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TITLE: Demyelination as a Target for Cell-Based Therapy of Chronic Blast-Induced Traumatic Brain Injury

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14. ABSTRACT The burden of traumatic brain injury (TBI) is expressed in the disabling behavioral and cognitive abnormalities noted in significant number of combat veterans. These clinical phenotypes suggest impairment in distributed cerebral functions dependent on the integrity of white matter (WM) tracts. In this proposal we explore mechanisms of mild blast trauma-associated demyelination, with the goal of testing a therapeutic strategy to enhance remyelination using human glial restricted progenitors (hGRPs; Q Therapeutics Inc.).					
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The burden of traumatic brain injury (TBI) is expressed in the disabling behavioral and cognitive abnormalities noted in significant number of combat veterans. These clinical phenotypes suggest impairment in distributed cerebral functions dependent on the integrity of white matter (WM) tracts. In a recent study, clinical and neuropathological features consistent with CTE were identified in humans following both sport-related and experimental blast TBI⁷. Taken together, these data suggest a model linking trauma, WM injury, and chronic neurodegeneration. Consequently, myelin damage could represent a biological pathway linking traumatic WM injury, cognitive impairment, and neurodegenerative disease. In this proposal, mechanisms of trauma-associated demyelination are explored, with the goal of testing a therapeutic strategy to enhance remyelination that uses glial restricted progenitors (GRPs)

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

Diffusion tensor imaging, learning and behavior, white matter abnormalities, blast traumatic brain injury, social interaction behavior

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

Specific task 1: Blast injury results in long-term alterations in white matter and is associated with a significant decrease in myelination, as evidenced by DTI and MTR MRI and by histopathology in a murine model.

Specific task 2: Stereotactic intracerebral transplantation of GRPs induces myelination and ameliorates behavioral deficits in a murine blast TBI model

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.

(a) Overall accomplishments:

During this reporting cycle we accomplished the following:

- Immunohistopathological studies for acute studies have been accomplished
- We have studied long term effect of blast exposure on white matter injury using DTI analysis in Wild-type (balb/c) animals
- Chronic DTI results have been analyzed and injury criterion have been established in Wild-type (Balb/c) animals
- Chronic behavior studies have been completed to estimate learning and memory for wild-type (balb/c) animals
- Chronic social interaction studies have been completed and are currently under analysis stage
- Immunohistochemistry of wild-type animals have been completed and are under analysis stage
- Chronic DTI data have been secured for immunodeficient (rag-2) mice and analysis is currently underway
- Chronic behavior studies have been completed for immunodeficient mice (rag-2) mice for learning, memory and social interaction

Accomplishments with specific tasks:

Specific task 1 : *Blast injury results in long-term alterations in white matter and is associated with a significant decrease in myelination, as evidenced by DTI and MTR MRI and by histopathology in a murine model.*

- **Subtask 1:**

(a) Accomplishments

Imaging and behavior assessment has been for acute changes have been accomplished in year 1. The established of injury severity in accordance with subtask 1 was accomplished in year 1. Histopathological assessment of brain has been assessed for inflammation using microglial marker, Iba1 and white matter injury using oligodendrocytes precursor marker, Olig-2.

(b) Reportable Outcomes:

1. Histopathological assessment (supervised by dr Janowski)

Microglial marker, IBA1: We have further analyzed acute inflammatory responses following the animal's exposure to different blast intensities. We have found a variety response in different brain regions (figure 2) with a general increase almost all brain regions except corpus callosum (*p < 0.05) when compared to control group.

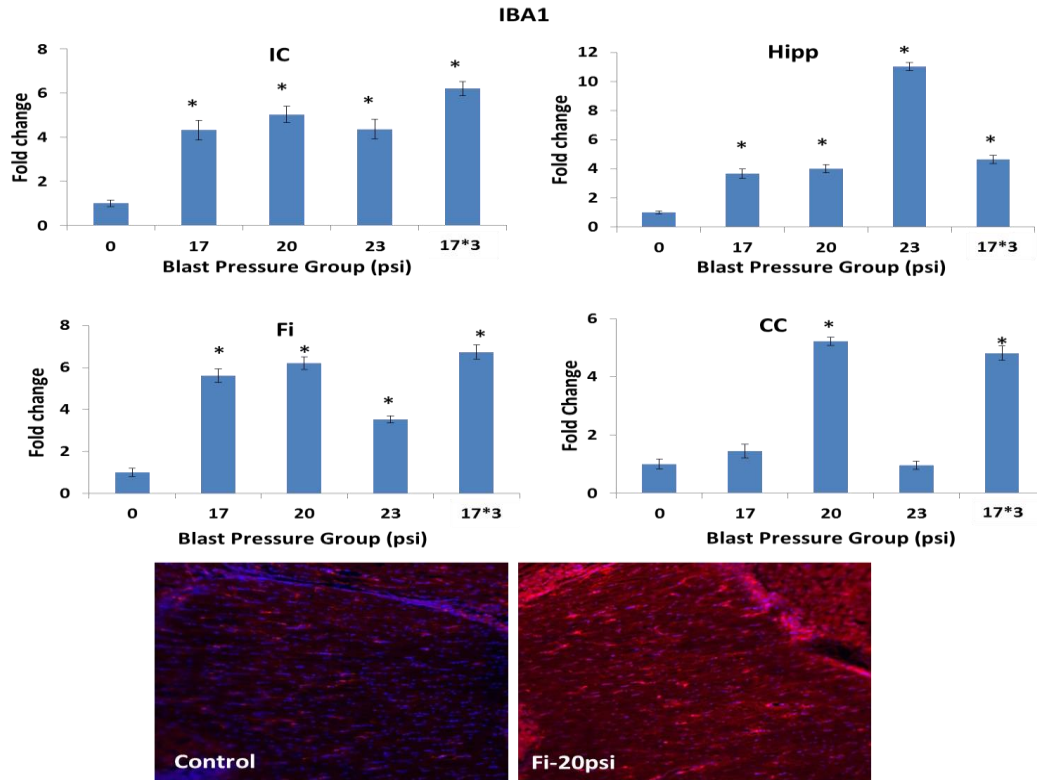


Figure 1: Microglial response in different regions of brain (Fi – Fimbria; IC – Internal Capsule; Hipp – Hippocampus; CC- Corpus Callosum) at 4 days following different intensities of blast overpressure exposure.

Oligodendrocytes precursors, Olig2: We have further analyzed acute loss of glia by quantifying oligodendrocytes following the animal's exposure to different blast intensities. We have found a variety response in different brain regions (figure 1) with a general decrease in olig-2 positive oligodendrocytes in 20 and 23 psi pressure groups in all brain regions that are evaluated (*p < 0.05) when compared to control group.

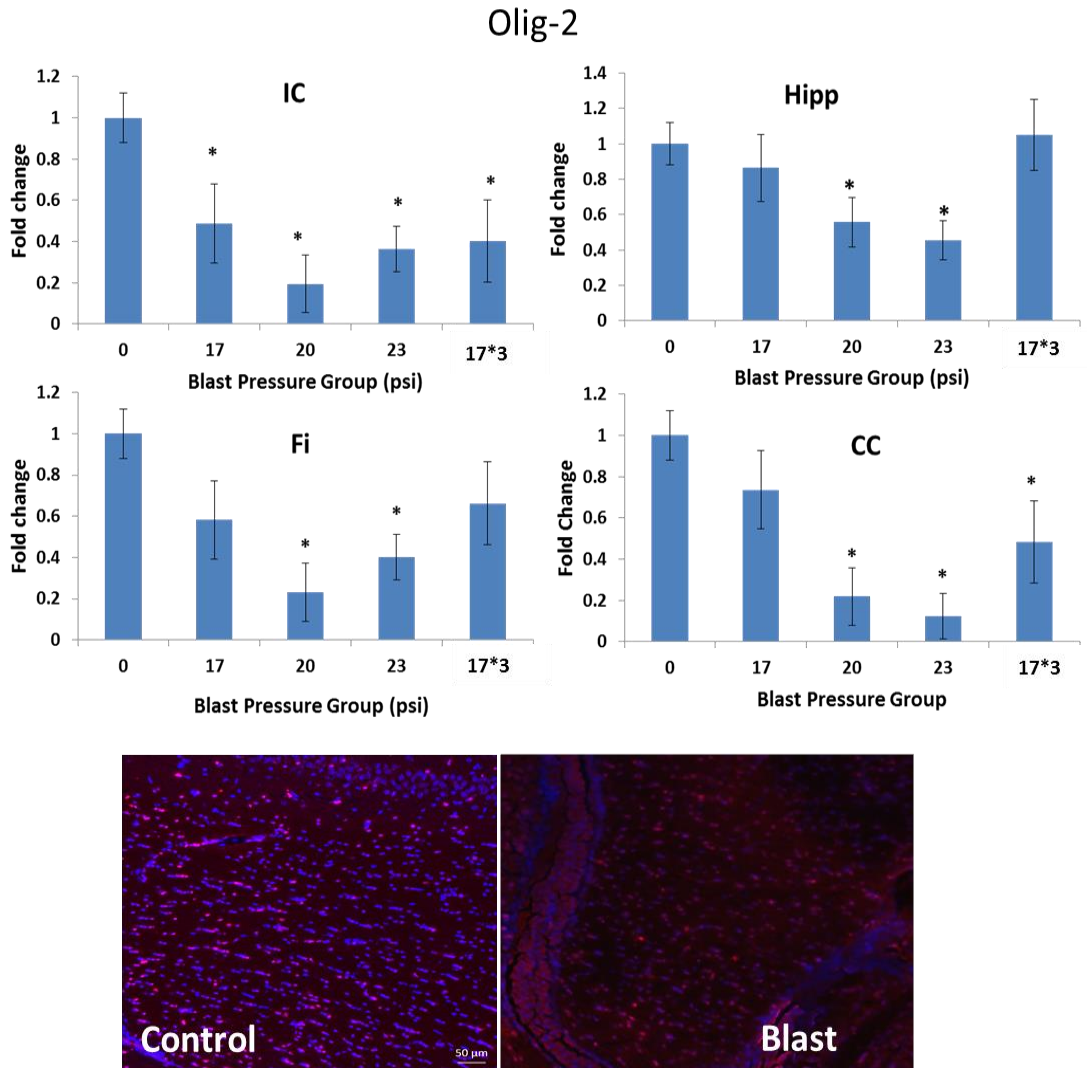


Figure 2: White matter injury (Olig-2, a marker of mature oligodendrocytes) in different regions of brain (Fi – Fimbria; IC – Internal Capsule; Hipp – Hippocampus; CC- Corpus Callosum) at 4 days following different intensities of blast overpressure exposure.

2. Serum biomarker assessment with injury severity (supervised by dr Janowski)

Sphingomyelins and dihydrosphngomyelins are the lipids that are particularly abundant in myelin sheaths. In order to understand the potential axonal injury in blood, we analyzed these lipids from plasma using mass spectroscopy. We have observed significant changes ($p < 0.05$) with selective fractions for the groups 17, 17*3 and 20 psi as shown in figure 3.

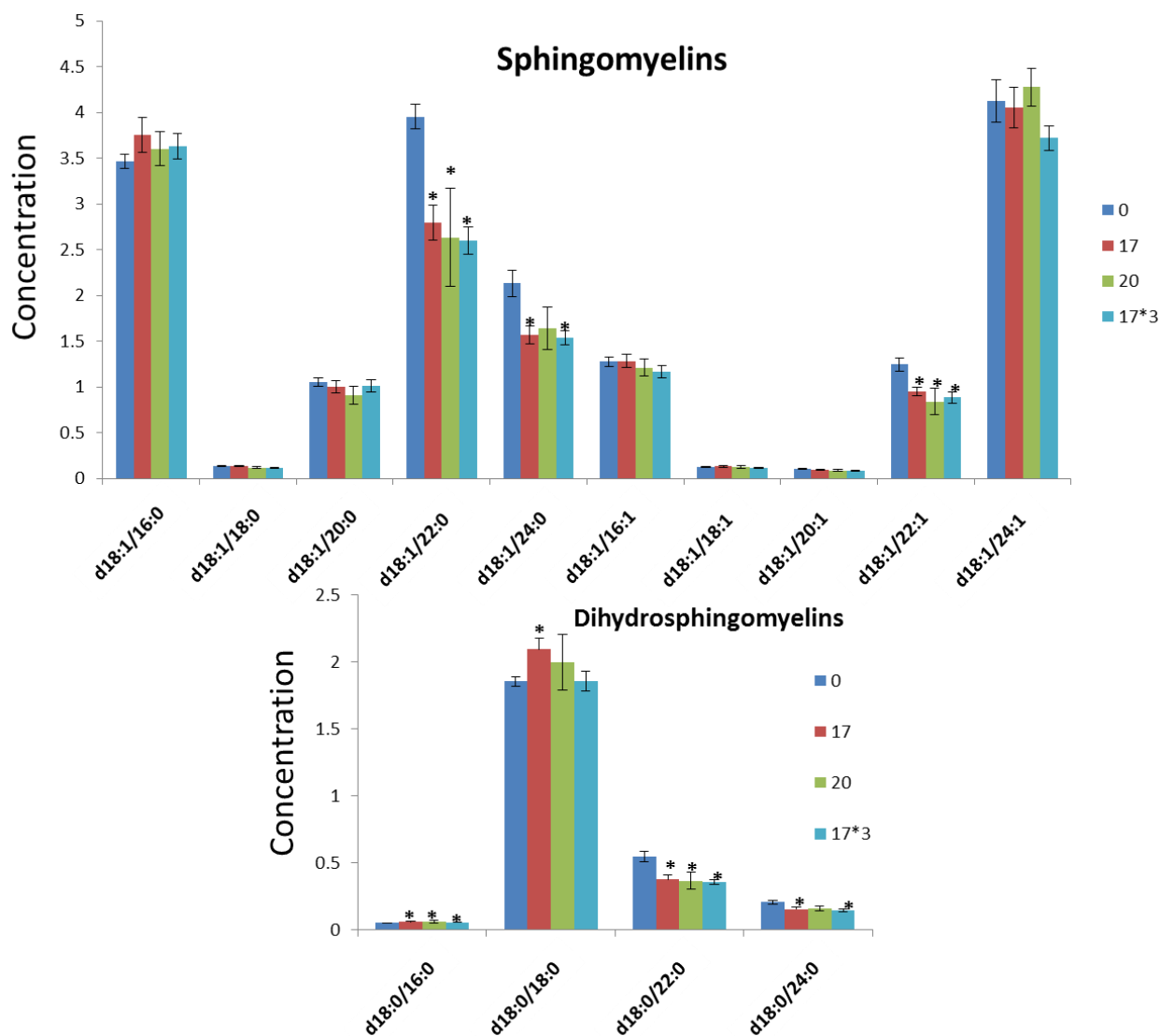


Figure 3: Changes in the sphingomyelins at 4 days following blast over pressure exposure (* $p < 0.05$).

Contrary to the changes in sphingomyelins, there are selective changes in dihydrosphingomyelins. While 17, 20 and 17*3 pressure groups have significant increase in d18:0/16:0 fraction, and 17psi has an significant increase in d18:0/18:0 fraction, and d18:0/22:0 fractions has significant decrease in all the pressure groups. This data shows certain metabolites fractions of dihydrosphingomyelins could be used for biomarkers that reflect the severity of the injury.

- **Subtask 2 – Non-invasive MR imaging of myelin (supervised by Dr. Walczak)**

(a) Accomplishments:

Successful completion of chronic non-invasive MR imaging of myelin using immunocompetent mice (balb/c), this data has been analyzed for all time points (1 day, 1 week, 1-4 months). In addition, we have secured the data immunodeficient animals (Rag-2), the data analysis have been completed for 1 day time point.

(b) Reportable outcomes:

Diffusion tensor imaging (DTI): Different regions of brain have shown changes in DTI, with diffuse injury in nature. Although, the specific regions are not similar among all the regions of brain that have been studied, blast overpressure had chronic implications on overall status of white matter injury. No changes have been observed in radial diffusivity following blast injury.

Axial diffusivity: Decrease in AD have been observed at 1 day time point in optic tract (OT), internal capsule (IC), fimbria (Fi). However, increase in AD have been observed in IC and OT at 1 month, 2 month and 3 month time points, while Fi is increased at 1 month and 2 month time-points. Only, corpus callosum (CC) has been increased at 4m time point as shown in Figure 4. No changes were observed at 1 week time-point.

Fractional anisotropy (FA): Fi have shown a decrease in 1 day and 4 month following blast overpressure exposure, CC have shown to be increased at 1 and 2 months, IC have shown to be increased at 3 month, while changes have been observed in OT following blast overpressure exposure.

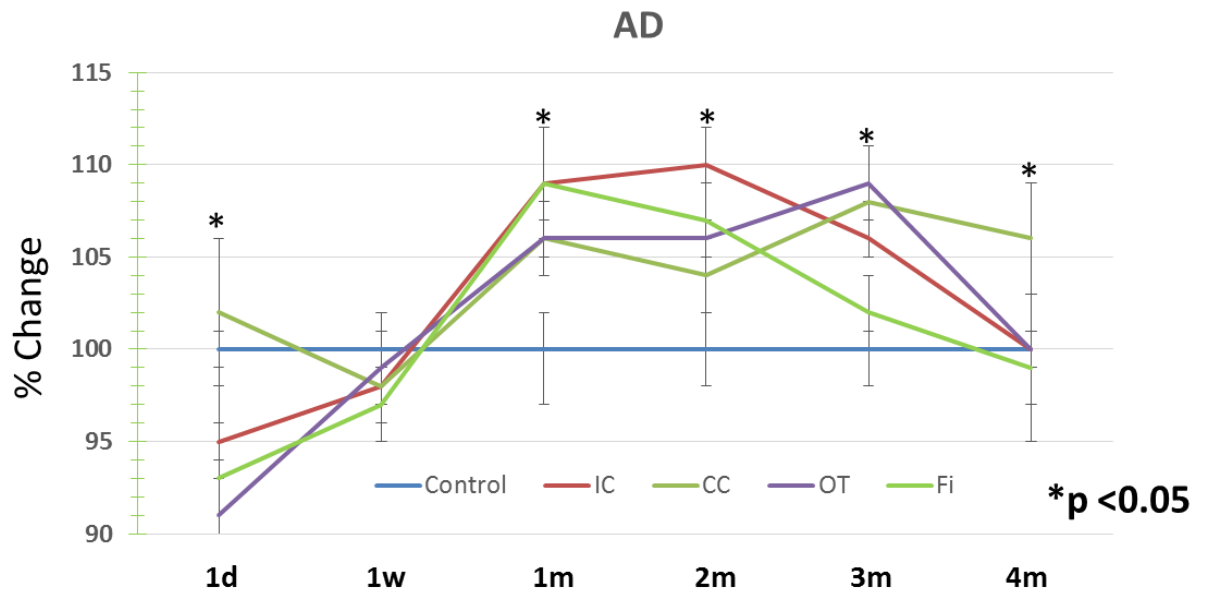


Figure 4. Summary of region specific changes and their time course following blast overpressure exposure at 17X2 psi.

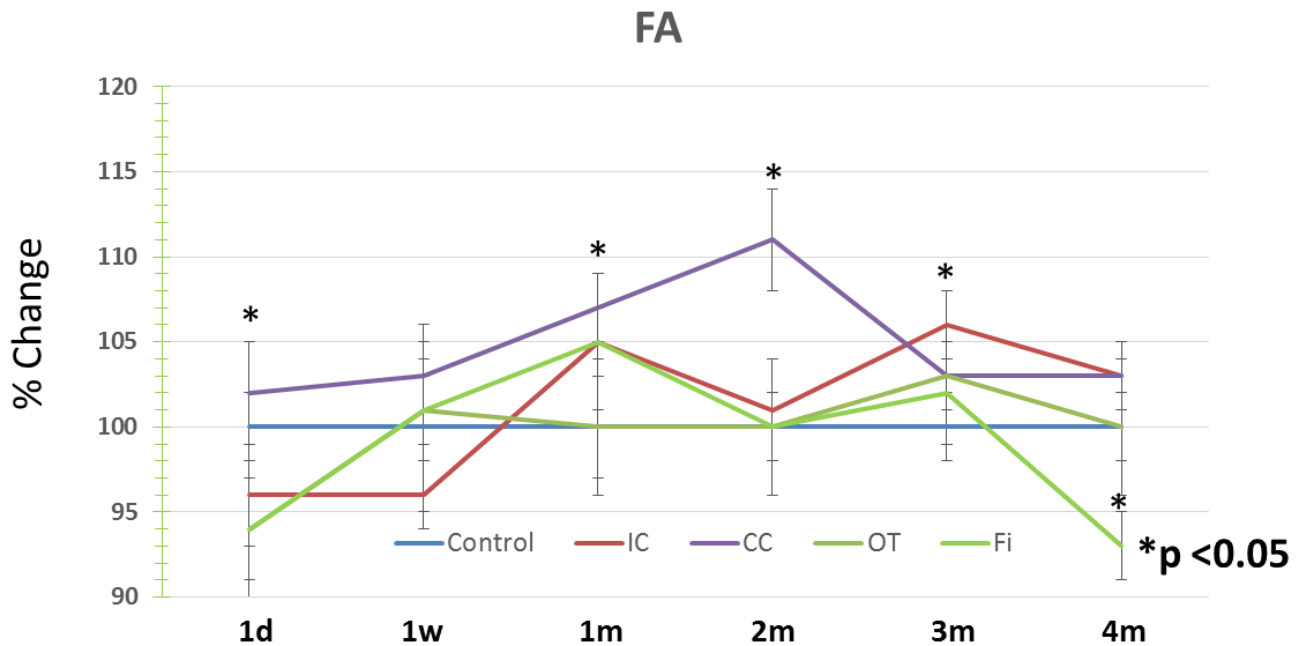


Figure 5: Summary of region specific changes and their time course following blast overpressure exposure at 17X2 psi.

• **Subtask 3 – Behavioral evaluation of blast injury (supervised by Dr. Janowski)**

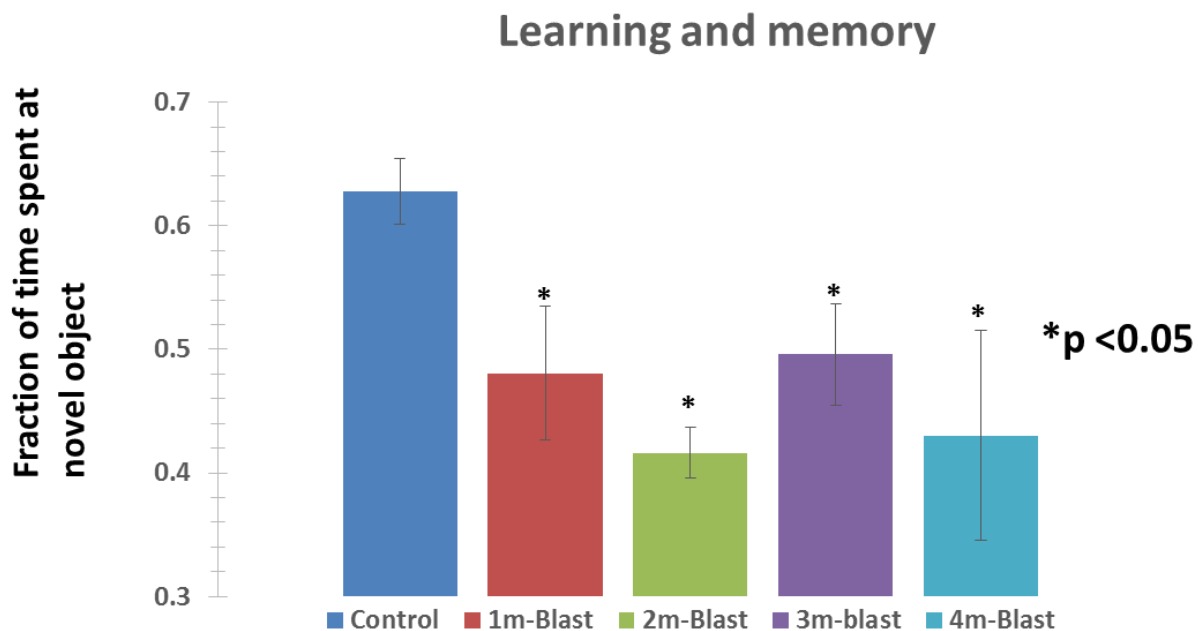
(a) Accomplishments:

Successful completion of chronic behavior testing for learning, memory and social interaction test for immunocompetent (Balb/c) mice. Data has been analyzed for learning and memory at all time points. Data has been analyzed for social interaction test at 1 month time point, while the rest of the data analysis is currently underway. Data have been acquired for immunodeficient (Rag-2) mice for learning, memory and social interaction and data analysis is currently underway.

(b) Reportable outcomes:

1. Learning and memory:

Significant reduction in learning and memory was observed at 1 month – 4 month in immunocompetent mice following blast overpressure. This depicts white matter injury as evidenced in DTI could result in cognitive impairment.



2. Social interaction:

No changes have been observed in social interaction at 1 month following blast overpressure in immunocompetent mice, while the rest of time point analysis is currently underway.

- **Subtask 4 – Neuropathological evaluation of blast injury**

(a) Accomplishments:

We have secured all the brains of all the animals and histopathological processing is currently underway.

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.

Nothing to Report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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

Nothing to Report

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

- 1 The analysis of MRI-DTI data and behavioral data to conclude the temporal demyelination for immunocompetent mice. In addition, preparation of manuscript for acute effects of varied pressure groups on mice will be drafted.
- 2 With the ongoing DTI data acquisition, we plan to identify a time-point that is best suited for stem cell therapeutics. This leads the way to the stem cell therapeutics for demyelination.

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

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.

Oligodendrocytes and myelin play important role in many neurological disorders including stroke, multiple sclerosis or even amyotrophic lateral sclerosis so knowledge about the status of myelin following TBI may bring important clues about myelin damage in these diseases.

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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:

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.

Nothing to report during this cycle

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.

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

Nothing to report

Significant changes in use of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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

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.*

Presentations and conference papers:

1. Thresholding injury in a murine model of blast traumatic brain injury: an imaging, behavioral, and pathological assessment. 2014 NIH TBI Workshop, Bethesda, Maryland
2. Mice model of blast traumatic brain injury injury: An imaging, behavior and pathological assessment. 2014 Summer Biomechanics, Bioengineering, and Biotransport Conference, Snowbird, Utah

- **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.

None

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were 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. 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;*
- *physical 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.)

Personnel

PI: Piotr Walczak MD/PhD: 2.4 months
Co-inv: Jeff Bulte PhD: 1 months
Post-doctoral fellow: Sujith Sajja PhD: 6 months

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.*

Collaborating organization: Walter Reed Army Institute of Research (WRAIR)

8. SPECIAL REPORTING REQUIREMENTS

NONE TO REPORT

9. APPENDICES: NONE TO REPORT