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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?

*Please note that this report is focused on the completion of the project under a no-cost extension (NCE). The overall goals and accomplishments of the project will be reviewed with special emphasis on the results of work completed during the past 12 months of NCE support.

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, tibia fracture and TBI.
Complete

ST2.4 Test effective doses of drugs in open field and rotarod paradigms.
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.
60% Complete

ST3.2 Measure the potency and efficacy of oral preparations of curcumin in pain and functional outcomes after incision and TBI.
Incomplete

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

ST4.3 Establish the role of descending facilitation in supporting nociceptive and spinal molecular changes after TBI+limb fracture in mice and rats.

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.

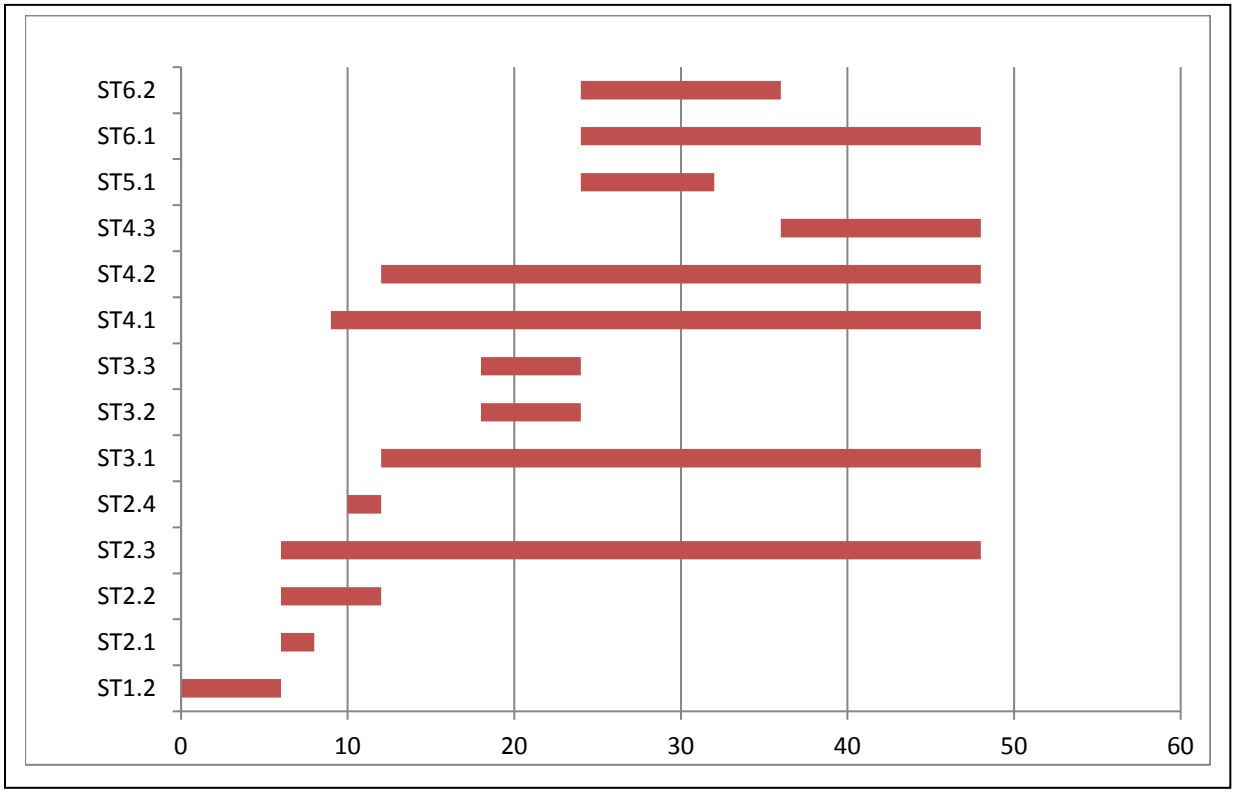
ST5.1 Measure changes in spinal cord HAT activity hypothesized to be caused by hindpaw incision, TBI or the combination. Determine if blockade of HAT activity reduces incision, TBI and incision/TBI-induced increases in the expression of spinal cord CXCR2.
Complete

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.
Complete

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

Gantt chart for project. Note the Y-axis lists the subtasks, and the X-axis displays the project months. This is the approved Gnatt chart for the project under the NCE.



What was accomplished under these goals

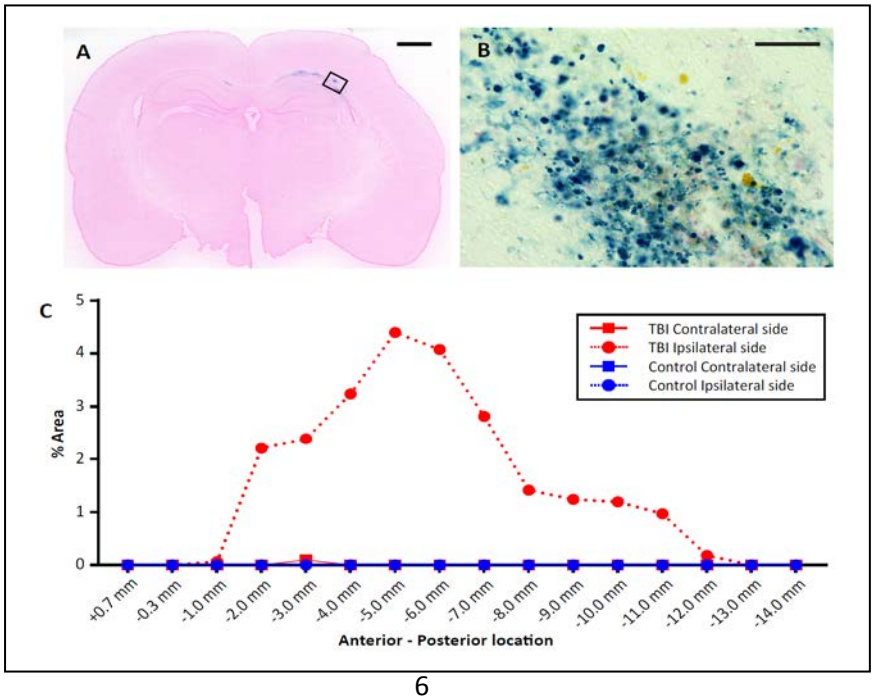
The following section of the report focuses on the accomplishments of the studies including the three scheduled years and the no-cost extension year. During this time there were 5 full-length publications and several poster presentations and conference lectures. Furthermore, the work has allowed the group to continue investigations in this field by making us competitive for VA awards and perhaps future DoD opportunities. Major findings are presented in graphical form with descriptions of additional accomplishments in text format. Annotations are made to the SOW and publications where relevant.

Rat TBI model:

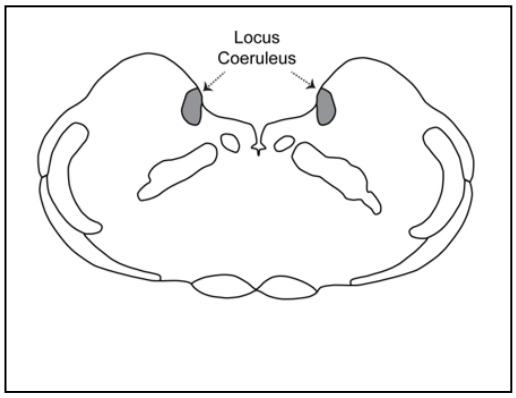
(The following studies support Specific Aim 1, Major Task 2)

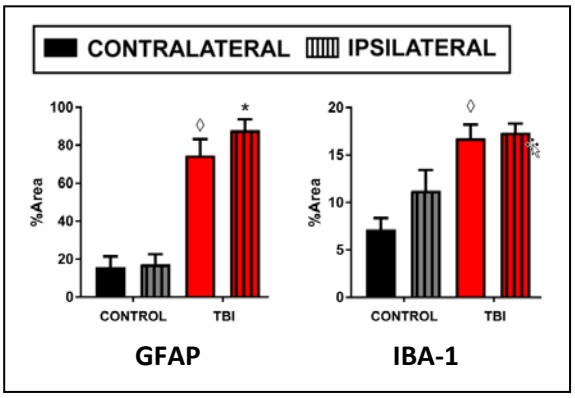
We greatly improved our understanding of the damage to the brain done in our rat lateral fluid percussion (LFP) model. It was made clear by discussions with experts in the field and comments from manuscript review that a more complete neuropathological characterization of the LFP model with focus on 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 additional manuscripts: Irvine and Clark, Pain Medicine 2018; Irvine et al., J. Neurotrauma, 2018.

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, and inhibitor of the epigenetic process of histone acetylation, 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.

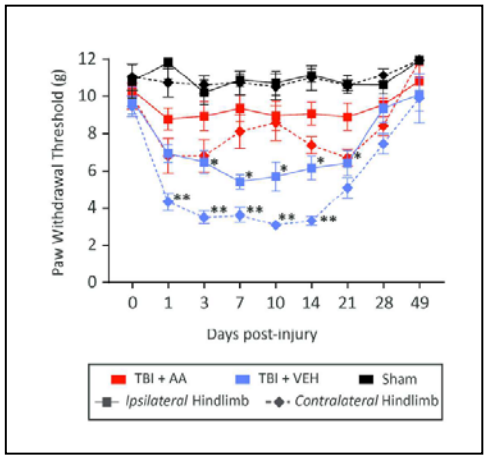




(The following studies support Specific Aim 1, Major Task 2)

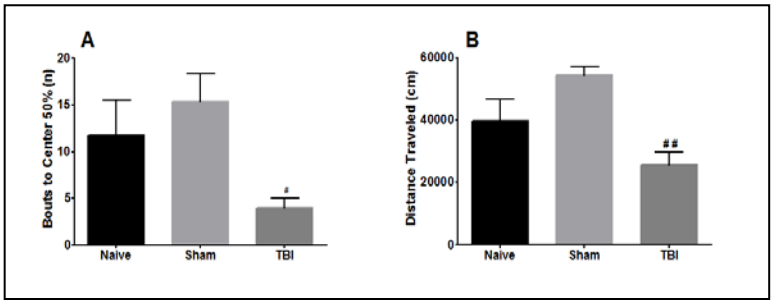
A major goal of the project involves the use of the rat LFP TBI model in which to study the effects of TBI on pain-related behaviors as well as the effects of the histone acetyltransferase (HAT) inhibitor anacardic acid (AA). Histochemical and immunohistochemical staining was used to examine the structural underpinnings of the effects. In addition, we looked at effects both 7 days after TBI when AA effects were maximal, and 28 days when allodynia resolves in TBI animals but latent sensitization remains suggesting more persistent changes in CNS functioning after TBI. The studies described below have been submitted for publication (Ferguson et al., 2017). A few of the key results are presented and discussed below.

To clearly demonstrate the effects of AA on pain sensitization, we gave AA to rats beginning immediately after TBI and continuing once daily for 7 days. Vehicle was given to other animals, and sham controls were used. The results presented below demonstrate that AA substantially prevented mechanical pain sensitization for the first several weeks after injury.



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

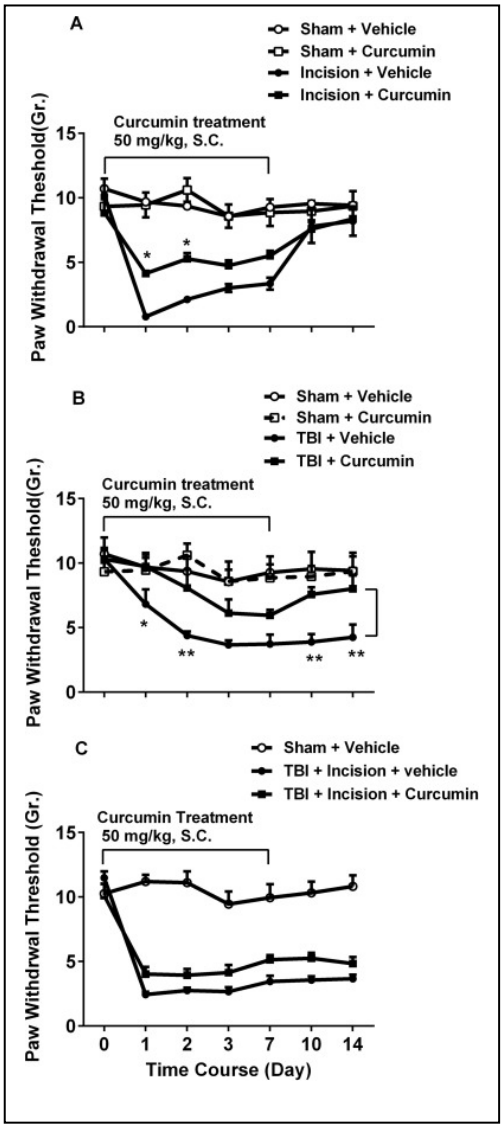
We went on to characterize our rats in terms of measures of anxiety-like behavior including open field and zero maze testing. The LFP-TBI rats showed anxiety-like behaviors in open field (panel A) and zero maze (panel B) testing. Scored for video recorded depression showed smaller changes.



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

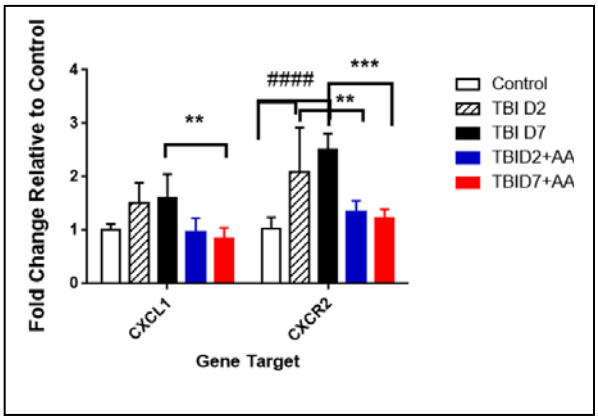
The injection of PGE2 into the hindpaws of rats after resolution of allodynia caused enhanced behaviors in TBI rats in comparison to sham TBI rats. This indicates that there is a latent nociceptive sensitization in TBI animals suggesting persistent changes in the CNS. These results helped to bolster our investigations into spinal gene expression changes outlined in the sections below.

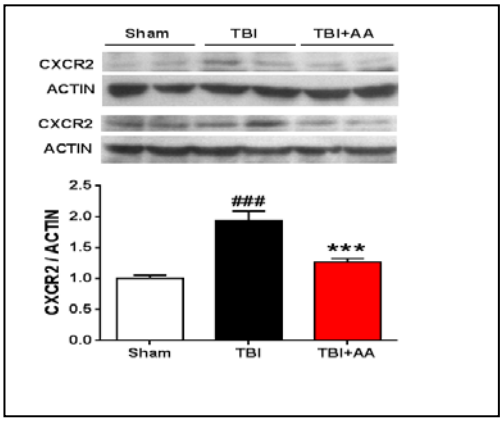
Also following the major goals for the application, we completed studies looking at the ability of additional medications altering epigenetic histone acetylation to change the histopathological and pain-related outcomes from rat LFP TBI. These results were published in 2017 (Liang et al., IBRO Reports, 2017). One of the key observations was that the HAT inhibitor curcumin did reduce histone acetylation in injured tissues and spinal cord after TBI, and resulted in less pain-related behaviors as shown in the figure below. Curcumin (50 mg/kg) was injected subcutaneously daily from day 0 to day 7 beginning immediately after TBI injuries. Nociceptive sensitivity was assessed using von Frey fibers daily prior to daily curcumin injections. These results were in keeping with the anacardic acid data. Further published observations showed that the deacetylation inhibitor SAHA prolonged pain sensitization and worsened some biochemical markers of neurotrauma in the TBI model.



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

While better characterizing our rat LFP model, we conducted CXCR2 expression studies on lumbar spinal cord tissue contralateral to the side of TBI (more sensitized). CXCR2 was selected as a spinal target as this is an epigenetically regulated gene, and preliminary data showed its spinal expression to be regulated by TBI. 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.

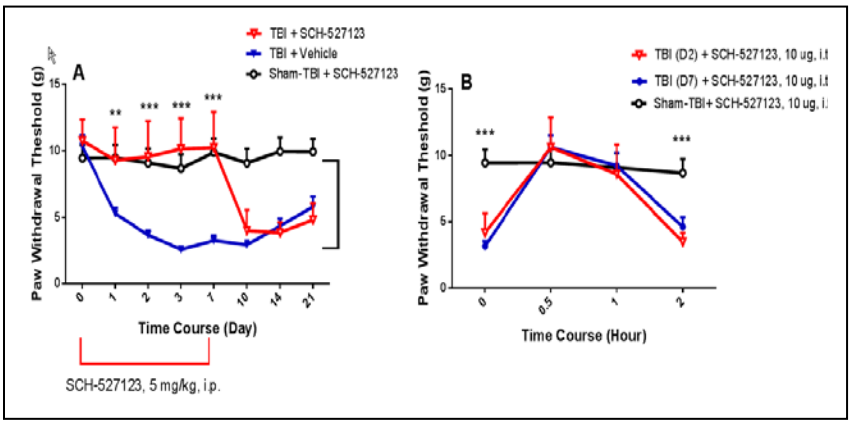




Additional findings include the identification of genes such as *BDNF* and *PDYN* that are up-regulated by TBI, but <u>not altered</u> by HAT inhibition. Thus it appears that CXCR2 may be a unique and viable target to provide pain relief in patients with histories of TBI. This helped bolster one of the study's main hypotheses that CXCR2 antagonists might be useful in reducing post-TBI pain.

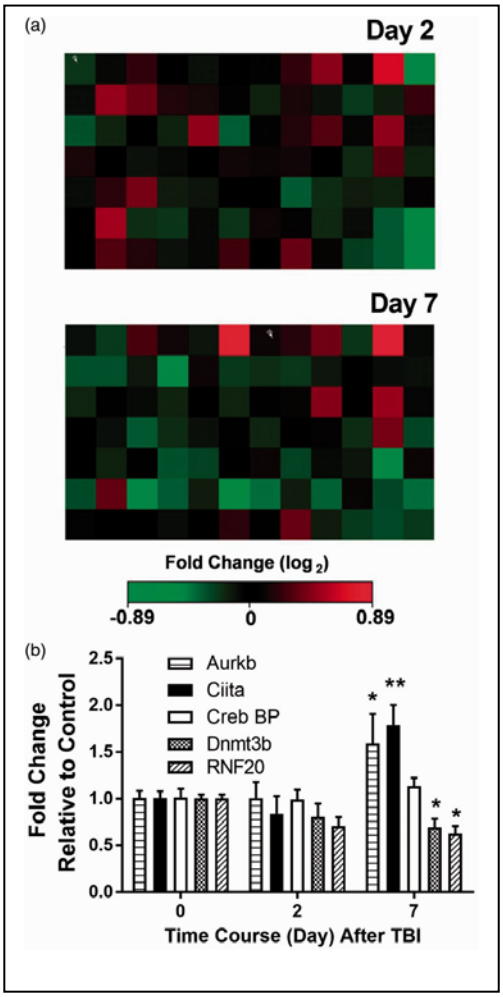
(The following studies support Specific Aim 2, Major Task 6)

A core portion of the project involved the determination of the effects of CXCR2 antagonists on nociceptive sensitization and other phenotypes after TBI. To that end we administered the selective CXCR2 molecule SCH-527123 both systemically and intrathecally to the TBI. 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. These studies were published recently, Liang et al., Molecular Pain, 2017.



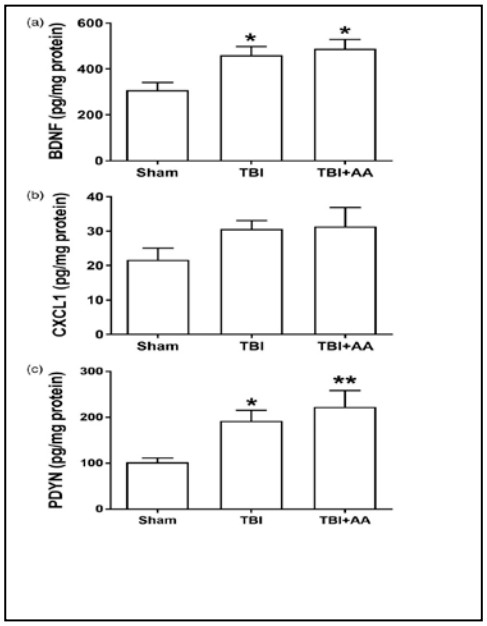
(The following studies support Specific Aim 2, Major Task 5)

In addition to the pharmacological characterization of HAT activity and effects on gene expression, an important task for the project was to identify the activation or changes in expression of epigenetically-related genes in spinal tissue after TBI. To that end we conducted a series of expression array experiments on spinal cord tissue at 2 time points after TBI. Those results showed the selective change in expression of only 4 epigenetic genes. One of the up-regulated genes, Ciita, is a known regulator of HAT activity thus providing significant mechanistic information to the project's results.



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

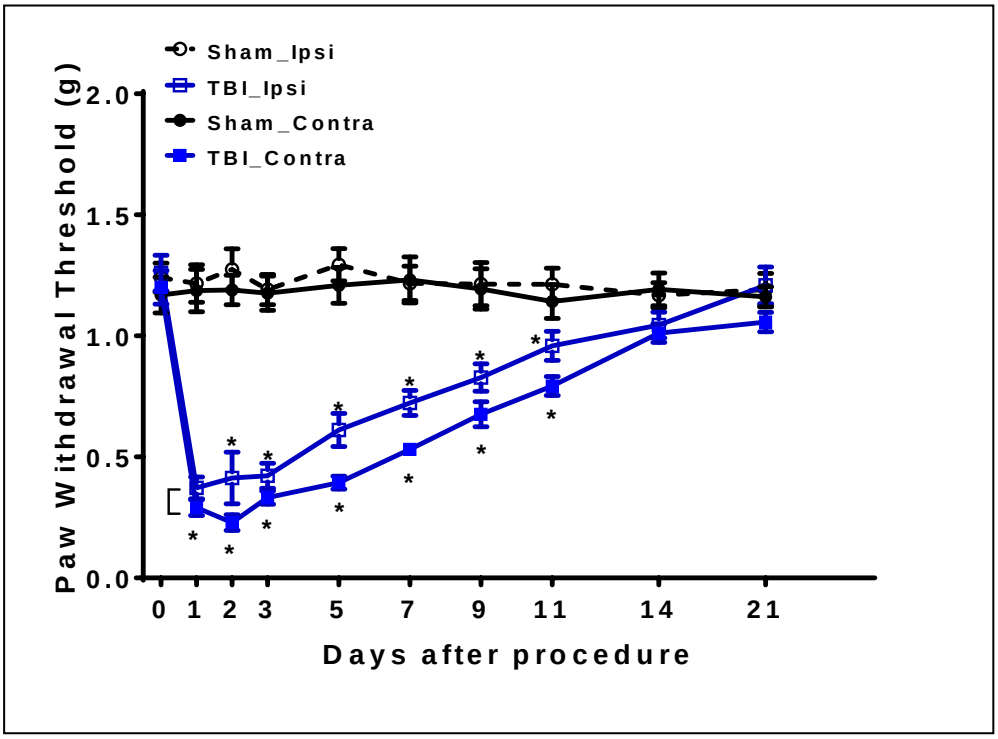
We were able to go on to demonstrate that this HAT-mediated regulation was not generalizable to all up-regulated spinal cord genes after TBI. Using ELISA assays, we showed that BDNF, CXCL1 and prodynorphin levels in spinal cord tissue were indeed elevated after TBI, but were not reduced by anacardic acid treatment as shown below.



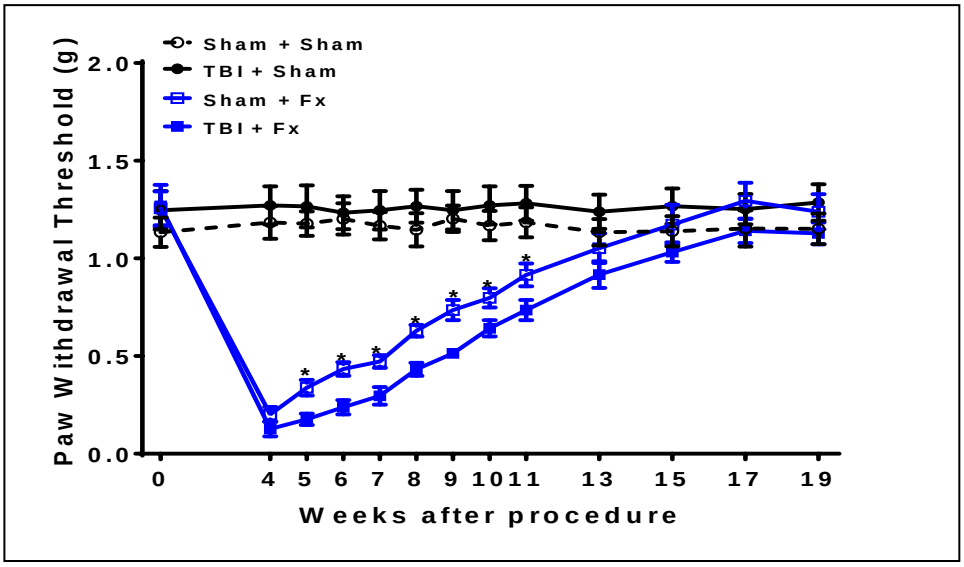
Mouse TBI model:

(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 studies 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.

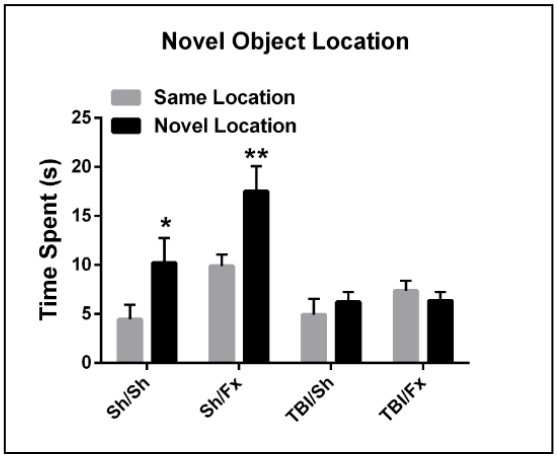


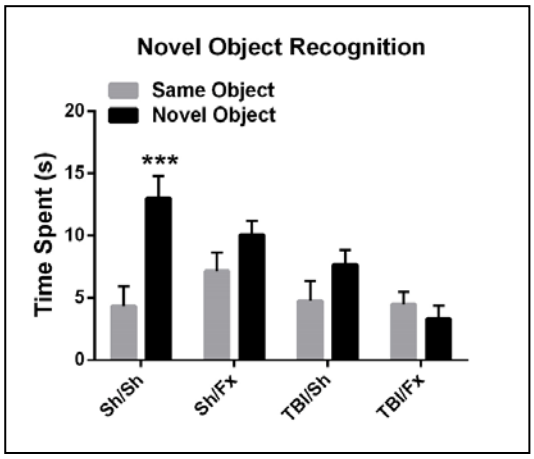
We went on to examine pain-related responses in animals having tibia fracture with or without TBI. TBI worsened this pain-related outcome. The data are provided in the figure below. Note limb fracture was accomplished within 18 hours of TBI and that all fracture animals had the fracture limb cast immobilized 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)

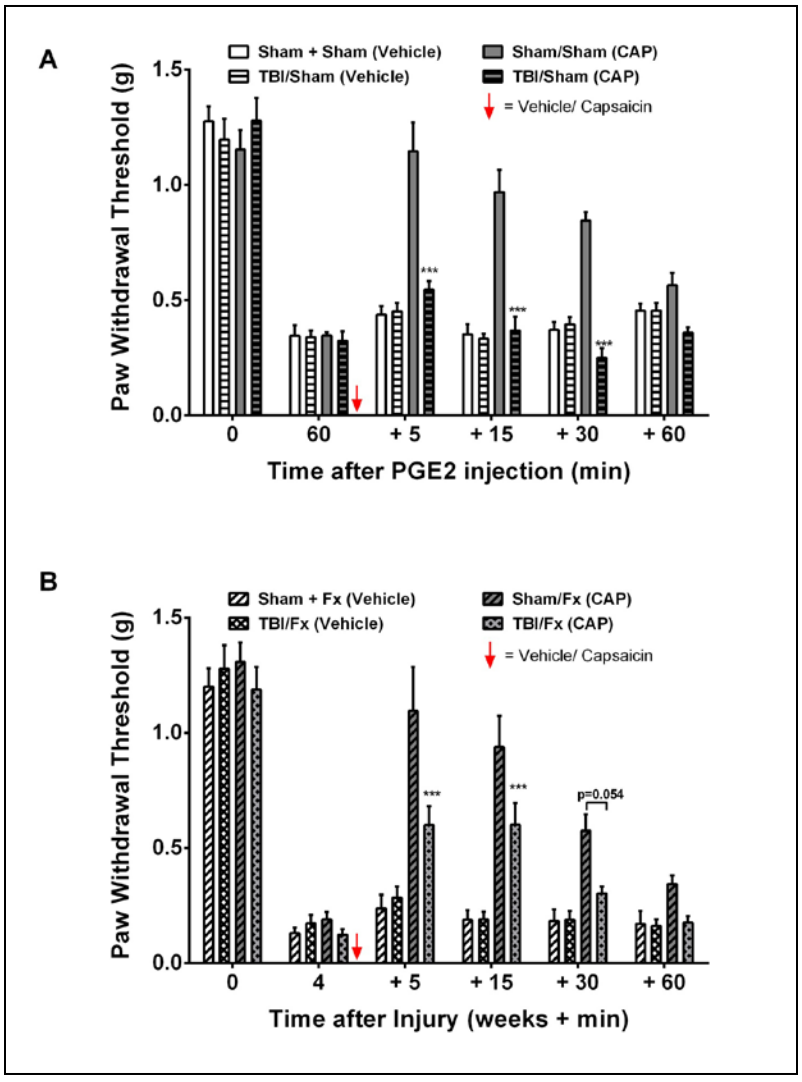
We then explored the interaction of TBI and fracture with memory in the mouse polytrauma model. We found that TBI further impacts spatial memory when added to limb fracture as suggested in the novel object location task shown below. On the other hand, non-spatial memory is diminished after fracture, 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. These and the remaining polytrauma results were published (Sahbaie et al. J. Pain, 2018).





(The following studies support Specific Aim 2, Major Task 4)

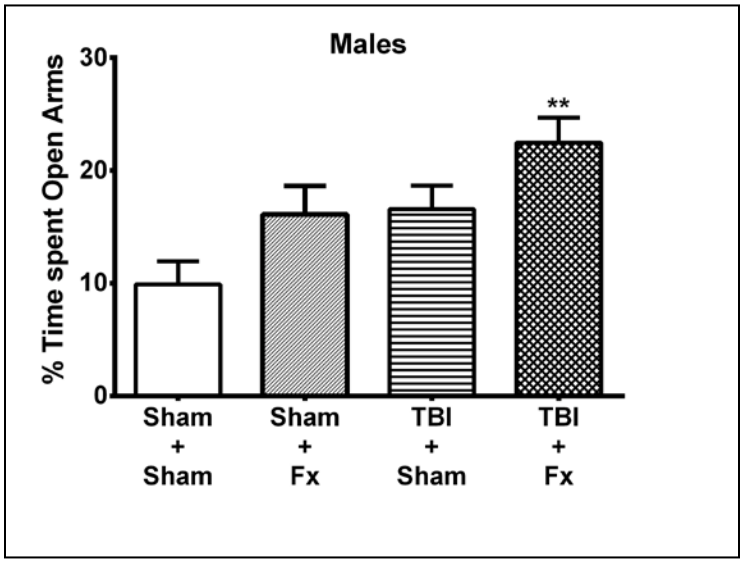
In addition to the nociceptive data, we have performed critical experimentation showing that descending inhibition, a major mechanism responsible for suppressing chronic pain implicated in TBI-related pain conditions, is almost completely lost after TBI or TBI and limb fracture. This is important information, as therapies such as with newer SSRI-class antidepressants might help patients with this particular type of deficit. In panel A below, mice having sustained mild closed head TBI have their hindpaws sensitized by the injection of PGE2. Descending inhibition is then assessed by injecting a single forepaw with capsaicin. Positive descending inhibition of pain is measured as the increase in withdrawal threshold observed in the previously sensitized hindpaw. In panel B we display data from the fracture-sensitized hindpaws of fracture-TBI and fracture alone mice. In this case, forepaw capsaicin injection reversed sensitization in the fracture alone animals, but not those with TBI. Again, this has implications for the treatment of pain in patients after polytrauma.



Mouse TBI model:

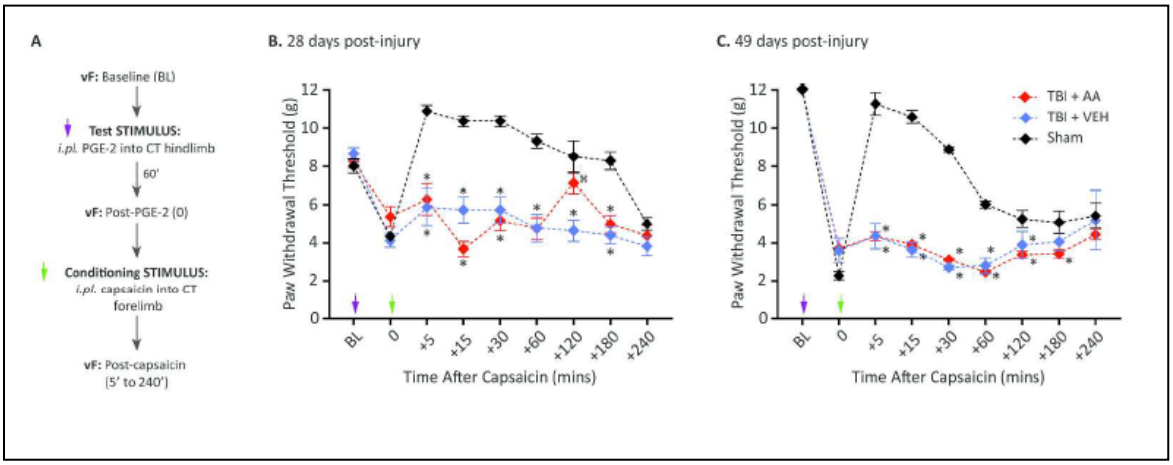
We also measured time spent in the open arms of a zero maze for male mice, as a measure of anxiety. As shown below, TBI plus fracture led to effects more serious than either injury individually.

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

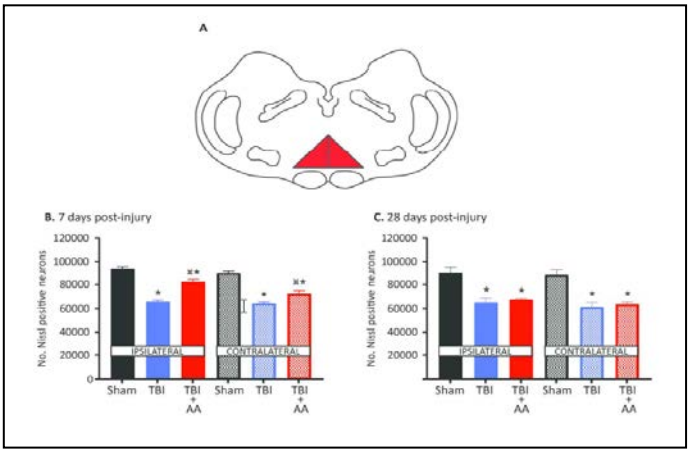


(The following studies support Specific Aim 2, Major Tasks 4)

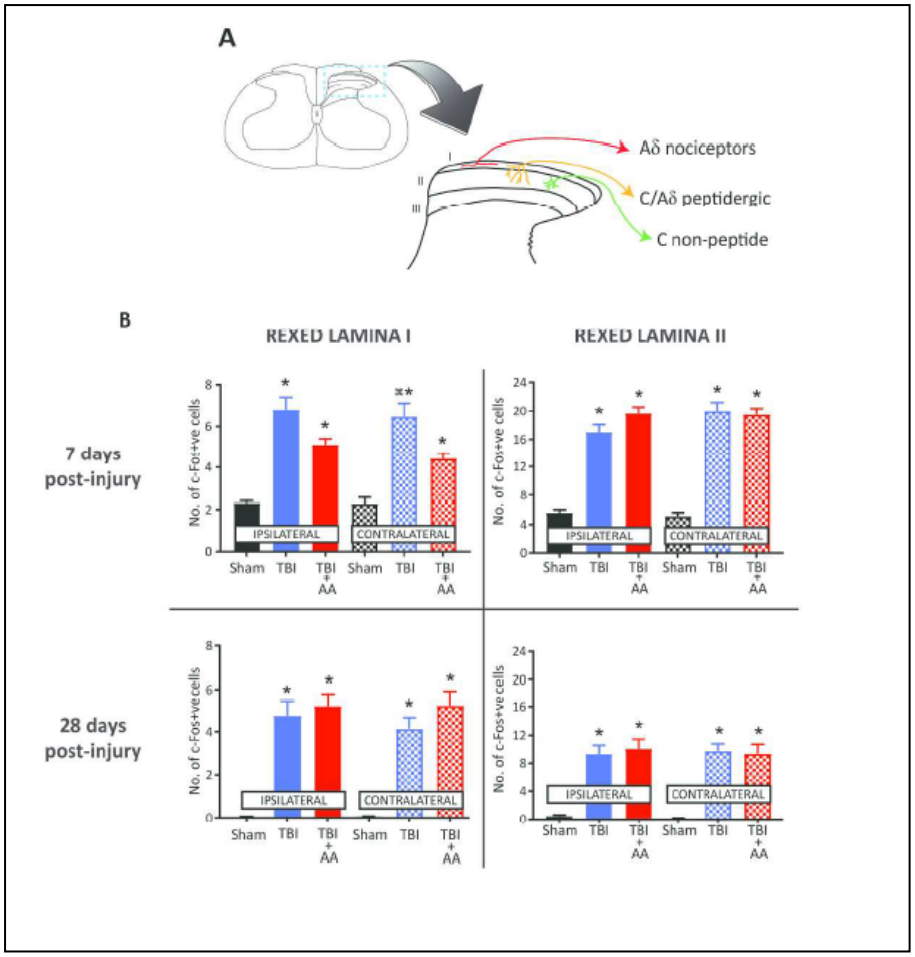
To support the case for TBI and diminished descending inhibition and to use the advantages of the rat LFP model, we pursued TBI effects on descending inhibition in this model as well. While it was clear that AA prevents much of the initial pain sensitization observed in this model (see above), we know that pain after TBI can be very problematic in its chronic form. Therefore, we measured the efficiency of descending inhibitory pain control in rats after recovery from sensitization. Deficient control of this type is strongly linked to chronic pain. The graphs below show that our protocol for measuring descending control of pain is robust in sham treated rats either 28 or 49 days from injury, but that TBI animals showed completely abolished descending pain control whether or not they were treated with AA immediately after TBI. Thus, AA is an excellent early phase analgesic, but one that cannot prevent longer term pain sensitization if given short term. Some of the following data are taken from our 2018 Irvine et al. paper in J. Neurotrauma.



To determine why we saw positive effects analgesic of AA at early time points, but no prevention of loss of descending inhibition 4 weeks after TBI, we examined the effects of AA on various measures of damage to CNS tissues at both early (7d) and late (28d) time points. These points were chosen as days on which AA was having strong effects (7d), or loss of effect (28d) after TBI. We examined cortical, subcortical, brainstem and spinal cord tissues. Below we first present data on cell loss in the RVM, a major descending regulatory control center in the brainstem that projects to the dorsal horn of the spinal cord to control the flow of nociceptive information. The results show that AA partially prevented cell loss in this center 7d after injury, but these effects were lost by 28 days.



We went on the look at changes in the expression of Fos, a marker of neuronal activation and surrogate for activation of nociceptive neurons in spinal cord tissue. As can be seen in the figure below, we again observed some effects of AA to prevent TBI-induced changes 7d after injuries, but these effects had disappeared by 28 days after injury. Together we feel that these and our other data demonstrate that the short-term suppression of HAT activity after TBI may moderate short term effects, but that long-term changes will require a more aggressive approach.

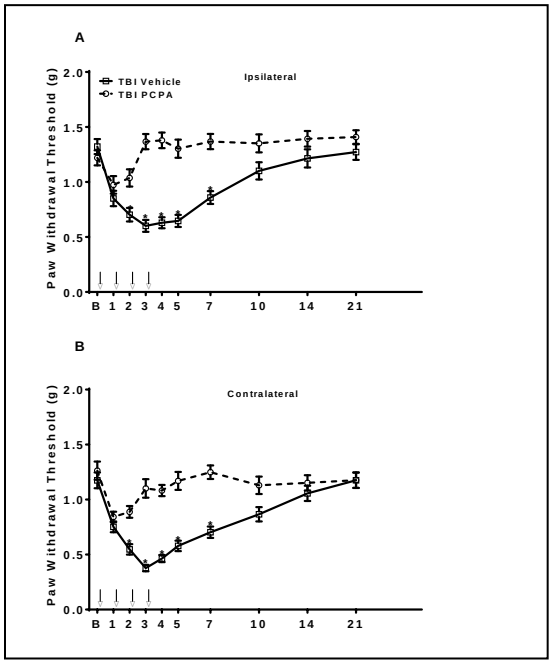


(The following studies support Specific Aim 2, Major Tasks 4 and 6)

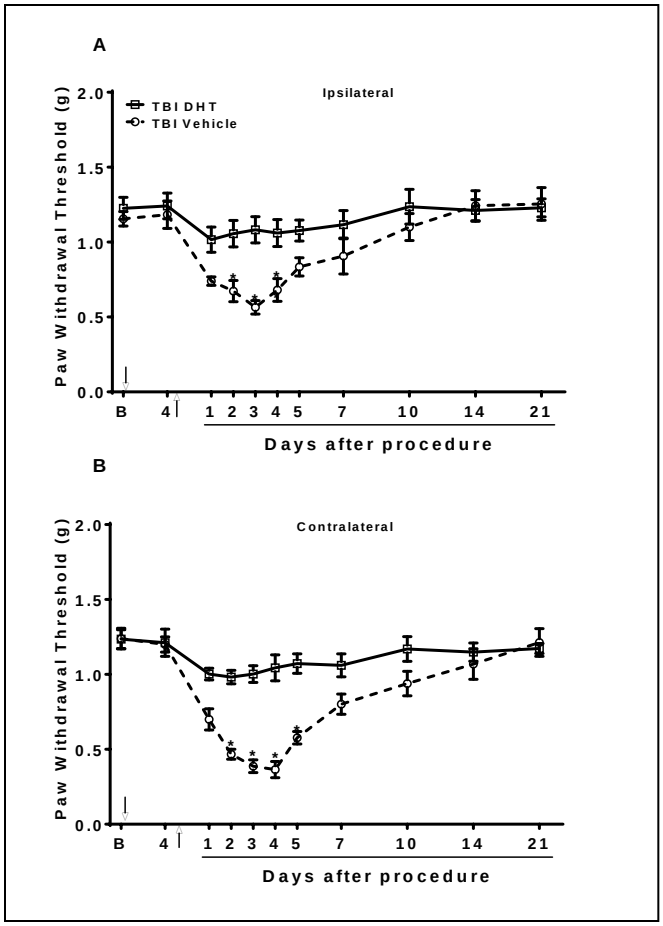
Having established that descending pain modulation functions aberrantly in mice and rats after TBI, we have gone on to examine the mechanisms potentially responsible. We have focused on the mouse TBI model as one in which we could understand the role of descending serotonergic facilitation of pain. The following are key findings from that work. In all cases the model used was the approved closed-head TBI model that causes about 2 weeks of nociceptive sensitization in mice.

Assessment of mTBI-induced mechanical hypersensitivity after systemic or spinal 5HT depletion

To assess the role of serotonin signaling in TBI-induced mechanical hypersensitivity, systemic 5-hydroxytryptamine (5-HT) depletion was initially carried out using two complementary pharmacological approaches. Once daily treatment for 4 days with p-chlorophenylalanine (PCPA) in TBI mice resulted in gradual but lasting recovery of mechanical sensitivity to baseline nociceptive threshold. Changes in sensitization contralateral to TBI appeared to be greater in magnitude than those observed ipsilateral consistent with our previous observations.



Further experiments investigated the effects of 5-HT depletion of spinal serotoninergic neurons using 5,7-dihydroxytryptamine. Mice treated with 5,7-DHT prior to TBI displayed significantly reduced mechanical hypersensitivity relative to vehicle-treated mice after TBI in both ipsilateral and contralateral limbs. Treatment with vehicle, PCPA or 5,7-DHT had no effect on basal paw withdrawal thresholds of sham mice at any timepoint after TBI. Immunohistochemical staining showed that serotonergic fibers were strongly depleted in these mice.



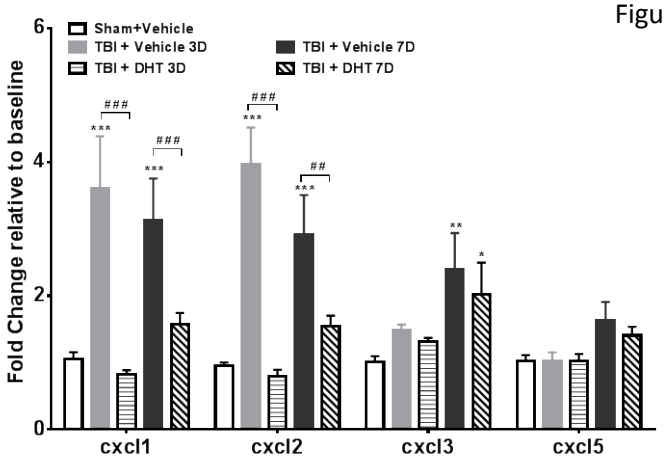
Assessment of CXCR2 ligand expression levels after mTBI and spinal 5HT depletion

As the CXC chemokine receptor 2 (CXCR2) was found to be involved in nociceptive sensitization in a model of rat TBI, we went on to determine if there were any expression changes of CXCR2 and its ligands after the mouse closed head model of mild TBI. Significant lumbar spinal CXCL1 and CXCL2 expression level increases were seen at the early (3 days) and later (7 days) time point following TBI, with some increases of the other CXCR2 ligands family members including the receptor itself (**Table 1**). Next, we looked at the effects of spinal 5HT depletion on the mRNA expression levels of those significantly increased ligands after TBI. Spinal application of 5,7-DHT prior to TBI attenuated increased CXCL1 (Day 3 and 7: $p<0.001$) and CXCL2 (Day 3: $p<0.001$, day 7: $p<0.01$) after injury compared to vehicle treated mice. No significant effect of 5HT depletion was seen for the CXCL3 expression levels at any time point after TBI compared to controls (following figure). The protein levels of the above-mentioned ligands followed gene expression patterns after serotonin depletion (data not shown), with the exception of CXCL3 at day 3 post injury where 5,7-DHT treatment reduced CXCL3 spinal levels ($p<0.05$) compared to vehicle controls.

	Time after Injury (day)		
	0	3	7
CXCL1	1.08 ± 0.16	2.74 ± 0.16***	2.42 ± 0.22**
CXCL2	1.09 ± 0.11	3.53 ± 0.67***	3.37 ± 0.61***
CXCl3	1.09 ± 0.12	2.00 ± 0.51	3.03 ± 0.37***
CXCL5	1.08 ± 0.18	1.93 ± 0.46	1.96 ± 0.24
CXCL7	1.08 ± 0.15	1.74 ± 0.35	1.82 ± 0.18
CXCR2	1.05 ± 0.09	2.02 ±0.26*	1.91 ± 0.11

Table 1. Gene expression changes in the CXC chemokine ligands family and their receptor in the lumbar spinal cord after closed head TBI. Data are presented as mean ± S.E.M fold change relative to baseline, n = 6/group, *p<0.05, **p<0.01, *** p<0.001 for comparison to baseline (Day 0).

Figure 4



Effects of CXCR2 and 5HT3 antagonist treatment on mTBI induced mechanical hypersensitivity

The effects of systemic and spinal CXCR2 antagonist SCH527123 on mechanical thresholds after TBI were assessed as CXCL1, 2 and 3 exert their effects by signaling through the chemokine receptor CXCR2. Systemic SCH527123 treatment for 7 days dose-dependently attenuated mechanical hypersensitivity after TBI with the 10mg/kg dose having a maximal reversing effect. Mechanical hypersensitivity after TBI remained attenuated during SCH527123 treatment but the effects dissipated upon cessation of drug administration. Spinal administration of SCH527123 on day 3 after TBI effectively restored

mechanical thresholds to baseline values, highlighting the importance of spinal CXCR2 and its chemokine ligands in maintenance of nociceptive sensitization after TBI.

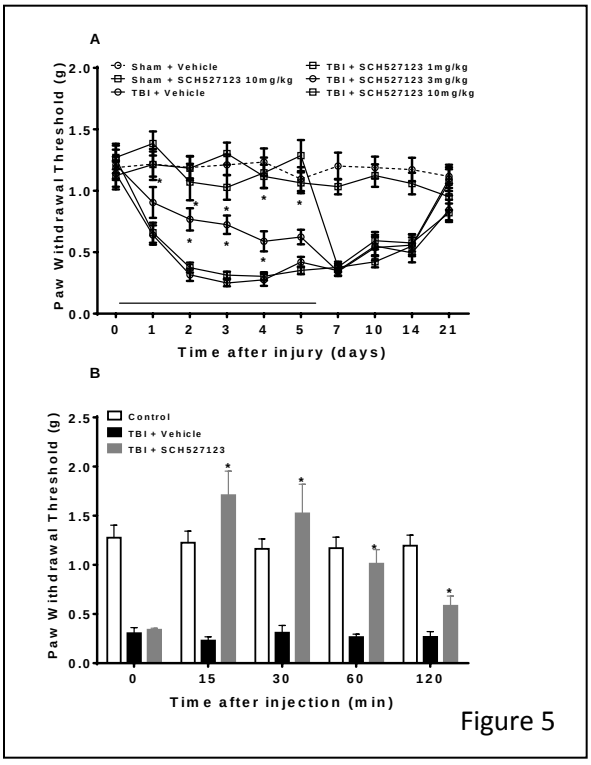


Figure 5

These and additional data related to the effects of the 5-HT3 antagonist ondansetron in blocking early-phase nociceptive sensitization after TBI will be submitted for publication in the near future. Additional studies in rats using the 5,7-DHT serotonin depletion technique provided similar results.

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?

*This is a final report. However, extensions of this work will be pursued through the utilization of funding from the Department of Veterans Affairs.

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. This is an area of growing interest as attention continues to focus on the etiology and treatment of chronic pain after injuries.

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. These spinal processes are likely activated secondary to the dysregulation of brainstem areas involved in endogenous pain modulation. 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. It is also possible for existing FDA-approved selective norepinephrine reuptake inhibitors to enter clinical trials for the management of pain 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 (Project total)
Journal publications

1. TBI-induced nociceptive sensitization is regulated by histone acetylation. 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* (2) June 2017, 14-23.

2. The Chemokine Receptor CXCR2 Supports Nociceptive Sensitization after Traumatic Brain Injury. Liang DY, Shi X, Liu P, Sun Y, Sahbaie P, Li WW, Yeomans DC, Clark JD. Mol Pain. 2017 Jan-Dec;13:1744806917730212.

3. Chronic Pain after Traumatic Brain Injury: Pathophysiology and Pain Mechanisms. Irvine KA and Clark JD. *Pain Medicine* Pain Med. 2018 Jul 1;19(7):1315-1333.

4. Nociceptive and Cognitive Changes in a Murine Model of Polytrauma. Sahbaie P, Tajerian M, Yang P, Irvine KA, Huang TT, Luo J, Wyss-Coray T, Clark JD. J Pain. 2018 Jun 30. pii: S1526-5900(18)30313-4.

5. Traumatic Brain Injury Disrupts Pain Signaling in the Brainstem and Spinal Cord. Irvine KA, Sahbaie P, Liang DY, Clark JD. J Neurotrauma. 2018 Jul 1;35(13):1495-1509.

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, 2010.

5. Characterization of nociceptive alterations and cognitive impairments in a preclinical model of polytrauma. Presented at Society for Neuroscience annual meeting at San Diego, CA on Nov. 15, 2016.

6. TBI Alters Serotonergic Descending Inhibition in a Murine Model of Traumatic Brain Injury. Peyman Sahbaie, De-Yong Liang, Karen Amanda Irvine and J. David Clark. 17th World Congress on Pain. September 16th, 2018 Boston, MA, USA

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

  Name: David J. Clark
  Project Role: PI
  Annualized calendar months: 2.4
  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: 1.2

  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: 3.2
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: 3.75
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: 3.2
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.

Name: Wenwu Li
Project Role: Research Associate
Annualized calendar months: 2.5
Contribution to Project: This person is responsible for a portion of the animal testing, and will perform a portion of the surgeries.

Name: Tianzhi Guo
Project Role: Research Associate
Annualized calendar months: 1
Contribution to Project: Conducts experiments in rats and analyzes data.

Effort listed for PI/Senior Key Personnel reflects the approved effort. Effort for staff reflects actual effort worked during this reporting period.

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

Nothing to report

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

Nothing to report

7. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS

QUAD CHARTS: See Attached

APPENDICES:

Targeting Epigenetic Mechanisms in Pain due to Trauma and TBI

MR130295
W81XWH-14-1-0579

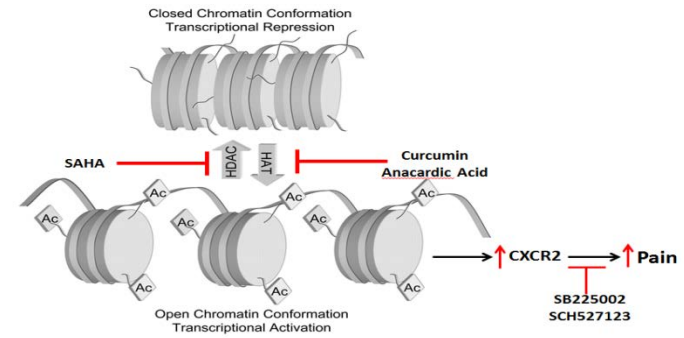
PI: David J. Clark **Org:** PAVIR **Award Amount:** $1,171,533

Study/Product Aim(s)

- 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
- 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

Approach

The objective of this project is to define the role of agents targeting epigenetic mechanisms in reducing pain and disability after trauma. We will employ incisional and fracture extracranial injuries in conjunction with TBI. In our first aim we systematically evaluate the efficacy of HAT inhibitors in reducing pain and disability in these models. We will study the interaction of TBI with peripheral trauma. In the second aim we examine the epigenetic control of genes such as CXCR2 in controlling pain and disability. The selected test compounds will be suitable for translational human studies.



Peripheral trauma and TBI converge on spinal neuron histone acetylation to control the expression of pain-related genes including CXCR2 ultimately supporting persistent pain. Curcumin and additional pharmaceuticals can be used to regulate this process.

The required animal models, testing procedures, staff and equipment in place. Our preliminary data show that HAT inhibitors effectively reduce pain and functional disability after surgery, and that the CXCR2 chemokine receptor is involved.

Timeline and Cost

Activities / CY	14	15	16	17
Pilot studies and pre-application				
HAT inhibitors and behavioral models				
Epigenetic studies – ChIP analysis				
CXCR2 antagonists, pain and disability				
Estimated Budget ($K)	**$100**	**$395**	**$387**	**$289**

Updated: 12/21/18

Goals/Milestones

CY14 Goal – Design study, initiate experiments
- Complete pre-application process, secure animal use approvals
- Initiate pain-related testing for selected HAT inhibitors

CY15 Goals – Complete studies focused on HAT inhibitors
- Complete pain-related testing for selected HAT/HDAC inhibitors
- Initiate complex behavior-related paradigms
- Initiate epigenetic studies

CY16 Goal – Complete major epigenetic studies
- Establish that incision and TBI regulate histone acetylation
- Identify involved cell types

CY17/18 Goals – Establish pain and disability-related roles of chemokines
- Conduct pharmacological testing of selective CXCR2 antagonists in incisional, TBI and combined models.
- Identify cytokines and receptors in spinal tissue regulated by TBI.

Budget Expenditure to Date

Projected Expenditure: $1,171,533
Actual Expenditure: $1,168,169