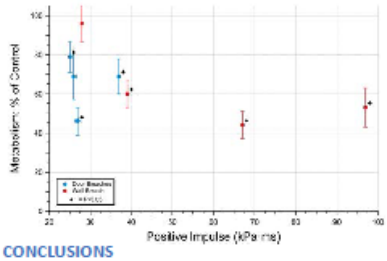
- All results are relative to pair matched controls
- Cell response measured as change in culture metabolism via MTT assay
- MTT is a colorimetric assay based on mitochondrial activity
- Figure to the right shows an example of per well results for a single blast condition
- Each color dot maps to a single cell pack well
- Black dots show summary data as mean ± SEM



RESULTS

- The below two graphs summarize the results from wall and door breach event exposures for the neuronal cell cultures
  - Each point represents one blast event (walls) or 2-4 blast events (doors)
  - Each point represents 6-16 blast exposed cultures (wells) and 11-16 control cultures; Data is mean ± SEM
  - A star indicates the blast exposed cells cultures were statistically different from pair-matched controls via Student's t-test

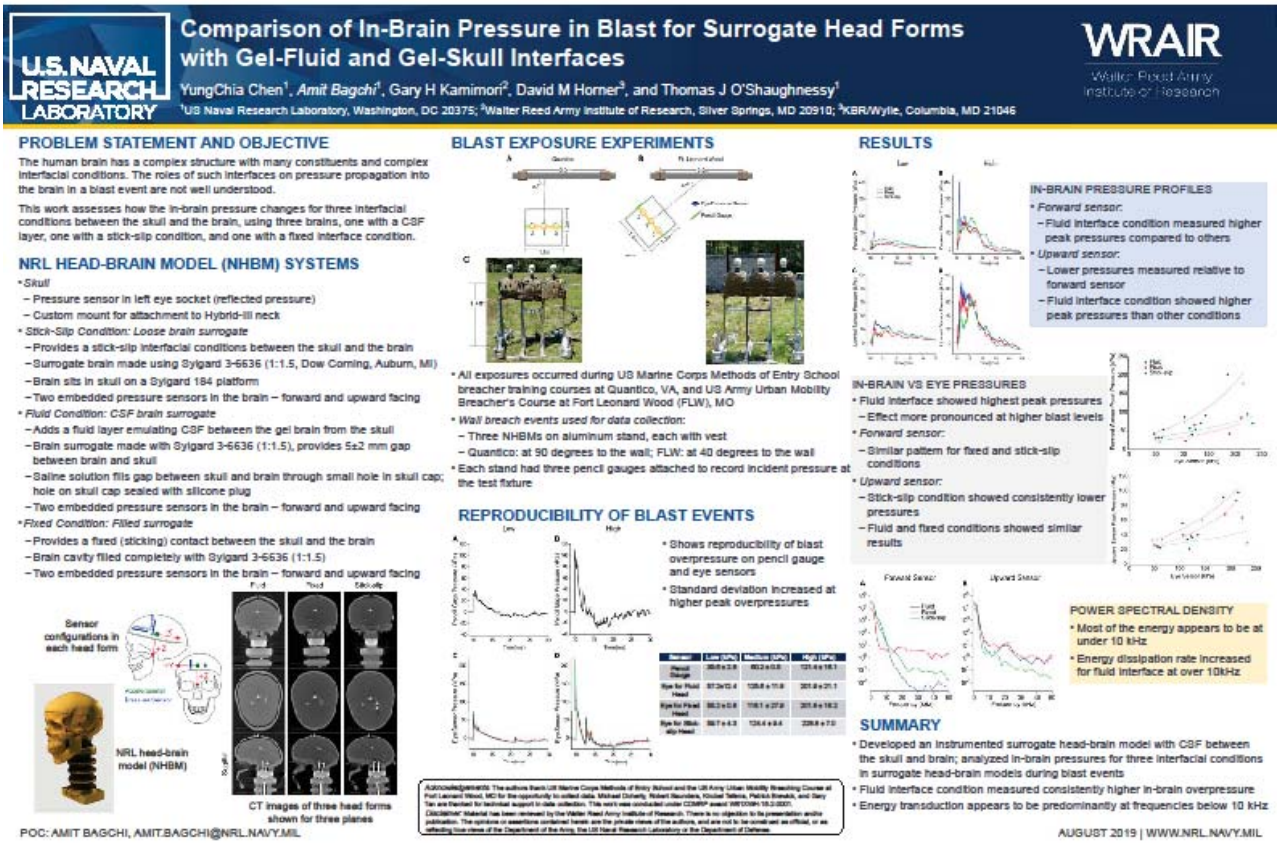




CONCLUSIONS

- There is higher variability in the cell response to live blasts than has been previously seen in shock tube experiments.
- There is a clear trend of decreasing culture metabolism vs. increasing peak pressure; there is no clear trend for positive impulse
- The threshold for cell response in this system is between 30 and 36 kPa peak incident pressure.

Appendix C: 2019 Military Health Sciences Research Symposium (MHSRS) Poster



Comparison of In-Brain Pressure in Blast for Surrogate Head Forms with Gel-Fluid and Gel-Skull Interfaces

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U.S. NAVAL RESEARCH LABORATORY

WRAIR Walter Reed Army Institute of Research

PROBLEM STATEMENT AND OBJECTIVE

The human brain has a complex structure with many constituents and complex interfacial conditions. The roles of such interfaces on pressure propagation into the brain in a blast event are not well understood.

This work assesses how the in-brain pressure changes for three interfacial conditions between the skull and the brain, using three brains, one with a CSF layer, one with a stick-slip condition, and one with a fixed interface condition.

NRL HEAD-BRAIN MODEL (NHBM) SYSTEMS

- *Skull*
  - Pressure sensor in left eye socket (reflected pressure)
  - Custom mount for attachment to Hybrid-III neck
- *Stick-Slip Condition: Loose brain surrogate*
  - Provides a stick-slip interfacial conditions between the skull and the brain
  - Surrogate brain made using Sylgard 3-6636 (1:1.5, Dow Corning, Auburn, MI)
  - Brain sits in skull on a Sylgard 184 platform
  - Two embedded pressure sensors in the brain – forward and upward facing
- *Fluid Condition: CSF brain surrogate*
  - Adds a fluid layer emulating CSF between the gel brain from the skull
  - Brain surrogate made with Sylgard 3-6636 (1:1.5), provides 5±2 mm gap between brain and skull
  - Saline solution fills gap between skull and brain through small hole in skull cap; hole on skull cap sealed with silicone plug
  - Two embedded pressure sensors in the brain – forward and upward facing
- *Fixed Condition: Filled surrogate*
  - Provides a fixed (sticking) contact between the skull and the brain
  - Brain cavity filled completely with Sylgard 3-6636 (1:1.5)
  - Two embedded pressure sensors in the brain – forward and upward facing

CT images of three head forms shown for three planes

BLAST EXPOSURE EXPERIMENTS

- All exposures occurred during US Marine Corps Methods of Entry School breacher training courses at Quantico, VA, and US Army Urban Mobility Breacher's Course at Fort Leonard Wood (FLW), MO
- *Wall breach events used for data collection:*
  - Three NHBMs on aluminum stand, each with vest
  - Quantico: at 90 degrees to the wall; FLW: at 40 degrees to the wall
- Each stand had three pencil gauges attached to record incident pressure at the test fixture

REPRODUCIBILITY OF BLAST EVENTS

- Shows reproducibility of blast overpressure on pencil gauge and eye sensors
- Standard deviation increased at higher peak overpressures

Sensor	Low (kPa)	Medium (kPa)	High (kPa)
Pencil Gauge	39.6 ± 2.8	89.2 ± 5.8	131.4 ± 18.1
Eye for Fluid Head	57.3 ± 10.4	126.8 ± 11.8	201.8 ± 21.1
Eye for Fixed Head	56.3 ± 5.8	116.1 ± 27.8	201.8 ± 16.2
Eye for Stick-slip Head	59.7 ± 4.3	124.4 ± 9.4	229.8 ± 7.0

Acknowledgements: The authors thank US Marine Corps Methods of Entry School and the US Army Urban Mobility Breaching Course at Fort Leonard Wood, MO for the opportunity to collect data. Michael Doherty, Robert Saunders, Kristel Tellera, Patrick Breslich, and Gary Tan are thanked for technical support to data collection. This work was conducted under CDMRP award W81XWH-18-2-0031.

RESULTS

IN-BRAIN PRESSURE PROFILES

- *Forward sensor:*
  - Fluid interface condition measured higher peak pressures compared to others
- *Upward sensor:*
  - Lower pressures measured relative to forward sensor
  - Fluid interface condition showed higher peak pressures than other conditions

IN-BRAIN VS EYE PRESSURES

- Fluid interface showed highest peak pressures
  - Effect more pronounced at higher blast levels
- *Forward sensor:*
  - Similar pattern for fixed and stick-slip conditions
- *Upward sensor:*
  - Stick-slip condition showed consistently lower pressures
  - Fluid and fixed conditions showed similar results

POWER SPECTRAL DENSITY

- Most of the energy appears to be at under 10 kHz
- Energy dissipation rate increased for fluid interface at over 10kHz

SUMMARY

- Developed an instrumented surrogate head-brain model with CSF between the skull and brain; analyzed in-brain pressures for three interfacial conditions in surrogate head-brain models during blast events
- Fluid interface condition measured consistently higher in-brain overpressure
- Energy transduction appears to be predominantly at frequencies below 10 kHz

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