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TITLE: Nano-pulsed Laser Optoacoustic Therapy for Pre-treatment and Post-treatment of Traumatic Brain Injury

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CONTRACTING ORGANIZATION: University of Texas Medical Branch, Galveston, TX

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14. ABSTRACT

Traumatic brain injury (TBI) represents both an acute and a chronic medical challenge among service members and veterans. The **purpose** of this research is to demonstrate, in a rodent model, that a unique non-invasive nano-pulsed laser optoacoustic therapy (NPLT) is a promising pre-treatment for military personnel at high risk of combat-related TBI and a promising treatment after combat-related TBI, with the **goal** of limiting onset and progression of neuropathology and cognitive impairment. As of 09/30/21, our data show that NPLT, applied 24 hours before blast-induced brain injury (bTBI), prevents vestibulomotor dysfunction (in a focal model of bTBI) and cognitive dysfunctions (in a diffuse model of bTBI). Surprisingly, when rats were subjected to repetitive bTBI (2 consecutive bTBI 48 hours apart), we found no significant vestibulomotor and cognitive dysfunctions. However, after repetitive bTBI, we found significant accumulation of microglia in the brain that was prevented by NPLT treatment. Further histopathological and biochemical analyses are in progress to determine whether neuroinflammation and/or neurodegeneration is detected in the brain after single or repetitive bTBI and the effect of NPLT on bTBI and Sham rats.

15. SUBJECT TERMS
Traumatic Brain Injury; Blast Injury; Non-invasive Therapy; Nano-pulsed Optoacoustic Laser Therapy; Neuroprotection

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1. INTRODUCTION:

Traumatic brain injury (TBI) represents both an acute and a chronic medical challenge among service members and veterans. We have reported that transcranial application of a unique non-invasive nano-pulsed laser optoacoustic therapy (NPLT) stimulates the expression of specific factors which increase neuronal survival after TBI. The purpose of this research is to demonstrate, in a rodent model, that NPLT is a promising pre-treatment for military personnel at high risk of combat-related TBI and a promising treatment after combat-related TBI, with the goal of limiting onset and progression of neuropathology and cognitive impairment.

2. KEYWORDS:

Traumatic Brain Injury; Blast injury; Non-invasive Therapy; Nano-pulsed Optoacoustic Laser Therapy; Neuroprotection

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- **Goal 1**- Preparing protocol of animal use for ACURO review and approval.
- **Goal 2:** Testing preventative NPLT efficacy for bTBI **(Aim 1)**
  - **Major Task 1:** NPLT treatment and blast TBI on 96 rats **(100% completed)**
  - **Major Task 2:** Behavioral assessments **(100% completed)**
  - **Major Task 3:** Biochemical and histological analyses **(30% completed)**
- **Goal 3:** Testing NPLT efficacy for repetitive bTBI **(Aim 2)**
  - **Major Task 4:** NPLT treatment and blast TBI on 48 rats **100% completed)**
  - **Major Task 5:** Behavioral assessments **(100% completed)**
  - **Major Task 6:** Biochemical and histological analyses **30% completed)**

What was accomplished under these goals?

Major activities related to Goal 2/ Major Task 1: NPLT treatment and blast TBI

Adult (2 months old) male, Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were randomized to receive NPLT or no treatment and further randomized to be subjected to blast TBI (bTBI) or Sham Injury (N=12 rats/experimental group)

NPLT treatment. NPLT was delivered transcranially to the shaved rat head through a 3 mm diameter, specially developed, fiber-optic bundle system positioned directly on the head and held in place using a

stereotaxic holder (Figure 1). To treat the entire brain, NPLT was delivered transcranially to two sites (one on each hemisphere, 5 minutes per site).

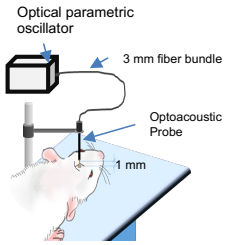


Figure 1. Nano-pulsed laser therapy (NPLT) will be delivered directly to shaved head under mild anesthesia. The optical parametric oscillator delivers the pulsed laser through a 3 mm fiber bundle attached to an optoacoustic probe that is placed noninvasively on top of the rat's head.

Blast TBI models.

Diffuse bTBI - In one set of experiments, blast TBI (bTBI) was produced by an Advanced Blast Simulator (ABS), a shock tube designed to circumvent some of the problems associated with experimental blast research. Specifically, the ABS is equipped with a reflected wave suppressor that prevents reflection of pressure waves (produced when the primary blast wave interacts with either the closed or the open end of the tube back) into the specimen chamber. Moreover, the divergent area driver chamber and expansion section of the ABS are designed to produce a shock waveform that closely reproduce blast injury in combatants (Figure 2).

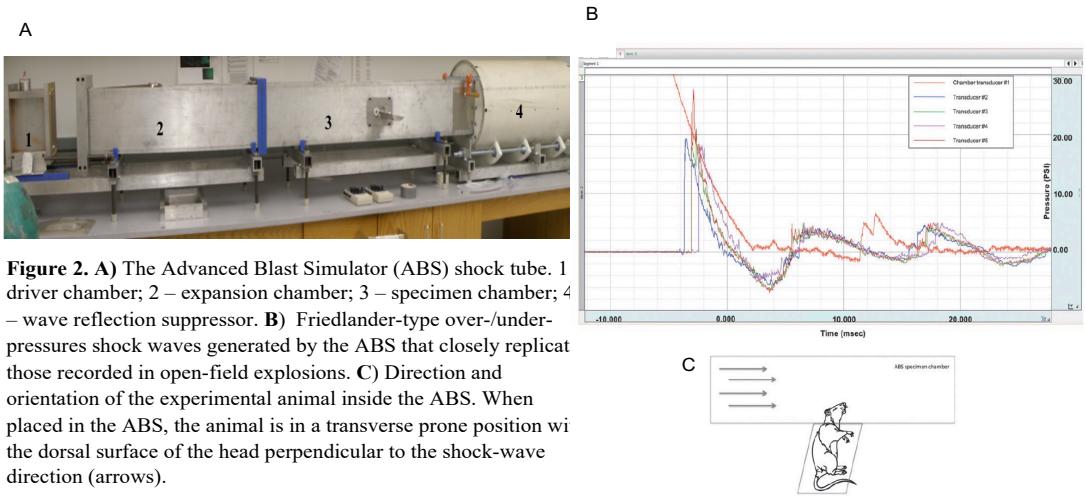


Figure 2. A) The Advanced Blast Simulator (ABS) shock tube. 1 driver chamber; 2 – expansion chamber; 3 – specimen chamber; 4 – wave reflection suppressor. **B)** Friedlander-type over-/under-pressures shock waves generated by the ABS that closely replicat those recorded in open-field explosions. **C)** Direction and orientation of the experimental animal inside the ABS. When placed in the ABS, the animal is in a transverse prone position wi the dorsal surface of the head perpendicular to the shock-wave direction (arrows).

Rats were anesthetized (4% isoflurane), intubated and ventilated (2.0% isoflurane) in O_2/room air (80:20) using a volume ventilator (Small Animal Ventilator, Harvard Apparatus, Inc., Holliston, MA). Core body temperature was monitored using a rectal thermometer (Thermalert Monitoring Thermometer, Physitemp Instruments, Inc., Clifton, NJ) and maintained within normal limits using a thermostatically controlled water blanket (Mul-T-Pad Temperature Therapy Pad, Gaymar Industries, Inc., Orchard Park, NY). After intubation, the scalp was shaved, foam plugs placed in each ear, and the animal was secured on the specimen tray with Velcro straps in a transverse prone position with the head supported at right angles to the direction of the shockwave by a leather sling suspended between two supports. When the specimen tray is placed in the ABS, only the rat's head is exposed to the shockwave (Figure 4C). After the rat was secured to the specimen tray, the isoflurane was temporarily discontinued, the ventilator hoses were detached but the rat remained intubated, the specimen tray was locked into the ABS and, at the return of a withdrawal reflex to paw pinch, the rat was subjected to bTBI (20-24 psi, 110-160 kPa) or sham injury. After injury, the animal was removed from the ABS, the duration of suppression of the righting reflex was recorded, the animal was reconnected to the ventilator, and anesthesia with isoflurane resumed. For all sham animals, the preparatory procedure previously stated was followed, but the rat was not subjected to bTBI.

Focal bTBI - In another set of experiments, bTBI was produced using a custom-made Vandenberg device using nail gun cartridges inserted into a detachable barrel. To prevent accidental activation, the device only fires when an operator simultaneously presses two switches, which requires both hands. A solenoid drives a metal bar to strike the firing pin against the cartridge. Ramset/Remington nail gun cartridges of 0.27 caliber with power level 4, where used for these studies. Under these conditions, the Vandenberg blast device produces a combined blast over/under pressure that is followed by a blunt impact caused by the venting gas jet. The dorsal surface of the head was shaved, and the rat was moved onto a 5 cm thick foam pad to minimize tertiary blast injuries. Using high-speed video recordings, we had previously confirmed that the force of the blast presses the rat into the

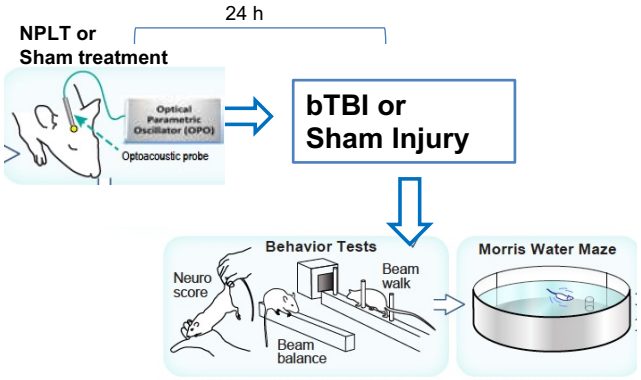
foam pad. To block both debris (e.g., unburned powder) and heat from reaching the animal, a 1.5 mm thick silicone rubber pad was placed on the head. Earplugs were inserted to protect the eardrums. The rat was positioned under the Vandenberg device, with the opening of the barrel 15 mm above the protective pad and directly over the right hemisphere of the brain. Isoflurane was discontinued and paw pinches were tested repeatedly (once per sec) until a withdrawal response was detected, at which point the blank cartridge was fired. For sham injury, the rats were positioned under the blast device, but the blank cartridge was not fired. Immediately after the firing of the blank cartridge, rats were removed from the blast device, placed in supine position, and monitored until they recovered the righting reflex. The time to recover the righting reflex was recorded.

Accomplishments related to Goal 2/Major Task 1: Animal work- treatments (NPLT or Sham), injuries (bTBI or Sham injury)- as detailed above, was completed on 96 rats (48 rats for the ABS studies and 48 rats for the Vandenberg device studies).

Major activities related to Goal 2/ Major Task 2: Behavioral assessments

Rats were acclimated to handling for five days and pre-trained to neurological, balance, and motor coordination tests for two days prior to receiving bTBI or sham injury. All behavioral measures were conducted by an observer blinded to the experimental groups. Gross vestibulomotor function and fine motor coordination were assessed on post-injury days (PIDs) 1 – 5 using a short neurological assessment, beam-balance and beam-walk tasks and cognitive function was assessed on PIDs 13 – 17 using a working memory version of the water maze (Figure 3).

Figure 3. Behavioral Assessments



Neuroscore. The following reflex tests were administered in order and repeated three times. A normal response received a score of 0 while an abnormal response received a score of 1 for each trial of each test for a total possible score of (7 x 3 = 21), the higher the score, the greater the deficit.

1. Forelimb Flexion Test (0-1)
2. Hind Limb Flexion Test (0-1)
3. Visually Triggered Placing Test (0-1)
4. Contact Triggered Placing Test, right (0 -1)
5. Contact Triggered Placing Test, left (0 -1)
6. Hind Paw Grasping Reflex Test, right (0-1)
7. Hind Paw Grasping Reflex Test, left (0-1)

Beam balance. Rats underwent one training session 24h before and one pre-assessment test on the day of the blast injury or sham procedure. The rats were trained to balance for 60 s on a short wooden beam (50 x 1.5 x 4 cm) raised 90 cm off the floor. Once the rats were able to remain on the beam, they were evaluated for three consecutive trials per session and rated using a six-point scale:

1. Balances with steady posture (grooms, climbs barrier)
2. Balances with unsteady posture (grasps sides of beam and/or has shaky movements)
3. Hugs the beam or slips or spins on the beam
4. Attempts to balance but falls off after 10 seconds.
5. Drapes over or hangs from the beam, falls off in less than 10 seconds
6. Falls off, making no attempt to balance or hang onto the beam

Beam walk. Animals were trained to traverse a wooden beam (100 x 2.5 x 4.0 cm) elevated 1 m above the floor. Four steel pegs were spaced at equal distances along the top and a darkened goal box was positioned at the far end of the beam. Once trained, the rats were timed during three consecutive trials, with time to reach the goal box as the primary endpoint. On the day of injury rats underwent a pre-injury assessment.

Working memory version of the Morris water maze test. Rats were placed in a tank filled with water to a level that was 2 cm higher than the hidden platform. Rats were assigned four starting points and four platform locations in a balanced order to avoid starting points too close to the platform. For Trial 1, rats were placed in the tank and allowed 120 s to find the platform. Once on the platform, the rats were allowed 15 s to rest and then were placed in the tank again from the same starting point to begin Trial 2. They were again allowed 120 s to find the platform. Rats were rested 4 min in a heated enclosure before starting a second pair of trials which used different platform and starting locations. Rats received four pairs of trials daily for five consecutive days. All rats received the same sequence of starting points and platform locations.

Accomplishments related to Goal 2/Major Task 2

In rats subjected to **diffuse bTBI** using the ABS device, we found no significant impairments in vestibulomotor function and fine motor coordination on PIDs 1-5 in any of the experimental groups (data not shown). We found a significant impairment in the water maze performance at PIDs 13 and 17 in the rats exposed to bTBI that was prevented by pretreatment with NPLT (Figure 4).

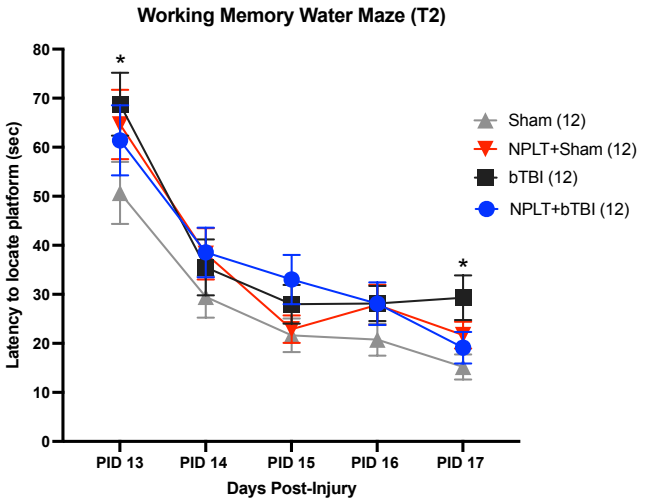


Figure 4. *Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days (F=35.28; p=9.3E-23), was detected, and a significant effect of treatment (F=3.48; p=0.017). Post-hoc comparisons reveal a significant difference between SHAM and TBI on Days 13 & 17 (*p<0.05).*

In rats subjected to **focal bTBI** using the Vandenberg device, we found a significant impairment in vestibulomotor function at PIDs 1 and 2 using the beam-balance test and on PID 1 on the beam-walk test. In both cases, NPLT pretreatment prevented bTBI-induced impairments (Figure 5). On the other hand, we did not detect significant impairments in fine motor coordination on PIDs 1-5 in any of the experimental groups (data not shown).

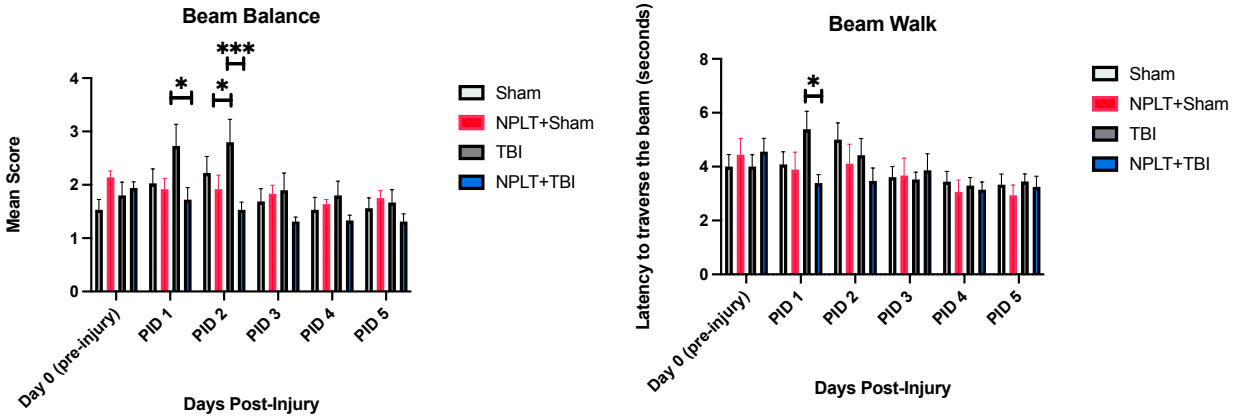


Figure 5. *Comparison between the four groups was performed using a 2-way ANOVA. Multiple comparison post-hoc analysis revealed significant differences between bTBI and NPLT+bTBI on PIDs 1 and 2 in the Beam Balance test and on PID 1 in the Beam Walk test. A significant difference between NPLT+Sham and bTBI was detected on PID2 in the Beam Balance test. *p<0.05; ***p<0.001.*

In the MWM test for cognitive function, we found no significance differences between any of the experimental groups (Figure 6).

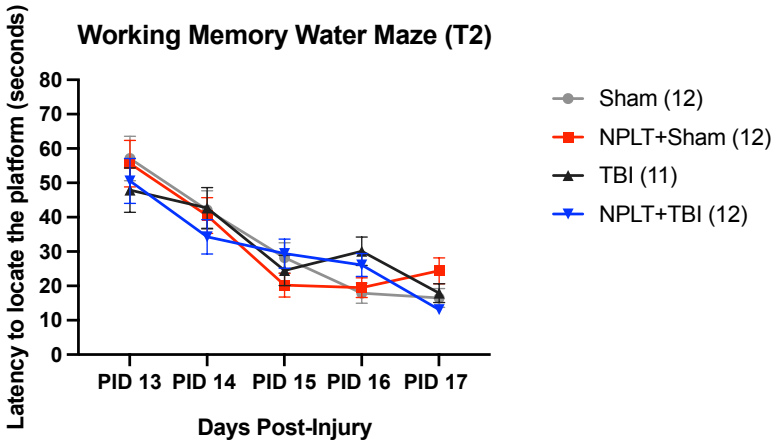


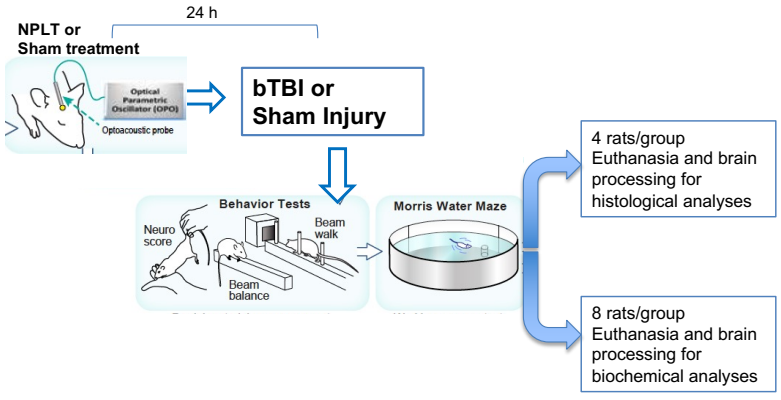
Figure 6. *Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days ($F=37.23$; $p<0.0001$), was detected, but not a significant effect of treatment ($F=1.1694$; $p=0.9170$).*

Major activity related to Goal 2/ Major Task 3: Biochemical and histological analyses

Rats were euthanized at the end of the behavioral assessment (PID 17). For immunohistological and immunofluorescence analysis, rats were anesthetized and perfused with saline followed by freshly prepared phosphate-buffered formaldehyde solution (pH 7.4). The brains were dissected and post-fixed in formaldehyde for 12–16 hours at room temperature, transferred to a phosphate buffered solution (PBS) and shipped to NeuroScience Associates (Knowxville, TN) for tissue processing and immunohistological staining using a patented Multibrain® technology. Briefly, 16 rat brains were embedded in one single block, sectioned on a microtome at 40 µm thickness in the coronal plane and collected every 480 µm throughout the entire cerebrum. The sections were adhered to glass slides and processed for amino cupric staining to reveal neurodegeneration and for immunohistochemistry staining of microglia markers (Iba1, for total microglia and CD68 for activated microglia). Slides were imaged with a BZ-X710 microscope (Keyence America, Itasca, IL) supported by the BZ-X analyzer software (Keyence America, Itasca, IL). Quantitative analyses were performed by an investigator who was blinded to the experimental groups using Image-J software.

For biochemical analyses, rats were euthanized using 2-3% isoflurane followed by decapitation and the brains immediately dissected out, frozen on dry ice and stored at -80 °C until further processing. (Figure 7).

Figure 7



Accomplishments related to Goal 2/ Major Task 3: Biochemical and histological analyses.

In the diffuse bTBI studies, using the ABS device, histological analyses showed absence of microglia activation, as measured by CD68 staining, in the brain of the rats from all experimental groups (data not shown). Quantification of Iba1 staining (a marker of microglia) showed some significant changes in the cortex and hippocampus (Figure 8).

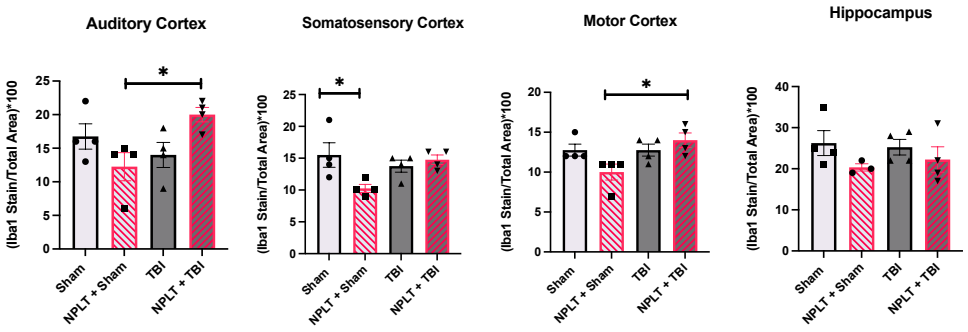


Figure 8. *Significanlty more total Iba1 staining was observed in the auditory cortex and motor cortex of NPLT+TBI rats as compared to NPLT+SHAM rats and in the somatosensory cortex of NPLT+SHAM rats as compared to SHAM rats. *p<0.05 one-way ANOVA with Tukey's muliplte comparison test. Data is mean+/-SEM*

Quantitative real time PCR was performed to analyze the expression of mRNA encoding for IL1β (an inflammatory cytokine). Our results show that IL1β expression was not significantly different between the experimental groups (Figure 9).

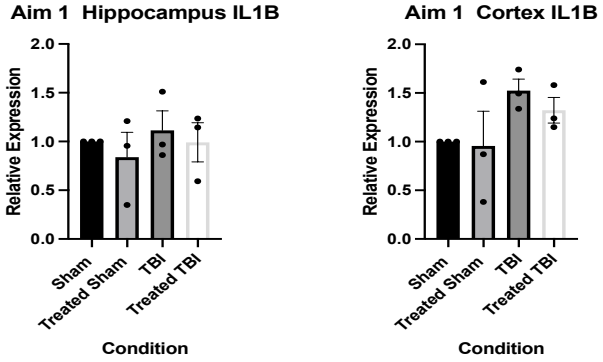


Figure 9. *The expression of IL1β mRNA was measured by qRT-PCR analysis. Data was normalized to GAPDH and relative to SHAM as mean +/- SEM. Comparisons among the groups was performed using ANOVA followed by Tukey's multiple comparisons test. Data is Mean +/-SEM.*

Major activity related to Goal 3/ Major Task 4: NPLT treatment and blast TBI

Adult (2-month-old) male Sprague-Dawley rats were randomized to receive NPLT or no treatment and further randomized to receive two consecutive blast TBIs (48 hours apart) or sham-injury. NPLT treatments and bTBI were performed as described above in Goal 2/Major Task 1.

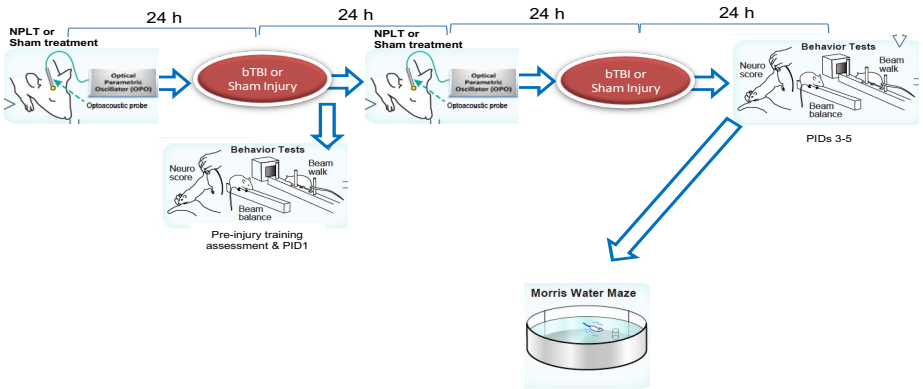
Accomplishments related to Goal 3/ Major Task 4: NPLT treatment and blast TBI

Animal work- treatments (NPLT or Sham), injuries (bTBI or Sham injury)- as detailed above was completed on 48 rats using the ABS device.

Major activity related to Goal 3/Major Task 5: Behavioral assessments

Behavioral assessment of neurological and cognitive function was performed as described above in Goal2/Major Task 2 (Figure 10).

Figure 10. Goal 3/Major Task 5



Major accomplishments related to Goal 3/Task 5: Behavioral assessments

In rats subjected to **diffuse bTBI** using the ABS device, we found no significant impairments in vestibulomotor and fine motor coordination on PIDs 1-5 in any of the experimental groups (data not shown). We found no significant impairment in the water maze performance at PIDs 13 and 17 (Figure 11).

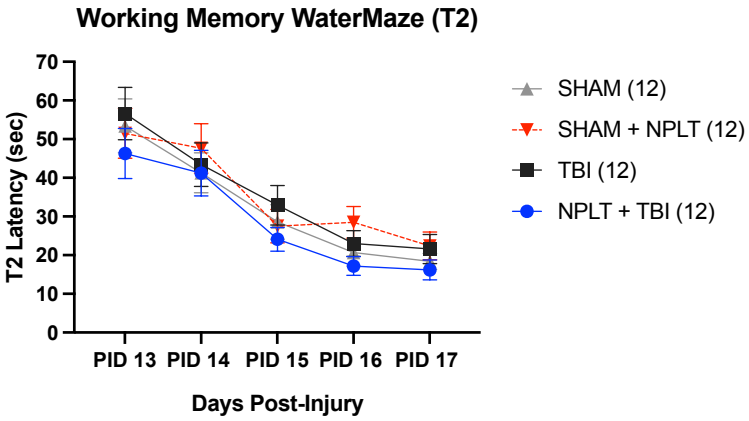
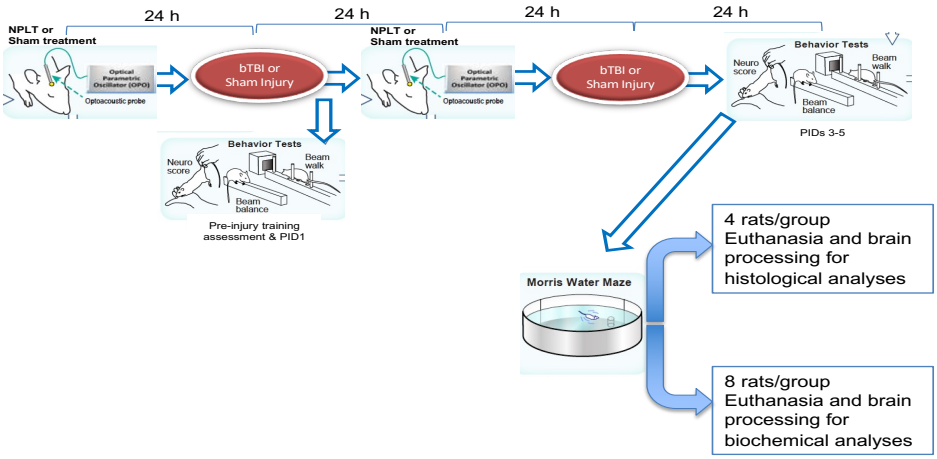


Figure 11. *Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days ($F=33.24$; $p<0.0001$), was detected, but not a significant effect of treatment ($F=2.037$; $p=0.1096$). Data is Mean+/-SEM*

Major activity related to Goal 3/Task 6: Biochemical and histological analyses

At the end of the water maze test rats were anesthetized with isoflurane and perfused with saline followed by freshly prepared phosphate-buffered formaldehyde solution (pH 7.4). The brains were dissected and post-fixed in formaldehyde for 12-16 hours at room temperature, transferred to a phosphate buffered solution (PBS) and stored at 4 °C until ready for processing for histological staining (Figure 12).

Figure 12. Goal 3/Major Task 6



Major accomplishments related to Goal 3/Task 6: Biochemical and histological analyses

In the diffuse bTBI studies, using the ABS device, histological analyses showed increased staining for the microglia marker Iba1 after bTBI in the hippocampus, cortex and thalamus that was prevented by NPLT (Figure 13).

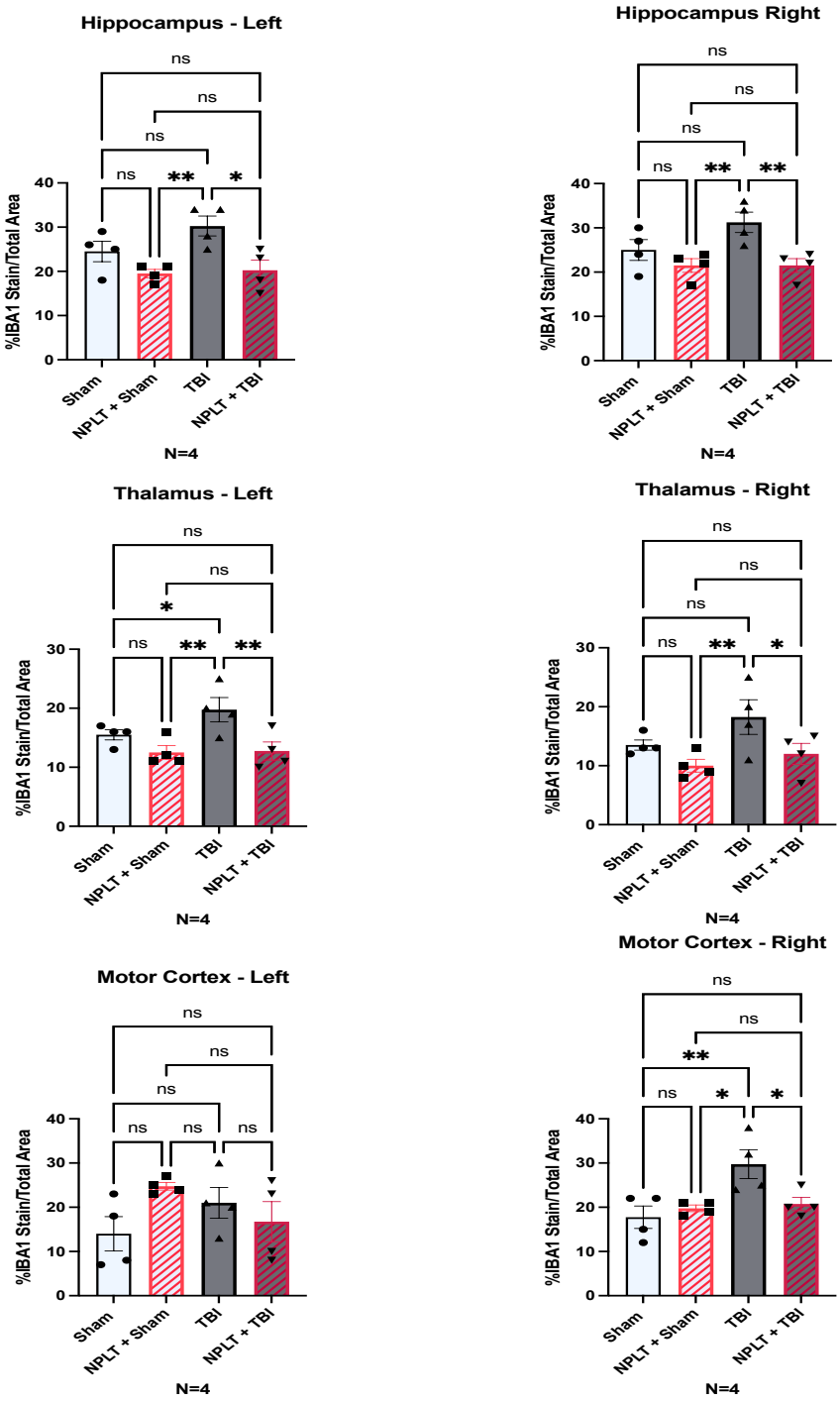


Figure 13. *Significantly more total Iba1 staining was observed in the brain of bTBI rats as compared to NPLT+bTBI and NPLT+Sham in hippocampus, thalamus, and right motor cortex. Significant differences in Iba1 were observed between bTBI and Sham rats in the left thalamus. *p<0.05; **p<0.01 two-way ANOVA with Fisher post-hoc test. Data is mean+/- SEM*

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

During the next annual reporting period, we plan to:

- Complete quantitative analyses of histopathological staining related to Aim 1 – Goal 2/Task 3
- Complete biochemical analyses for Aim1 – Goal 2/Task3
- Perform animal work- treatments (NPLT or Sham), injuries (bTBI or Sham injury) – using the Vandenberg device for focal bTBI as detailed in Aim 2 – Goal 2/Task 4
- Perform behavioral testing and statistical analyses as detailed in Aim 2 – Goal 3/Task 4
- Perform histopathological staining and analyses as detailed in Aim 2 – Goal 3/Task 5
- Perform biochemical analyses as detailed in Aim 2 – Goal 3/Task 6

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

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

Not Applicable

Significant changes in use or care of vertebrate animals

A request for major amendment to the animal protocol for the addition of 96 rats was submitted and approved by ACURO on 6/08/2021.

Significant changes in use of biohazards and/or select agents

Not Applicable

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

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

Nothing to Report

Other publications, conference papers and presentations.

The following abstract was submitted and accepted for poster presentation at the 38th Annual National Neurotrauma Symposium July 11-14, 2021 Virtual Conference:

- N. Gupta, K.M. Johnson, I. Petrov, Y. Petrov, R. Esenaliev, S.L. Sell, D.S. DeWitt, D.S. Prough, MA Micci Nano-Pulsed Laser Therapy Prevents Working Memory Dysfunction in Rats Subjected to Blast-Induced Neurotrauma. *Journal of Neurotrauma,* Vol. 38, No. 14, published online Jul 2021

- **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: *Maria Micci*
Project Role: *Principal Investigator*
ORCID ID: 0000-0001-6689-2994
Nearest person month worked: *2.4*
Contribution to Project: Dr. Micci has planned, coordinated and directed the experimental work. She has prepared the technical report and communicated with the DoD.

Name: *Stacy L. Sell*
Project Role: *Co-Investigator*
UTMB ID: *047383*
Nearest person month worked: *2.85*
Contribution to Project: Dr. Sell has performed work related to the behavioral studies of the project.

Name: *Helen Hellmich*
Project Role: *Co-Investigator*
UTMB ID: *097512*
Nearest person month worked: *2.85*
Contribution to Project: Dr. Hellmich has performed work related to the molecular studies of the project.

Name: *Rinat Esenaliev*
Project Role: *Co-Investigator*
UTMB ID: *059206*
Nearest person month worked: *0.6*
Contribution to Project: Dr. Esenaliev has worked in the area of NPLT treatment oversight.

Name: *Irene Petrov*
Project Role: *Co-Investigator*
UTMB ID: *162553*
Nearest person month worked: *0.6*
Contribution to Project: Dr. I. Petrov has worked in the area of NPLT treatment administration
Funding Support:

Name: *Yuriy Petrov*
Project Role: *Co-Investigator*
UTMB ID: *160886*
Nearest person month worked: *1.2*
Contribution to Project: Dr. Y. Petrov has worked in the area of NPLT treatment administration.

Name: *Nikita Gupta*
Project Role: *Graduate Student*
UTMB ID: *254221*
Nearest person month worked: *12*
Contribution to Project: Ms. Gupta has performed work in the area of animal handling, bTBI/sham injury administration and behavioral testing.

Name: *Kathia Johnson*
Project Role: *Research Associate*
UTMB ID: *136690*
Nearest person month worked: *6*
Contribution to Project: Ms Johnson performed work in the areas of animal ordering, animal handling, behavioral studies, euthanasia and tissue collection and storage.

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

One active grant (R56 AG063405) for Dr. Micci has closed. See appendices for current other support for Dr. Micci. This change has not impacted Dr. Micci's effort on this project.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS:

Nano-pulsed Laser Optoacoustic Therapy for Pre-treatment and Post-treatment of Traumatic Brain Injury

DM180663; Year 2/Annual Report

W81XWH-19-1-0522

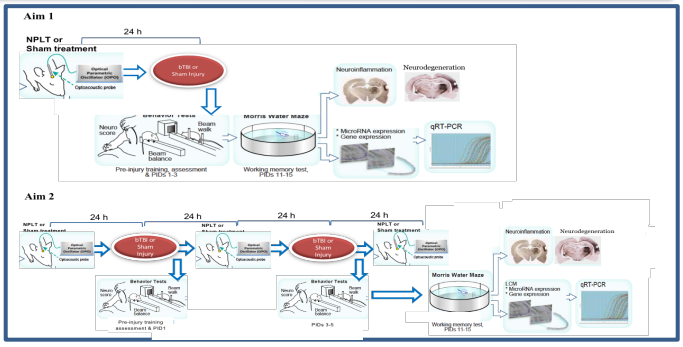
PI: Maria-Adelaide Micci **Org:** University of Texas Medical Branch, Galveston **Award Amount:** $552,721.00 ($350,000 direct cost)

Study Aims

- **Aim 1** will test whether NPLT can be used as preventive treatment before experimental TBI to reduce/delay neuropathology and cognitive impairment.
- **Aim 2** will test whether NPLT after each of multiple experimental TBIs will effectively mitigate neuropathology and cognitive impairments.

Approach

These aims will be accomplished by using an established rat model of blast TBI (bTBI). NPLT will be delivered to the intact rat head before bTBI (Aim 1) and after each bTBI in a repetitive injury paradigm (Aim 2). Two weeks after the last bTBI, neurocognitive outcome will be assessed, and rats will be euthanized for assessment of neuroinflammation and neurodegeneration in comparison to un-injured rats and sham-treated bTBI rats using ANOVA. We will focus our analyses on the cortex (frontal and parietal) and hippocampus, areas critically involved in learning, memory and executive functions, and among the earliest and most affected brain areas in TBI.



Accomplishment – During this reporting period we have performed animal work, behavioral assessments, histopathological and biochemical analyses as detailed in Aims 1 and 2 of the proposal.

Timeline and Cost

Activities CY	19	20	21
ACURO Approval			
Aim 1-NPLT treatment and bTBI			
Aim 1- behavioral tests/biochemical and histological analyses			
Aim 2-NPLT treatment and bTBI			
Aim 2- behavioral tests/biochemical and histological analyses			
Estimated Budget ($K)	**$53,500**	**$196,500**	**$100,000**

Updated: October 28, 2021

Goals/Milestones

CY19 Goal – Preparing protocol of animal use for ACURO review

☑ ACURO approval

☑ ACURO approval of amendment

CY20 Goals – Testing preventative NPLT efficacy for bTBI (Aim 1)

☑ Complete NPLT treatment and blast TBI on 96 rats

☑ Complete behavioral assessments on 96 rats

– Testing NPLT efficacy for repetitive bTBI (Aim 2)

☑ Complete repetitive blast TBIs and NPLT treatments on 48 rats

☑ Complete behavioral assessments on 48 rats

CY21 Goals – Testing preventative NPLT efficacy for bTBI (Aim 1)

☑ Complete histological staining

☐ Complete quantitative analysis of histological staining

☐ Complete biochemical analyses

– Testing NPLT efficacy for repetitive bTBI (Aim 2)

☑ Complete histological staining

☐ Complete quantitative analysis of histological staining

☐ Complete biochemical analyses

Comments/Challenges/Issues/Concerns- Nothing to Report.

Budget Expenditure to Date

Projected Expenditure: $327,000; Actual Expenditure: $327,982.02

9. APPENDICES

Other Support

Micci, Maria

Current

W81XWH1910522 Micci (PI) 09/30/19-03/31/21 2.04 cal mths
Dept of Defense
"Nano-Pulsed Laser Optoacoustic Therapy for Pretreatment and Post-Treatment of Traumatic Brain Injury"
Goal: To demonstrate in a rodent model that a unique, non-invasive, nanopulsed laser optoacoustic therapy (NPLT) is a promising pre-treatment for military personnel at high risk of combat-related TBI and a promising treatment after combat-related TBI, with the goal of limiting onset and progression of neuropathology and cognitive impairment.
Aims: 1) To test whether NPLT can be used as a preventive treatment before experimental TBI to reduce/delay neuropathology and cognitive impairment; 2) To test whether NPLT after each of multiple experimental TBIs will effectively mitigate neuropathology and cognitive impairments.
Role: Principal Investigator
Contact: Kevin R. Moore, 820 Chandler St., Fort Detrick, MD 21702, 301-619-7101, kevin.r.moore88.civ@mail.mil
Overlap: None

R01 AG069433 Taglialatela (PI) 08/01/20-04/30/25 3.60 cal mths
National Institutes of Health
"Promoting Brain Resilience to Alzheimer's Neuropathology"
Goal: The overall objective in this application, is to evaluate the link between NSC (and their released exosomes) and increased synaptic resilience to the toxic actions of tau oligomers as a function of aging, the strongest AD risk factor.
Aims: To obtain the overall objective, we will pursue three specific aims that will evaluate the efficacy of NSC-exo in promoting synaptic resilience to Tau oligomers (Aim 1), determine the invoved miRNA cargoes and their impact on key synaptic proteins (Aim 2) and evaluate the impact of aging on such protective mechanisms as a function of decreasing numbers of resident NSC and their released exosomes (Aim 3).
Role: Principal Investigator
Contact: Bradley C. Wise; Email: wiseb@nia.nih.gov Phone: 301-496-9350
Overlap: None

Ended

R56 AG063405, ended 6/30/21