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TITLE: “Central Mechanisms and Treatment of Blast-Induced Auditory and Vestibular Injuries”

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14. ABSTRACT The study is to utilize our well-defined shock tube simulation of mild blast-induced traumatic brain injury (bTBI) in rodents to characterize interrelated biomechanical and pathophysiological mechanisms of blast-induced central auditory processing disorders (CAPDs) and central vestibular injuries (CVIs) and to develop an early therapeutic intervention for hearing loss and balance disorder mitigation. The major objectives of the proposed studies and relevant research sub-gaps are: 1) Verify the time course of hearing loss and balance disorders induced by blast exposure and define plasma and CSF TDP-43 as a biomarker related to blast-induced central auditory/vestibular deficits; 2) Characterize blast induced biochemical, functional and morphological alterations in central auditory/vestibular systems and establish that blast-induced altered expression of TDP-43 and its BDPs in these structures play a key pathophysiological mechanism leading to secondary injuries.						
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1. INTRODUCTION:

With widespread use of improvised explosive devices in recent military conflicts, blast-induced traumatic brain injury (bTBI) and neurosensory dysfunction have emerged as key military medical issues. Auditory and vestibular disorders are particularly prevalent, and the debilitating consequences of these injuries likely progress with age. A comprehensive understanding of the structural and molecular components of the injury is essential for the development of the most appropriate therapies for auditory and vestibular deficits resulting from blast exposure. Existing data indicate that both the inner ear and the structures in the brain responsible for auditory and vestibular function are at high risk of injury following blast exposure. The ongoing study utilizes an Advanced Blast Simulator (ABS) to recreate these injuries in rodents in the laboratory. Through comprehensive assessments of the resultant auditory and vestibular deficits using a battery of functional tests in conjunction with characterizations of the underlying biochemical and anatomical changes in these structures, the interrelated biomechanical and pathophysiological mechanisms responsible for blast-induced central auditory processing disorders (CAPDs) and central vestibular injuries (CVIs) are being elucidated and will provide therapeutic targets for hearing loss and balance disorder mitigation.

2. KEYWORDS:

Mouse, blast, injury, neuronal connectivity, auditory cortex (AU), medial geniculate nucleus (MGN), TDP-43, single cell RNA sequencing (scRNA-seq)

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.

1) Verification of the time course of central auditory processing disorders and vestibular injuries induced by blast exposure and definition of time-dependent changes in TDP-43 in plasma and CSF as a biomarker related to blast-induced central auditory/vestibular deficits; 2) Characterization of blast injury to primary auditory cortex and brainstem/cerebellum associated with CAPDs and CVIs and definition of blast-induced altered expression of TDP-43 as a key pathophysiological mediator leading to the secondary central auditory and vestibular processing injuries.

Milestones: **Year 1:** Obtain IACUC and ACURO approval of animal use protocol, define time-course of blast-induced auditory function deficits, and define the role of TDP-43 in neuronal development. **Year 2:** Assess time-course of vestibular functional disruptions, determine TDP-43 levels in serum and CSF, examine morphological alterations in specific neurons in AU, identify blast impaired functional connection between MGN and AU, and examine the regulation of TDP-43 target genes. **Year 3:** Examine morphological alterations of Purkinje neurons in the cerebellum and demonstrate blast impairments of functional connections between FL and VeN.

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.

Major activities

In this reporting period, we have been focused on two major studies: 1) Determine the gene expression changes in the neuron that responded to overexpression of TDP-43 using scRNA-seq technology; 2) Determine the changes of synaptic proteins in the auditory cortex following blast exposure using immunohistochemistry and Western blot technique.

Specific Objectives

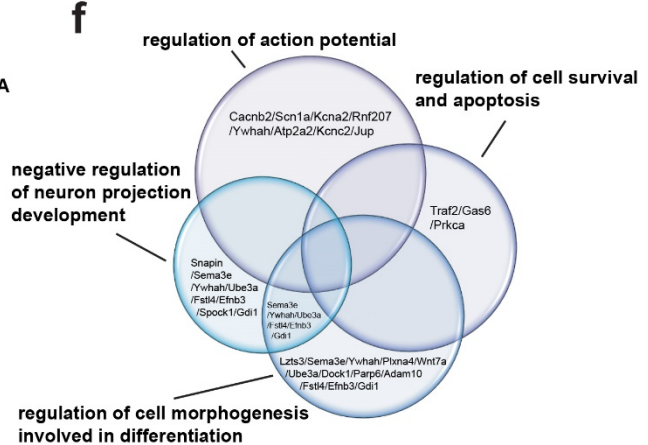
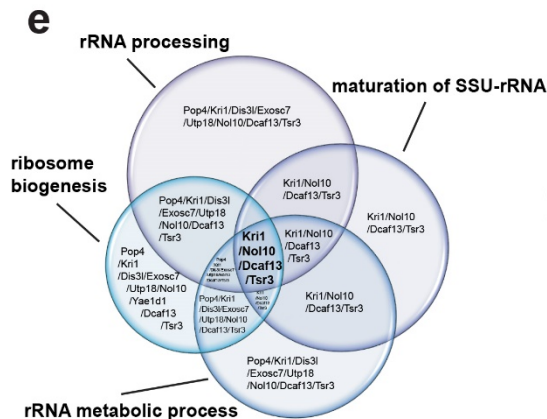
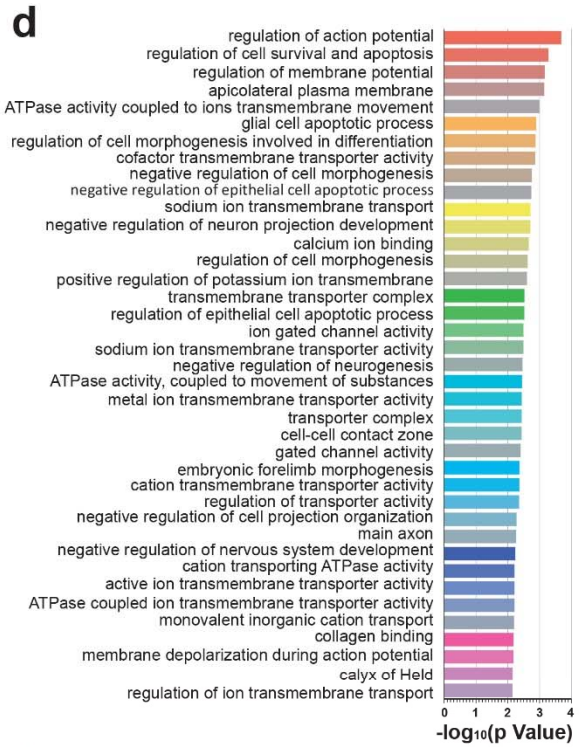
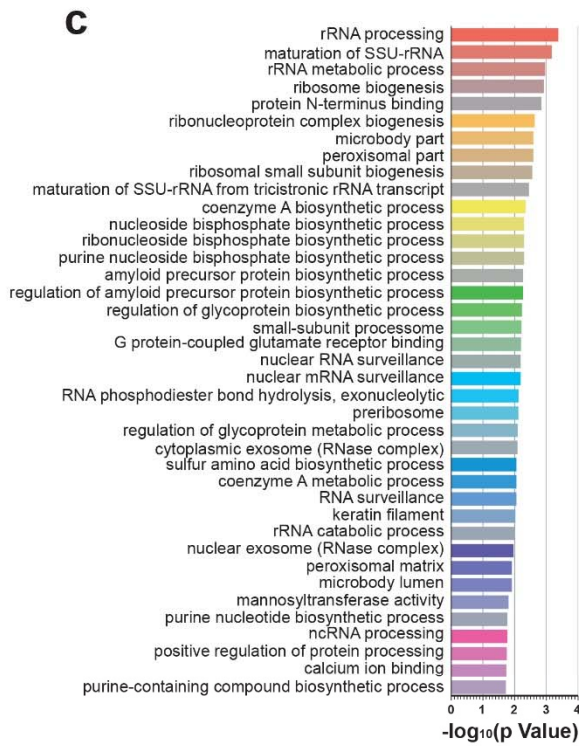
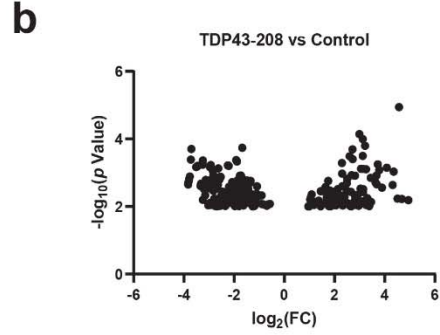
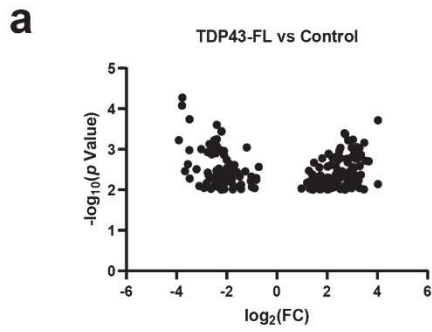
1. Submitted final report for animal use protocol 16-PN-20S, and obtained the approved new animal use protocol 19-PN-13S from WRAIR/NMRC IACUC
2. Submitted and obtained approval of a new animal use appendix for research from ACURO
3. Microinjection of AAV-CAG-TDP43-GFP, AAV-CAG-TDP43(208)-GFP and AAV-CAG-GFP into medial geniculate nuclei (MGN) of CBA/J mice, respectively at WRAIR
4. Transportation of mice on time from WRAIR to LIBD
5. Selection of green fluorescent protein enabled monitoring of a single neuron within MGN region using single cell patch technique, performance of single cell RNA sequencing (scRNA-seq) and data analyzing at LIBD
6. Investigation of changes in synaptic proteins in the cortex of CBA mice at 1 day and 28 days after blast injury
7. Evaluation of morphological changes in AU region using Thy1-RFP mice

Significant results, including major findings, development, or conclusions

To investigate specific objective #4, to examine the regulation of TDP43 target genes in individual neurons within the MGN, we employed single-cell RNA sequencing assay combined with whole-cell patch-clamp recording to investigate the mRNA expression alterations triggered by upregulation of TDP-43. The following methods have been technically included.

- A. *Brain virus injections*: Mice were anesthetized with isoflurane (4% induction, 1.5–2.0% maintenance) and heads were carefully secured in the stereotaxic apparatus (David Kopf instruments, Inc). After establishing a sterile operation field, craniotomy holes of 1 – 2 mm diameter were made in the skull by a dental drill over the region of interest. The aav-GFP reagent was administered into the medial geniculate nuclei (MGN) using a NANOFIL syringe with a 33 G needle (World Precision Instruments, Inc). The scalp was sutured and sealed with tissue glue.
- B. *Single neuron collection*: Mice were euthanized at 28d after microinjection. The brain was quickly removed and slices containing MGN (400 μ m) were cut as coronal sections using a vibrating blade microtome (Leica VT1000S, Leica Systems) in ice-cold slicing buffer bubbled with 95% O₂ and 5% CO₂. Slices were then transferred to a holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF) for 30 min at 34°C and for another 30 min at 22°C for recovery, and then transferred to a submersion recording chamber continually perfused with 32°C oxygenated ACSF (rate: 2 ml/min). GFP-positive MGN neurons were visualized with DIC using an Olympus BX51 microscope, and each single cell was harvested by a whole-cell recording pipette for single-cell RNA sequencing experiment. Three mice were used for each group, aav-cag-TDP43-gfp, aav-cag-□TDP43-gfp or aav-cag-gfp (control).
- C. *Cell processing for RNA extraction*: Five cells were processed as a single sample using the SMART-Seq HT Kit accorded to the manufacturer's instruction. A total of 32 samples including control, TDP43-FL and truncated TDP43-208 were processed.
- D. *RNA-seq and analysis*: Library constructs and RNA sequencing were performed using an Illumina HiSeq 2000. Bioinformatics Analyses94 software was used to identify specific signaling pathways that are upregulated in each cell population following altered TDP-43 expression at the LIBD/JHU.

The overall RNA-seq results showed that, compared to the control neuron, 206 differentially expressed genes (DEGs) were identified in the TDP43 full length (TDP43-FL) overexpressed neuron, and 261 DEGs were identified in the truncated TDP43 (TDP43-208) overexpressed neuron ($p < 0.01$, Fig. a-b). Gene set enrichment analyses demonstrated that DEGs in TDP43-FL vs control were mostly significantly enriched in RNA binding processing (Fig. c), including 'rRNA processing' ($p = 4.18e-04$, GO: 0006364), 'maturation of SSU-rRNA' ($p = 6.67e-04$, GO: 0030490), 'rRNA metabolic process' ($p = 1.08e-03$, GO: 0016072) and 'ribosome biogenesis' ($p = 1.18e-03$, GO: 0031347). The differential expression and GO analyses together suggested that Kri1 ($p = 6.36e-03$), Nol10 ($p = 4.44e-03$), Dcaf13 ($p = 1.02e-03$) and Tsr3 ($p = 4.76e-03$) were among the most biologically relevant genes for these RNA binding processing (Fig. a and e). In regard to TDP43-208 vs control, gene set enrichment analyses revealed that DEGs were mostly significantly enriched in 'regulation of action potential' ($p = 2.08e-04$, GO: 0001508), 'regulation of cell survival and apoptosis' ($p = 5.25e-04$, GO: 0034350), 'regulation of cell morphogenesis involved in differentiation' ($p = 1.33e-03$, GO: 0010769) and 'negative regulation of neuron projection development' ($p = 1.96e-03$, GO: 0010977).



Our findings indicate that blast exposure increases expression of TDP43 in brain tissue, which could also increase truncated TDP-43 fragments. Our electrophysiological data have shown that blast exposures impair the synaptic transmission in MGN-AU projections. Combined with the single-cell RNA-seq analyses, these results implicate that blast-induced central auditory processing disorders might be attributable to dysfunction of MGN-AU projections, which is caused by blast-induced overexpression of TDP43 and truncated TDP43 fragments leading to dysfunctional rRNA binding, impaired neuronal axonal development and neuronal firing, as well as neuronal survival and apoptosis.

To determine blast-induced molecular changes in auditory cortex (AU), we investigated synaptic protein expression in both CBA/J and Thy1-RFP mice. The following methods have been technically included.

- A. *Brain virus injections:* Mice received a stereotaxic microinjection of AAV-CAG-GFP into MGN of Thy1-RFP mice that express spectral variants of red fluorescent protein (RFP) at high levels in motor and sensory neurons and subsets of central neurons.
- B. Blast exposure: Mice were subjected to tightly coupled double blast exposures at 1 month after GFP injection.
- C. *Pathology:* Brains of mice were dissected after euthanasia and fixed in 4%PFA solution at the designated days post-injury. Coronal brain sections (40 - 100 μ m) were prepared using a vibrating microtome (Leica VT-1000S). Brain sections were processed for immunostaining.
- D. *Western blot:* The protein extracts (20 – 30 μ g) were fractionated by an SDS-PAGE Electrophoresis System and were electrophoretically transferred to polyvinylidene difluoride membranes using an iBlot apparatus according to the manufacturer's recommended protocol (Thermo Fisher Scientific). The membranes were probed with the primary antibodies, followed by the appropriate HRP-conjugated secondary antibody. The protein bands were detected by the Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific).

As we have known, the numbers and shapes of dendritic spines correlate with the strength of synaptic transmissions that are associated with the function of particular neural networks. In our early study, Thy1-YFP (stock 003782) mice were used to determine blast-induced morphological changes. Data demonstrated in the year 2 report that numbers of dendritic spines in auditory cortex increased significantly at 4h and 7d after blast exposure. The results indicated the synaptic transmissions in excitatory neurons were quite sensitive to blast insult. Also, electrophysiological data showed the MGN-AU projections in the AU were significantly reduced at 1, 3 and 7 days after blast exposures (Year 3 report).

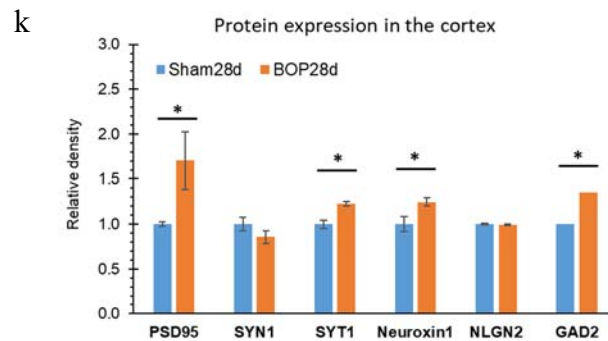
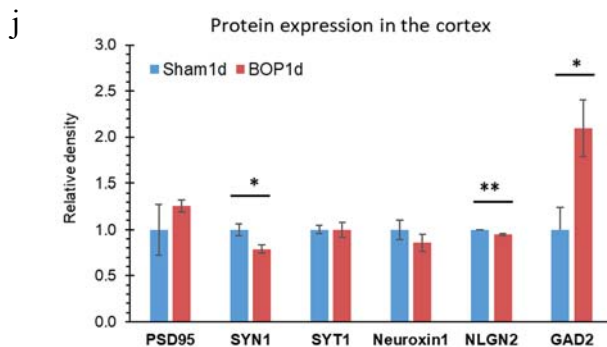
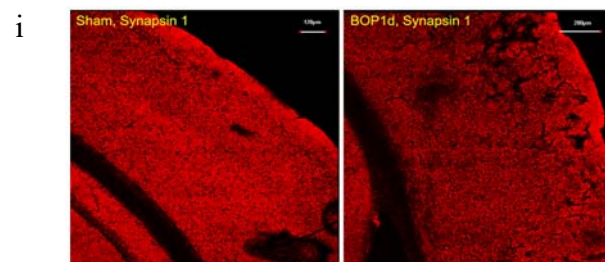
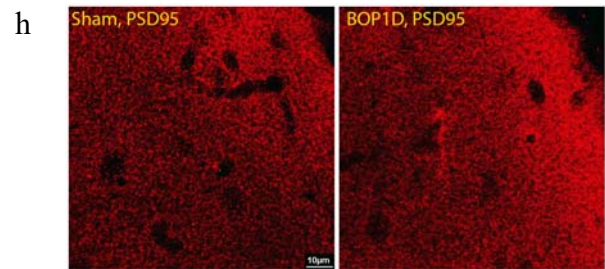
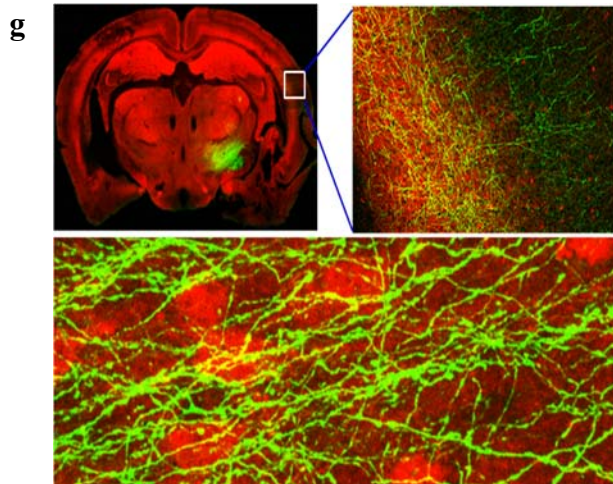
To determine the changes in synaptic proteins, we injected aav-cag-GFP into the MGN of Thy1-RFP (stock 00791) mice, then examined the pathology of neural networks in the AU region (Fig. g). Thy1-RFP (stock 00791) was recommended by Jackson Laboratories who discontinued Thy1-YFP (stock 003782). The visualized fluorescence in the AU region of Thy1-RFP mice was not the same as that in Thy1-YFP. Additional time was required to determine the optimal way to analyze images. In this reporting period, antibodies to PSD95 (Fig. h), Synapsin 1 (Fig. i), Synaptotagmin 1, Neuroxin 1, Neuroligin 1 and GAD2 were used for immunohistochemistry on brain sections of CBA/J mice. To avoid biased pathological analyses, we validated assessments of expression of these synaptic proteins in the cortical region by the Western blot technique.

Data presented in the figure j were for 1 day and k were for 28 days post injury and were compared to paired sham controls. The dendritic spine is the site of the postsynaptic density (PSD)

of excitatory synapses and is tightly regulated by synaptic proteins that influence glutamatergic transmission. PSD95 protein is involved in the recruitment and stabilization of glutamate receptors and is a major regulator of the maturation of glutamatergic synapses. PSD95 also influences the size and density of dendritic spines during neurodevelopment and can have appreciable effects on synaptic connectivity and activity. Compared to the sham controls, PSD95 in the cortex increased significantly at 28 days post blast exposure (PBE), but only slightly at 1 day PBE.

Neurons release neurotransmitters or hormones into the extracellular space by exocytosis in response to a rise of intracellular Ca^{2+} levels near the release site. Synaptotagmin 1 (SYT1), a synaptic vesicle membrane glycoprotein, is essential for the Ca^{2+} dependent triggering of membrane exocytosis. It acts as the Ca^{2+} sensor in the regulation of exocytosis and neurotransmitter release. SYT 1 may have a regulatory role in membrane interactions during the trafficking of synaptic vesicles at the active zone of the synapse. A significant increase of SYT1 in the cortex was verified at 28 days PBE.

Synapsin 1 (SYN1) encodes neuronal phosphoprotein that is associated with the involcytoplasmic surface of synaptic vesicles. Family members are characterized by common protein domains and are implicated in synaptogenesis and the modulation of neurotransmitter release, with proposed potential roles in several neuropsychiatric diseases.



SYN1 plays a role in regulation of axonogenesis and synaptogenesis. It serves as a substrate for several different protein kinases and its phosphorylation may function in the regulation of this protein in the nerve terminal. Neurexins act predominantly at the presynaptic terminal in neurons and play essential roles in neurotransmission and differentiation of synapses. Neuroxin 1 decreased significantly at 28 days PBE. Neuroligin 2 (NLGN2), a cell adhesion protein on the postsynaptic membrane, is mainly concentrated at inhibitory synapses. It mediates the formation and maintenance of synapses among neurons. Significant decreases of NLGN2 were found at 1 day and 7 days PBE (data not shown). Neurexins bound to neuroligins can form trans-synaptic complexes at excitatory and inhibitory synapses that are involved in synapse specification, establishment, maturation and plasticity. Importantly from a medical point of view, impairments caused by mutations in the neurexin-neuroligin complex lead to an imbalance of excitatory to inhibitory activity in neuronal circuits which has been implicated in the pathomechanisms of neuropsychiatric disorders.

Glutamate decarboxylase 65 (GAD2), expression in the GABAergic neurons is localized to nerve terminals and synapses. GAD2 is predominantly found in an inactive state. Our data showed that GAD2 increased significantly at acute and chronic injury phases after blast exposure. The results reveal that blast injury can activate GABA neurotransmission.

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.

During the report year, PIs, Research Associates and technicians learned how to analyzing single cells RNA-sequence data, as well as immunohistochemistry on brain sections.

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.

- Platform presentation, entitled “Impairment of functional connectivity in the brain of mouse following repeated blast exposures might contributes to blast-induced hearing deficits” at the National Capital Area TBI Research Symposium (NCATBI) in March 2019
- Platform presentation, entitled “Blast-induced abnormalities in long-range connectivity between medial geniculate nuclei and auditory cortex: Pre-clinical studies on the mechanisms of blast related auditory” at Military Health System Research Symposium (MHSRS), in August 2019

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.

Further pathological analysis is necessary for making definitive conclusions regarding the changes in synaptic plasticity following blast injury.

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).

Nothing to report at this point

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.

Nothing to report at this point

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.*

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.*

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:

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.

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.

Unanticipated technical challenges were encountered and were successfully addressed with experimental modifications but as a result, fewer recordings were possible.

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.

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

None.

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

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).*

Nothing to report at this stage, although multiple manuscripts are in preparation for peer review during the upcoming 6 months.

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).

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.*

- Platform presentation, entitled “Impairment of functional connectivity in the brain of mouse following repeated blast exposures might contribute to blast-induced hearing deficits” at the National Capital Area TBI Research Symposium (NCATBI) in March 2019
- Platform presentation, entitled “Blast-induced abnormalities in long-range connectivity between medial geniculate nuclei and auditory cortex: Pre-clinical studies on the mechanisms of blast related auditory” at Military Health System Research Symposium (MHSRS), in August 2019

- **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.

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.

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.

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.*

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).

Dr. Joseph B. Long, no change

Dr. Ying Wang, no change

Dr. Yanling Wei, no change

Ms. Donna Wilder, 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.

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.*

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Nothing to report.

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.