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TITLE: Targeting Epigenetic Mechanisms in Pain due to Trauma and Traumatic Brain Injury (TBI)

PRINCIPAL INVESTIGATOR: David J. Clark, MD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Persistent pain after TBI, trauma to the extremities and in the situation where both types of injury exist is highly problematic. For example, persistent pain after surgery and other forms of soft tissue injury occurs in up to 50% of patients, and as many as 85% of those with TBI experience ongoing pain. Battlefield trauma, motor vehicle accidents and sports-related injuries are particularly likely to involve TBI, peripheral trauma or both. Disability due to pain and other causes is very high amongst such patients. We have no effective approaches to reducing the likelihood of developing chronic pain after TBI or peripheral injuries, and the mechanisms supporting such pain are poorly understood. Recent advances have suggested, however, that epigenetic changes occurring in the dorsal horn of the spinal cord after either brain or peripheral trauma may support chronic pain. Our work to-date has established a rodent model of TBI in combination with injury to a limb as a model for addressing this clinical problem. We have established the severity and time course of pain-related changes after TBI and incision. Critically, we have demonstrated that histone deacetylase inhibitors greatly exacerbate the pain problems while agents that block histone acetylation reduce the pain-related changes. Additional evidence suggests that changes in the levels of genes in the spinal cord along with brain-level changes after TBI may be responsible. These observations suggest novel approaches to treatment.

15. SUBJECT TERMS
Traumatic Brain Injury, Chronic Pain, Epigenetic, Chemokine, Disability, Analgesia, Spinal Cord

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1. **INTRODUCTION:**

Persistent pain after TBI, trauma to the extremities and in the situation where both types of injury exist is highly problematic. For example, persistent pain after surgery and other forms of soft tissue injury occurs in up to 50% of patients, and as many as 85% of those with TBI experience ongoing pain. Battlefield trauma, motor vehicle accidents and sports-related injuries are particularly likely to involve TBI, peripheral trauma or both. Disability due to pain and other causes is very high amongst such patients. We have no effective approaches to reducing the likelihood of developing chronic pain after TBI or peripheral injuries, and the mechanisms supporting such pain are poorly understood. Recent advances have suggested, however, that epigenetic changes occurring in the dorsal horn of the spinal cord after either brain or peripheral trauma may support chronic pain. Specifically, the acetylation of histone proteins with spinal cord dorsal horn neurons leads to the sustained up-regulation of pain-related chemokine receptor CXCR2 thereby supporting chronic pain. The objective of this project is to define the role of agents targeting epigenetic mechanisms in reducing pain and disability after trauma, particularly in the setting of TBI. This objective is closely in alignment with the pain management focus area of the CRMRP Neurosensory Research Award program. Specifically, these studies involve, 1) applied research on alternative non-opioid analgesic drugs, 2) strategies for management of acute and chronic pain under the care of a clinician in non-deployed settings (specifically in patients with TBI), and 3) research studies to evaluate novel analgesics and mechanisms of pain in relevant animal models. At the completion of the proposed studies we will have addressed our project's main objective using multiple approaches. We will have a refined mechanistic understanding of how tissue trauma, TBI and the combination lead to the experience of chronic pain. We will also have preclinically evaluated the complementary approaches of using HAT or chemokine signaling inhibition to reduce chronic pain and disability after TBI and soft tissue trauma.

2. **KEYWORDS:** Traumatic Brain Injury, Chronic Pain, Epigenetic, Chemokine, Disability, Analgesia, Spinal Cord

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The approved project was accompanied by a Gantt chart listing major specific tasks (ST's). The headings below refer to those tasks. The following summary of major goals reflects the status of the project to-date.

Specific Aim 1: To evaluate the hypothesis that histone acetyl transferase (HAT) inhibitors reduce pain and disability after surgical incision, TBI and the combination of the two injuries.

Major Task 1 (Pre-experimental animal approval)

ST1.1 Local IACUC Approval:
Complete
ST1.2 DoD ACURO Approval:
Complete

Major Task 2: Establish the roles of HAT inhibitors on simple measures of nociception after incision and TBI

ST2.1 Measure effects of HAT inhibitors on nociceptive sensitization after incision
Complete
ST2.2 Measure effects of HAT inhibitors on nociceptive sensitization in TBI model
Complete
ST2.3 Measure effects of HAT inhibitors on nociceptive sensitization after incision and TBI
Complete

Major Task 3: Establish the roles of HAT inhibitors on more complex pain and functional measures as well as the efficacy of oral preparations of HAT inhibitors after incision and TBI

ST3.1 Measure effects of HAT inhibitors on complex pain behaviors (CPP, PGE2) and gait changes after incision and TBI.
30% Complete

ST3.3 Measure the efficacy of curcumin on pain, cognitive and mood in a mouse closed-head model of TBI +/- limb fracture.
50% Complete

Specific Aim 2: To evaluate the hypothesis that HAT inhibitors block incision-related epigenetic histone acetylation in control and TBI model animals thereby normalizing expression of key pain-related genes

Major Task 4: Identify the type and cellular location of epigenetic changes in spinal cord tissue after incision and TBI, and the relationship of those changes to CXCR2 expression

ST4.1 Establish spinal cord sites and cell types displaying enhanced histone acetylation (AcH3K9) in the settings of incision/TBI.
Complete

ST4.2 Establish spinal cord sites and cell types displaying enhanced CXCR2 expression and co-localization with histone acetylation in the settings of incision/TBI
Complete

Major Task 5: Identify changes in spinal cord HAT activity and the consequences of those changes in terms of reducing pain and functional impairment after incision and TBI

ST5.1 Measure changes in spinal cord HAT activity hypothesized to be caused by hindpaw incision, TBI or the combination. Determine if blockade of HAT activity reduces incision, TBI and incision/TBI-induced increases in the expression of spinal cord CXCR2
50% Complete

Major Task 6: Examine specifically the efficacy of a selective CXCR2 antagonist in reducing pain and functional impairment after incision and TBI

ST6.1 Determine the efficacy of selective CXCR2 antagonists in reducing pain-related behaviors in the incisional, TBI and combination models
75% Complete

What was accomplished under these goals?

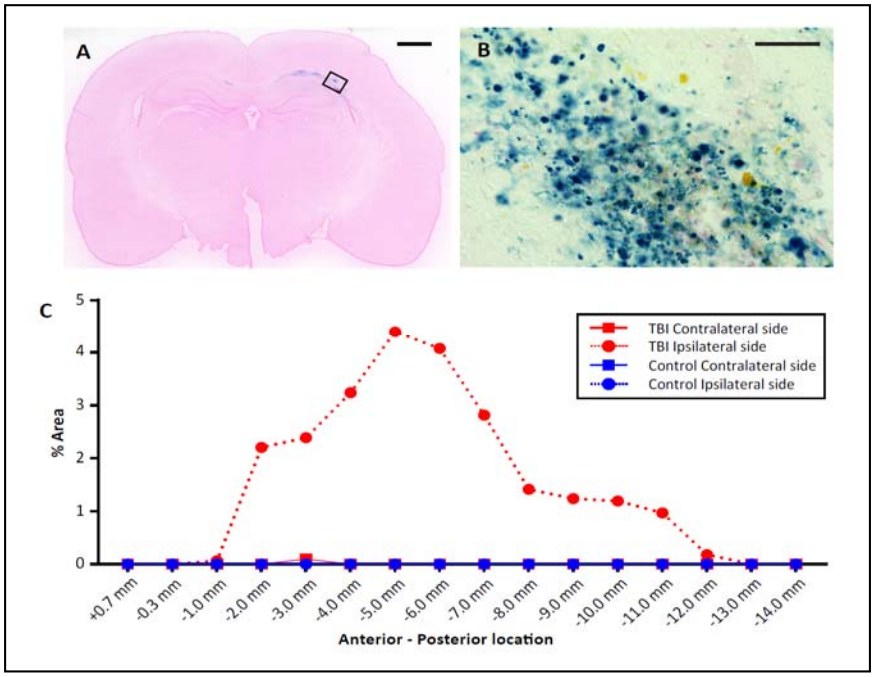
The following section of the report focuses on the accomplishments of the previous year including the outcome of studies presented as partially complete in the previous annual report. Major findings are presented in graphical form with descriptions of additional accomplishments in text format.

Rat TBI model:

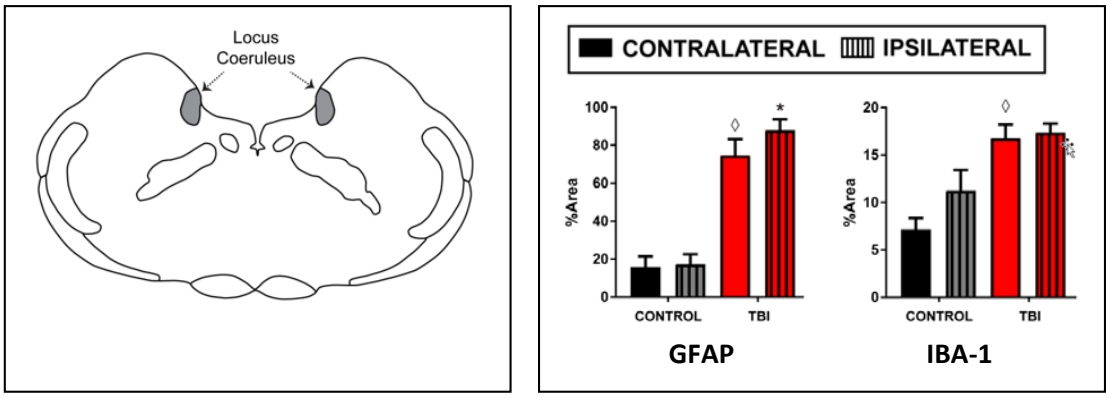
(The following studies support Specific Aim 1, Major Task 2)

We greatly improved our understanding of the damage to the brain done in our rat lateral fluid percussion (LFP) model. It was made clear by discussions with experts in the field and comments from manuscript review that a more complete neuropathological characterization of the LFP model with focus on pain centers was needed. We therefore harvested a set of tissues from experimental rats and conducted histological and immunohistochemical analysis to document the extent of injury and the effects of a candidate HAT inhibitor anacardic acid as outlined in the approved application. Components of the following findings have been included in presentations and our first manuscript now accepted for publication.

In addition to collection of Neurological Severity Scores (NSS) previously reported, staining of rat brain sections for hemosiderin (microbleeding), IgG (BBB breakdown) and amyloid precursor protein (APP) were completed. These indicate that significant ipsilateral>contralateral damage occurs in the LFP model. However, the damage was characterized as mild in nature, and largely localized to areas near the point of cortical impact. Additional analyses included an analysis of the effects of anacardic acid on these neuropathological changes. It was not observed that the anacardic acid treatments blocked the TBI-related histological changes. The results for hemosiderin staining are provided below.

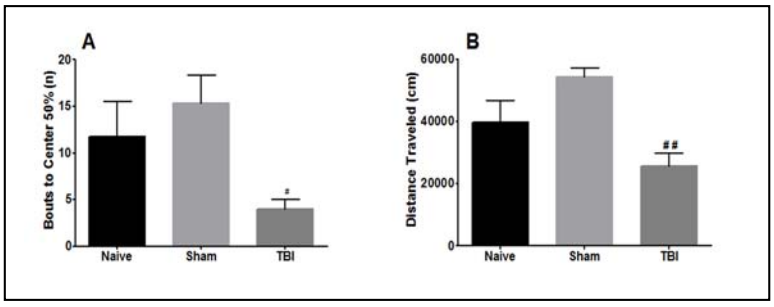


We also looked for evidence of neuroinflammation in centers important in the descending modulation of nociception as well as the cortex and thalamus. This involved staining for GFAP (activated astrocytes) and IBA-1 (activated microglia). Evidence for activation in many of these centers was found 7 days post-injury when nociceptive sensitization was strong as shown below using the LC data as an example. Additional analysis showed that the administration of the HAT inhibitor anacardic acid did reduce astrocytic neuroinflammation after TBI in some centers, but had little effect on microglial activation.



(The following studies additionally support Specific Aim 1, Major Task 2)

We went on to characterize our rats in terms of measures of anxiety-like behavior including open field and zero maze testing. The LFP-TBI rats showed anxiety-like behaviors in open field (panel A) and zero maze (panel B) testing.

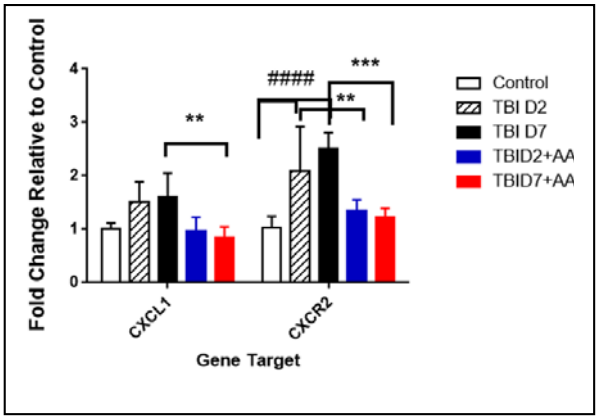


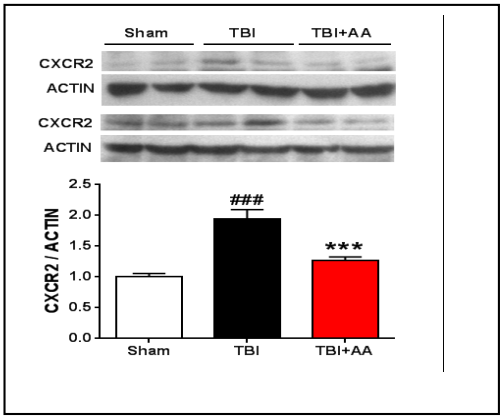
(The following studies support Specific Aim 1, Major Task 3)

The injection of PGE2 into the hindpaws of rats after resolution of allodynia caused enhanced behaviors in TBI rats in comparison to sham TBI rats. This indicates that there is a latent nociceptive sensitization in TBI animals suggesting persistent changes in the CNS.

(The following studies support Specific Aim 2, Major Tasks 4 and 5)

While better characterizing our LFP model, we conducted CXCR2 expression studies on lumbar spinal cord tissue contralateral to the side of TBI (more sensitized). We found that CXCR2 was expressed at higher levels after TBI, and that the histone acetyltransferase (HAT) inhibitor arachidonic acid blocked that expression 2 and 7 days after TBI. These results were obtained for both mRNA and protein measurements as shown below.

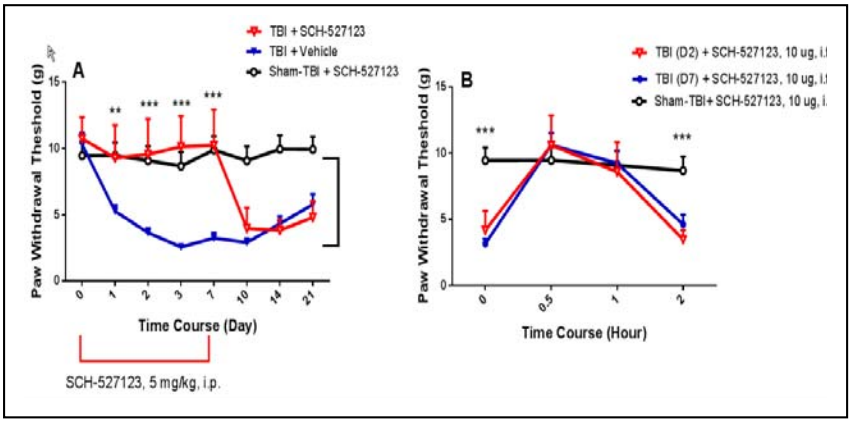




Additional findings include the identification of genes such as *BDNF* and *PDYN* that are up-regulated by TBI, but not altered by HAT inhibition. Thus it appears that CXCR2 may be a unique and viable target to provide pain relief in patients with histories of TBI.

(The following studies support Specific Aim 2, Major Task 6)

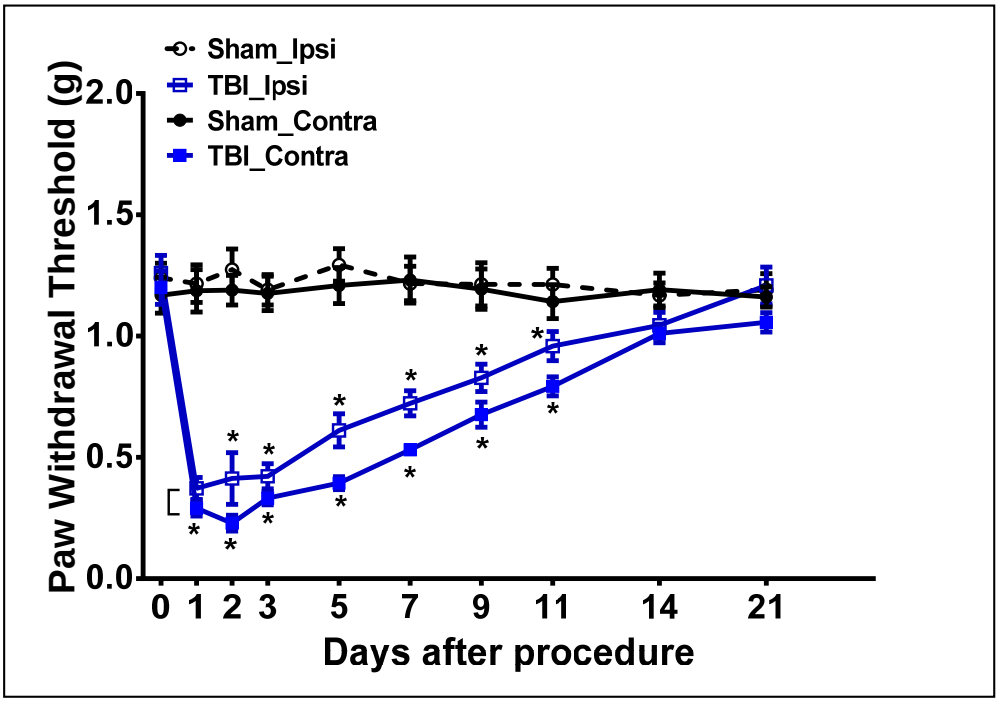
A core portion of the project involves the determination of the effects of CXCR2 antagonists on nociceptive sensitization and other phenotypes after TBI. To that end we have administered the selective CXCR2 molecule SCH-527123 both systemically and intrathecally to the TBI mice. This drug has been used in human trials and may therefore be a good candidate for translation. As can be seen below, the drug was efficacious in reducing mechanical allodynia in rats after TBI when administered by both routes. Additional dose-response studies are ongoing.



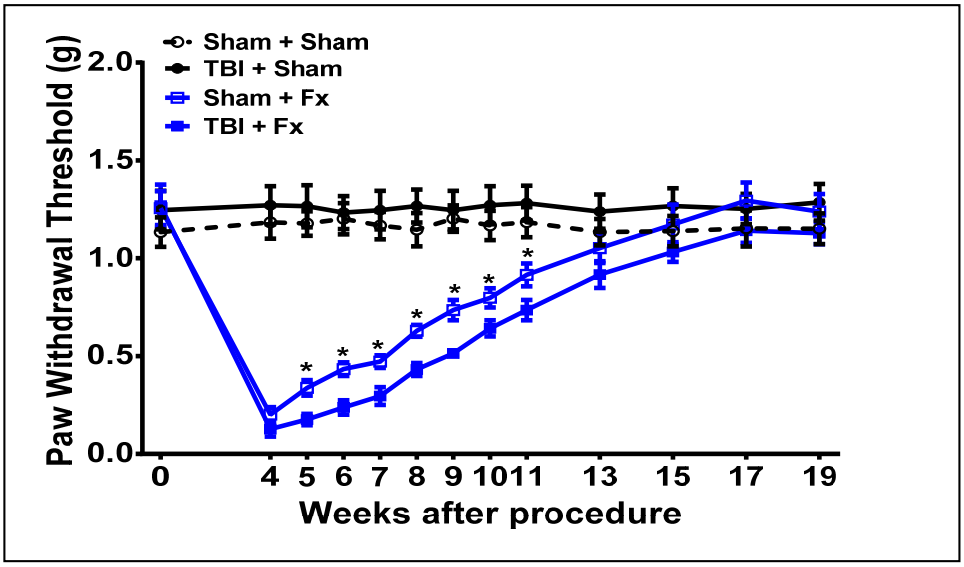
Mouse TBI model:

(The following studies support Specific Aim 1, Major Task 3)

A project modification approved by the DoD, ACURO and our local committees during the course of the past year was the addition of a mouse polytrauma model to the project. This allowed the much more rapid collection of data using a model with enhanced relevance to the TBI/polytrauma population. This model uses closed head TBI and, in some mice, tibial fracture. First, we characterized nociceptive sensitization in mice after TBI. The results presented below are from mice in limbs both ipsi- and contralateral to TBI. TBI was induced using a single impact to skull with impactor velocity 5.8 m/s, duration 200 ms. As can be seen in the figure, nociceptive sensitization is induced for about 2 weeks. As can be noted from the data in the figure, mice rapidly develop mechanical allodynia. The allodynia is slightly more robust in the limb contralateral to TBI, and sensitization resolves over about 2 weeks. This, then, is a viable model for the study of TBI-induced pain sensitization.

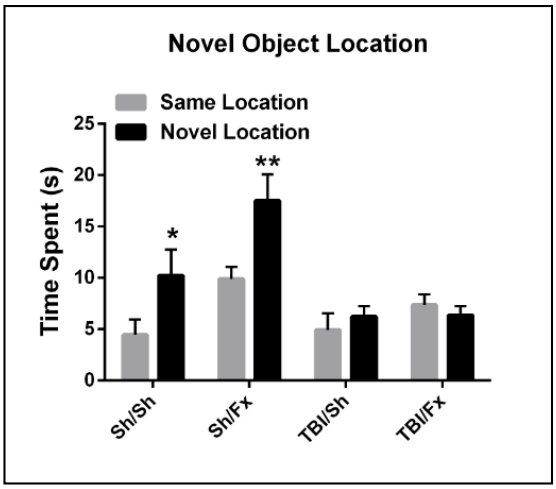


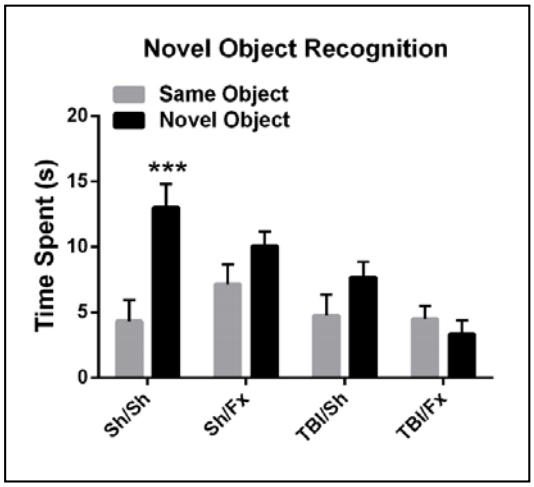
We went on to examine pain-related responses in animals having tibia fracture with or without TBI. TBI worsened this pain-related outcome. The data are provided in the figure below. Note limb fracture was accomplished within 18 hours of TBI and that all fracture animals had the fracture limb cast immobilized for three weeks before nociceptive measurements took place. These data show that we have a viable model of polytrauma which was the goal of the effort.



(The following studies additionally support Specific Aim 1, Major Task 3)

We have explored the interaction of TBI and fracture with memory in the mouse polytrauma model. We have found that TBI further impacts spatial memory when added to limb fracture as suggested in the novel object location task shown below. On the other hand, non-spatial memory is diminished after fracture, and no additional effects of TBI could therefore be detected. Additional behavioral measurements suggest that the mice with TBI display a hyperactivity phenotype after TBI similar to some patients, and that TBI + fracture leads to the most severe phenotype.





Challenges – While our work has generally gone smoothly, we have been challenged in one area related to **Specific Aim 1, major Tasks 2 and 3**. We have needed to move beyond the consideration of TBI interacting with peripheral trauma using just the rat hindpaw incisional model. There was little additional nociceptive sensitization found when incisions were made in TBI rats at the time of TBI. Furthermore, the use of HAT inhibitors was less effective when TBI and incision were performed in rats in comparison with either lesion alone. These results are included in our accepted manuscript listed in the appropriate section below. This, however, was one of the reasons for bringing in the mouse model, and the mouse model is working very well in showing interactions between TBI and limb injuries.

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

The project was not generally designed to provide professional development opportunities. However, the learning of new methods and familiarization of staff with a new area of science and medicine does represent a benefit of the work completed.

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

The major work left to be done on the project falls under specific tasks (from the Statement of Work) 5.1, 6.1 and 6.2.

ST5.1 - Measure changes in spinal cord HAT activity hypothesized to be caused by hindpaw incision, TBI or the combination. Determine if blockade of HAT activity reduces incision, TBI and incision/TBI-induced increases in the expression of spinal cord CXCR2.

We have collected and are processing samples from spinal cord that will be used to screen for changed expression of known HAT and HDAC enzymes known to affect histone acetylation. With these data we will proceed to protein measurements and HAT enzyme activity experiments.

ST6.1 - Determine the efficacy of selective CXCR2 antagonists in reducing pain-related behaviors in the fracture, TBI and combination models.

We have excellent data using systemic and intrathecal CXCR2 antagonists, but we plan to use oral preparations of these drugs and establish dose-response relationships as these will be pharmacologically useful and make the work more rigorous. We will also use the drugs in tests of anxiety and gait as these are involved in polytrauma.

ST6.2 - Determine the efficacy of selective CXCR2 antagonists in reducing pain-related behaviors in the mouse TBI and combination fracture/TBI mice.

We will test the same antagonists found to be effective in the rat model in the more polytrauma-relevant mouse model. Nociception, anxiety and gait studies will be the main focus

4. **IMPACT:**

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

Prior to this time there was very little understanding of the mechanisms linking TBI to pain, a major cause of disability after TBI. There was no explanation for why pain might be worse at sights distant from the head after TBI. This constitutes a fundamental contribution to the discipline.

What was the impact on other disciplines?

The field of pain research had very little information to this point explaining how injury to the CNS could result in pain. Here we have demonstrated that CNS injury in the form of TBI leads to fundamental changes in spinal nociceptive processing. This is a novel idea for a related set of disciplines, and helps to explain pain in other types of CNS injury and possibly neurodegenerative disease.

What was the impact on technology transfer?

It is possible that CXCR2 antagonists could be repurposed for use as analgesics after TBI.

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

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:**

Publications, conference papers, and presentations (Past 1 year)
Journal publications

1. Epigenetic Regulation of Chronic Pain after Traumatic Brain Injury. De-Yong Liang, Peyman Sahbaie, Karen-Amanda Irvine, Yuan Sun, Xiaoyou Shi, Anders Meidahl, Tian-Zhi Guo, Peng Liu, David C. Yeomans and J. David Clark. *IBRO Reports* (In Press).

2. Chronic Pain after Traumatic Brain Injury: Pathophysiology and Pain Mechanisms. Karen-Amanda Irvine and J. David Clark. *British Journal of Anesthesia* (Submitted).

Other publications, conference papers, and presentations

1. Epigenetic Mechanisms and TBI. De-Yong Liang, Peyman Sahbaie, Yuan Sun, Xiaoyou Shi, Anders Meidahl, Tian-Zhi Guo, Peng Liu, David C. Yeomans and J. David Clark. VA Palo Alto TBI Research Symposium. Palo Alto, CA, March, 2016.
2. Traumatic Brian Injury (TBI) and Pain. J. David Clark. Wake Forrest University Pain Research Symposium. Winston-Salem, NC, August, 2016.
3. Epigenetic Mechanisms and TBI. De-Yong Liang, Peyman Sahbaie, Yuan Sun, Xiaoyou Shi, Anders Meidahl, Tian-Zhi Guo, Peng Liu, David C. Yeomans and J. David Clark. Stanford University Department of Anesthesiology Annual Awards Dinner. Palo Alto, CA, June, 2016.
4. Epigenetic Regulation of Chronic Pain after Traumatic Brain Injury. De-Yong Liang, Peyman Sahbaie, Karen-Amanda Irvine, Yuan Sun, Xiaoyou Shi, Anders Meidahl, Tian-Zhi Guo, Peng Liu, David C. Yeomans and J. David Clark. International Association for the Study of Pain Congress. Yokohama, Japan, September, 2106

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: David J. Clark
Project Role: PI
Annualized calendar months: 2
Contribution to Project: This person is the project PI and administratively oversaw the completion of the regulatory requirements, the purchase of equipment, and the initiation of experimentation.

Name: David C. Yeomans
Project Role: Co-I
Annualized calendar months: 1

Contribution to Project: This person co-directs the experimentation. He reviews the progress of the experiments, provides scientific input and trouble-shoots scientific and technical issues.

Name: Deyong Liang
Project Role: Investigator
Annualized calendar months: 9
Contribution to Project: This person conducts the majority of the rat experimentation. He has performed the TBI surgeries as well as the incisional model. He orders the animals and plans experiments. He processes and presents the data generated.

Name: Peyman Sahbaie
Project Role: Research Associate
Annualized calendar months: 6
Contribution to Project: This person led the effort to acquire and set-up the TBI device. He is responsible for a portion of the animal testing, and will perform a portion of the surgeries.

Name: Karen-Amanda Ferguson
Project Role: Research Associate
Annualized calendar months: 4
Contribution to Project: This person has completed all of the neuropathological and immunohistochemical studies that are a part of this study. In addition, she will in the coming year take on the more complex animal testing protocols.

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

The PI, Dr. Clark has received a new award from the NIH:

R01 NS094438 PIs Clark, Kingery 9/1/16-8/31/17 1.8 CM
NIH/NINDS
Neural Immunoregulation of Post-Traumatic Autoimmunity

The major goals of this projects are to: 1) to map post-fracture changes in dendritic cell antigen recruitment, maturation, trafficking and adaptive immune responses in skin, lymph nodes, and sciatic nerve, and spinal cord, 2) to determine whether passive-transfer autoimmunity occurs when immunoglobulin obtained from the fracture mice or from CRPS patients is injected into other mice, potentially rekindling CRPS-like sequelae in post-fracture mice with resolving CRPS symptoms, and in addition, to use mouse and CRPS patient antibodies to identify regionally restricted autoantigens fracture mouse skin, nerve, cord, and fracture callus, and in CRPS patient skin, and 3) to determine whether sensory neuropeptide or sympathetic adrenergic signaling is required for the development of post-traumatic autoimmune responses.

- **What other organizations were involved as partners?**

Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

QUAD CHARTS: See Attached

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. ***DO NOT RENUMBER PAGES IN THE APPENDICES.***