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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.

15. SUBJECT TERMS
Traumatic brain injury

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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, auditory cortex (AU), medial geniculate nucleus (MGN), cerebellum, proteomics

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 the reporting period of year 5, our major activities were 1) controlled blast exposures of mouse treatment groups, 2) assessment of auditory brainstem responses (ABR) following blast exposures, 3) determination of blast-induced molecular changes in the cortex, middle brain and cerebellum, respectively, and 4) evaluation of blast-induced morphological changes in the brain.

Specific Objectives

1. Investigation of changes in cortical proteomics after blast exposures
2. Investigation of changes in cerebellar proteomics at after blast exposures
3. Determination the effects of blast exposure on synaptic proteins
4. Determination the effects of blast exposure on neurotrophins and their receptors
5. Determination of blast-induced alterations in synaptic morphologies

Significant Results

1. Characterized the proteomes of the mouse cerebellum at ages 10, 14 and 18 weeks
2. Identified blast-induced differential expression of proteins in the cerebellum at 1, 28 and 60 days after injury
3. Identified blast-induced differential expression of proteins in the cortex at 1, 7 and 28 days post injury
4. Investigated the effects of blast exposure on axonal branch and synaptic boutons
5. Validated blast-induced protein changes in the cortical region that are associated with synaptic plasticity and neurotrophic factors

Significant results, including major findings, development, or conclusions

1. Proteomic characterization of the proteomes in the cortex and cerebellum of mouse

To identify the effects of blast exposure on the proteomic profile in the mouse brain, a comparative proteomic analysis of the proteins at a global level was performed by using a mass spectrometry-based quantitative proteomics technology. A total of 36 mice were separated randomly into experimental treatment groups including tightly coupled double blast exposures (B) and sham controls (C). The investigation time intervals were 1, 7, 28 and 60 days after injury. The sham control groups were C1 (n=6), C28 (n=6) and C60 (n=3). The blast groups were B1 (n=6), B7 (n=6), B28 (n=6) and B60 (n=3). Brain tissues, cerebral cortex (ct) and cerebellum (cb) were processed for protein extraction and TMT-multiplex labeling. The peptide fractionation and nanospray LC/MS-MS analysis were performed by Poochon Scientific (Frederick, Maryland).

1) Identification of differential expression of proteins in the cerebeluum after blast injury

We have quantitatively identified a total of 4993 proteins in the cerebellum of mouse among six experimental groups. There were C1 (n=5), B1 (n=5), C28 (n=5), B28 (n=6), C60 (n=3) and B60 (n=3). The proteomic profiles were illustrated in the heat maps (Fig.1 a and b). Fig 1b shows the relative abundance of 414 proteins identified across 6 groups of 27 samples which are ranked (fold change >10%, $p < 0.05$, n=3) in C60 group comparing to C28 group. Very interestingly, the proteomic pattern of B28 was remarkablely different from that of C28, but was similar to that of C60 and B60. This finding potentially indicates that blast injury can accelerate aging related protein expressions.

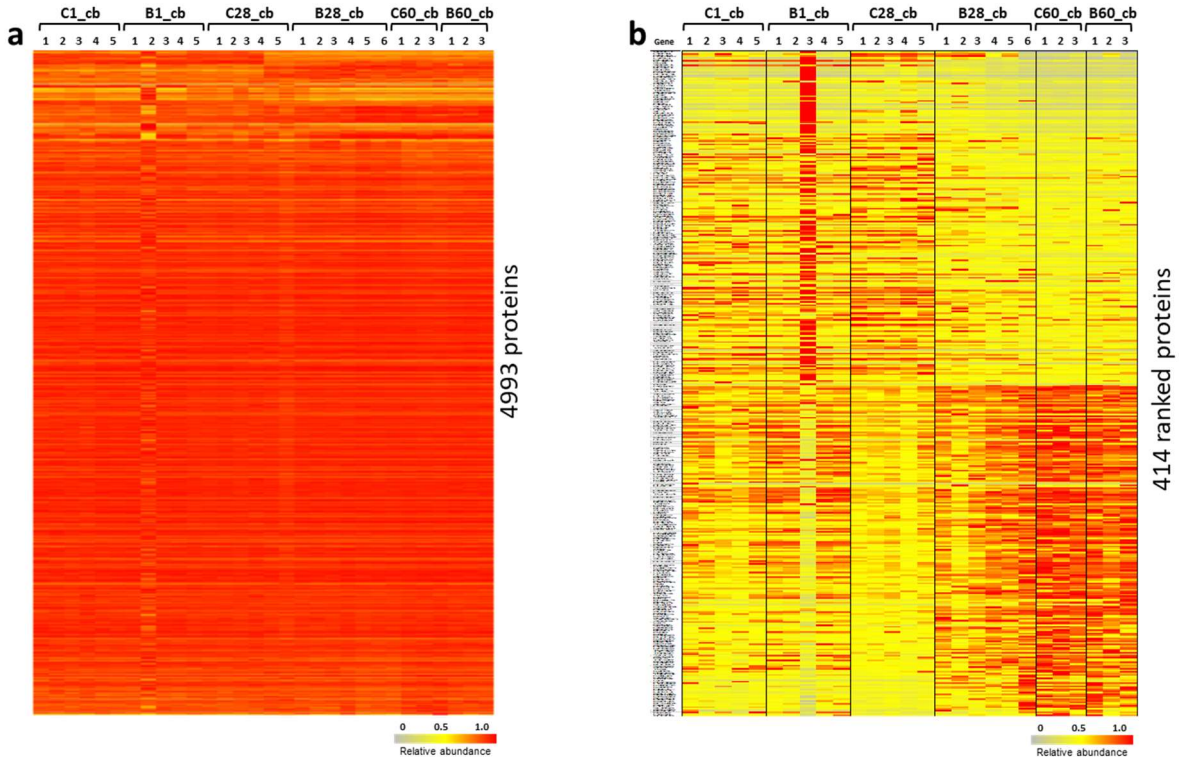

Fig.1. Heat map depicting the proteomes of mouse's cerebellum. (a) the relative abundance of 4993 proteins, (b) the relative abundance of ranked 414 proteins identified across 6 groups of 27 samples. The color key indicates the relative abundance of each protein (0 to 1.0).

To identify the variation of proteins in mouse's cerebellum, the relative aboundance of 4993 proteins were analyzed. Volcano plots (Fig.2) showed that 9 proteins were changed by at least 10%, $p<0.05$ in the B1 group comparing to the C1 group (Fig.2a), while 204 proteins changed in B28 vs C28 (Fig.2b), and 23 proteins changed in B60 vs C60 (Fig.2c).

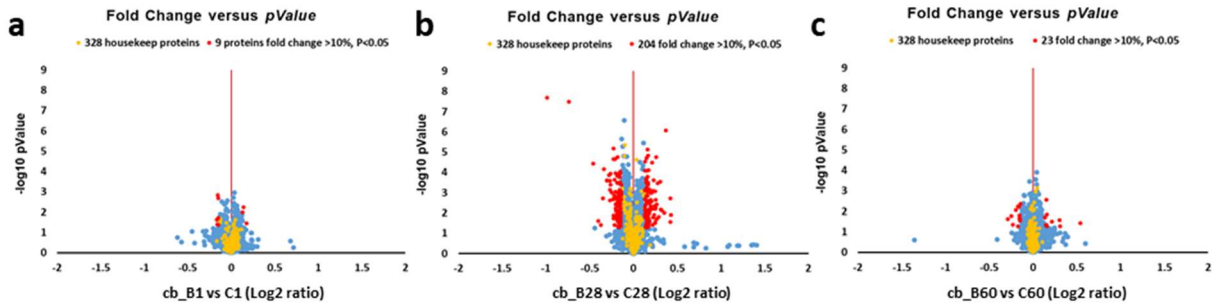

Fig.2. Volcano plots for the log2(fold change) and –log10 (p value) of all proteins in B vs C at 1, 28 and 60 days after blast exposure.

The distribution of the differentially expressed proteins (DEPs) is presented by a Venn diagram (Fig.3), 117 DEPs (2.34% of the total proteins) were identified with 69 (58.97%) up-regulation and 48 (41.03%) down-regulated proteins in B1 vs C1. In contrast, 1079 DEPs (21.61% of total proteins) were identified with 469 (43.47%) up-regulation and 610 (56.53%) down-regulated proteins in B28 vs C28. And 249 DEPs (4.99% of total proteins) were identified with 119 (40.48%) up-regulation and 175 (59.52%) down-regulated proteins in B60 vs C60. There are 29 proteins are shared between D1 and D28.

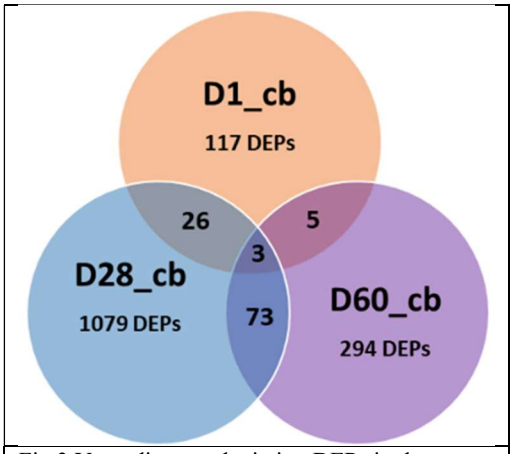

Fig.3 Venn diagram depicting DEPs in the cerebellum at 1, 28 and 60 days post-blast.

We also identified the DEPs among C1, C28 and C60 groups, which included three ages (10, 14 and 18 wks) of normal control mice. Volcano plots showed that 83 proteins were changed by at least 10%, $p<0.05$ in the C2 group comparing to the C1 group (Fig.4a), while 369 proteins changed in C60 vs C1 (Fig.4b). Four particular proteins, Hmgb2, Cdh13, Ly6h and Camkv, have been identified among those three age groups (Fig.5).

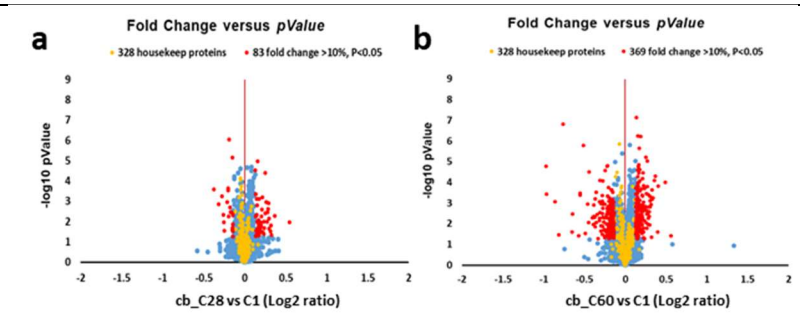

Fig.4. Volcano plots for the log2(fold change) and –log10 (p value) of all proteins in C28 vs C1 and C60 vs C1.

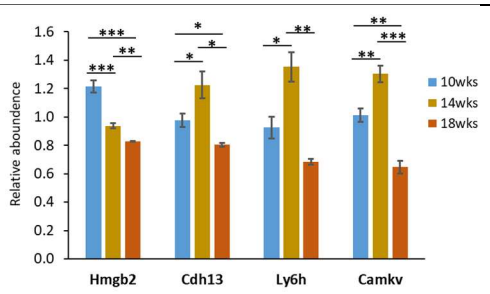

Fig. 5. Age related DEPs in the cerebellum of sham mouse, *p<0.05, **p<0.005, ***p<0.001

2) *Identification of differential expression of proteins in the cortex after blast injury*

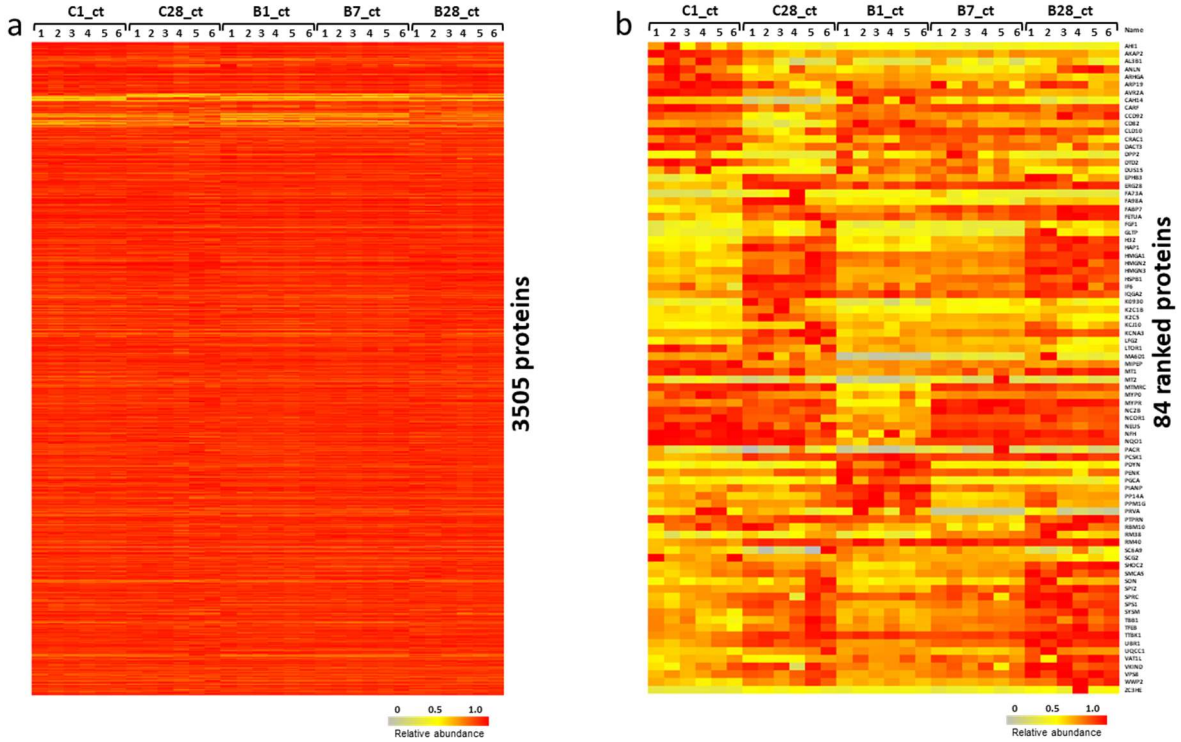

Fig.6. Heat map depicting the proteomes of mouse's cortex. (a) the relative abundance of 3505 proteins, (b) the relative abundance of ranked 84 proteins identified across 5 groups of 30 samples. The color key indicates the relative abundance of each protein (0 to 1.0).

To identify the protein signature related to the blast injury, a total of 6069 proteins were quantitatively identified in this study and 3505 proteins were quantitatively identified in all 30 samples. Protein abundance in the cortical region of mice were compared among five experimental groups (C1, B1, B7, C28 and B28, 6 mice for each group). The proteomic profiles are illustrated in the heat maps (Fig.6 a and b). Fig. 6b shows the relative abundance of 84 proteins identified across 5 group of 30 cortical samples which are changed by at least 25% in one treatment in comparison with C1 (fold change >25%, p-Value <0.05, n=6) .

The distribution of the differentially expressed protein (DEPs) is presented as a Venn diagram (Fig.7), 1399 DEPs (39.91% of the total proteins) were identified with 627 (44.82%) up-regulated and 772 (55.18%) down-regulated proteins in B1 vs C1. In contrast, 1546 DEPs (44.11% of total proteins) were identified with 804 (52.01%) up-regulated and 742 (47.99%) down-regulated proteins in B7 vs C1. Finally, 555 DEPs (15.83% of total proteins) were identified with 252 (45.41%) up-regulated and 303 (54.59%) down-regulated proteins in B28 vs C28. There are 125 proteins are shared among D1, D7 and D28.

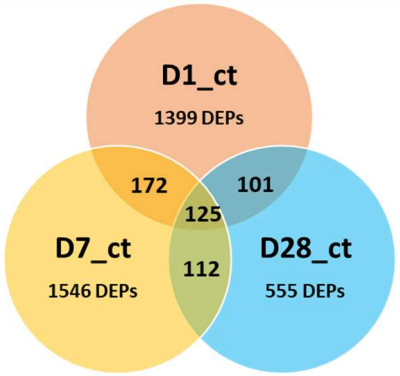

Fig.7. Venn diagram depicting DEPs in the cortex at 1, 7 and 27 days post-blast.

Volcano plots showed that 26 proteins were changed by at least 1.25-fold in the blast-exposed mice (B1 group comparing to the C1, $p<0.05$, Fig.8a), while 19 proteins changed significantly in B7 vs C1 (Fig.8b), and 38 proteins changed in B28 vs C28 (Fig.8c).

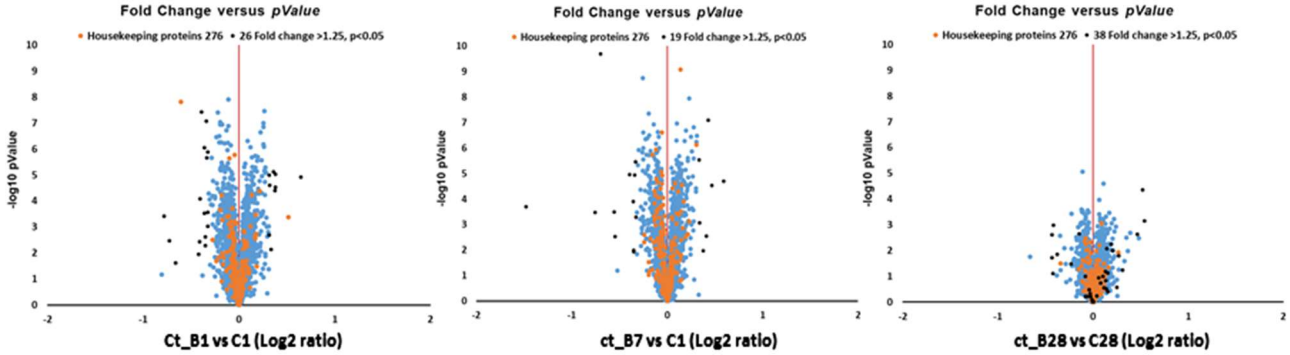

Fig.8. Volcano plots for the log2(fold change) and –log10 (p value) of all proteins in B vs C at 1, 7 and 28 days after blast exposure.

2. Determine the effects of blast exposure on axonal plasticity

In the previously reported work, we found that blast exposure significantly impaired the long-range functional connectivity between the medial geniculate nuclei (MGN) and auditory cortex (AU) at 1, 3 and 7 days post-injury using whole-cell patch-clamp electrophysiological recording. Consistent with the functional changes, dendritic morphogenesis was also altered following blast exposure. Data showed that total numbers of dendritic spines and postsynaptic structures in the AU region were increased at 4 hrs post-injury, and the changes were predominantly in the immature types of stubby and thin spines, so that the ratio of mushroom type spines decreased.

We next questioned whether presynaptic boutons and branches are impacted by the shockwaves. A group of 10 Thy1-RFP mice was used for this investigation. Briefly, all mice received injection of 1 ul AAV-CAG-GFP in the MGN. After 4 weeks recovery, mice were selected randomly into a blast treatment group or a sham group. At designated time points, mice were euthanized and fixed in 4%PFA solution. Mouse brains were then dissected and cut coronally. The brain sections (80 μm) were mounted on glass slides. Images of the auditory cortex (AU) in each section were prepared using an Olympus confocal microscope and contained 30 – 50 areas with 40 z-stack pictures for each area. Imaris software was used for analyzing axonal plasticity.

Mice exposed to the shockwaves (3 days) showed that axons in the AU region (green color, Fig.9) increased in branch level (23%) and branch depth (100%), but decreased with regard to the number of synaptic knobs (24%) and terminal bouton volume (35%) comparing to the sham control. The result from colocalization analysis (merged colors, Fig.9) between synaptic bouton (green) and post-synaptic neurons (red) indicated the colocalized area was less than 18% in blasted mouse.

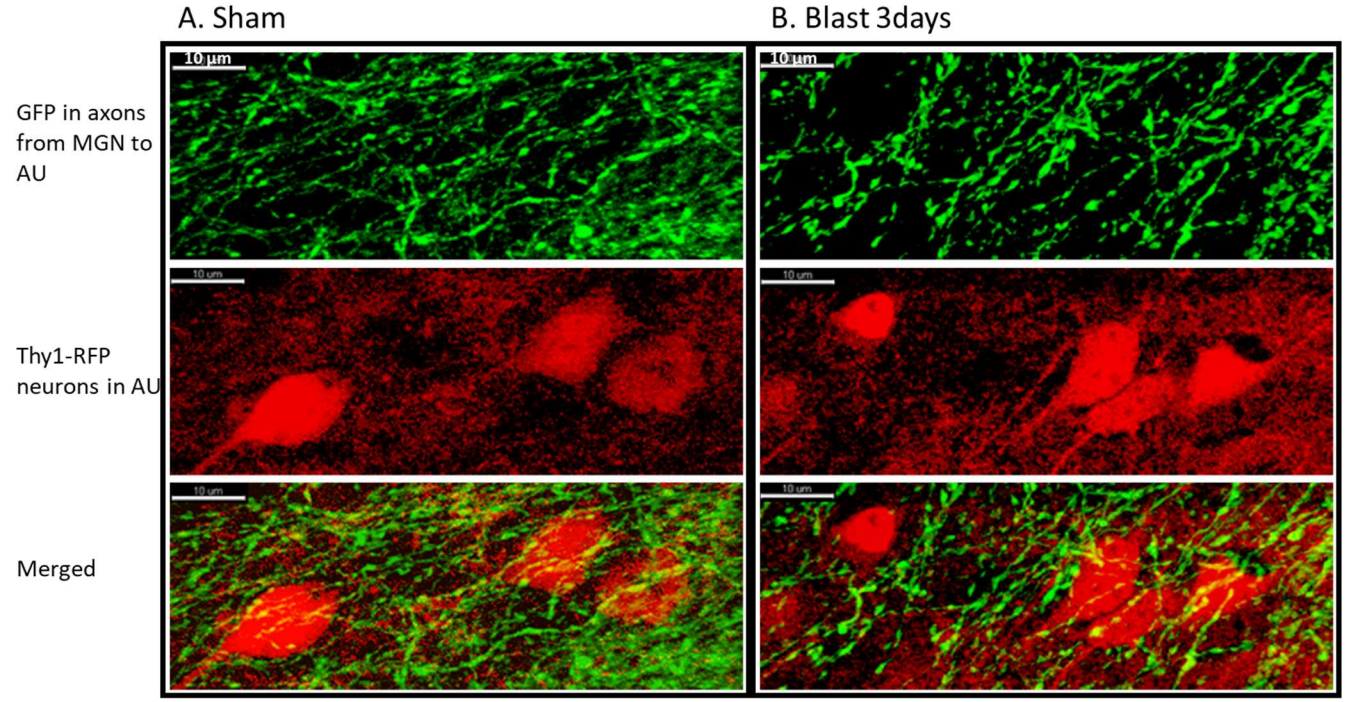

Fig.9.Representative cryostat sections (80 µm thick) of sham and at 3 days post-blast.

3. Validation of blast-induced DEPs in the cortex by western blot or ELISA

In the reporting period, we also used Western blotting or ELISA analysis to validate some of DEPs resulted from proteomic analysis. Those were presynaptic and postsynaptic protein expression, as well as neuron-derived neurotrophic factor in the cortex region.

- The postsynaptic density (PSD) serves as a signaling apparatus. It has been proposed to concentrate and organize neurotransmitter receptors in the synaptic cleft. Compared to the sham controls, PSD95 (DLG4), PSD93 (DLG2) and DLG3 increased significantly at 28 days post-blast. They also increased slightly at an acute phase, but no statistic differences were observed. PSD97 was unchanged throughout. As PSD-95/SAP90-binding proteins, DLGP3 and DLGP4 increased ($p < 0.01$) at 1 day post-blast, but no changes for DLGP1 and DLGP2 were detected.

- Neuroligin (NLGN) is a cell adhesion protein on the postsynaptic membrane that mediates the formation and maintenance of synapses between neurons. NLGN1 localizes at excitatory synapses, NLGN2 at inhibitory synapses and NLGN3 at both.
- NLGN2 and NLGN3 decreased ($p < 0.005$) at acute phase, but no changes in NLGN1 were seen. Reduction in the levels of neuroligins 1, 2 and 3 results in a strong reduction of inhibitory input but little reduction in excitatory input.
- The N-methyl-D-aspartate receptors (NMDA) are also associated with synaptic plasticity. NMDARs (also named GluN or GRIN) are composed of two subunits. Ca2+ flux through GluN is thought to be critical in synaptic activities. Compared to the sham controls, GluN2B increased ($p < 0.005$) at 1 days but decreased at 28 days post-blast. There were no changes in GluN1 and GluN2A.
- The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is a non-NMDA-type receptor for glutamate. AMPARs are composed of four types of subunits endcoded by genes GRIA (also named GluA or GluR). AMPARs are integral to synaptic plasticity at many postsynaptic membranes that mediate fast synaptic transmission in the CNS. Compared to the sham controls, GluA2 and GluA3 decreased ($p < 0.05$) at 28 days post-blast, but no changes in GluA1 and GluA4.
- Synaptotagmins (SYT) serve as sensors for calcium ions in the process of vesicular trafficking and exocytosis. SYT1 localized in the membrane of the pre-synaptic axon terminal binds to Ca2+ and participates in triggering neurotransmitter release. Compared to the sham controls, SYT1 decreased significantly at 1 and 7 days post-blast, while SYT2 increase increased ($p<0.05$) at 28 days post-blast.
- Synapsins (SYN) bind synaptic vesicles to components of the cytoskeleton which prevents them from migrating to the presynaptic membrane and releasing neurotransmitter. Compared to the sham controls, SYN1 decreased significantly ($p < 0.001$) at both acute phase and chronic phase, while SYN2 decreased ($p<0.05$) at 28 days post-blast. SYN1 decreased significantly ($p < 0.001$) at both acute phase and chronic phase, while SYN2 decreased ($p<0.05$) at 28 days post-blast.
- Synaptophisin (SYP) is a synaptic vesicle glycoprotein, and was unchanged following blast exposure.
- Neurotrophic factors (NTFs) comprise a family of biomolecules that support the growth, survival, and differentiation of both developing and mature neurons. Compared to the sham controls, neurotrophic tyrosine kinase receptor type 2 (NTRK2) decreased at 7 days post-blast, but increased at 28 days post-blast. BDNF decreased in both acute and chronic phases.

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 analyze dendritic spines using Imaris software.

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."

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

Continue pathological analysis 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.

Because of the prolonged COVID-19 pandemic situation, everyone was ordered to telework from March through June. The animal experiments and molecular assays were resumed in July when WRAIR issued a 25% personnel return to the workplace directive.

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, a manuscript is in preparation.

  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.*

Nothing to report.

- **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.*

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.