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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. 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 both single and repetitive bTBI, we found that NPLT prevented neuroinflammation and loss of myelin in the brain. Taken together our results suggest that NPLT might be an effective preventative treatment for combat soldiers at high risk of bTBI.

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

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TABLE OF CONTENTS

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

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

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

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.

- **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 **(100% 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 **100% completed)**

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 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).

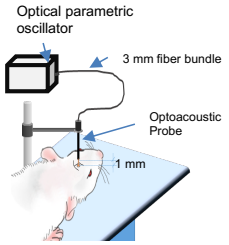


Figure 1. Nano-pulsed laser therapy (NPLT) will be delivered directly to shaved head under mild anesthesia. The optical parametric oscillator delivers the pulsed laser through a 3 mm fiber bundle attached to an optoacoustic probe that is placed noninvasively on top of the rat's head.

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).

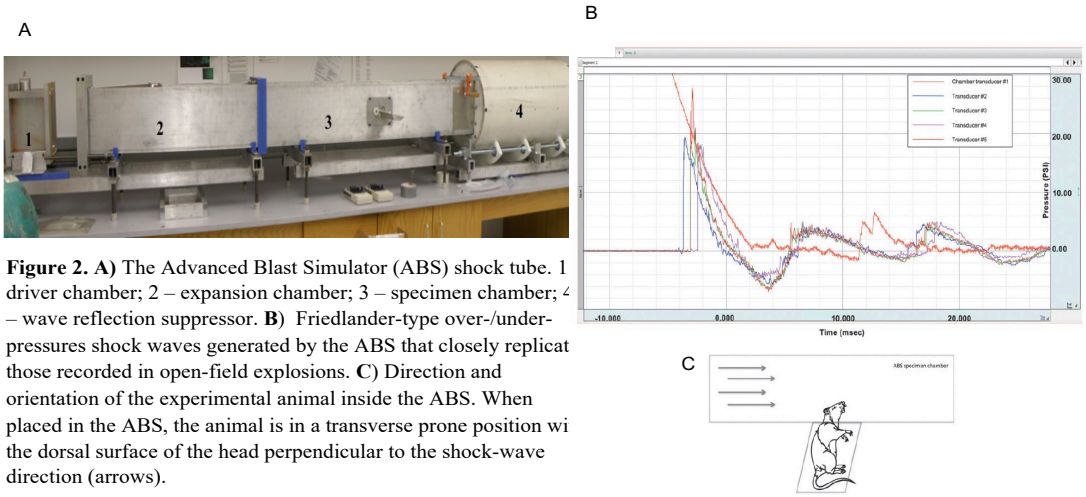


Figure 2. A) The Advanced Blast Simulator (ABS) shock tube. 1 driver chamber; 2 – expansion chamber; 3 – specimen chamber; 4 – wave reflection suppressor. **B**) Friedlander-type over-/under-pressures shock waves generated by the ABS that closely replicat those recorded in open-field explosions. **C**) 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

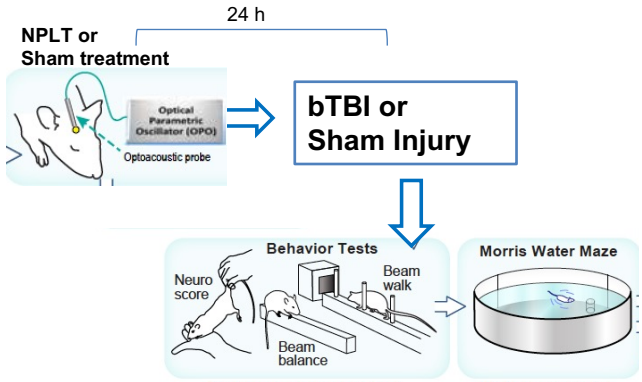
foam pad. To block both debris (e.g., unburned powder) and heat from reaching the animal, a 1.5 mm thick silicone rubber pad was placed on the head. Earplugs were inserted to protect the eardrums. The rat was positioned under the Vandenberg device, with the opening of the barrel 15 mm above the protective 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).

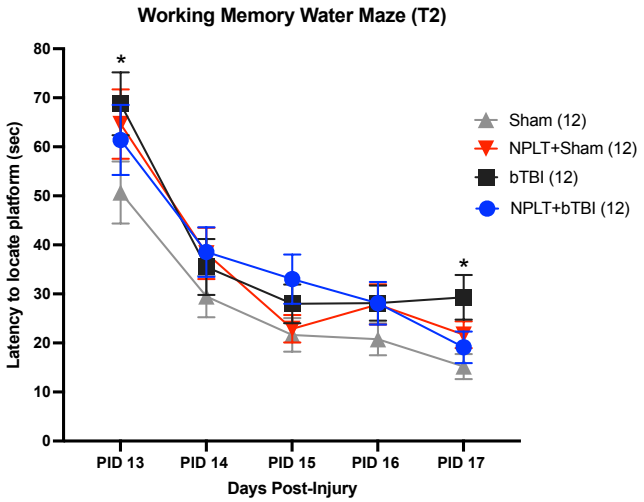


Figure 4. *Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days (F=35.28; p=9.3E-23), was detected, and a significant effect of treatment (F=3.48; p=0.017). Post-hoc comparisons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

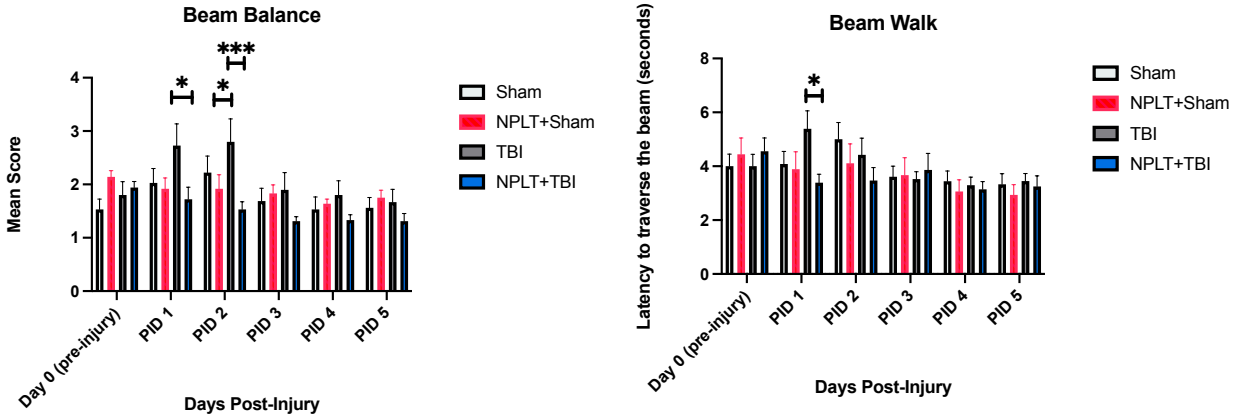


Figure 5. *Comparison between the four groups was performed using a 2-way ANOVA. Multiple comparison post-hoc analysis revealed significant differences between bTBI and NPLT+bTBI on PIDs 1 and 2 in the Beam Balance test and on PID 1 in the Beam Walk test. A significant difference between NPLT+Sham and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

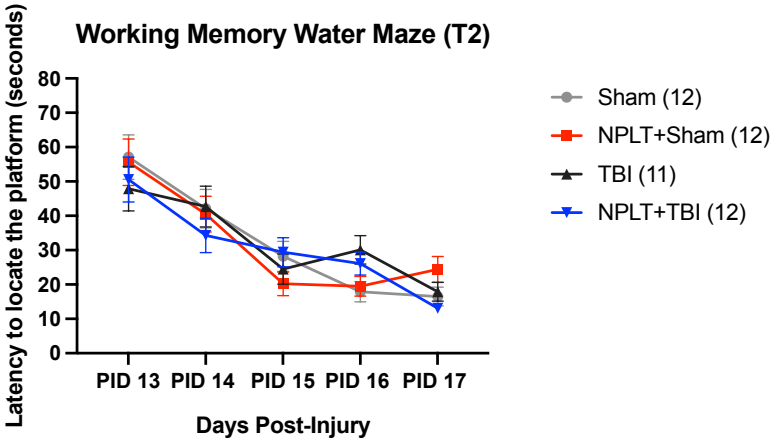


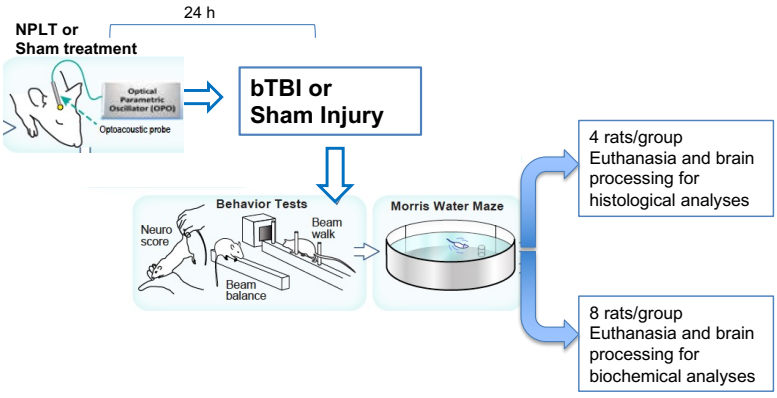
Figure 6. *Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days ($F=37.23$; $p<0.0001$), was detected, but not a significant effect of treatment ($F=1.1694$; $p=0.9170$).*

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 Solochrome staining of myelin. They were also processed for immunohistochemistry staining of microglia markers (Iba1, for total microglia, CD68 for activated microglia and GFAP for astrocytes). 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: *Analysis of histopathological changes in the brain.*

To determine the effect of bTBI and NPLT pre-treatment on neuroinflammation 17 days post a single Blast TBI we performed immunofluorescence analysis using a specific antibody against Iba1, a marker of microglia, and a specific antibody against GFAP, a marker for astrocytes. To assess myelination, we used a specific antibody against Solochrome Cyanine (Figure 8).

In the diffuse bTBI studies, using the ABS device, histological analyses showed a significant decrease of myelin in the corpus callosum of rats subjected to TBI as compared to Sham rats and TBI rats treated with NPLT (Figure 9).

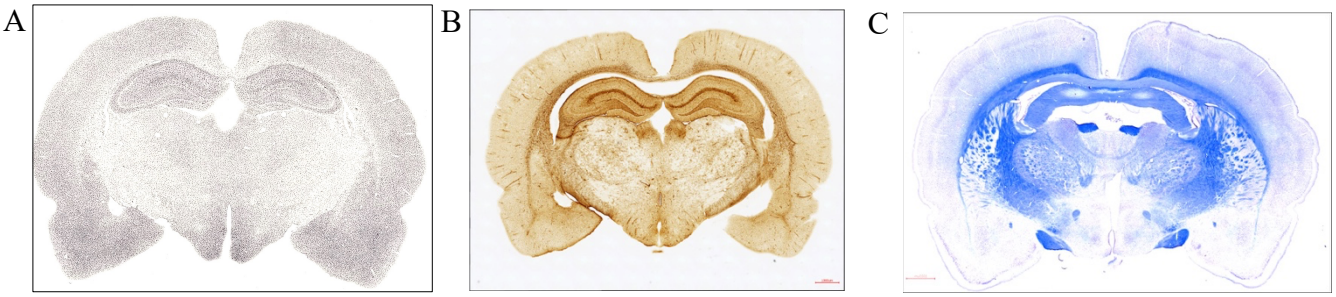


Figure 8. Representative images of Iba1 (A), GFAP (B) and Solochrome stain (C) for myelin in rat brain sections.

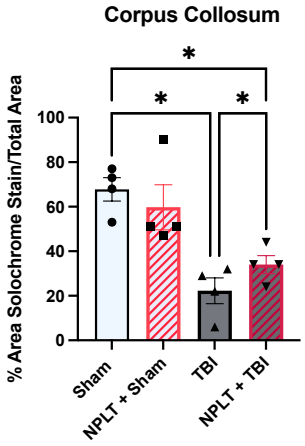


Figure 9. Quantification of Solochrome stain for myelin in rat brain sections. Area occupied by the stain was calculated and expressed as percentage of total corpus callosum area. Data is expressed as mean +/- SEM. Comparisons among the groups was performed using ANOVA followed by Fisher's test. *p<0.05

Quantitative analysis of immunohistological staining for microglia revealed decreased Iba1 staining in the NPLT+Sham group in the right Auditory Cortex (Figure 10), left and right Motor Cortex (Figure 11), left and right Somatosensory Cortex (Figure 12), right Corpus Collosum (Figure 13), right Hippocampus (Figure 14). Of these brain regions, a decrease in Iba1 cell count in the NPLT+Sham and TBI group was observed in the left and right Motor Cortex (Figure 11, Panel C, D, G, and H), left and right Somatosensory Cortex (Figure 12, Panel D and H), and right and left Corpus Collosum (Figure 13, Panel C, D, G, H). The left Corpus Collosum at bregma -4.44 reveals a decrease in the TBI and NPLT+TBI group (Panel B) when compared to the NPLT+Sham group, and a decrease in cell count in all groups on average and bregma -4.44 (Panel C, and D) when compared to Sham. Notably, an increase in cell surface area is observed in the Corpus Collosum in the TBI group when compared to the Sham group (p=.043). Lastly, a similar trend is observed in the left Thalamus at bregma -3.00 (Figure 15, Panel B), with a decrease in Iba1 staining in the NPLT+Sham group when comparted to the NPLT+TBI group. In the right thalamus, at bregma -3.00 there is a decrease in Iba1 cell count, but no other significant differences can be seen in the Thalamus.

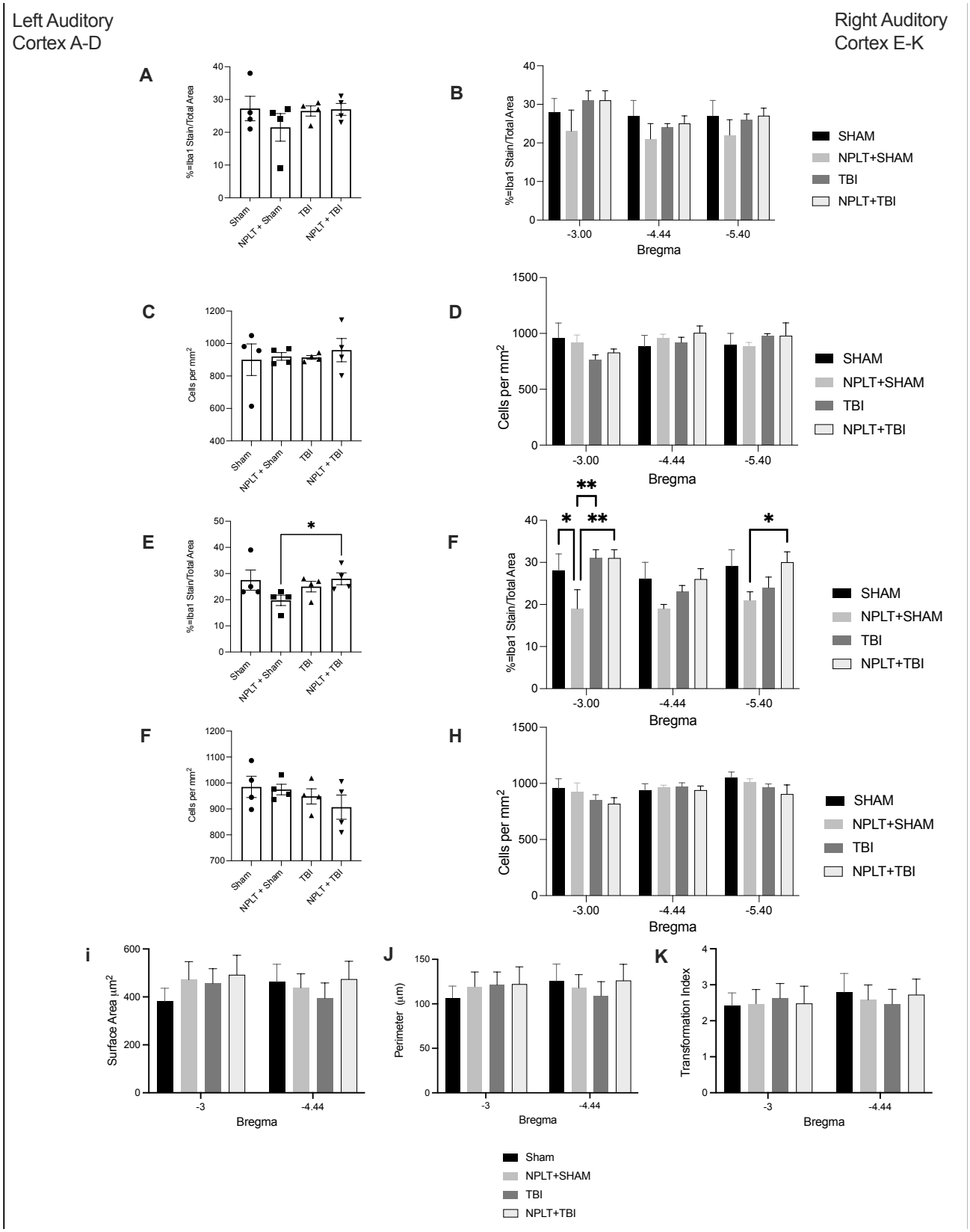


Figure 10: Blast TBI after NPLT increases Iba1 staining in the right auditory cortex when compared with NPLT only animals. Graphs a-b depicting Iba1 percent stain (a, b) and Iba1 cell count (c, d) in the left auditory cortex. Graphs e-h depicting Iba1 percent stain (e, f) and Iba1 cell count (g, h) in the right auditory cortex. **(a)** No changes observed in Iba1 percent stain average of all bregma in the left auditory cortex. **(b)** No changes observed in Iba1 percent stain in bregma - 3.0, -4.44, and -5.40. **(c)** No changes observed in Iba1 cell count average of all bregma **(c)** or **(d)** in Iba1 cell count amongst bregma -3.0, -4.44, and -5.40. **(e)** NPLT+Sham animals show a decrease in Iba1 percent stain compared to NPLT+TBI animals *(p=.048, one-way ANOVA).* **(f)** At bregma -3.00 and -5.40 NPLT+Sham have decreased Iba1 percent staining *(-3.00: Sham vs NPLT+SHAM p=.0359, NPLT+SHAM vs. TBI p=.006, NPLT+SHAM vs. NPLT+TBI p=.006), (-5.40 NPLT+SHAM vs. NPLT+TBI p=.035), two-way ANOVA)*, but no changes are observed in **(g, h)** in cellular count. Cell surface area **(i),** perimeter **(j),** and transformation index **(k)** values show no significant differences.

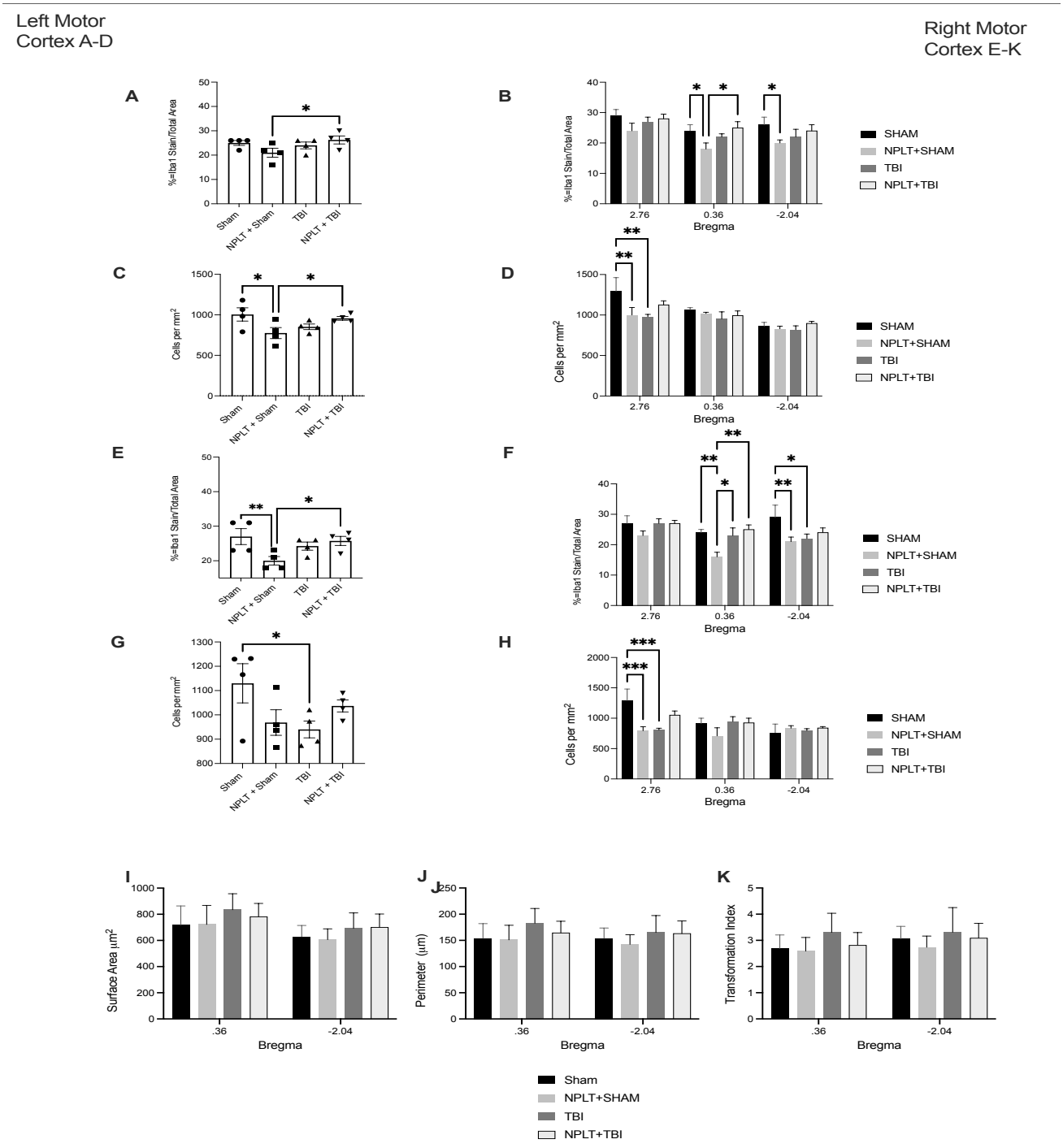


Figure 11: Motor Cortex: A decrease is observed in NPLT+SHAM and TBI animals in Iba1 cell count at bregma 2.76, but only NPLT+SHAM animals show a reduction in Iba1 percent stain at bregma .36. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (c, d) in the left motor cortex. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right motor cortex. (**A)** A significant difference between NPLT+SHAM and NPLT+TBI *(p=.030, one-way ANOVA)* is observed in Iba1 percent stain average of all bregma in the left motor cortex. **(B)** NPLT+SHAM shows a decrease at bregma .36 in Iba1 percent stain when compared to SHAM *(p=.035 two-way ANOVA)* and NPLT+TBI *(p=.0152, two-way ANOVA).* **(C)** A significant difference between NPLT+SHAM and sham *(p=.015, one-way ANOVA)*, NPLT+TBI *(p=.045, one-way ANOVA)* is observed in Iba1 cell count average of all bregma in the left motor cortex. **(D)** A decrease in Iba1 cell count is observed in the NPLT+SHAM *(p=.005, two-way ANOVA)* and TBI *(p=.003, two-ANOVA)* when compared to Sham a bregma .36. **(E)** In the right motor cortex, there is an average decrease of the NPLT+SHAM group when compared to the Sham *(p=.009, one-way ANOVA)* and NPLT+TBI *(p=.02, one-way ANOVA)*. **(F)** At bregma .36 a significant decrease is seen between NPLT+SHAM and SHAM *(p=.006, two-way ANOVA)* and NPLT+TBI *(p=.003, two-way ANOVA).* At bregma -2.04 there is a decrease when compared to the SHAM group in the NPLT+SHAM *(p=.007, two-way ANOVA)* and TBI *(p= .016, two-way ANOVA).* **(G)** A significant decrease is observed in average cell count in the TBI group when compared to the Sham group *(p=.025, one-way ANOVA).* **(H)** At bregma 2.76, a significant decrease in IBA1 cell count is observed between Sham and NPLT+SHAM *(p=.0006, two-ANOVA)* and TBI *(p=.0008, two-way ANOVA)*. No significant differences were seen in cell surface area **(I)**, cell perimeter **(J),** and transformation index **(K)** at bregma .36 and -2.04 in the right motor cortex.

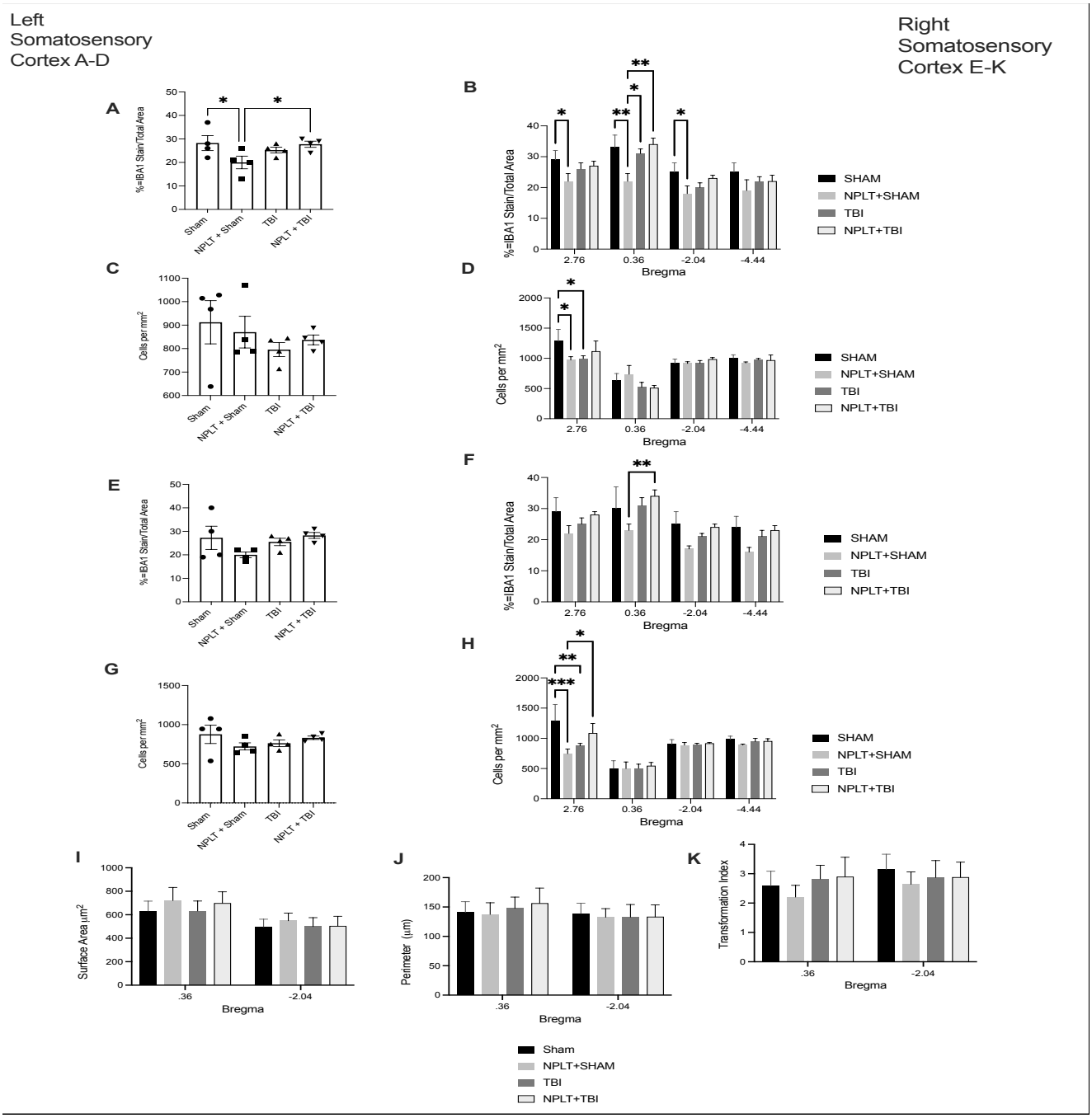


Figure 12: Single application of NPLT decreases IBA1 percent stain and cell count, however TBI and NPLT+TBI groups do not significantly differ in Iba1 percent stain in the somatosensory cortex. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (c, d) in the left somatosensory cortex. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right somatosensory cortex. **(A)** A decrease in Iba1 percent stain on average amongst bregma are observed in NPLT+Sham when compared to Sham *(p=.024, one-way ANOVA)* and NPLT+TBI *(p=.032, one-way ANOVA)* in the left somatosensory cortex. **(B)** At bregma 2.76 in the left somatosensory cortex there is a decrease in Iba1 percent stain in the NPLT+Sham group when compared to the sham animals *(p=.049, two-way ANOVA)*. At bregma .36 a significant decrease is seen between NPLT+SHAM and SHAM *(p=.002, two-way ANOVA)*, NPLT+*TBI (p=.001, two-way ANOVA),* and TBI *(p= .012, two-way ANOVA).* At bregma -2.04 there is a decrease in NPLT+SHAM *(p=.048 two-way ANOVA)* when compared to Sham. **(C)** No significant differences were seen in the Iba1 cell count average of all bregma in the left somatosensory cortex. **(D)** Decreases in NPLT+SHAM *(p=.019, two-way ANOVA)* and TBI *(p=.029, two-way ANOVA)* when compared to SHAM in Iba1 cell count are seen at bregma 2.76 in the left somatosensory cortex. **(E)** In the right somatosensory cortex, no differences were seen in the average Iba1 stain percentage, but at bregma .36 **(F)** we can see a decrease in Iba1 stain percentage in NPLT+SHAM when compared to NPLT+TBI *(p= .01, two-way ANOVA).* No significant differences are observed in the average Iba1 cell count; however, a **(H)** decrease in cell count can be observed between SHAM and NPLT+SHAM *(p=.00003, two-way ANOVA)*, SHAM and TBI (p=.004, two-way ANOVA), and NPLT+SHAM and NPLT+TBI *(p=.0109, two-way ANOVA).* Although not significant, it is notable to see the increase in surface area **(I)** in the NPLT+SHAM group when compared to the SHAM and TBI groups. However, the lack of difference in perimeter between SHAM and SHAM+NPLT as well as the increase in the TBI and NPLT+TBI groups when compared to sham should be noted.

Left Corpus Collosum A-D

Right Corpus Collosum E-K

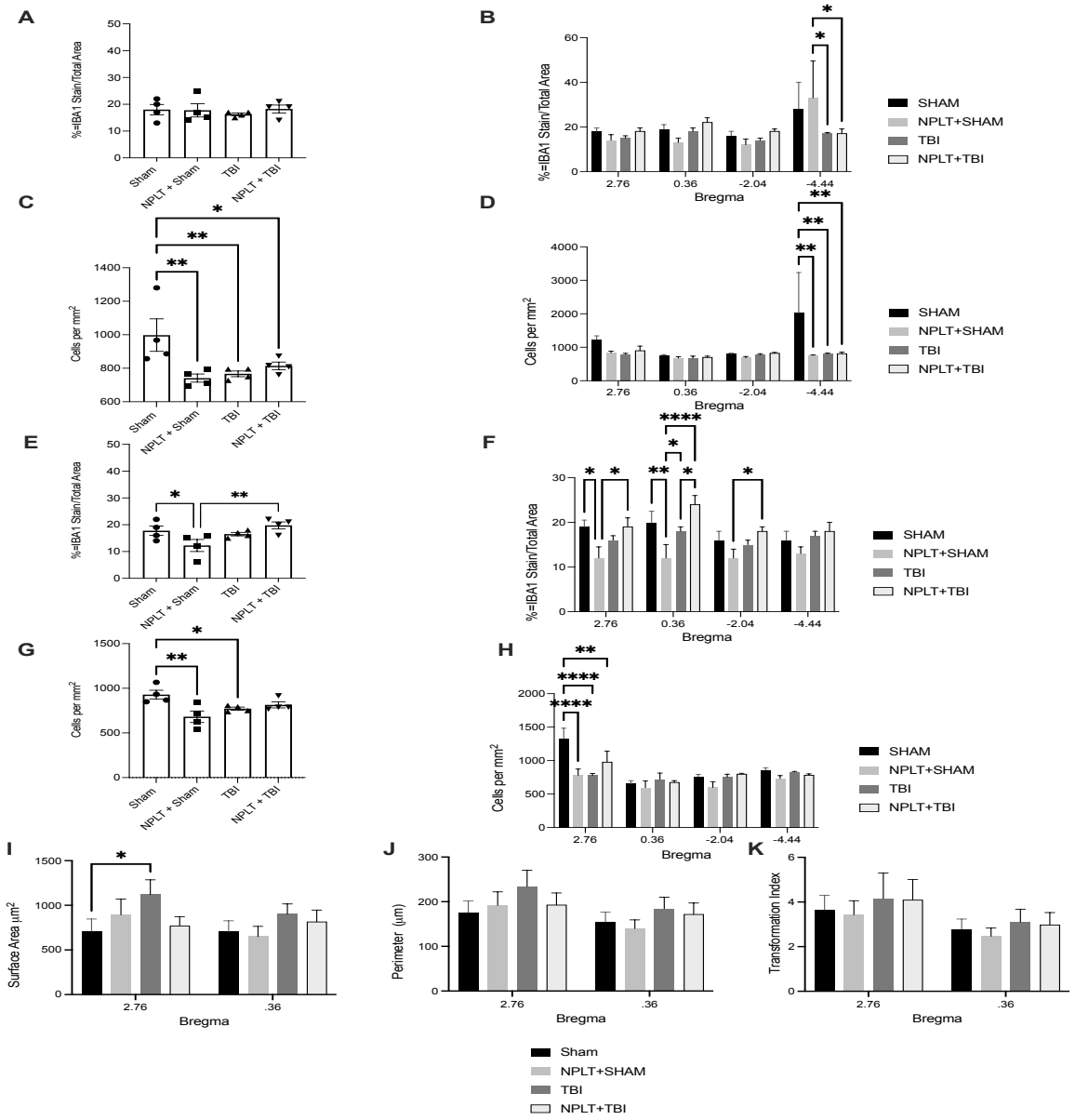


Figure 13: Single NPLT application reduces Iba1 expression and cell count in Sham animals in the corpus collosum. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left corpus collosum. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right corpus collosum. **(A)** No significant differences are observed in the average Iba1 percent stain, however **(B)** at bregma -4.44 *(p=.039, two-way ANOVA)* TBI and NPLT+TBI *(p=.039, two-way ANOVA)* have decreased Iba1 staining when compared to NPLT+TBI. The insignificant decrease in the NPLT+SHAM group and increase in the NPLT+TBI group at the other bregma levels should be noted. **(C)** On average, when compared to sham, NPLT+SHAM *(p=.004, one-way ANOVA), TBI (p= .008, one-way ANOVA),* and NPLT+TBI *(p=.02, one-way ANOVA)* show significantly decreased Iba1 cell count in the left corpus collosum. This is most likely driven by the same seen at bregma -4.44 **(D)** (Sham vs. NPLT+SHAM *(p=.005, two-way ANOVA*), SHAM vs. NPLT+TBI *(p=.007, two-way ANOVA)* SHAM vs. TBI *(p=.007, two-way ANOVA).* **(E)** There is a decrease in Iba1percent stain average in the right corpus collosum between NPLT+SHAM and Sham *(p=.031, one-way ANOVA)*, and NPLT+SHAM and NPLT+*TBI (p=.006, one-way ANOVA)*. **(F)** At bregma 2.6 there is a decrease in Iba1 percent stain in the NPLT+Sham group when compared to the Sham *(p=.01, two-way ANOVA)* and NPLT+TBI *(p=.01, two-way ANOVA).* At bregma .36 a significant decrease is seen between NPLT+SHAM and SHAM *(p=.003, two-way ANOVA)*, NPLT+TBI *(p=.0001, two-way ANOVA),* and TBI *(p=.026, two-way ANOVA)* as well as an increase in NPLT+TBI when compared to the TBI group *(p=.026, two-way ANOVA).* At bregma -2.04 there is a significant reduction in NPLT+Sham in Iba1 stain when compared to the NPLT+TBI *(p=.026, two-way ANOVA)* group. **(G)** On average we see a reduction in the NPLT+SHAM *(p=.002, one-way ANOVA)* and TBI *(p=.029, one-way ANOVA)* group when compared to the Sham group in the right corpus collosum. **(H)** At bregma 2.76 there are significant decreases in Iba1 stain between SHAM and NPLT+SHAM *(p=.0001, two-way ANOVA),* TBI *(p=.0001, two-way ANOVA),* and NPLT+TBI *(p=.002, two-way ANOVA)*. There is a significant increase in surface area in the right corpus collosum between Sham and TBI (p=.043, one-way ANOVA) **(I).** Although not significant there is also an increase in perimeter **(J)** and transformation index ration **(K)** in the TBI group in the right corpus collosum.

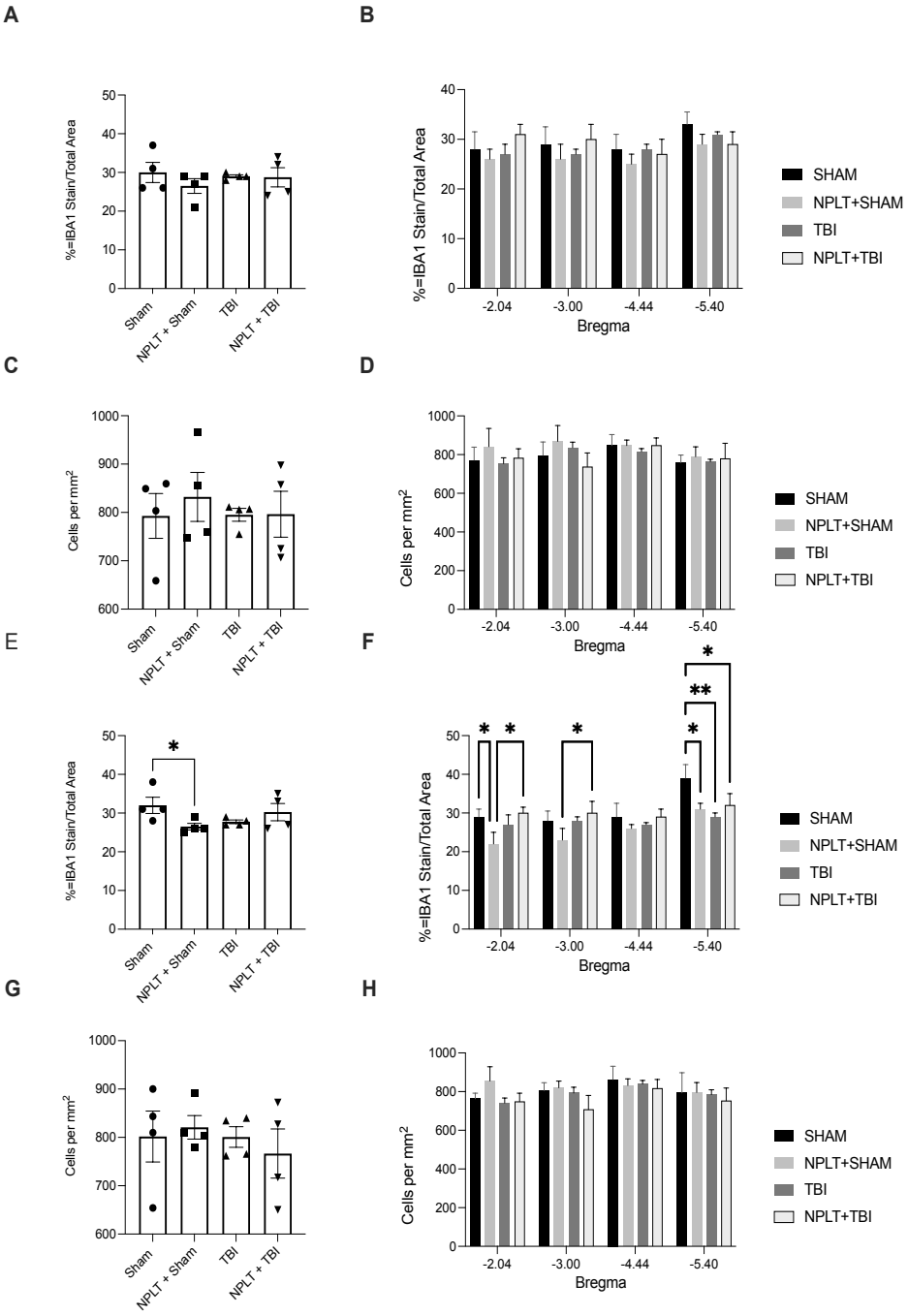


Figure 14: A single NPLT application decreases Iba1 staining but does not alter Iba1 cell count when compared to NPLT+TBI in the hippocampus. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left hippocampus. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right hippocampus. **(A)** No significant differences in average Iba1 percent stain in the left hippocampus. **(B)** When broken down by bregma level no significant differences in Iba1stain were observed in the left hippocampus. **(C)** No significant differences are observed in the average Iba1 cell count in the left hippocampus. **(D)** No significant differences are observed in any bregma level in Iba1 cell count in the left hippocampus. **(E)** There is a significant decrease as measured by average Iba1 percent stain in the NPLT+Sham group when compared to the Sham group *(p=.032, one-way ANOVA)* in the right hippocampus. **(F)** At bregma -2.04 there is a significant decrease in NPLT+SHAM when compared to Sham (p=.04, two-way ANOVA) and NPLT+TBI *(p=.02, two-way ANOVA)* group. At bregma -3.00 there is a decrease seen in NPLT+Sham *(p=.04, two-way ANOVA)* when compared to the NPLT+TBI group. At bregma -4.44 there is an insignificant decrease in the NPLT+Sham and TBI group. At bregma - 5.40 there is a decrease in Iba1 percent stain in groups NPLT+Sham *(p=.02, two-way ANOVA)*, TBI *(p=.004, two-way ANOVA)*, and NPLT+TBI *(p=.04, two-way ANOVA)*. **(G)** There are no significant changes in average Iba1 cell count in the left hippocampus. **(H)** There are no significant changes in average Iba1 cell count in the left hippocampus.

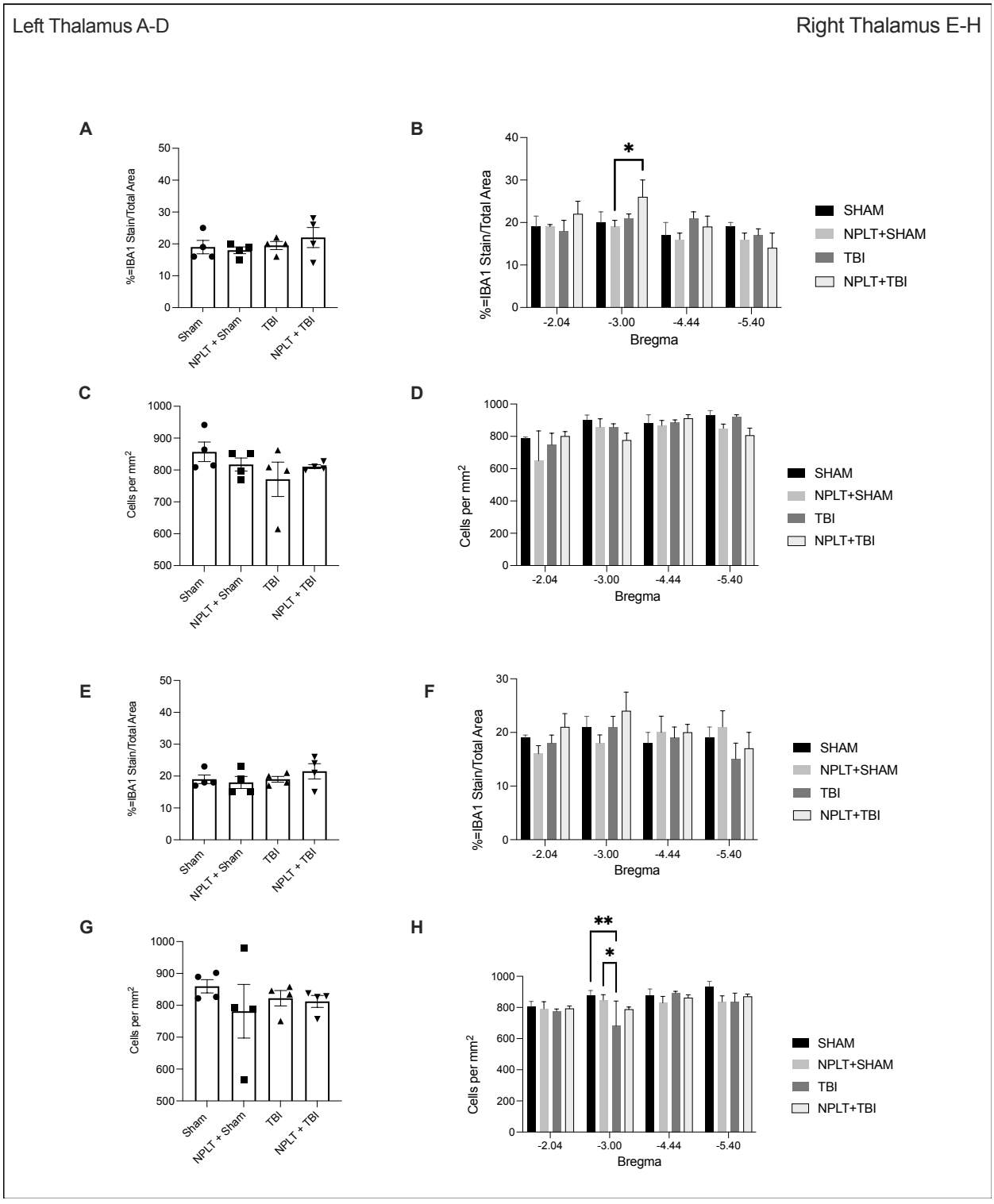


Figure 15: Application of NPLT increases GFAP staining but reduces GFAP cell count in the hippocampus. Mild blast injury after NPLT increases staining and insignificantly increases cell count in the hippocampus. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left thalamus. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right thalamus. **(A)** No significant changes were observed in average Iba1 percent stain in the left thalamus. **(B)** At bregma -3.00 there is a decrease in NPLT+Sham Iba1 staining when compared to NPLT+TBI *(p=.036, two-way ANOVA)*. **(C)** No significant changes were observed in the average Iba1 cell count in the left thalamus. **(D)** No significant changes were observed in the Iba1 cell count across all bregma levels in the left thalamus. **(E)** In the right thalamus no significant changes were observed in the Iba1 stain percent. **(F)** No significant changes were observed in the Iba1 cell count across all bregma levels in the right thalamus, however there is an insignificant decrease in NPLT+Sham groups at bregma -2.04 and -3.00. **(G)** No significant changes were observed in the average Iba1 cell count in the right thalamus. **(H)** At bregma -3.00, there is a significant decrease in the TBI group Iba1 cell count when compared to the Sham *(p=.008, two-way ANOVA)* and NPLT+Sham *(p=.025, two-way ANOVA)* group.

Quantification studies of the GFAP stain revealed the left and right Auditory cortex to have decreased GFAP staining and increased GFAP cell count in the NPLT+TBI group at bregma -4.44 (Figure 16, Panel B, D, and F). A similar trend is observed in the left Motor Cortex, where a decrease in GFAP staining in the NPLT+TBI and TBI groups is observed but a significant increase in TBI GFAP cell count and insignificant increase in the NPLT+TBI GFAP cell count is observed (Figure 17). GFAP quantification in the left and right Somatosensory cortex showed a decrease in GFAP staining in the NPLT+TBI group (Figure 18, Panel A, B, and F) but little to no changes in GFAP cell count. In the left and right Corpus Collosum, the TBI and NPLT+TBI groups showed decreased GFAP staining and increased GFAP cell count (Figure 19 Panel A-H). In the left and right Hippocampus, a decrease in GFAP staining in the NPLT+TBI groups is seen (Figure 20 Panel A, B, E, and F), while GFAP cell count increases are only seen significantly in the TBI group in the left Hippocampus (Figure 21, Panel G). GFAP quantification in the left and right Thalamus revealed decreased NPLT+TBI GFAP staining (Figure 21, Panel A, B, E, and F), with little to no changes in GFAP cell count.

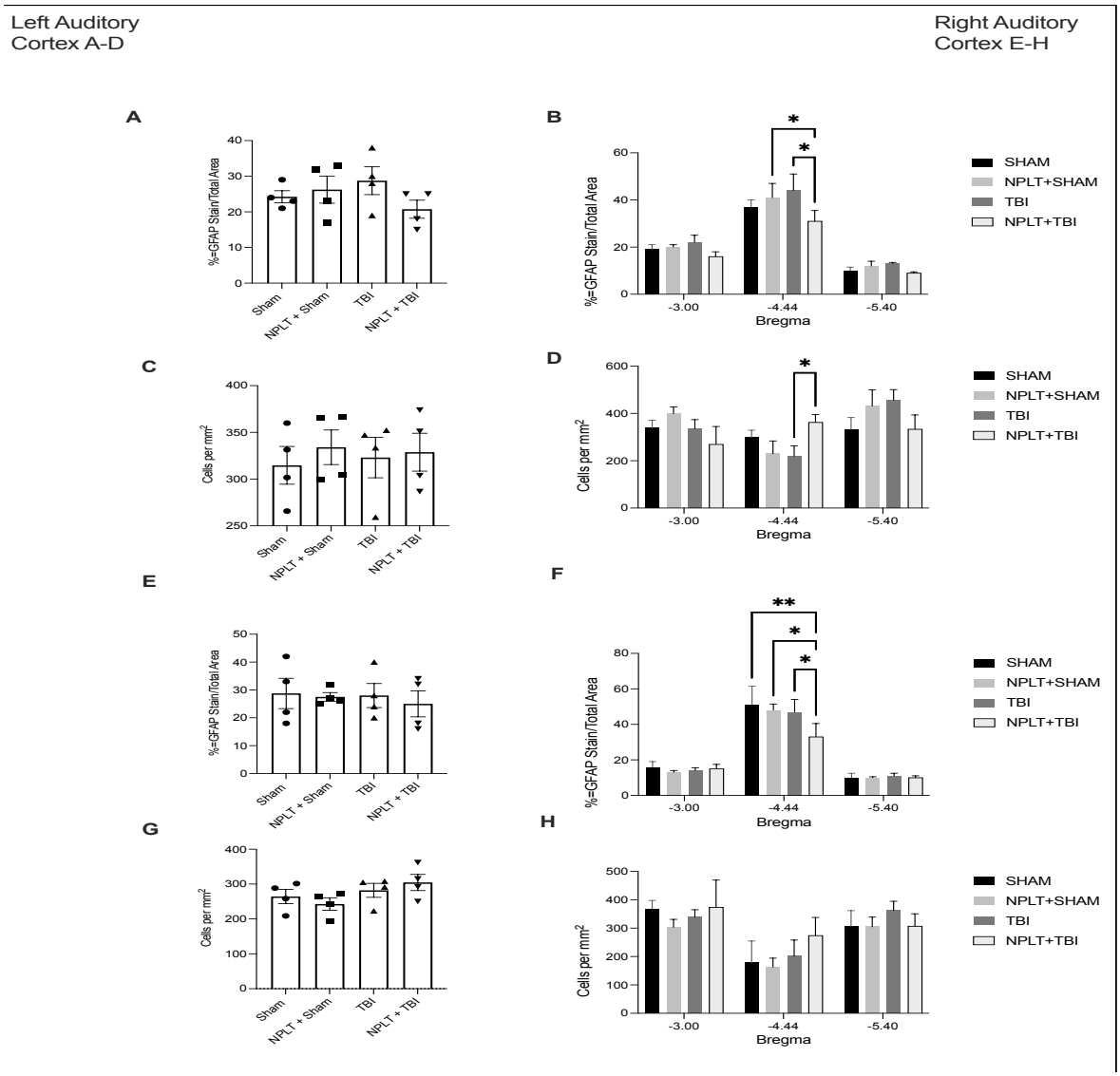


Figure 16: Single application of NPLT prior to bTBI decreases GFAP percent stain in the right and left auditory cortex at bregma -4.44 and increases GFAP cell count in the left auditory cortex. Graphs a-b depicting GFAP percent stain (a, b) and GFAP cell count (c, d) in the left auditory cortex. Graphs e-h depicting GFAP percent stain (e, f) and GFAP cell count (g, h) in the right auditory cortex. **(a)** No changes observed in GFAP percent stain average of all bregma in the left auditory cortex. **(b)** At bregma -4.44 NPLT+TBI animals have significantly decreased GFAP percent stain compared to NPLT+SHAM *(p=.044, 2-way ANOVA)* and TBI animals *(p=.010, 2-way ANOVA*). **(c)** No changes observed in Iba1 cell count average of all bregma, however **(d)** at bregma -4.44 an increase in GFAP cell count is observed in the NPLT+TBI animals when compared to TBI *(p=.046, 2-way ANOVA).* **(e)** No changes observed in GFAP percent stain average of all bregma in the right auditory cortex or in the cell count average of all bregma **(g)** and **(h)** cell count amongst all bregma levels. In graph **(f)** a decrease in GFAP stain at bregma -4.44 right auditory cortex in NPLT+TBI when compared to SHAM *(p=.009),* NPLT+SHAM *(p=.027),* and TBI *(p=.038)* by 2-way ANOVA is observed.

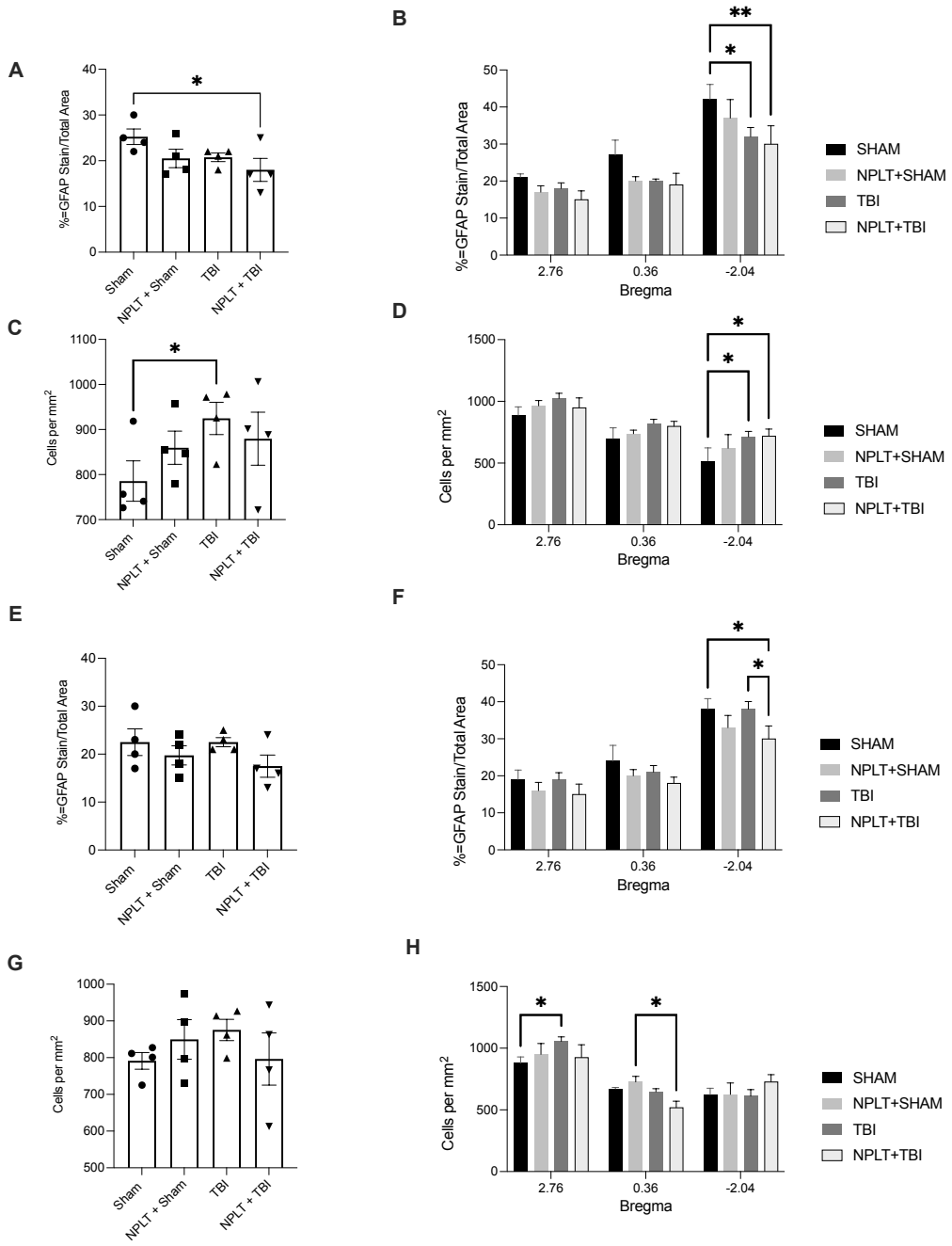


Figure 17: Single application of NPLT prior to bTBI and bTBI decreases GFAP percent stain when compared to sham in the left motor cortex, however little difference between TBI and NPLT+TBI groups. Graphs A- B depicting GFAP percent stain (A,B) and GFAP cell count (C, D) in the left motor cortex. Graphs E-H depicting GFAP percent stain (E, F) and GFAP cell count (G, H) in the right motor cortex. **(A)** A decrease in GFAP percent stain on average amongst bregma are observed in NPLT+TBI when compared to Sham *(p=.024, one-way ANOVA).* **(B)** At bregma -2.04 significant decreases are seen between SHAM and TBI *(p=.026, two-way ANOVA)* and NPLT+ TBI *(p=.009, two-way ANOVA)*. Notably, differences in the opposite trends can be in SHAM vs. TBI *(p=.046, two-way ANOVA)* and SHAM vs. NPLT+TBI *(p=.039, two-way ANOVA)* in GFAP cell count **(D)**. **(C)** A significance difference is observed in GFAP average cell count between TBI and Sham *(p=.05, one-way ANOVA).* **(E, G)** No differences were observed in average GFAP percent stain and cellular count in the right motor cortex. Differences in bregma can be seen **(F)** at -2.04 between SHAM and NPLT+TBI *(p=.043, two-way ANOVA)* and TBI and NPLT+TBI *(p= .040, two-way ANOVA)*. Bregma 2.76 shows a significant difference between Sham and TBI in GFAP cell count *(p=.049, two-way ANOVA)* **(H)** and at bregma .36 NPLT+TBI cell count is decreased when compared to NPLT+SHAM *(p=.021, two-way ANOVA)*.

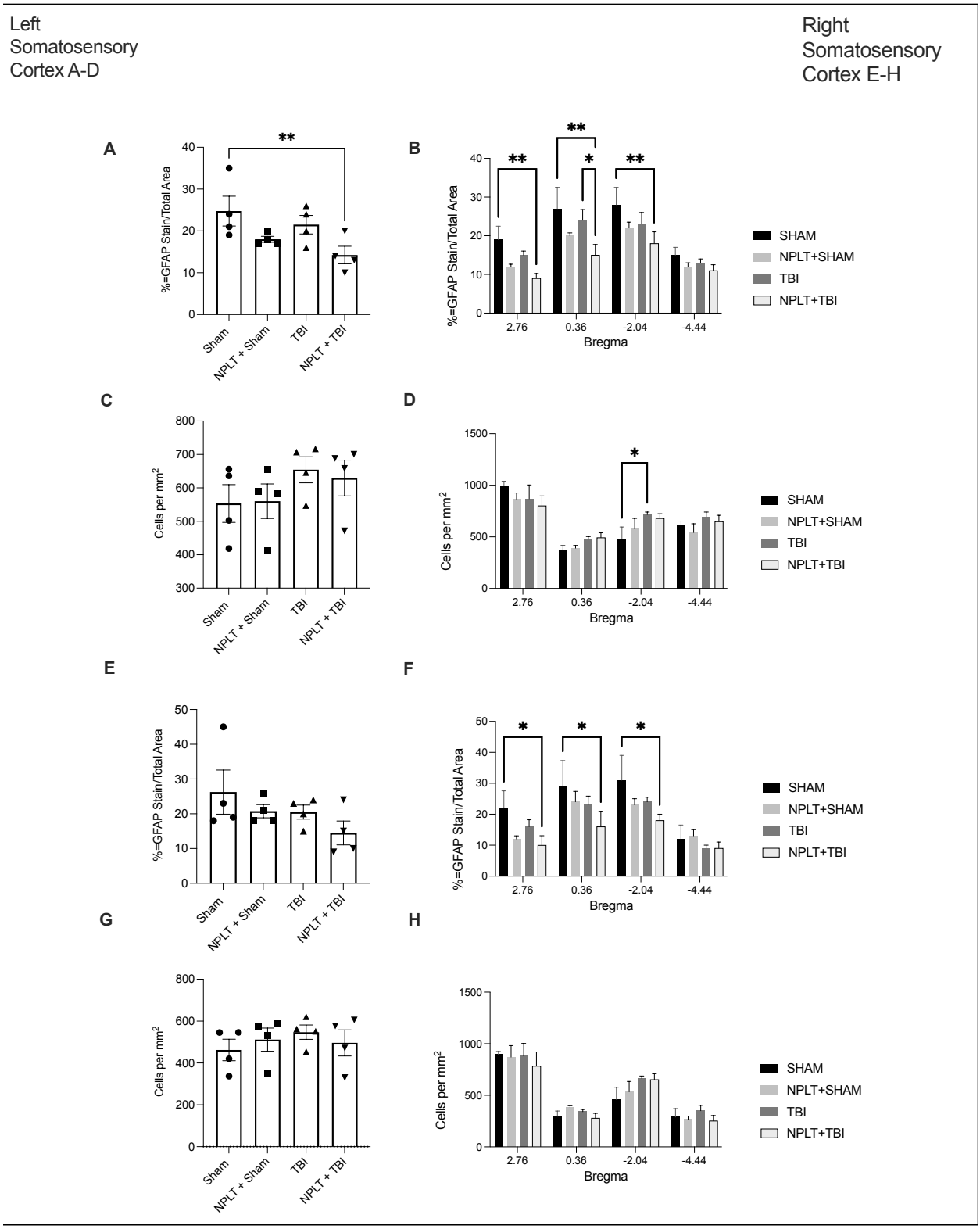


Figure 18: Application of NPLT followed by a single bTBI causes decreased GFAP percent stain when compared to sham in the somatosensory cortex, however no changes in cell count are observed. Graphs A-B depicting GFAP percent stain (A, B) and GFAP cell count (C, D) in the left somatosensory cortex. Graphs E-H depicting GFAP percent stain (E, F) and GFAP cell count (G, H) in the right somatosensory cortex. **(A)** A decrease in GFAP stain percent in NPLT +TBI when compared to the Sham *(p=.008, one-way ANOVA)* is observed in the left somatosensory cortex. **(B)** At bregma 2.76,.36, -2.04 there is a decrease in GFAP stain in the NPLT+TBI group when compared to Sham *(p=.009, p=.002, =.019, two-way ANOVA).* At bregma .36 there is a decrease in NPLT+ TBI GFAP stain when compared to TBI *(p=.019, two-way ANOVA).* **(C)** There were no significant changes observed in the average GFAP cell count in the left somatosensory cortex. **(D)** At bregma -2.04 there is a significant increase in GFAP cell count in TBI when compared to Sham *(p=.023, two-way ANOVA)*. **(E)** There are no significant differences in the average GFAP stain percentage in the right somatosensory cortex. **(F)** At bregma 2.76, ,36, and -2.044 there is a decrease in NPLT+TBI stain when compared to the Sham group in the right somatosensory cortex *(p=.041, p=.028, p=.028, two-way ANOVA)*. (G) There were no significant changes observed in the average GFAP cell count in the right somatosensory cortex. (H) No significant differences were observed in any bregma in the right somatosensory cortex.

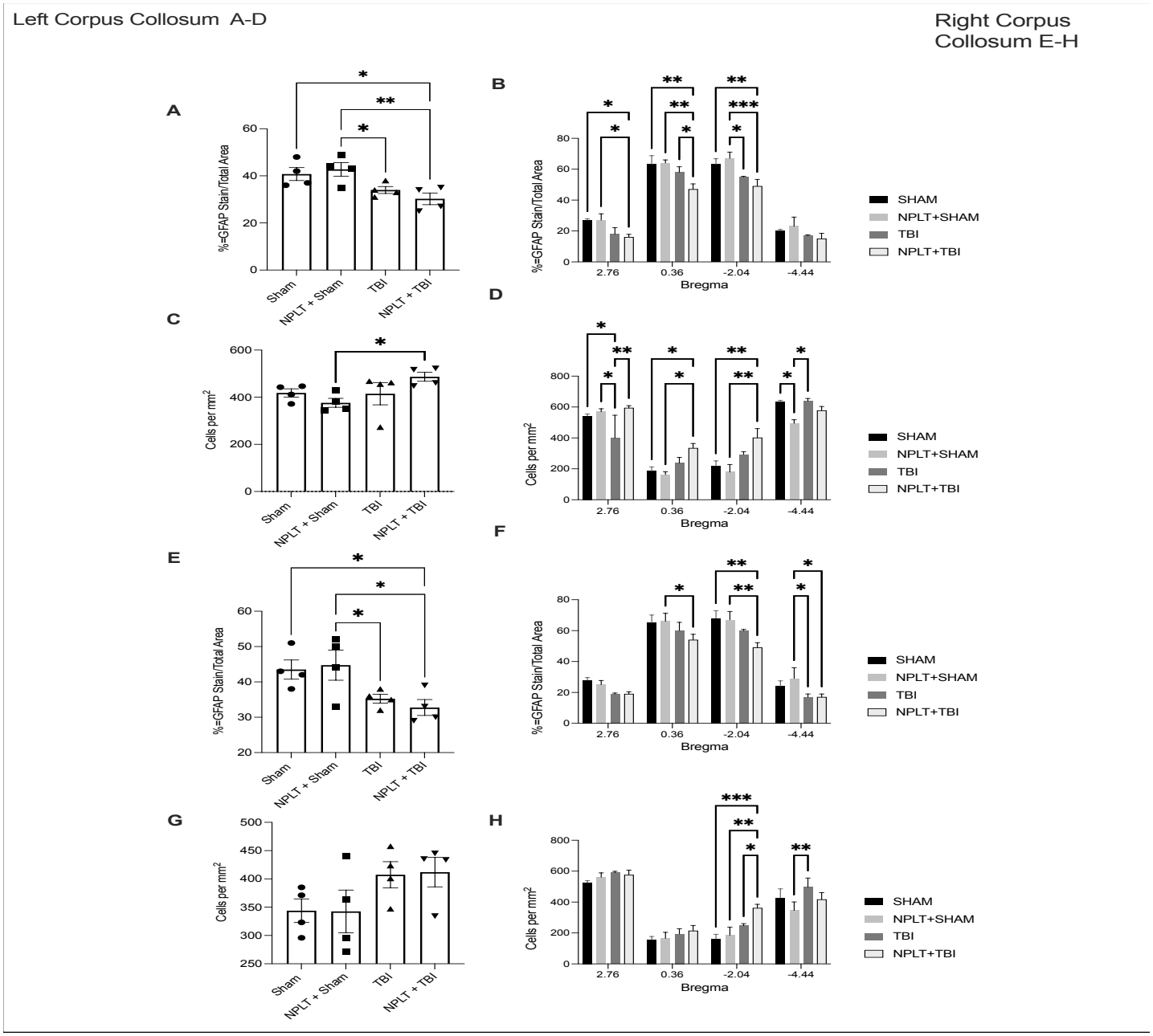


Figure 19: Mild blast TBI after NPLT application reduces GFAP staining but increases GFAP cell count in the corpus collosum. Graphs A-B depicting GFAP percent stain (A, B) and GFAP cell count (C, D) in the left corpus collosum. Graphs E-H depicting GFAP percent stain (E, F) and GFAP cell count (G, H) in the right corpus collosum. **(A)** Significant decreases in average GFAP percent stain can be seen in TBI *(p=.027, one-way ANOVA)* and NPLT+TBI *(p=.003, one- way ANOVA)* when compared to NPLT+Sham in the left somatosensory cortex. A decrease in NPLT+TBI compared to Sham is also seen *(p=.01, one-way ANOVA)*. **(B)** A decrease in GFAP stain in NPLT+TBI when compared to Sham and NPLT+Sham is observed in bregma 2.76 *(p=033, p=.033)* .36*(p=.002) (p.001=)*, and -2.04 *(p=.007) (p=.0008)* by two-way ANOVA. At bregma .36 there is a decrease in GFAP stain in NPLT+TBI compared to TBI *(p=.021, two-way ANOVA)* and at bregma -2.04 there is a reduction in GFAP stain in TBI compared to NPLT+Sham *(p=.021, two-way ANOVA)*. **(C)** There is an increase in average GFAP cell count in NPLT+TBI when compared to NPLT+Sham *(p=.018, two-way ANOVA)*. **(D)** At bregma 2.76, there are significant reductions seen in GFAP cell count between groups Sham and TBI *(p=.038),* NPLT+Sham and TBI *(p=.013)*, and NPLT+TBI and TBI *(p=.004)* by two-way ANOVA. At bregma .36 and -2.04, there are significant increases between group Sham and NPLT+TBI *(p=.028, p=.007)* and NPLT+Sham and NPLT+TBI *(p=.012, p=.001)* by two-way ANOVA. At bregma -4.44 there is a reduction in GFAP cell count in NPLT+Sham when compared to Sham (*p=.038, two-way ANOVA)*, and NPLT+Sham when compared to TBI *(p=.032, two-way ANOVA)*. **(E)** In the right corpus collosum, average GFAP stain is reduced in NPLT+TBI when compared to Sham *(p=.02, one-way ANOVA)* and NPLT+Sham (*p=.011, one-way ANOVA)*. There is also a reduction seen in TBI when compared to NPLT+Sham *(p= .035, one-way ANOVA)*. **(F)** At bregma .36 there is a reduction in GFAP staining in NPLT+TBI when compared to NPLT+Sham *(p=.034, two-way ANOVA)*. At bregma -2.04 there is a reduction in NPLT+TBI when compared to Sham *(p=.001, two-way ANOVA)* and NPLT+Sham *(p=.002, two-way ANOVA)*. At bregma -4.44 There is a reduction in GFAP stain in TBI *(p=.034, two- way ANOVA)* and NPLT+TBI *(p=.034, two-way ANOVA)* when compared to NPLT+Sham. **(G)** There are no significant changes were observed in right corpus collosum average GFAP cell count. **(H)** A bregma -2.04 there is an increase in GFAP stain in NPLT+TBI when compared to Sham *(p=.0005, two-way ANOVA)*, NPLT+Sham *(p=.001, two-way ANOVA)*, and TBI *(p=.035, two-way ANOVA)*. At bregma -4.44 there is an increase in TBI GFAP stain when compared to NPLT+Sham *(p=.006, two-way ANOVA)*.

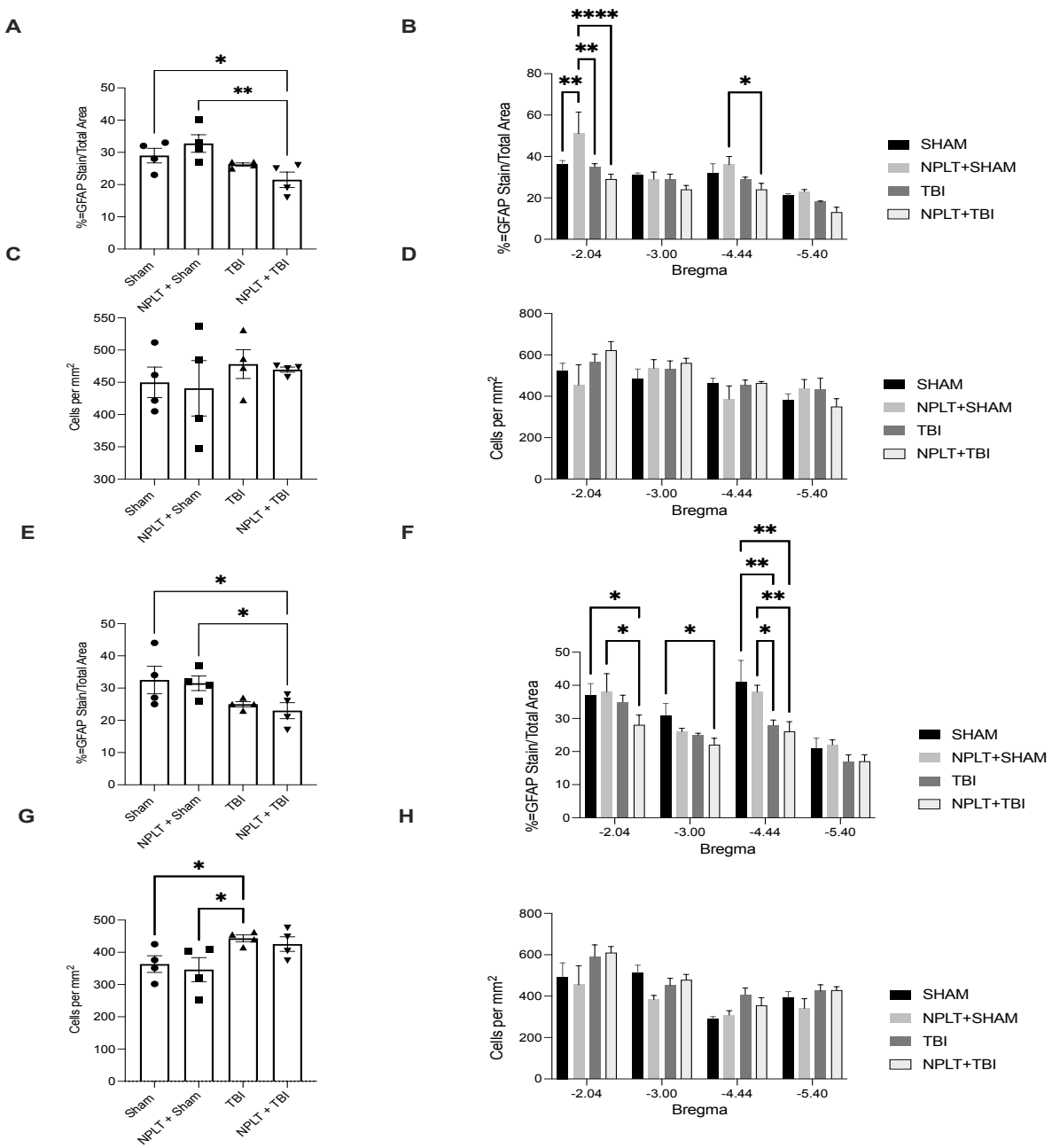


Figure 20: Application of NPLT increases GFAP staining but reduces GFAP cell count in the hippocampus. Mild blast injury after NPLT increases staining and insignificantly increases cell count in the hippocampus. Graphs A-B depicting GFAP percent stain (A, B) and GFAP cell count (C, D) in the left hippocampus. Graphs E-H depicting GFAP percent stain (E, F) and GFAP cell count (G, H) in the right hippocampus. **(A)** A decrease is observed in NPLT+TBI when compared to Sham *(p=.029, two-way ANOVA)* and NPLT+Sham *(p=.003, two-way ANOVA)* as measured by average GFAP stain percentage in the left hippocampus. **(B)** At bregma -2.04 there is an increase in GFAP stain in NPLT+Sham compared to Sham *(p=.004, two-way ANOVA)*, TBI *(p= .002, two-way ANOVA)*, and NPLT+TBI *(p<.0001, two-way ANOVA)*. At bregma -4.44 there is a decrease in the NPLT+TBI group when compared to the NPLT+Sham group *(p=.02, two-way ANOVA)*. **(C)** There are no significant differences seen in the average GFAP cell count in the left hippocampus. **(D)** There are no significant differences seen in GFAP cell count across bregma - 2.40 to bregma 5.40. **(E)** There is a reduction in average GFAP stain in the NPLT+TBI group when compared to Sham (p=.031, one-way ANOVA) and NPLT+Sham *(p=.049, one-way ANOVA)*. **(F)** At bregma -2.04 there is a reduction in GFAP count in NPLT+TBI when compared to Sham *(p=.042, two-way ANOVA*) and NPLT+Sham *(p=.025, two-way ANOVA)*. At bregma -3.00, there is a reduction in the GFAP+TBI compared to Sham *(p=.042, two-way ANOVA)*. At bregma -4.44 there is a reduction in TBI when compared to Sham *(p=.004, two-way ANOVA)* and NPLT+Sham *(p=.025, two-way ANOVA)*. There is also a reduction in NPLT+TBI compared to Sham *(p=.001, two-way ANOVA)* and NPLT+Sham *(p=.007, two-way ANOVA)*. **(G)** There is a reduction in average GFAP cell count in TBI when compared to the Sham *(p=.049, two-way ANOVA)* and NPLT+Sham *(p=.02, two-way ANOVA)*. (H) No significant differences are seen in the GFAP cell count amongst across bregma -2.04 to bregma -5.40.

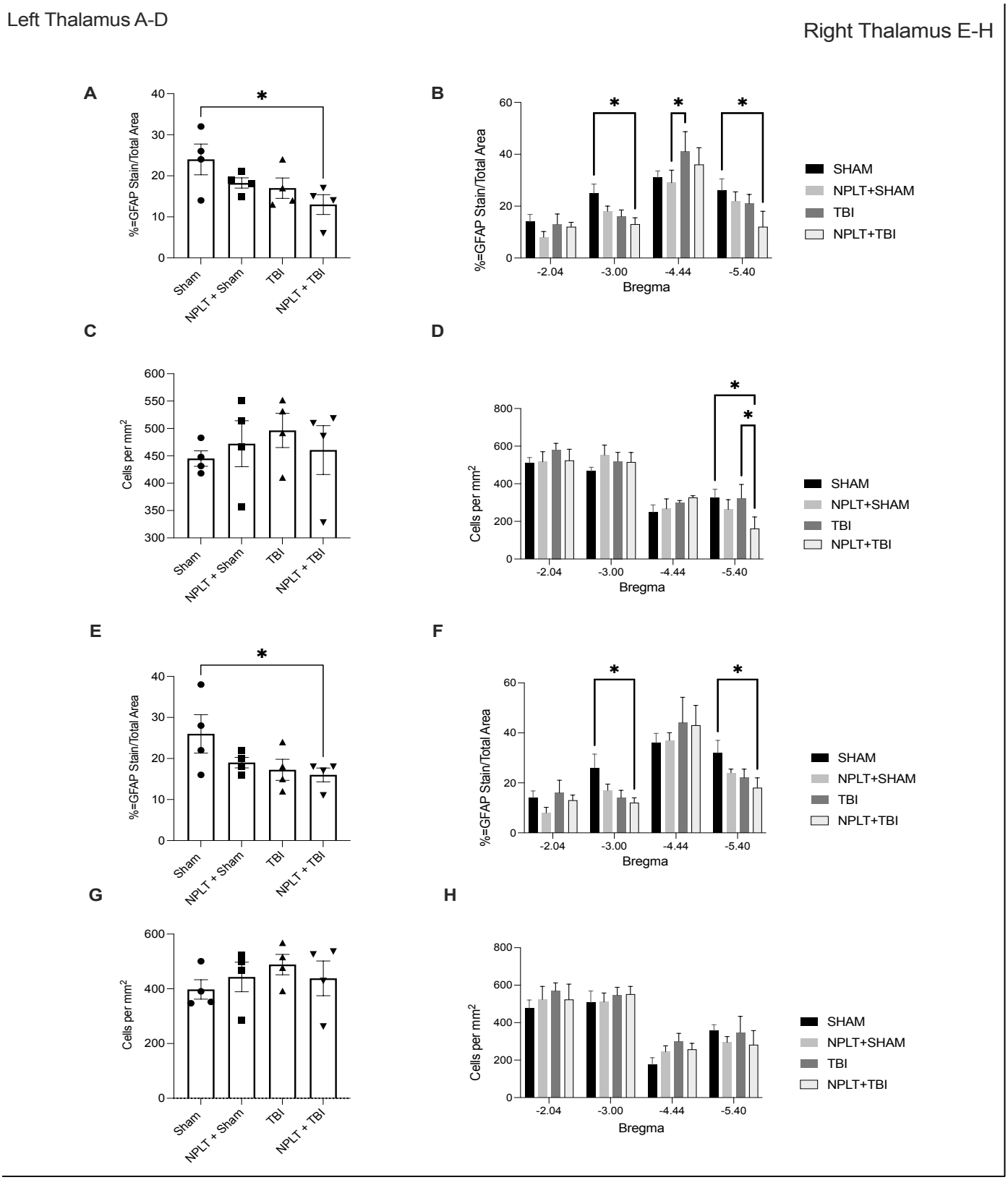


Figure 21: Application of NPLT prior to mild blast injury reduces GFAP staining but no changes are observed in GFAP cell count in the thalamus. Graphs A-B depicting GFAP percent stain (A, B) and GFAP cell count (C, D) in the left thalamus. Graphs E-H depicting GFAP percent stain (E, F) and GFAP cell count (G, H) in the right thalamus. **(A)** There is a reduction in NPLT+TBI GFAP average stain percent when compared to Sham *(p=.011, one-way ANOVA)* in the left thalamus. **(B)** At bregma -3.00 and -5.40 there is a reduction in NPLT+Sham when compared to Sham *(p= .045, p= .02, two-way ANOVA)*. At bregma -4.44 there is an increase in GFAP staining in TBI when compared to NPLT+Sham *(p=.045, two-way ANOVA)*. **(C)** There are no significant differences seen in average GFAP cell counts. **(D)** At bregma -5.40 there is a reduction in NPLT+TBI GFAP cell count when compared to Sham *(p=.017, two-way ANOVA)* and TBI *(p=.018, two-way ANOVA)*. **(E)** In the right thalamus there is a reduction in average GFAP stain percent in NPLT+TBI when compared to Sham *(p=.030, one-way ANOVA)*. **(F)** At bregma -3.00 and bregma -5.40 there is a reduction in GFAP stain in NPLT+TBI when compared to Sham *(p=.037, p=.037, two-way ANOVA)*. (G) There are no significant differences seen in average GFAP cell counts. (H) There are no significant changes seen in the GFAP cell count from bregma -2.04 to bregma -5.40.

Quantitative real time PCR was performed to analyze the expression of mRNA encoding for BDNF (a neurotrophic factor) and IL1β (an inflammatory cytokine). Our results showed that BDNF and IL1β expression was not significantly different between the experimental groups (Figure 22).

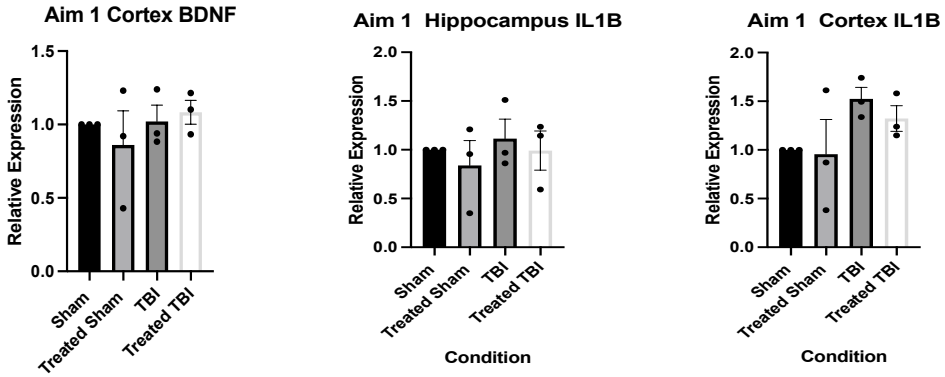


Figure 22. The expression of BDNF and IL1β mRNA was measured by qRT-PCR analysis. Data was normalized to GAPDH and relative to SHAM as mean +/- SEM. Comparisons among the groups was performed using ANOVA followed by Tukey's multiple comparisons test. Data is Mean +/- SEM.

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

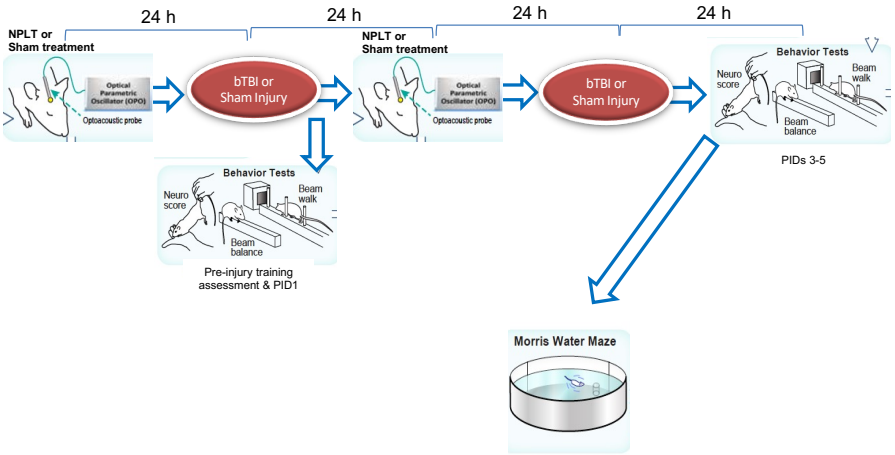
Adult (2-month-old) male Sprague-Dawley rats were randomized to receive NPLT or no treatment and further randomized to receive two consecutive blast TBIs (48 hours apart) or sham-injury. NPLT treatments and bTBI were performed as described above in Goal 2/Major Task 1.

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 23).



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

In the Beam balance test, we found no significant differences in scores between the groups on any of the post injury days (PIDs) 4-8 and no significant differences between PIDs 4-8 and pre-injury assessment scores on Day -2 were observed within groups (Figure 24). Animals who received multiple NPLT applications and blast injuries showed an increased in latency in the Beam walk assessment (Figure 25, Panel B) on PID 6 (p=.036) when compared to day -2 latencies. In the Morris Water Maze (T2) (Figure 26), animals who received multiple

Blast injuries, without NPLT treatment has insignificantly higher latencies in locating the platform when compared to treated and injured animals on PIDs 10, 12, 13.

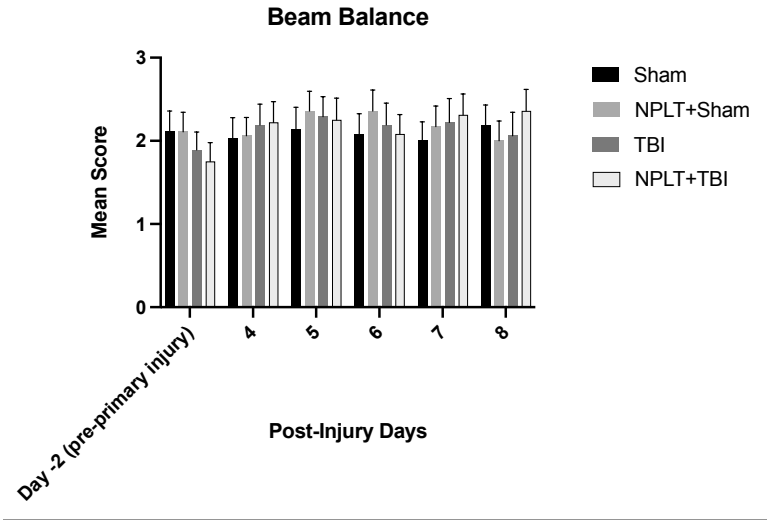


Figure 24. Comparison of Trial 2 latencies between the four groups was performed using a Two factor ANOVA (Treatment, Days) with replication (5 Days). An overall significant effect of Days ($F=33.24$; $p<0.0001$), was detected, but not a significant effect of treatment ($F=2.037$; $p=0.1096$). Data is Mean+/-SEM

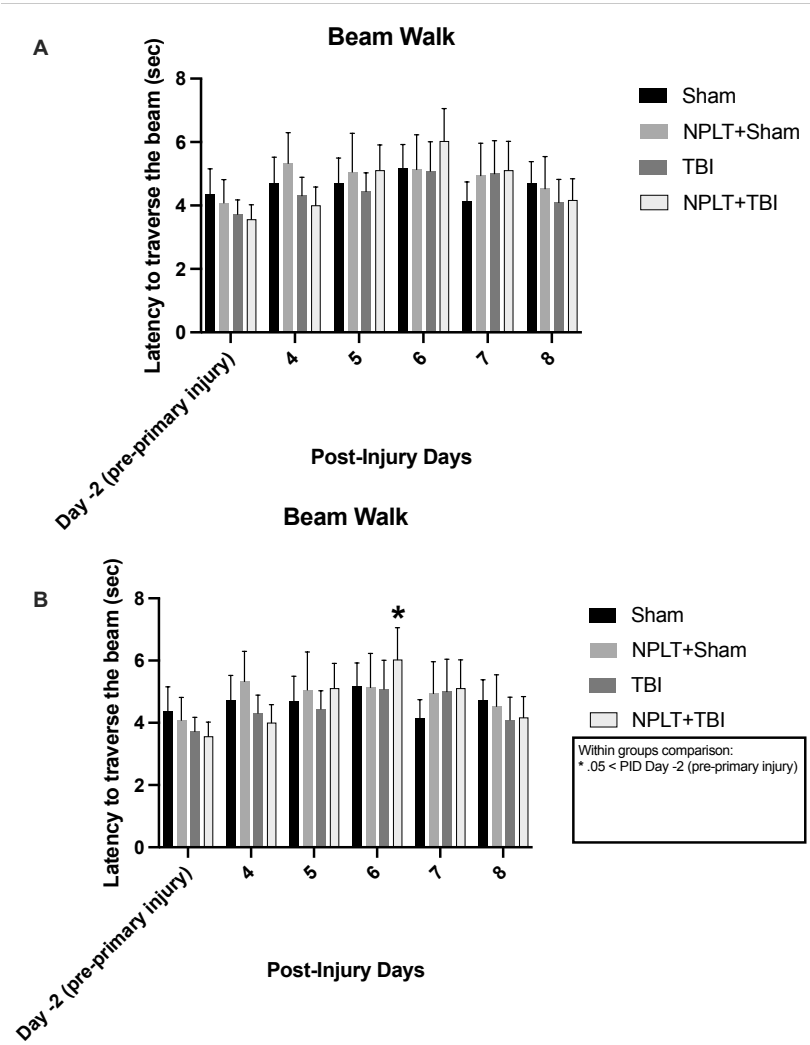


Figure 25. **No significant differences were observed between the groups (n=12/group) on any post injury day. There is a significant increase in NPLT+TBI on PID 6 when compared to NPLT+TBI on Day -2.** Graph **A** compares the latency to traverse the beam between groups on each post injury day. No significant differences were seen in graph A. Graph **B** compared latency to traverse the beam between post injury days and within the groups. There is a significant increase in NPLT+TBI on PID 6 when compared to NPLT+TBI on Day -2 ($p=.036$, two-way ANOVA).

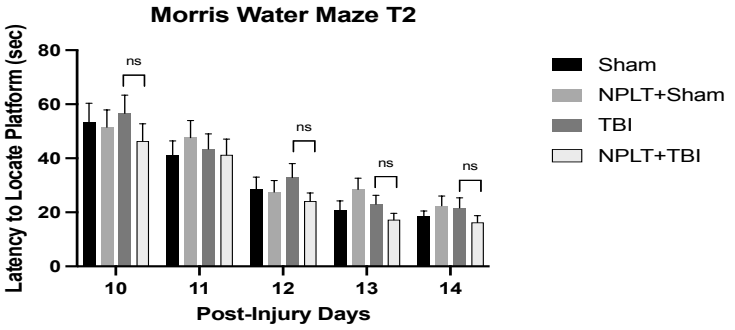
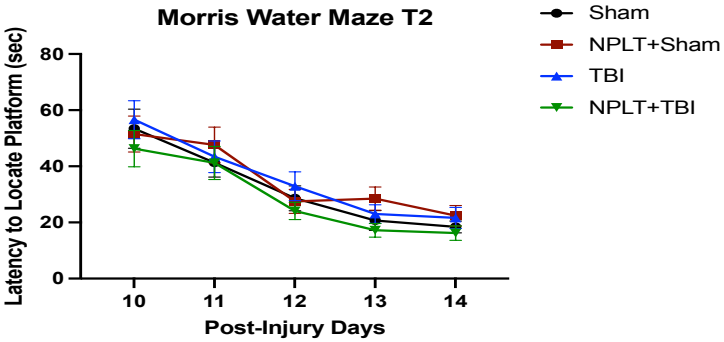
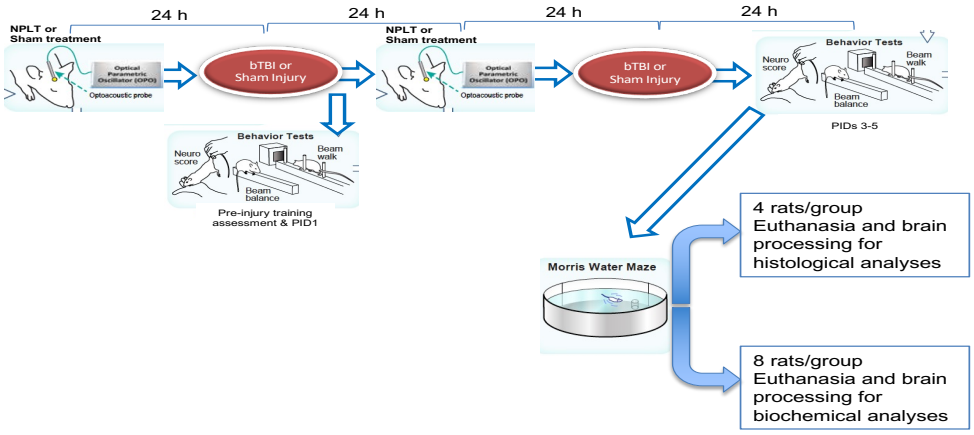


Figure 26. NPLT+TBI shows an insignificant trend towards decreased latency to locate the platform during the second trial for Morris Water Maze. Graph A depicts the latency to locate the platform in trial 2 during the Morris Water Maze and compares the latency between groups on each PID. Graph B depicts the same presented as a line graph to indicate a downward sloping curve. There are no significant differences seen amongst the four groups on any PID. On days 10,12,13,14 there is an insignificant decrease in latency in NPLT+TBI



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 27).



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

To determine whether multiple NPLT treatments can reduce inflammation 13 days post multiple blast TBI we analyzed inflammation by immunofluorescence using a specific antibody against Iba1, a marker of microglia, and a specific antibody against GFAP, a marker for astrocytes. To assess myelination, we used a specific antibody against Solochrome Cyanine.

Quantification studies revealed in the, right and left Auditory Cortex, there is an increase in Iba1 staining and decrease in Iba1 cell count in the TBI group when compared to all other groups (Figure 28 Panel A-H). Morphology studies showed an insignificant increase in the TBI and NPLT+TBI groups in cellular surface area (I), perimeter (J), and Transformation Index (K). The left and right Hippocampus showed similar patterns as the TBI group showed increased Iba1 staining and decreased microglial cell count (Figure 33, Panel A-H). Similar patterns are seen in the right and left Thalamus (Figure 34, Panel B and F), right and left Motor Cortex (Figure 29, Panel B and F), where an increase in Iba1 staining in the TBI group is observed. In the Auditory Cortex and

Hippocampus, the differences seen in the TBI group (increase in Iba1 staining and decrease in Iba1 cell count) are most significant at bregma -4.44. At bregma -4.44 in the Thalamus there is a decrease in Iba1 staining in the TBI group, but unlike the Auditory Cortex and Hippocampus, no changes in cell count are observed at bregma -4.44. In the left Somatosensory Cortex (Figure 30, Panel F), and left Corpus Collosum (Figure 31, Panel F) bregma 4.44 reveals an increase in Iba1 staining in the TBI group, but no differences were seen in cell count. Notably, in the left and right Somatosensory Cortex and right and left Corpus Collosum at bregma .36 an increase in Iba1 staining in the NPLT+Sham group, while no changes are seen in cell count. However, there is an insignificant increase in right cellular surface area in the Corpus Collosum (Figure 31, Panel I) at bregma -2.04.

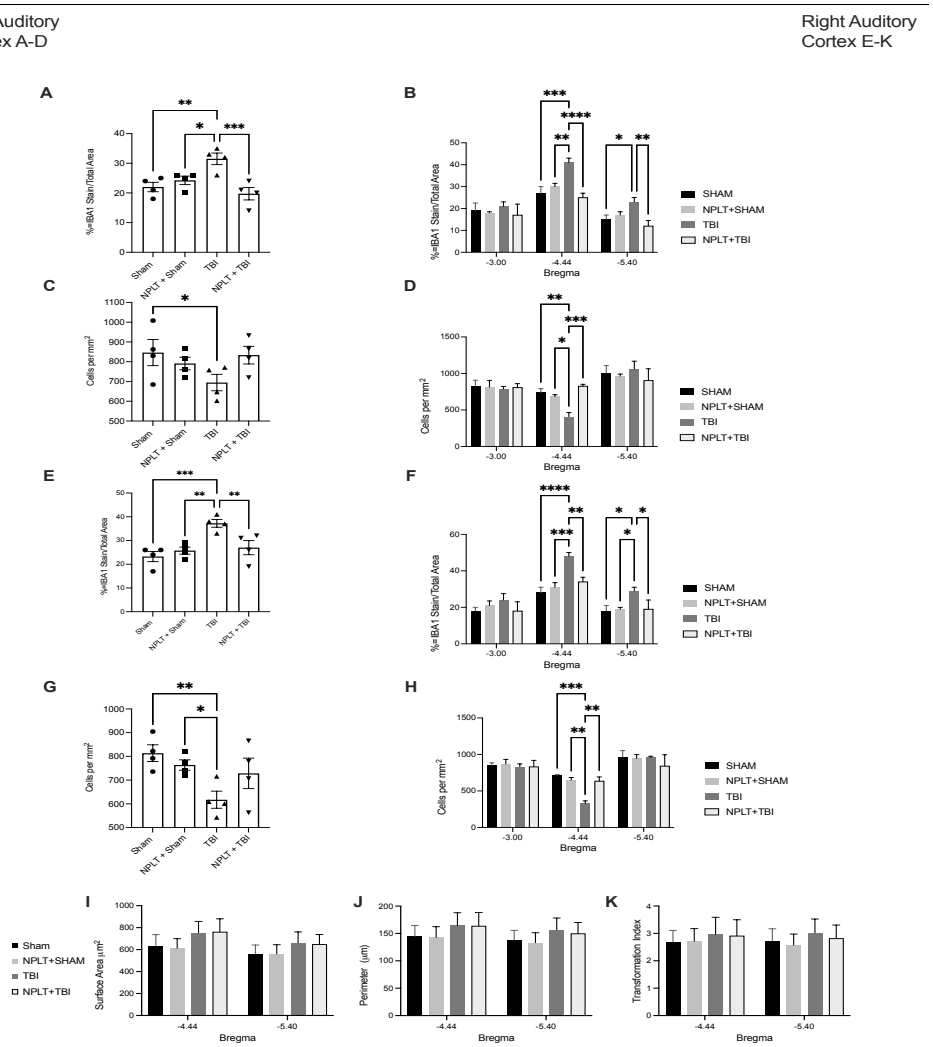


Figure 28: Multiple blast injuries increase Iba1 percent stain but decrease Iba1 cellular count, while multiple NPLT applications with or without blast injury show no differences when compared to sham. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left auditory cortex. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right auditory cortex. (A) In the left auditory cortex, TBI shows the highest average percent Iba1 stain when compared to sham (p=.002, one-way ANOVA), NPLT+Sham (p= .014, one-way ANOVA), and NPLT+TBI (p=.0005, one-way ANOVA). (B) At bregma level - 4.44 and increase in Iba1 percent stain is observed in TBI when compared to sham (p=.0004, two- way ANOVA), NPLT+Sham (p= .004, two-way ANOVA), and NPLT+TBI (p<.0001, two-way ANOVA). At bregma -5.40 an increase in Iba1 percent stain in TBI is observed when compared to sham (p=.032, two-way ANOVA) and NPLT+TBI (p= .004, two-way ANOVA). (C) A decrease in average Iba1 cell count was observed in TBI when compared to sham (p=.044, one-way ANOVA). (D) At bregma -4.44 a decrease in Iba1 cell count is observed in TBI when compared to sham (p=.005, two-way ANOVA), NPLT+Sham (p=.018, two-way ANOVA), and NPLT+TBI (p=.0006, two-way ANOVA). (E) In the right auditory cortex, an increase in the average bregma Iba1 percent stain is seen in TBI when compared to sham (p=.0006, one-way ANOVA), NPLT+Sham (p=.003, one-way ANOVA), and NPLT+TBI (p=.005, one-way ANOVA). (F) At bregma -4.44, there is an increase in Iba1 percent stain observed in TBI when compared to sham (p<.0001, two-way ANOVA), NPLT+Sham (p=.0004, two-way ANOVA), NPLT+TBI (p= .003, two-way ANOVA). At bregma -5.40 there is an increase in Iba1 percent stain in TBI when compared to sham (p=.015, two-way ANOVA), NPLT+Sham (p=.027, two-way ANOVA), and NPLT+TBI (p=.027, two-way ANOVA). (G) There is a decrease in average Iba1 cell count in TBI when compared to Sham (p=.007, one-way ANOVA) and NPLT+Sham (p=.030, one-way ANOVA). (H) At bregma -4.44, there is a decrease in Iba1 cell count in TBI when compared to Sham (p=.0003, two-way ANOVA), NPLT+Sham (p=.002, two-way ANOVA), and NPLT+TBI (p=.003, two-way ANOVA). Non-significant increases in surface area (I), perimeter (J), and transformation index (K) are seen in the TBI and NPLT+TBI when compared to Sham and NPLT+Sham.

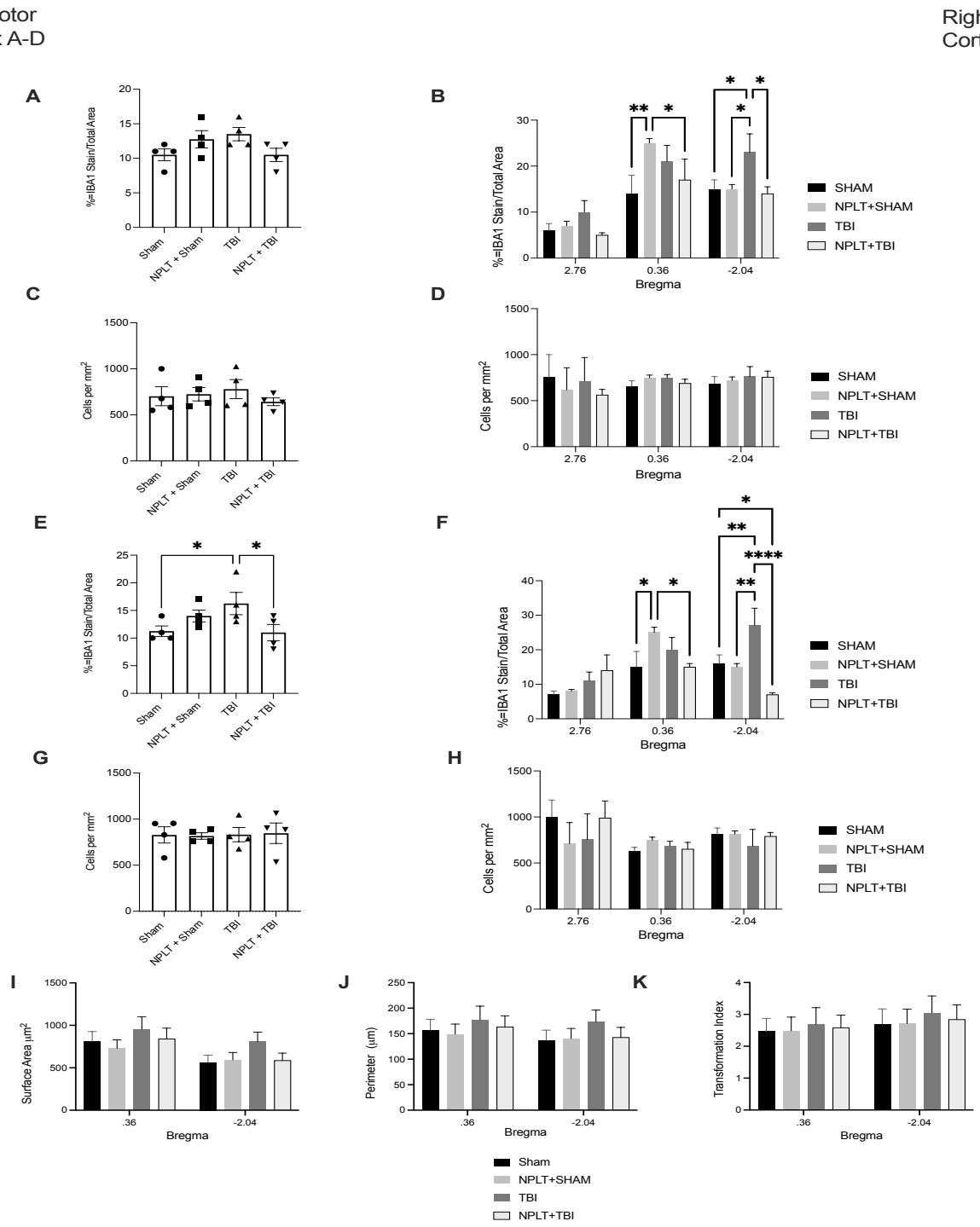


Figure 29: Motor Cortex: At bregma -2.04, multiple blast injuries cause an increase in Iba1 stain, while two blast TBI's treated with NPLT application show no differences to sham animals. At bregma .36 NPLT+Sham show slight increases in Iba1 stain. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left motor cortex. Graphs E- H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right motor cortex. (A) There are no differences seen in average Iba1 percent stain in the left motor cortex. (B) At bregma .36 there is an increase in Iba1 percent stain in NPLT+Sham when compared to Sham (p=.005, two- way ANOVA) and NPLT+TBI (p=.038, two-way ANOVA). At bregma -2.04, there is an increase in Iba1 percent stain in TBI when compared to Sham (p=.038, two-way ANOVA), NPLT+Sham (p=.038, two-way ANOVA), and NPLT+TBI (p=.020, two-way ANOVA). (C) No differences observed in the Iba1 average cell count. (D) No differences were observed in Iba1 cell count at any bregma. (E) In the right motor cortex, there is an increase in Iba1 percent stain in TBI when compared to Sham (p=.030, one-way ANOVA) and NPLT+TBI (p=.024, one-way ANOVA). (F) At bregma .36, there is an increase in Iba1 percent stain seen in NPLT+Sham when compared to sham (p=.017, two-way ANOVA) and NPLT+TBI (p= .017, two-way ANOVA). At bregma there is an increase in Iba1 percent stain in TBI when compared to sham (p=.009, two-way ANOVA), NPLT+Sham (p=.005, two-way ANOVA), and NPLT+TBI (p<.0001, two-way ANOVA). There is also an increase in NPLT+TBI when compared with sham (p=.031, two-way ANOVA). (G) No differences were observed in the average Iba1 cell count in the right motor cortex. (H) There are no significant differences seen in the Iba1 cell count at any bregma. Non-significant increases in surface area (I) and perimeter (J) are seen in the TBI when compared to Sham, NPLT+Sham, and NPLT+TBI in the right motor cortex. (K) There are no differences seen in the transformation index at bregma .36 and -2.04 in the right motor cortex.

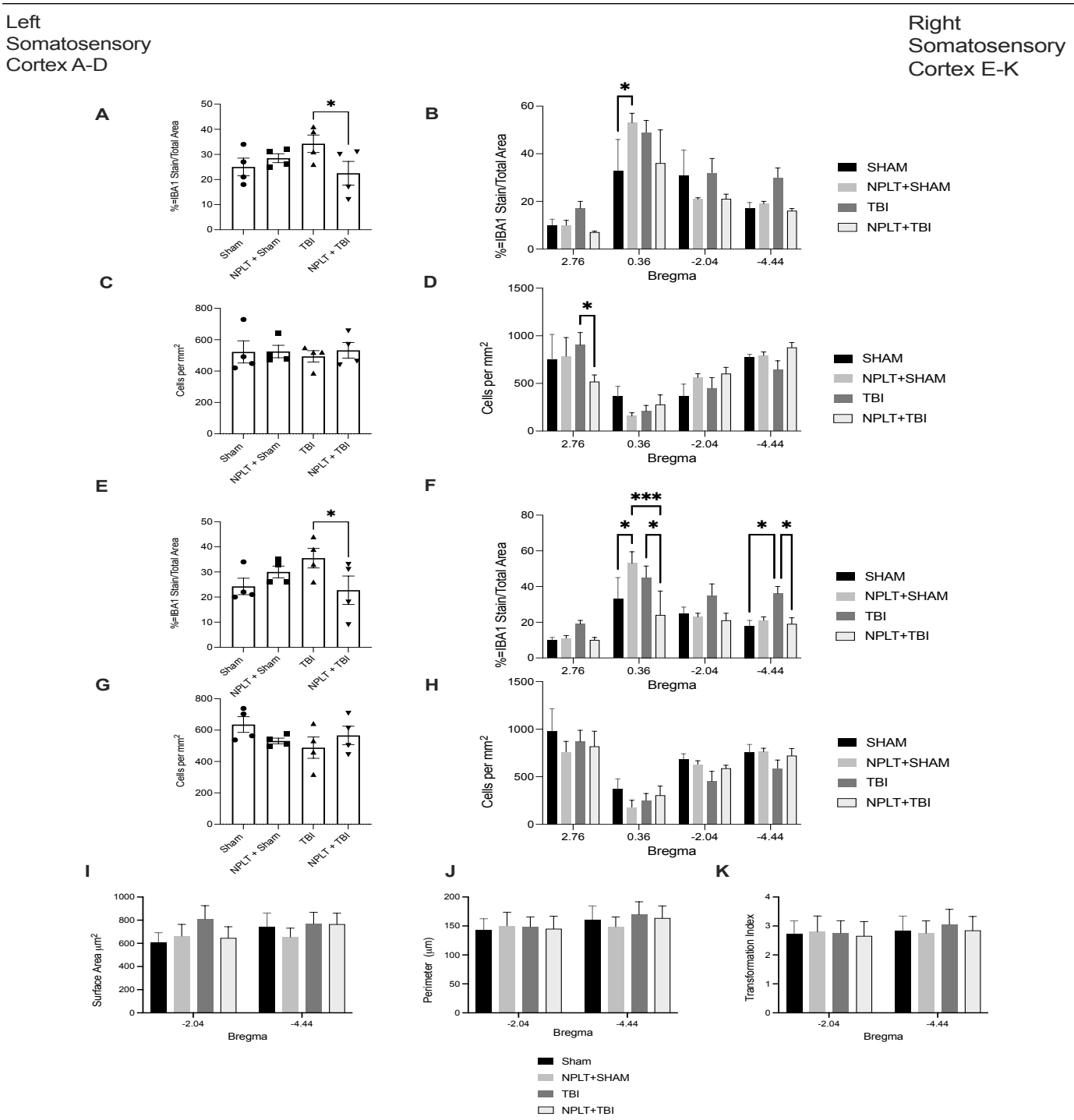


Figure 30: Multiple blast TBI's show an increase in Iba1 percent stain, but little to no changes in cell count in the somatosensory cortex. Multiple NPLT applications rescue the effects of multiple blast TBIs. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left somatosensory cortex. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right somatosensory cortex. (A) An increase in average Iba1 percent stain is seen in TBI when compared to NPLT+TBI (p=.037, one-way ANOVA). (B) At bregma .36, there is an increase in Iba1 percent stain in NPLT+Sham when compared to Sham (p=.025, two- way ANOVA). (C) No significant differences are observed in the average Iba1 cell count. (D) At bregma 2.76 there is a decrease in Iba1 cell count in NPLT+TBI when compared to TBI (p=.019, two-way ANOVA). (E) An increase in average Iba1 percent stain is observed in TBI when compared to NPLT+TBI (p=.042, one-way ANOVA). (F) At bregma .36, there is an increase in NPLT+Sham when compared to Sham (p=.018, two-way ANOVA), and NPLT+TBI (p=.0009, two-way ANOVA). There is also an increase in Iba1 percent stain in TBI when compared to NPLT+TBI (p=.014, two-way ANOVA). At bregma -4.44 TBI is increased in Iba1 percent stain when compared to Sham (p=.033, two-way ANOVA) and NPLT+TBI (p=.043, two-way ANOVA). (G) No significant differences are observed in the average Iba1 cell count. (H) No significant differences were observed in Iba1 cell count at any bregma. (I) There are no significant differences observed in surface area in the right somatosensory cortex, but there is an insignificant increase in TBI at bregma -2.04 and there is an insignificant increase in TBI and NPLT+TBI at bregma -4.44. (J) There are no significant differences seen in cell perimeter, and at bregma -4.44 there is an increase in perimeter in TBI and NPLT+TBI. (K) There are no significant differences seen in cell transformation index, and at bregma -4.44 there is an increase in transformation index in TBI and NPLT+TBI.

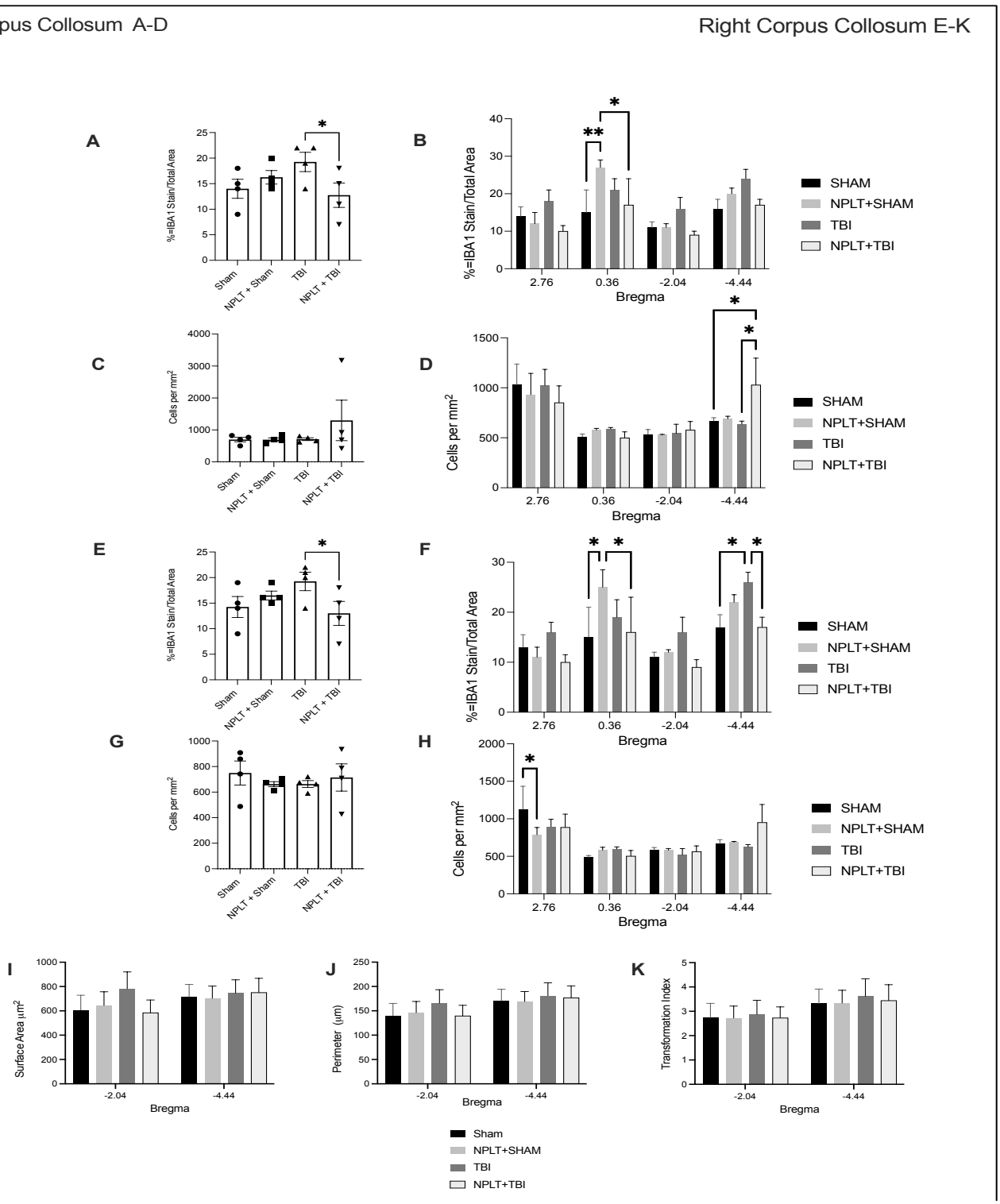


Figure 31: Multiple NPLT applications on multiple blast TBI rescue Iba1 percent staining while Iba1 cell count remains unchanged in the corpus collosum. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left corpus collosum. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the corpus collosum. **(A)** There is an increase in average Iba1 staining when compared to NPLT+TBI (p=.032, one-way ANOVA). **(B)** At bregma .36 an increase in Iba1 stain in NPLT+Sham when compared to sham (p=.009, two-way ANOVA), and NPLT+TBI (p=.028, two-way ANOVA). **(C)** No significant differences are observed in the average Iba1 cell count. **(D)** At bregma -4.44, an increase in Iba1 cell count is observed in NPLT+TBI when compared to Sham (p=.041, two-way ANOVA) and TBI (p=.028, two-way ANOVA). **(E)** An increase in average Iba1 staining was observed in TBI when compared to NPLT+TBI (p=.034, one-way ANOVA). **(F)** At bregma .36 there is an increase in Iba1 staining in NPLT+Sham when compared to Sham (p=.028, two-way ANOVA) and NPLT+TBI (p=.046, two-way ANOVA). **(G)** No significant differences are observed in average Iba1 cell count in the right corpus collosum. **(H)** At bregma 2.76, there is a decrease in Iba1 cell count in NPLT+Sham when compared to Sham (p=.049, two-way ANOVA). No significant differences are observed in (I) surface area, (J) perimeter, (K) and transformation index in the right corpus collosum. However, there is an insignificant increase in the TBI surface area at bregma -2.04

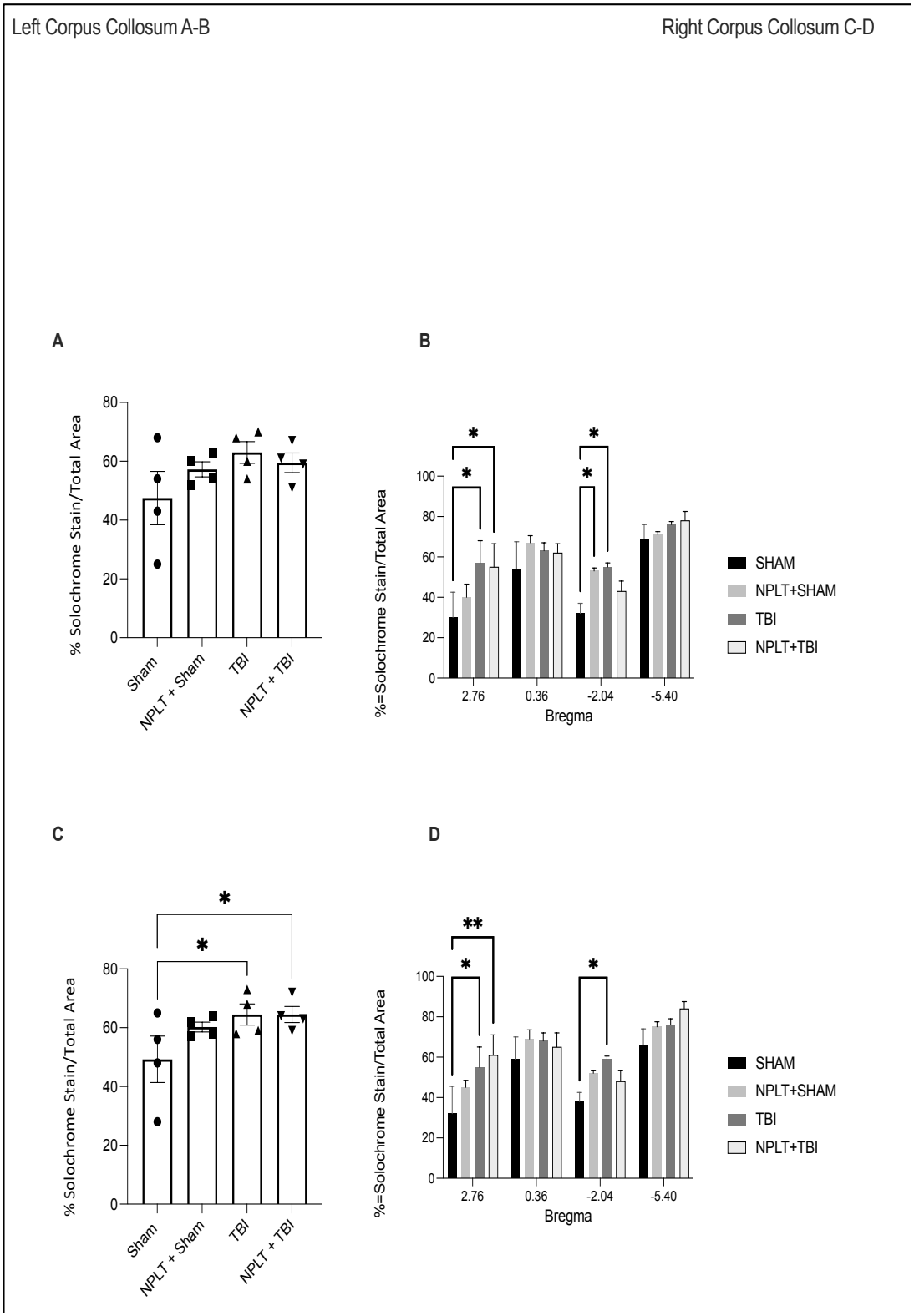


Figure 32: Multiple blast TBI's increase Solochrome staining, however multiple NPLT applications show no significant effect. Graphs A and B depict Solochrome percent stain in the left corpus collosum and graphs C and D depict Solochrome percent stain in the right corpus collosum. (A) No significant differences are seen in average Solochrome staining in the left corpus collosum. (B) At bregma 2.76 there is an increase in Solochrome staining in TBI (p=.010, two- way ANOVA) and NPLT+TBI (p=.016, two-way ANOVA) when compared to Sham. At bregma -2.04, there is an increase in Solochrome staining in TBI (p= .027, two-way ANOVA) and NPLT+Sham (p=.043, two-way ANOVA) when compared to Sham. (C) An increase in average Solochrome staining in TBI (p=.038, one-way ANOVA) and NPLT+TBI (p=.038, one-way ANOVA) when compared to Sham in the right corpus collosum. (D) At bregma 2.76, there is an increase in Solochrome staining in TBI (p=.021, two-way ANOVA) and NPLT+TBI (p=.004, two- way ANOVA) when compared to Sham. At bregma -2.04, TBI shows an increase in Solochrome staining when compared to Sham (p=.035, two-way ANOVA).

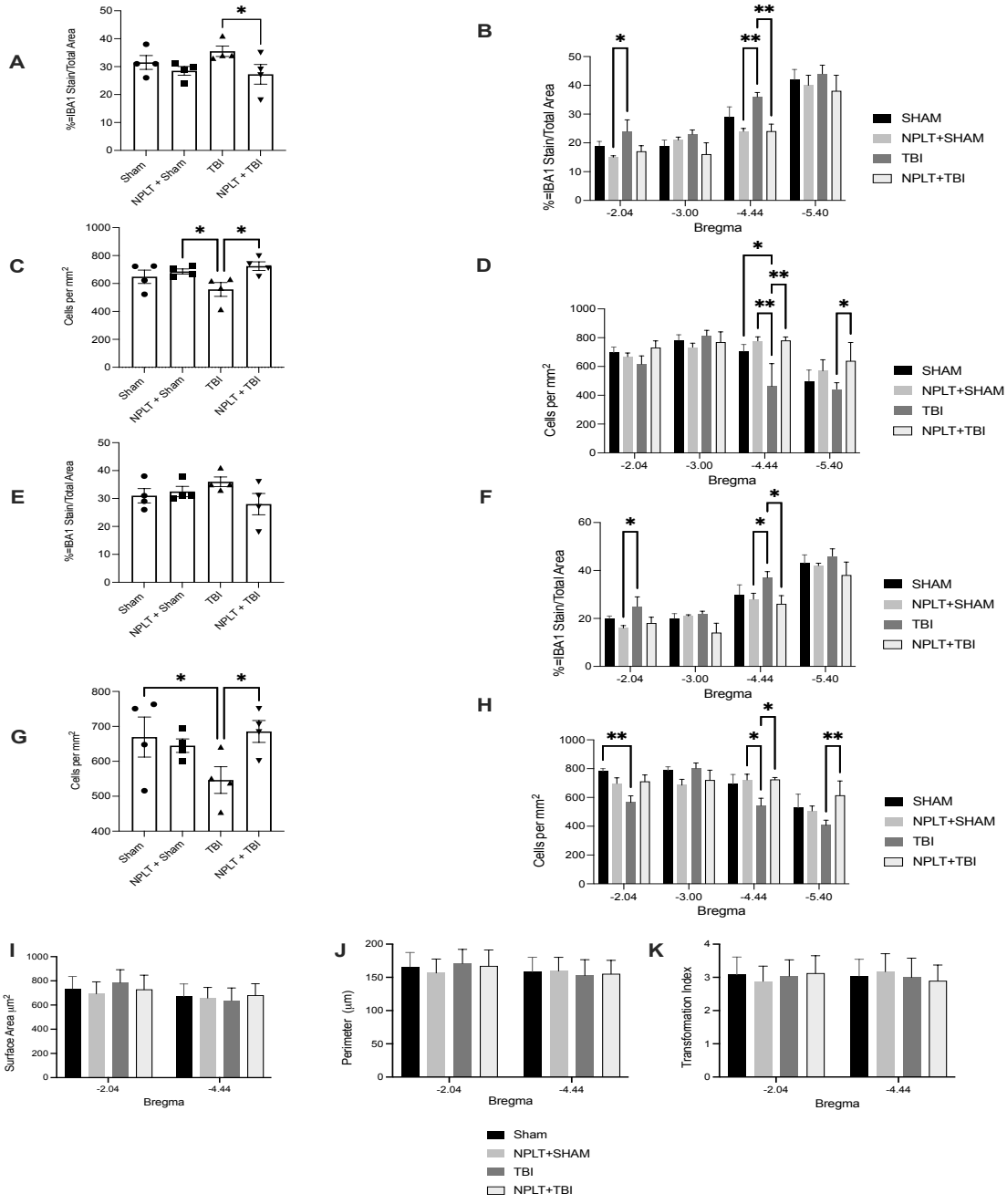


Figure 33: At bregma -2.04 and -4.44, multiple blast TBIs increase Iba1 percent stain and decrease cell count while multiple NPLT applications rescue these effects in the hippocampus. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left hippocampus. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right hippocampus. (A) There is an increase in average Iba1 staining in TBI when compared to NPLT+TBI (p=.036, one-way ANOVA) in the left hippocampus. (B) At bregma -2.04 there is an increase in Iba1 staining in TBI when compared to NPLT+Sham (p=.031, two-way ANOVA). At bregma -4.44 there is an increase in Iba1 staining in TBI when compared to NPLT+Sham (p=.005, two-way ANOVA) and NPLT+TBI (p=.005, two-way ANOVA). (C) There is a significant decrease in average Iba1 cell count in TBI when compared to NPLT+Sham (p=.038, two-way ANOVA) and NPLT+TBI (p=.011, two-way ANOVA). (D) At bregma -4.44 there is a significant decrease in Iba1 cell count in TBI when compared to Sham (p=.015, two-way ANOVA), NPLT+Sham (p=.002, two-way ANOVA), and NPLT+TBI (p=.002, two-way ANOVA). At bregma -5.40 there is a decrease in Iba1 cell count when compared to NPLT+TBI (p=.050, two- way ANOVA). (E) No significant differences in average Iba1 staining are observed in the right hippocampus. (F) At bregma -2.04 there is an increase in Iba1 staining in TBI when compared to NPLT+Sham (p=.036, two-way ANOVA). At bregma -4.44 there is an increase in TBI staining when compared to NPLT+Sham (p=.036, two-way ANOVA) and NPLT+TBI (p=.011, two-way ANOVA). (G) There is a significant decrease in average Iba1 cell count in TBI when compared to Sham (p=.046, one-way ANOVA) and NPLT+TBI (p=.027, one-way ANOVA). (H) At bregma -2.04 a significant decrease in Iba1 cell count in TBI when compared to Sham (p=.006, two-way ANOVA). At bregma -4.44 a significant decrease in Iba1 cell count is observed in TBI when compared to NPLT+Sham (p=.021, two-way ANOVA) and NPLT+TBI (p=.020, two-way ANOVA). At bregma -5.40 a significant decrease in Iba1 cell count is observed in TBI when compared to NPLT+TBI (p=.008, two-way ANOVA). No significant differences are seen in surface area (I), perimeter (J), and transformation index (K) in the right hippocampus.

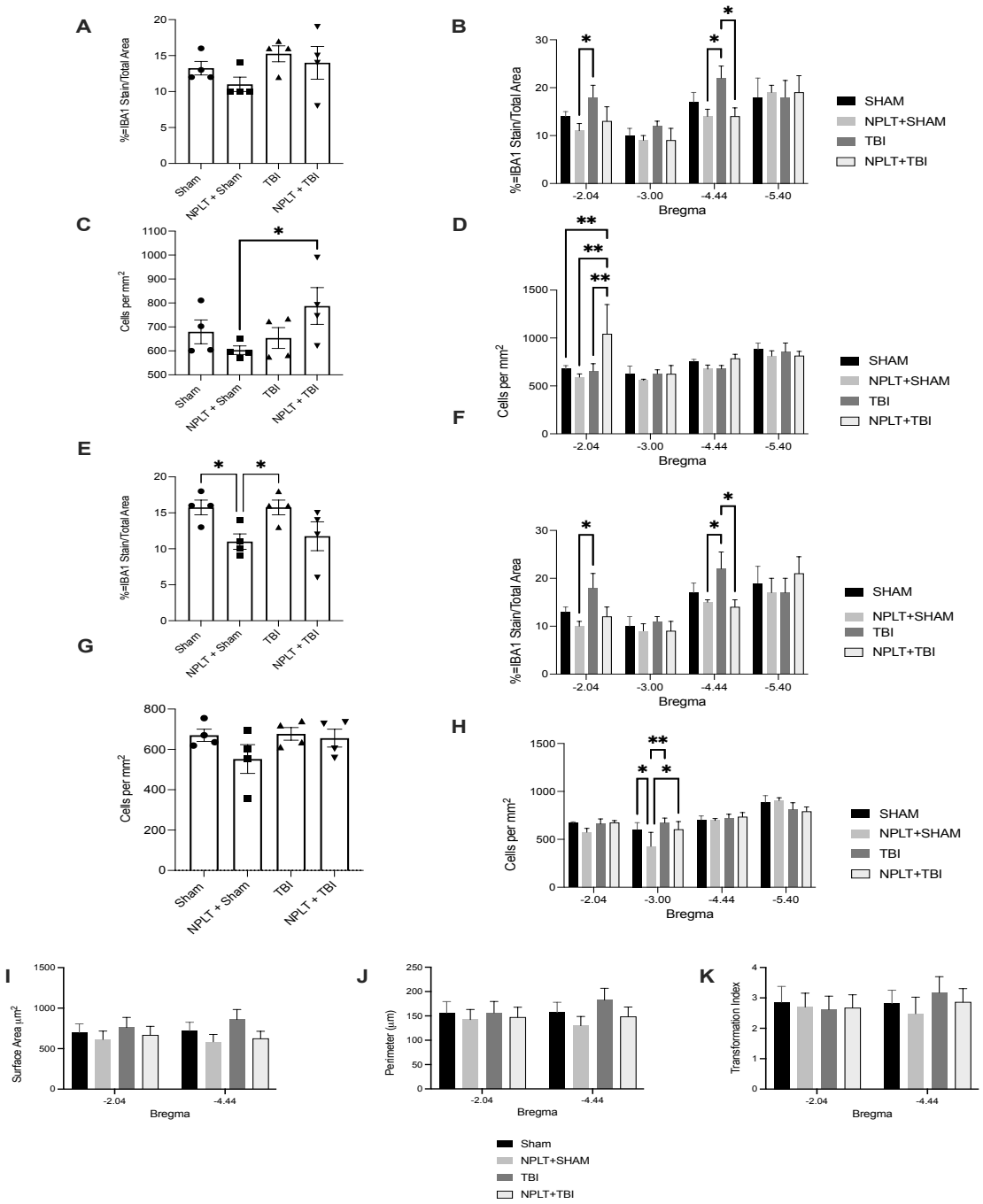


Figure 34: Multiple blast TBI's significantly increase Iba1 staining at bregma -4.44, while no significant difference is seen in cell count. Multiple NPLT applications rescue the effects of multiple blast TBI's. Graphs A-B depicting Iba1 percent stain (A, B) and Iba1 cell count (C, D) in the left thalamus. Graphs E-H depicting Iba1 percent stain (E, F) and Iba1 cell count (G, H) in the right thalamus. (A) No significant differences are observed in the average Iba1 staining in the left thalamus. There is an insignificant decrease in NPLT+Sham. (B) At bregma -2.04, there is an increase in Iba1 staining in TBI when compared to NPLT+Sham (p=.040, two-way ANOVA). At bregma -4.44, there is an increase an Iba1 staining observed in TBI when compared to NPLT+Sham (p=.020, two-way ANOVA) and NPLT+TBI (p=.014, two-way ANOVA). (C) An increase in average Iba1 cell count is observed in NPLT+TBI when compared to NPLT+Sham (p=.026, one-way ANOVA). (D) At bregma -2.04, there is an increase in Iba1 cell count in NPLT+TBI when compared to Sham (p=.009, two-way ANOVA), NPLT+Sham (p=.002, two- way ANOVA), and TBI (p=.006, two-way ANOVA). (E) A significant decrease in average Iba1 staining is observed in NPLT+Sham when compared to Sham (p=.029, one-way ANOVA), and TBI (p=.029, two-way ANOVA) in the right thalamus. (F) At bregma -2.04 there is an increase in Iba1 staining in TBI when compared to NPLT+ Sham (p=.019, two-way ANOVA). At bregma -4.44 there is an increase in Iba1 staining in TBI when compared to NPLT+Sham (p=.040, two- way ANOVA) and NPLT+TBI (p=.020, two-way ANOVA). (G) No significant differences are seen in the average cell count in the right thalamus. (H) At bregma -3.00 there is a significant decrease in Iba1 cell count observed in NPLT+Sham when compared to Sham (p=.046, two-way ANOVA), and TBI (p=.006, two-way ANOVA), and NPLT+TBI (p=.045, two-way ANOVA). No significant differences in cellular surface area (I), perimeter (J), and transformation index (K) are found in the right thalamus. However, at bregma -4.44 there is an insignificant increase in TBI cellular surface area (I), perimeter (J), and transformation index (K).

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

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.

Nothing to Report

4. **IMPACT:** D*escribe 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).

Traumatic brain injury (TBI) represents both an acute and a chronic medical challenge among service members and veterans. As noted by the Defense and Veterans Brain Injury Center (DVBIC), "the high rate of TBI and blast-related concussion events resulting from current combat operations directly impacts the health and safety of individual service members and subsequently the level of unit readiness and troop retention."
The results of our research are expected to make an **important and original contribution** to advancing solutions relevant to **military health and medicine. Using NPLT for non-invasive brain stimulation of individuals at risk of being exposed to TBI represents an innovative "game changer" approach,** leading to improved outcomes for Service members, Veterans, and the public (e.g., athletes before a sporting event).
Our proprietary system is safe, easy to use, stress-free for the subject, and operates at levels of optical energy that are eye- and skin-safe. We have confirmed experimentally, using highly sensitive, micro-thermocouples, that transcranial delivery of NPLT does not produce measurable tissue heating in the brain and that the amplitude of the optoacoustic (ultrasound) waves generated during NPLT is below well-established safety limits for ultrasound exposure of tissues (peak optoacoustic wave amplitudes do not exceed 0.05 bar). **Translation of NPLT to human patients will therefore be easily accomplished once we have completed the proposed experimental work.**
The impacts of TBI are felt within each branch of military service and throughout both the Department of Defense (DoD) and the Department of Veterans Affairs (VA) health care systems. For this reason, the development of an enhanced TBI treatment to decrease morbidity and improve immediate and long-term outcomes, here proposed, has the **potential to benefit multiple medical research program areas such as Military Operational Medicine, Combat Casualty Care and Clinical and Rehabilitative Medicine.**

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

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

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

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:*

Changes in approach and reasons for change
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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

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

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: *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).*

  - 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. Federal support acknowledged.
  - N. Gupta, K.M. Johnson, A. Grant, I. Petrov, Y. Petrov, R. Esenaliev, S.L. Sell, D.S. DeWitt, D.S. Prough, MA Micci Transcranial Administration of Nano Pulse Laser Therapy for Blast Induced Neurotrauma J. Neurotrauma *in preparation for submission.* Federal support acknowledged.

  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.*

  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)**
  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. 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).

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?
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

QUAD CHARTS:

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

DM180663; Year 2/Quarter 9 Report

W81XWH-19-1-0522

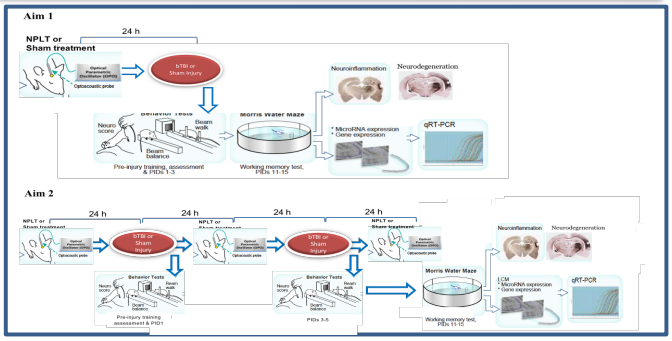
PI: Maria-Adelaide Micci **Org:** University of Texas Medical Branch, Galveston **Award Amount:** $552,721.00 ($350,000 direct cost)

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 –We have performed histopathological and biochemical analyses as detailed in Aims 1 and 2 of the proposal.

Timeline and Cost

Activities CY	19	20	21	22
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**	**$87,000**	**$13,000**

Updated: July 26, 2022

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: $350,000; Actual Expenditure: $350,000