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

PRINCIPAL INVESTIGATOR: Dr. Joseph Long

RECIPIENT: The Geneva Foundation
Tacoma, WA 98402

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Fort Detrick, Maryland 21702-5012

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The Geneva Foundation
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U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

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.

15. SUBJECT TERMS
Traumatic brain injury

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

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

Mouse, blast, injury, neuronal connectivity, auditory cortex (AU), medial geniculate nucleus (MGN), flocculus (FL), lateral vestibular nuclei (VeN), TDP-43.

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 to define 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 to define 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

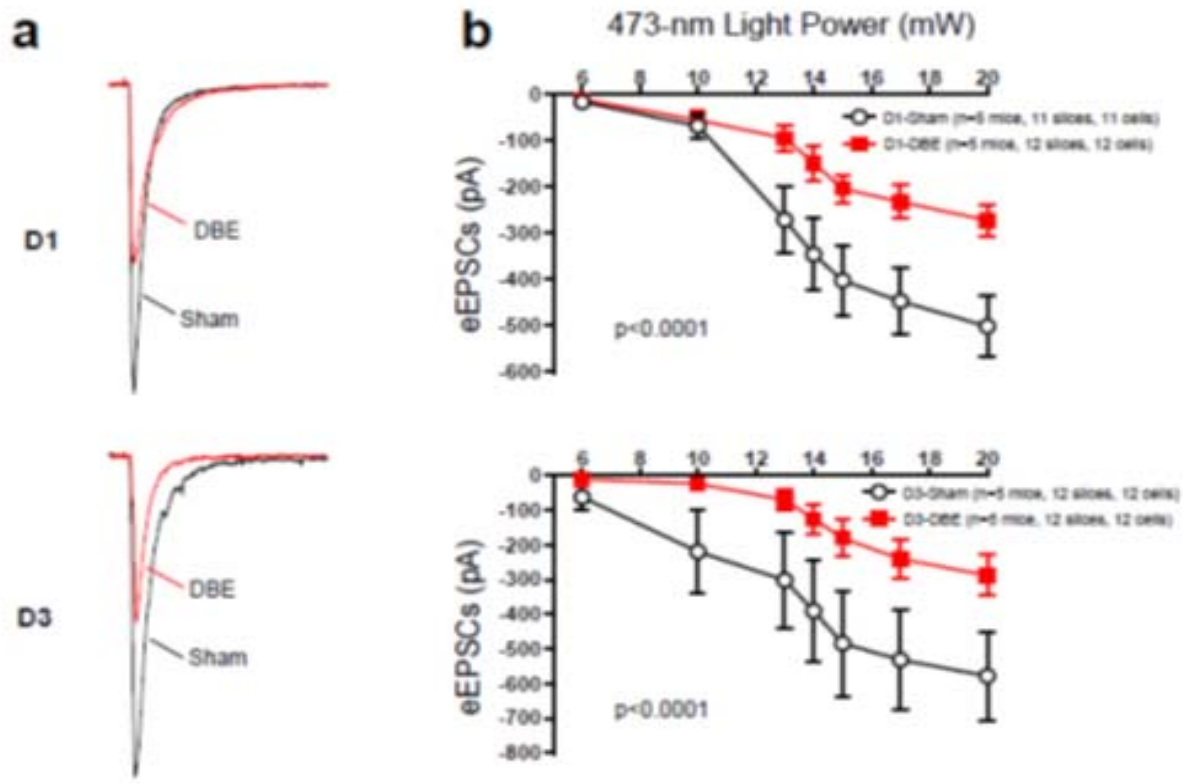
We have made the significant progress in revealing molecular, cellular and neural circuit mechanisms underlying blast-induced central auditory processing disorders (CAPDs) and central vestibular injuries (CVIs). With widespread use of improvised explosive devices in modern combat, blast-induced traumatic brain injury (bTBI) and related health issues have emerged as key military medical concerns. Our goal has been to utilize a well-defined shock tube simulation of mild bTBI in mice to characterize interrelated biomechanical and pathophysiological mechanisms of blast-induced CAPDs and CVIs, and to verify the time course of CAPDs and CVIs induced by blast exposure, as well as to define plasma and CSF TDP-43 as a biomarker related to blast-induced central auditory/vestibular deficits. In order to identify whether the bTBI mice exhibit these impairments, we have performed *in vitro* brain slice whole-cell patch recordings, microinjection of viruses into the medial geniculate nuclei (MGN) and cerebellar flocculus (FL), optogenetic studies in *in vitro* brain slices.

Specific Objectives

1. To apply the auditory brainstem responses (ABRs) as a quantitative measurement for hearing loss assessment and rotarod testing for impaired vestibulmotor functional assessment in a bTBI mouse model, and to measure the time course of CAPDs and CVIs induced by blast exposure as well as to define plasma and CSF TDP-43 as a biomarker related to blast-induced central auditory/vestibular deficits.
2. To microinject MGN with AAV-CAG-ChR2(H134R)-GFP and deliver repetitive blast exposures to injected mice, to apply optogenetics assay with whole-cell patch-clamp electrophysiological recording to analyze whether the synaptic alterations in AU neurons occurred in synapses driven by inputs originating from MGN.
3. To microinject FL with AAV-L7-ChR2(H134R)-GFP and deliver repetitive blast exposures to injected mice, to use optogenetics assay with whole-cell patch-clamp electrophysiological recording to analyze whether the synaptic alterations between FL and lateral vestibular nuclei (LVe) projection which plays a critical role in body balance regulation.
4. To apply single-cell RNA sequencing assays to examine the regulation of TDP-43 target genes in the individual neurons of MGN and FL, to investigate the mRNA expression alterations triggered by upregulation of TDP-43 in mouse model.

Significant results, including major findings, development, or conclusions

To investigate specific objective #2, we applied the optogenetics assay in combination with whole-cell patch-clamp electrophysiological recording. First, mice received a microinjection of adeno-associated virus encoded with CAG-ChR2-YFP genes into the MGN. After 4 weeks recovery from the surgery, mice were then anesthetized and received either closely coupled repeated blast exposures (peak static pressure of 16 psi and 4 msec positive phase duration) in the advanced blast simulator or sham handling. Brain slices (300 μm) were prepared and used for whole-cell patch-clamp recordings at 1, 3 and 7 days and 1 month post-injury. Recording was done under visual guidance using an Olympus BX51 microscope equipped with both transmitted light illumination and epifluorescence illumination. The blue light-induced EPSCs were recorded from layer IV neurons in the AU with a holding potential at -70 mV. Data showed that input–output relationships between stimulation intensity and EPSCs at the MGN-AU projections in the AU were significantly reduced at 1, 3 and 7 days after blast exposures (two-way ANOVA, $p<0.0001$, $p<0.0001$, and $p=0.0193$, respectively). There were no significant changes at 1 month (28 – 30 days) after the repeated blast exposures, indicating an apparent recovery (i.e. plasticity) from abnormal circuits among neurons and glia. These results reveal that closely coupled shockwaves impaired sound signals processing centers which potentially contribute to hearing deficits in acute and sub-acute injury phases. In addition to highlighting perturbations potentially underlying blast-induced auditory dysfunction, these findings also provide potential insights into optimal therapeutic window after blast exposures in patients.



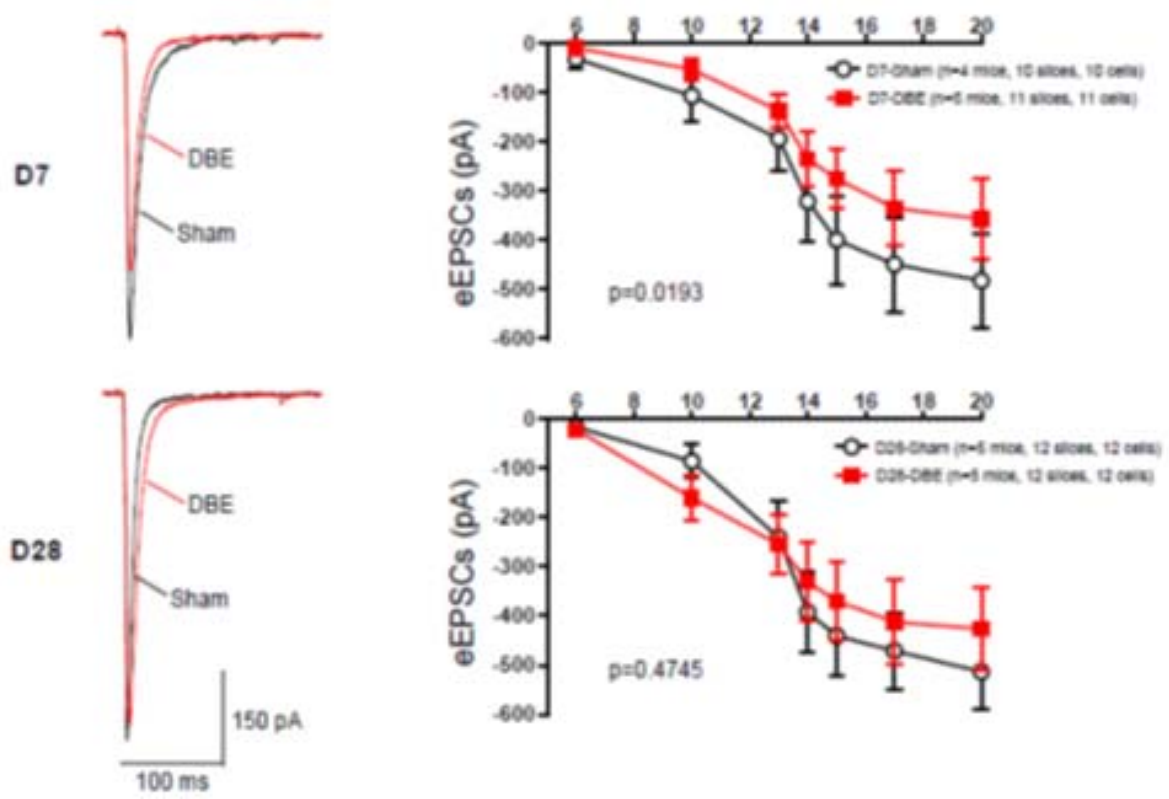


Figure. Impaired MGN-AU synaptic transmission in the blast mouse model

To investigate specific objective #3, we also employed whole-cell recording and optogenetic assay in in vitro brain slices to examine the functional connection of FL-LVe projection after blast exposure (shown in figure below). To selectively activate Purkinje cells, we injected FL (5.68 mm posterior to the Bregma, 2.90 mm lateral to the midline and 4.30 mm below the brain surface) with an adeno-associated virus, AAV DJ-L7-ChR2(H134R)-eYFP (UPENN core facility), that expressed channelrhodopsin-2 (ChR2) under the control of the Purkinje cell-specific L7/pcp-2 promoter, which activate neurons in response to 473-nm light. Mice were anesthetized with isoflurane in air, and a 1 µl sample of virus was slowly pressure-injected into the FL. After 4 weeks fully recovery, mice received repetitive blast exposures or sham handling and were then euthanized at a variety of time points (1d, 3d, 7d, 14d and 28d) after bTBI. Briefly, the brains were quickly removed after anesthetization with isoflurane, and FL-LVe slices (300 µm) were cut in coronal sections using a vibrating blade microtome (Leica VT1000S, Leica Systems) in ice-cold slicing buffer bubbled with 95% O2 and 5% CO2 (in mM: 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 4.0 MgCl2, 10 glucose and 250 sucrose). The slices were incubated in a Ringer solution (ACSF bubbled with 95% O2 and 5% CO2) containing (in mM) 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgSO4, 26 NaHCO3 and 10 glucose, pH 7.4, at room temperature for about 1 hour recovery before whole-cell patch recordings. To record inhibitory postsynaptic currents (IPSCs), a different Cs+-based intracellular solution (134 mM CsCl2, 2 mM MgCl2, 2 mM Na2-ATP, 0.5 mM Na2GTP, 5 mM Na2-phosphocreatine, 1 mM EGTA; 10 mM HEPES, and 0.3% biocytin, pH 7.25) was used in the presence of 50 µM CNQX (Sigma) and 50 µM D-APV (Sigma) to block AMPA currents and NMDA currents, respectively. Whole-cell recordings were intended from cell bodies of LVe neurons with optogenetic stimulation. However, an in figure above, only a few sparse neurons located in LVe were found within white matter of the brain stem although there the FL bundle with eYFP projecting to LVe was evident. Consequently, it was very difficult to establish a successful whole-cell patch

recording configuration and we were ultimately forced to abandon this portion of the study due to technical limitations with this electrophysiological approach despite a considerable investment of time and resources. To investigate functional connectivity between FL and LVe after bTBI in future studies, it might be more fruitful to employ brain imaging methods such as fiber photometry or/and inscopix system with GCAP6 as alternative approaches.,

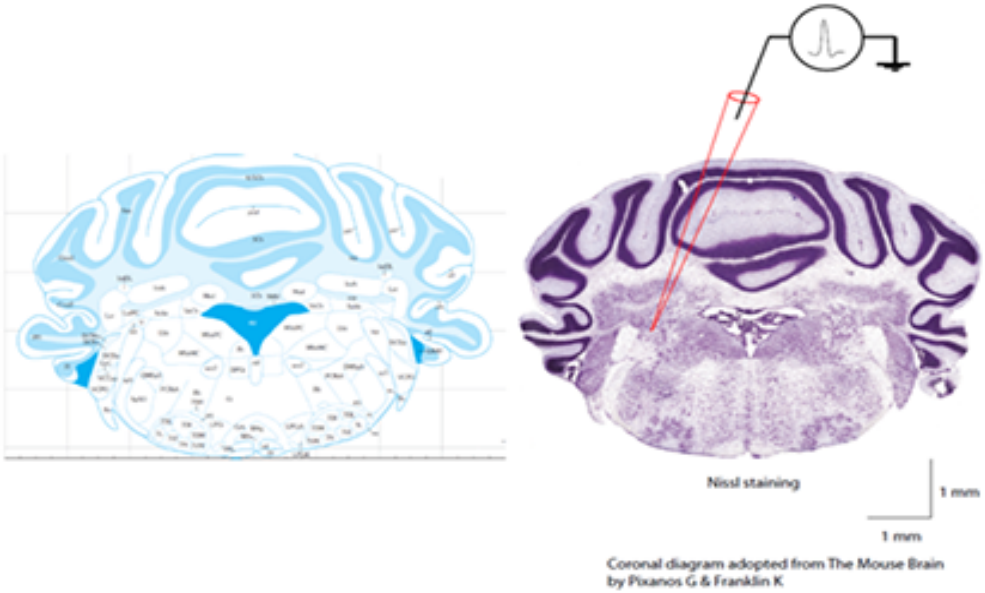


Figure Whole-cell patch clamp recording from lateral vestibular nucleus

To investigate specific objective #4, we employed a single-cell RNA sequencing assay combined with whole-cell patch-clamp recording to investigate the mRNA expression alterations triggered by upregulation of TDP-43. WT mice were initially anaesthetized with 2-5% isoflurane and maintained under anesthesia with 2-3% isoflurane when placed in a stereotaxic frame. Craniotomies were made to allow AAV encoded TDP-43 with GFP to be injected bilaterally into the MGN. AAV encoded GFP served as control was also injected into MGN. Mice were maintained to recover for 4 weeks after injection to allow maximal viral expression. Mice were euthanized at 28d after injection and the brains were quickly removed after anesthetization with isoflurane. Slices containing MGN (400 μm) were cut in coronal section 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 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 neurons in the MGN were visualized with DIC using an Olympus BX51 microscope, and each single cell was harvested by whole-cell recording pipette for single-cell RNA sequencing experiment. We collected 32 samples for single-cell RNAseq. Each sample contained five cells obtained from MGN and there were three different groups, including control, TDP43-full length and TDP43-208, respectively. We employed SMART-Seq HT Kit to process these samples to STOPPING POINT and stored at -20°C for next steps.

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 and developed the capability to execute the experiment to assess functional connectivity following blast insult. Althrough the stereotaxic brain microinjection system was set up a year ago, the successful ratio for getting GFP and ChR2 expression in the specific cells at MGN was consistently very low. The potential confounding issues included the design of DNA sequences, virus-related concentration and efficiency, injection speed and volume, as well as the brain positioning accuracy and the time reqired for the specific protein expression. The second large technical obstacle was performing a patch-claim recording on a brain slice from a mouse whose age exceeded 12 weeks.

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.

Nothing to report.

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."

Continue examing the pathological changes induced by blast injury using immunohistochemistry; Continue analyzing the single cell RNAseq data.

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).

- During the report period, we successfully obtained strong signals in the AU after injecting accurately AAV-CAG-ChR2(H134R)-GFP into MGN using modifed parameters of microinjection, since there was no available brain map for the age of 4-week CBA mouse.
- We have made successfully electrophysiology with optogenetics assay on the age of 12-week mouse, although the whole-cell-patch-clamp recording is technically difficulty on brain slice when mouse's age is over 8 weeks.
- The significant decrease in neurotransmission between MGN-AU projections after blast exposure determined by optogenetics with whole-cell patch recordings revealed molecular, cellular and neural circuit mechanism underlying blast-induced central auditory processing disorders.
- AAV-△L7-ChR2(H134R)-YFP was generated and producted after generation of truncated L7 (△L7) promoter from 3000 bp to 1000 bp which was verified by pathology.
- Adeno-associated virus carrying genes of TDP43-GFP and △TDP43-GFP (a truncated TDP43) with either CAG or L7 promoters were generated successfully.
- We have collected GFP positive neurons from the MGN at 4 weeks after injecting the AAV-CAG-TDP43-GFP, AAV-CAG-△TDP43-GFP and AAV-CAG-GFP (control), respectively by whole-cell recording pipette.
- The total of 32 samples in which each replicate contained five cells have been processed RNA isolation using SMART-Seq HT Kit and stored at -20°C for single-cell RNAseq analysis.

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.

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

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."

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.

WRAIR had another renovation and therefore the blast lab stopped work orders from September - November 2018. These were in addition to the stop work orders that had already put the project significantly behind, particularly subtask 3. A one year NCE was submitted and approved by the sponsor.

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 overcome with experimental modifications but as a result, fewer recordings were possible.

Changes that had a significant impact on expenditures

Nothing to report.

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

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.

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

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

• Presentation of a poster entitled "Structural plasticity in brain auditory signal processing centers following blast wave exposure in mice" at the National Capital Area TBI symposium in Bethesda, MD in Mar 2018. • A poster presentation, entitled "Dendritic structural plasticity may contribute to blast exposure-induced auditory dysfunction in mice" at the 3rd Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS on Neurotrauma and Critical Care Tononto, Canada in August 2018.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Project Role	Percent Effort	Organization
Dr. Joseph Long	PI	10%	WRAIR
Ying Wang	Co-PI	30%	WRAIR

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name	Location	Contribution

Lieber Institute for Brain Development at Johns Hopkins University	Maryland	Collaboration

8. SPECIAL REPORTING REQUIREMENTS

A Quad Chart is attached

9. APPENDICES:

Posters are attached

Central mechanisms and treatment of blast-induced auditory and vestibular injuries

MR141274

W81XWH-16-2-0002

PI: Joseph B. Long **Org:** WRAIR/The Geneva Foundation **Award Amount:** $1,476,364

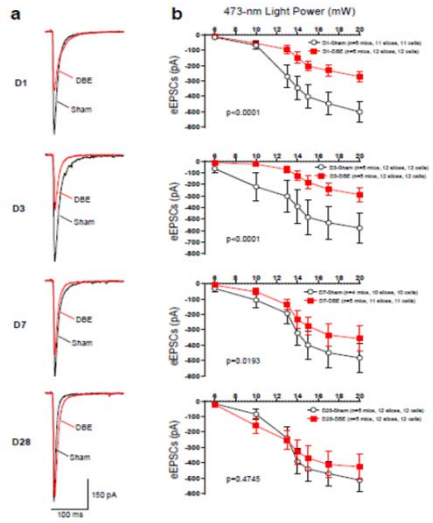
Study/Product Aim(s)

The etiology of blast-induced hearing loss and balance disorders is largely undefined. There are no FDA-approved drugs for treatment. This study utilizes a well-characterized, high fidelity rodent blast injury model to evaluate central auditory processing disorders (CAPDs) and central vestibular injuries (CVIs) and target disrupted TDP-43 and PERK-eIF2α-ATF4 signaling as a likely therapeutic means to mitigate blast-induced auditory and vestibular dysfunction.

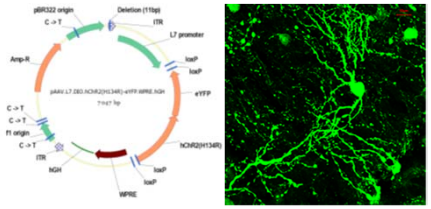
Approach

Blast TBI model: repetitive blast overpressure exposures to mice
Functional assessment: ABR, DPOAE, VsEP and Rotarod
New technology: optogenetics with whole-cell patch recording to uncover the impaired functional connection between brain regions; CRISPR/Cas9 gene editing and Single cell RNA-seq assay
Pathology: silver staining, immunohistochemistry on transgenic mice for specific neuronal plasticity and morphology
Define biomarkers: Western blotting and ELISA

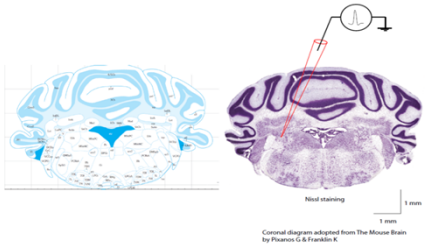
Impaired MGN-AU synaptic transmission following blast exposure



AAV-△L7-ChR2-YFP verification



Whole-cell patch clamp recording from lateral vestibular nucleus



Timeline and Cost

Activities CY	16	17	18
Verify blast-induced CAPDs and CVIs, define TDP-43 levels in serum and CSF			
Verify blast-induced morphological alterations in central auditory system and vestibular system			
Estimated Budget ($K)	$500	$500	$476

Updated: (02/2/2019)

Goals/Milestones

CY16 Goal
- ☑ Approval of animal use protocol
- ☑ Time-course of blast-induced auditory function deficits
- ☑ Define the role of TDP-43 in neuronal development

CY17 Goal
- ☑ Time-course of vestibular function assessment
- ☑ Determine TDP-43 levels in serum and CSF
- ☑ Morphological examination on specific neurons in AU
- ☑ Blast impaired functional connection between MGN and AU
- ☑ Examine the regulation of TDP-43 target genes

CY18 Goal
- ☑ Morphological examination on Purkinjie neurons in the cerebellum
- ☑ Blast impaired functional connection between FL and Lat

Budget Expenditure to Date

Projected Expenditure: $ 1,428,162.21

Actual Expenditure: $ 1,059,071.21

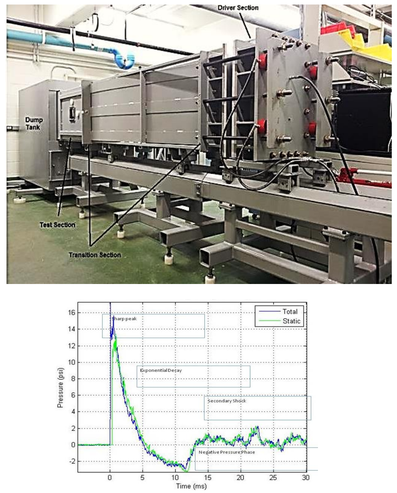
exposure to blast is still unclear. Accordingly, our present study aims to determine the molecular events in the inner ear and brain auditory signal processing centers after blast exposure and to identify potential therapeutic strategies against blast-induced hearing impairments.

Methods

Animal TBI model. Models of blast-induced TBI in mouse and rat have been used in this study. Briefly, isoflurane anesthetized CBA mouse (male, 25g) or Thy1-YFP mice (male, 26g) were secured in an Advanced Blast Simulator (ABS) in a prone position with the right side facing the oncoming shockwave. The blast overpressure (peak static pressure of 16 psi with a 4 msec positive phase duration) was generated by Valmex membrane rupture in the ABS. Animals for double blast exposures received two 16 psi blast shockwaves with a 2 min interval separating the two blasts. Sham animals were handled similarly without exposure to the blast.

Auditory functional assessment. A time-course of blast effects on auditory function was assessed by analyzing auditory brainstem response (ABR) under Ketamine/Dexdomitor anesthesia.

Figure 1. Advanced blast simulator



Pressure recording from tip/side gauge

RNA extraction and sequencing. After euthanasia, the cochlea was dissected from frozen temporal bone of rat. Total RNA was isolated using the RNeasy lipid tissue mini kit (Qiagen, Germanton, MD) according to the manufacturer's instructions. RNA concentration and quality were determined by spectrophotometric absorbance (NanoDrop ND-1000, Thermo Fisher) and RNA integrity number (RIN > 7.0) was confirmed using a 2100 Bioanalyzer (Agilent technologies). Library construction was generated using the Illumina following the manufacturer's protocol. Library concentration was quantified by Qubit 2.0 fluorometer (Life Technologies) and qPCR. Libraries were applied to the Illumina HiSeq 2000.

Bioinformatics analysis. Raw data (raw reads) of fastq format were firstly processed through in-house Perl script to clean data with high quality. HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. Differential expression analysis between two groups was performed using the DESeq2 R package. The resulting p-values were adjusted using the Benjamin and Hochberg's approach for controlling the False Discovery Rate (FDR). Genes with an adjusted p-value <0.05 found by DESeq2 were assigned as differentially expressed gene group. Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with corrected p-value less than 0.05 were considered significantly enriched by differential expressed genes.

Dendritic spines analysis. Animal was perfused with 4% PFA after euthanasia. The brains of Thy1-YFP mouse was immersed in cold PBS and sliced in 100-µm-thick coronal sections on a microtome (Leica VT1000S). Confocal images (20 z-stacks) were taken under Olympus Fluoview FV1200. Collapsed z-stacks were analyzed using ImagePro and Neuron Studio program. Spines were classified either as mushroom, stubby or thin type according to spine neck length and head size, referring to established parameters by Harris et al. (1992)

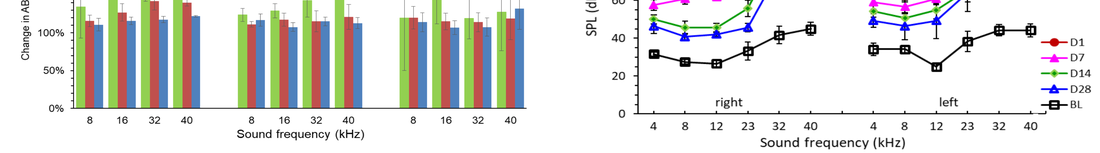


Figure 2. Auditory functional impairment following blast exposure. (a) ABR latency change is the ratio of latency at 7 and 28 days post exposure compared to its baseline in mice. (n=5). (b) Time-course changes in thresholds to the sound frequency stimuli in rats exposed to a double-blast treatment. Compared to high frequency (40 kHz) hearing loss after blast exposure, low frequency (8 kHz) hearing recovered early after insult. (n=6)

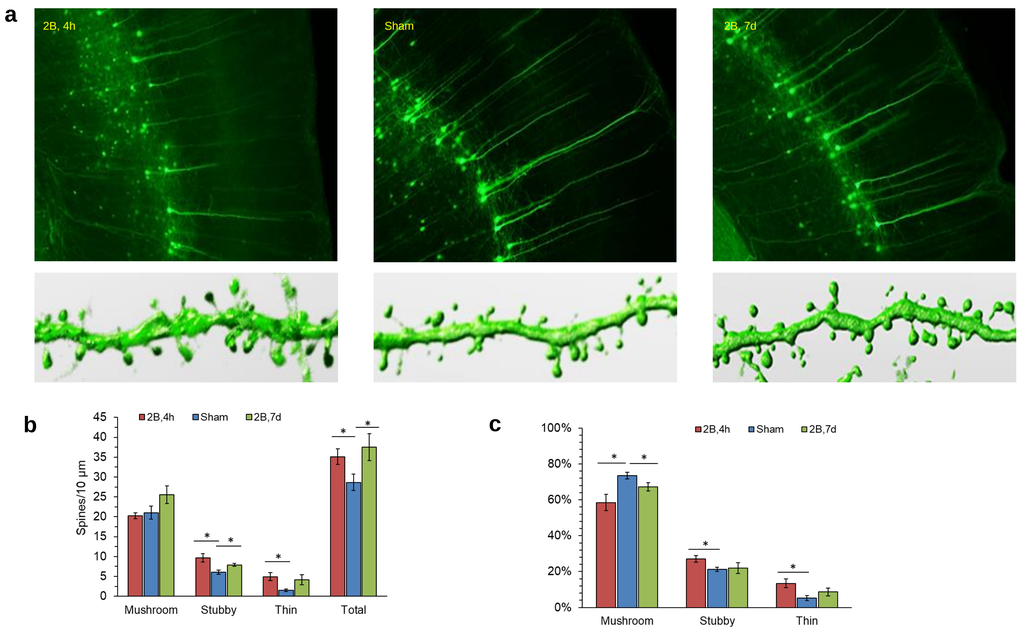


Figure 3. **Blast exposure altered dendritic spines in the auditory cortex. (a)** Representative photomicrograph depicts dendritic spines in the auditory cortex, (b) significant increase in total number of dendritic spines, particularly stubby and thin types at 4 h post-injury, (c) significant decrease in the percentage of mushroom type at 4 h and 7 days post-injury, n = 5, * p < 0.05.

Conclusions

In this study, we systematically characterized the transcriptome in the inner ear of blast-exposed rodents at acute and chronic phases by RNA-Seq. With such an informative resource, we can now better understand the pathological processes of blast-induced injury to cochleae through integrative analysis of the gene expression data. We also analyzed the morphology of dendritic spines in auditory cortex which correlate to the strength of synaptic transmissions that are associated with the function of neural networks following injury. The results revealed that the central auditory signal processing system was affected by blast shockwaves, which might also be responsible for hearing loss. Blast shockwave injury to ear and CNS leads to transcriptional and morphological changes that affect auditory function and govern regenerative processes. Further study of altered gene expression may provide insights into new methods for treating or preventing blast-induced auditory deficits.

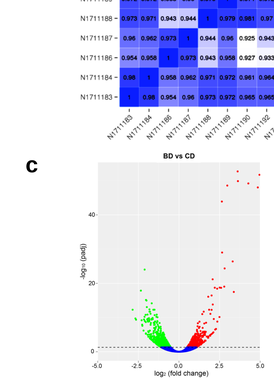


Figure 4. Heat maps and coefficient between sample groups (clusters) of genes. from large to small. Expe exposure, 1d) and BM (blas up (red) and down (blue) re 0.05. (e) Venn diagram of th

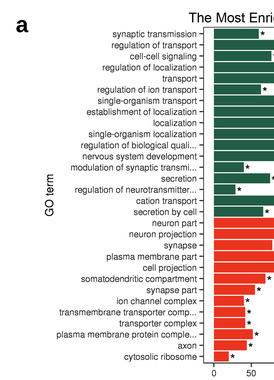


Figure 5. GO enrichment an regulation of cation channel and cell-cell signaling were presentation was significant

Acknowledgm

This work was supported W81XWH-16-2-0002.

Disclaimer

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Structural Plasticity in Brain Auditory Signal Processing Centers Following Blast Wave Exposure in Mice

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INTRODUCTION

Blast exposure-induced auditory deficit is one of the most common disabilities in military personnel and is thought to result at least in part from disrupted connectivity among brain auditory signal processing centers. However, the role of auditory neural signal processing abnormalities in the evolution of auditory impairment and long-term disability has not been extensively characterized. To uncover neurobiological mechanisms underlying these injuries, we have investigated the changes in brain structures involved in auditory signal processing using a mouse model of blast-induced auditory injury. The impact of blast injury on dendritic morphogenesis in auditory cortex that are associated with the function of neural networks was observed using Thy1-YFP mice. Expression of proteins synaptophysin, PSD-95, GFAP and Iba-1 in CNS were determined by immunohistochemistry. Data collectively indicated that blast shockwaves influence neurotransmission, membrane transport and glial cell proliferation in auditory signal processing centers at acute and/or sub-acute phase after injury. Cortical and brainstem injuries after blast exposure likely play vital roles in auditory dysfunction.

METHODS

Animal TBI model. Animal TBI model. Isoflurane anesthetized Thy1-YFP and CBA mice (male, 27±2 g) were exposed to blast overpressure (peak static pressure, in 4 msec positive phase duration) in the Advanced Blast Simulator (Fig.1). Experimental groups were single blast pressure with 4 psi (B-4), 8 psi (B-8), 16 psi (B-16), double (2B) and 3-repetitive exposures with 16 psi (3B) that subjects received exposures to 1 or 2 additional blast shockwaves, respectively, with a 2 min interval separating blast exposures during which time additional isoflurane anesthesia was delivered. Noise controls were placed outside of the blast chamber after anesthesia. Sham controls were handled similarly without exposure to the blast.

Auditory functional assessment. A time-course of blast effects on auditory function was assessed by analyzing auditory brainstem response (ABR) under Ketamine/Dexdomitor anesthesia.

Immunohistochemistry. The brain tissue was cut in 30-µm-thick coronal sections on a cryostat microtome. The slices were performed permeabilization with 0.2% triton-100 in PBS, non-specific blocking with 5% goat serum, incubation with primary antibodies (SYP, PSD-95, GFAP and Iba-1) and secondary antibodies. Images were taken under Olympus AX80 microscope.

Dendritic spines analysis. Animal was perfused with 4% PFA after euthanasia. The brains of Thy1-YFP mouse was immersed in cold PBS and sliced in 100-µm-thick coronal sections on a microtome (Leica VT1000S). Confocal images (20 z-stacks) were taken under Olympus Fluoview FV1200. Collapsed z-stacks were analyzed using ImagePro and Neuron Studio program. Spines were classified either as mushroom, stubby or thin type according to spine neck length and head size, referring to established parameters by Harris et al. (1992).

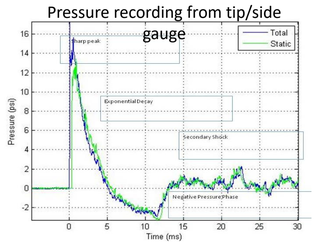

Fig. 1. Advanced blast simulator

RESULTS

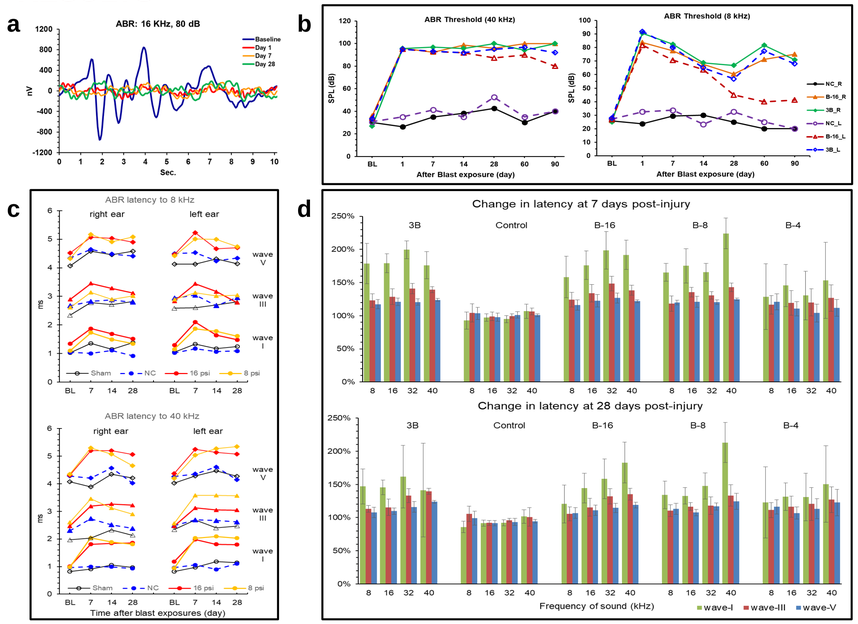

Fig. 2. ABR impairment following blast exposure. (a) Reduction in wave amplitudes after blast exposure, (b) elevation in thresholds to the sound of 8 and 40 KHz stimuli, (c) latency changes in the wave I, III and V, (d) changes in ratio of latency that compared to its baseline among experimental groups.

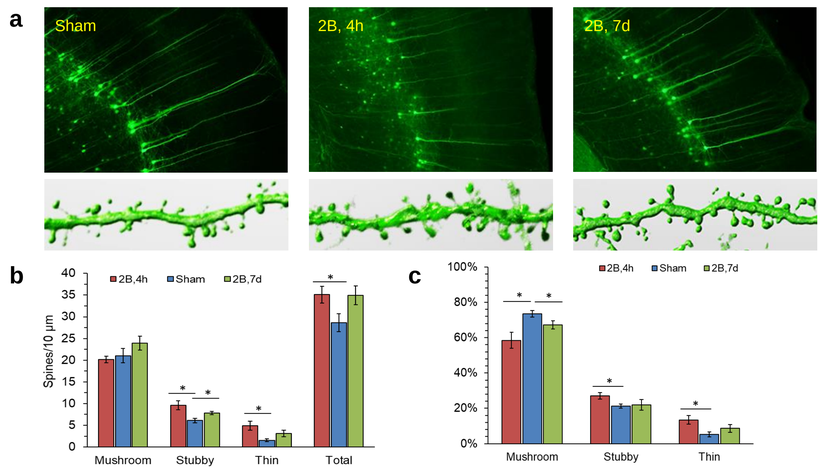

Fig. 3. Blast exposure altered dendritic spines in the auditory cortex. (a) Representative photomicrograph depicts dendritic spines in the auditory cortex, (b) significant increase in total number of dendritic spines, particularly stubby and thin types at 4 h post-injury, (c) significant decrease in the ratio of mushroom type at 4 h and 7 days post-injury, n = 5, * p < 0.05.

RESULTS

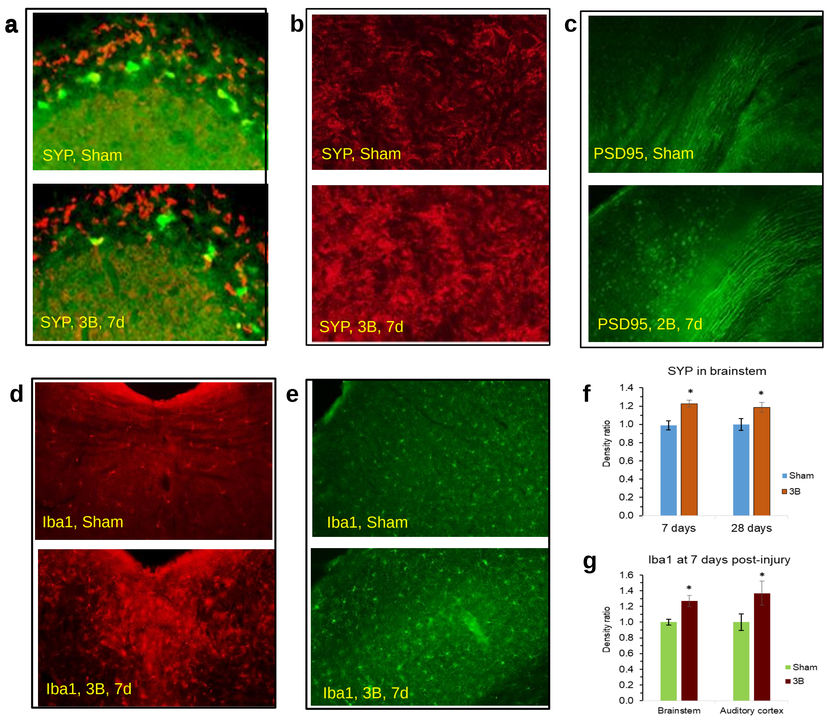

Fig. 4. Blast-induced SYP, PSD95 and Iba1 changes in CNS. Images depict immunohistochemistry on sections of cerebellum (a), brainstem (b, c, d) and auditory cortex (e). Compared to the sham controls, SYP (f) and Iba1 (g) increased significantly in the brainstem, n = 3, * p < 0.05.

CONCLUSIONS

- Blast shockwave exposure impaired the whole spectrum hearing, and its effect on high frequency hearing was more severe.
- Blast exposure injured the signal generation and transduction from inner ear through brainstem.
- Morphological changes in dendritic spines at excitatory synapses within the auditory cortex were evident and revealed blast-induced brain injury during early stages of synapse formation and synaptic transmissions in neural networks.
- Blast shockwaves influenced neurotransmission, membrane transport and glial cell proliferation in auditory signal processing centers at acute and sub-acute phase after exposure.

ACKNOWLEDGMENTS

This work was supported by CRMRP awards W81XWH-15-2-0024 and W81XWH-16-2-0002.

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