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14. ABSTRACT

Post-traumatic headache is one of the most common symptoms following mild traumatic brain injury, and can persist for months after the initial trauma. The most severe and long lasting posttraumatic headaches are usually classified as migraine; and are a major cause of disability. The mechanisms by which head trauma leads to migraine are currently unclear, due partially to a lack of predictive animal models. The studies proposed in this application will examine the consequence of mild traumatic brain injury within a mouse migraine model. A closed-head model of brain injury will be used, which has been shown previously to reflect the most common type of mild traumatic brain injury in humans. Migraine-like pain will be evoked by nitroglycerin, which is a known migraine trigger in humans, and causes migraine-associated symptoms in mice. Mice will be tested 2, 4 and 12 weeks after injury with different doses of nitroglycerin, which has been shown to produce varying levels of migraine pain. Mice will be treated repeatedly with nitroglycerin to model the effects of chronic migraine. Recovery from chronic migraine pain will also be measured to determine if brain trauma delays recovery time. A separate group of mice will undergo the same treatment, and will be used to measure proteins that are important mediators of migraine attacks.

15. SUBJECT TERMS

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1. INTRODUCTION:

Post-traumatic headache is the most common, and one of the most debilitating consequences of traumatic brain injury. Over the last 10 years, the incidence of post-traumatic headache has skyrocketed in the military, and is a leading cause of long term disability. Clinically, the more severe and chronic forms of post-traumatic headache are migraine. The mechanism by which traumatic brain injury leads to migraine is currently unclear, partly due to a lack of predictive animal models. The goal of this proposal is to develop a novel mouse model which reflects the relationship between mild traumatic brain injury and migraine; and to use this model to identify key molecular targets that are responsible for this link. This model will be a combination of two existing models; the closed head weight drop model of mild traumatic brain injury, and the nitroglycerin migraine model. Nitroglycerin (NTG) is a known migraine trigger in humans, and produces hyperalgesia in mice that is associated with migraine pain. In addition, chronic nitroglycerin results in a basal hypersensitivity which reflects chronification of migraine. Mild traumatic brain injury will be induced in mice by weight drop onto their intact cranium. To determine the long term consequences of mild traumatic brain injury on migraine susceptibility, separate groups of animals will be tested 2,4 and 12 weeks post-injury. Mice will be tested with chronic intermittent vehicle, low, or high dose nitroglycerin, and nitroglycerin-induced mechanical hyperalgesia, and long-term basal hypersensitivity and recovery will be determined. The predicted outcome is that mild traumatic brain injury will affect either the sensitivity to migraine pain (increased pain at lower doses of nitroglycerin), and/or increased recovery time from basal hypersensitivity. In addition, separate groups of mice will undergo similar treatment, and peripheral and brain tissue will be harvested for gene expression studies. Specifically, changes in two neuropeptides associated with migraine (CGRP and PACAP) will be examined in trigeminal ganglia, nucleus caudalis, hypothalamus, cortex, and amygdala. The expectation is that there will be a synergistic increase in one or both of these molecules in the combined injury-migraine groups, and that these changes will correspond with pain behaviors. This model has important clinical implications, and will be used to test new treatments for post-traumatic headache, and to identify novel drug targets in the future.

2. KEYWORDS:

headache, migraine, mild traumatic brain injury, hyperalgesia, mouse, model, calcitonin gene related peptide, pituitary adenylate cyclase associated polypeptide, PCR, trigeminovascular system

3. ACCOMPLISHMENTS:

What is the major goals of the project?

Specific Aim 1: In mice, does mild traumatic brain injury result in increased susceptibility to migraine-associated pain?	**Months**	**Personnel**	**% Completed**
Major Task 1: Perform TBI and determine changes in NTG model of migraine			
Subtask 1: Obtain approval for animal protocols from UIC and DoD	1-4	Dr. Pradhan	100
Subtask 2: Perform mTBI on mice	5-7	Dr. Zhang	100
Subtask 3: Test mice 2 weeks post-TBI in chronic NTG model	5-7	Ms. Tipton/Segura	100
Subtask 3: Test mice 4 weeks post-TBI in chronic NTG model	6-9	Ms. Tipton/Segura	80
Subtask 4: Test mice 12 weeks post-TBI in chronic NTG model	8-11	Ms. Tipton/Segura	80
Subtask 5: Analyze the data	6-12	Dr.Pradhan	80
Milestone(s) Achieved: determine if TBI results in increased sensitivity to NTG-induced migraine pain and/or longer duration of hypersensitivity	12		
Specific Aim 2: Is there a synergistic enhancement of headache-related neuropeptides in a combined mTBI-migraine model?			
Major Task 2: Perform TBI in mice and test in NTG model			
Subtask 1: Perform mTBI on mice	9-11	Dr. Zhang	100
Subtask 2: Test mice 2, 4 and 12 weeks post-TBI in chronic NTG model	9-15	Ms. Tipton/Segura	65
Subtask 3: Harvest tissue from treated animals	9-15	Dr. Zhang	65
Subtask 4: Isolate RNA and perform gene expression studies	9-18	Dr. Zhang	65
Subtask 5: Analyze the data	10-18	Dr. Pradhan	65
Milestone(s) Achieved: Gene expression studies completed in combo TBI-migraine model	18		

What was accomplished under these goals?

1) Major Activities – We have essential completed Major Task 1. We have established the closed head weight-drop mTBI model in our lab, and tested mice 2, 4 and 12 weeks post-TBI in the nitroglycerin (NTG) migraine model. We have also partially completed Major Task 2. We have collected tissue from animals at the 2 and 12 week time point, and gene expression studies are almost completed for the earlier time point.

For all experiments mice underwent mTBI with the closed head weight drop model in which they were anesthetized with isoflurane and a 30g weight was dropped onto their cranium from a 80 cm long tube. Sham animals were anesthetized with isoflurane, but were not hit. Mice were tested at three time points post-mTBI: 2, 4 and 12 weeks. Animals were tested for mechanical sensitivity to the known human migraine trigger, nitroglycerin (NTG). On test days, baseline mechanical responses (basal thresholds) were determined and mice were challenged with either vehicle, low dose (0.1 mg/kg) or high dose (10 mg/kg) NTG, and tested again 2h later (post-treatment thresholds). Animals were tested every second day for 9 days (5 test sessions). Following the last test day, mice were tested until their basal responses recovered to naïve levels.

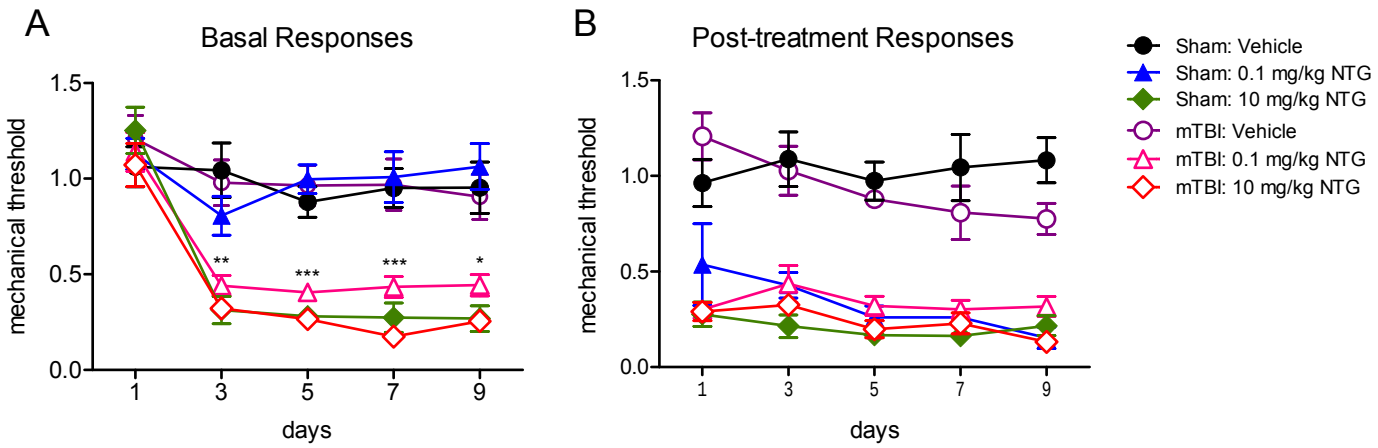

Figure 1: Mice tested 2 weeks post-mTBI show an increased sensitivity to low-dose NTG. Mice were tested every other day for 9 days. Basal responses (A) were determined following which mice were injected with vehicle, low (0.1 mg/kg IP), or high (10 mg/kg IP) doses of NTG, and tested again 2h later (B). We compared the low dose groups using 2-way RM ANOVA with Holm-Sidak post-hoc analysis, factors of injury and time. There was a significant effect of injury, time and interaction ($p<0.001$) for basal responses. For post-treatment responses there was a significant effect of time, but not injury or interaction. n=8-12/group, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

We chose to test two doses of NTG because we have previously shown that a high dose (10 mg/kg) produces both an acute pain, 2h post-injection (post-treatment threshold); and a progressive basal hypersensitivity over treatment days (basal thresholds). In contrast, the low dose (0.1 mg/kg) of NTG only produces the acute hyperalgesia, but does not produce chronic hypersensitivity. Our previous work has shown that the acute hyperalgesia reflects an acute migraine attack, and the chronic hypersensitivity is a model of migraine chronification.

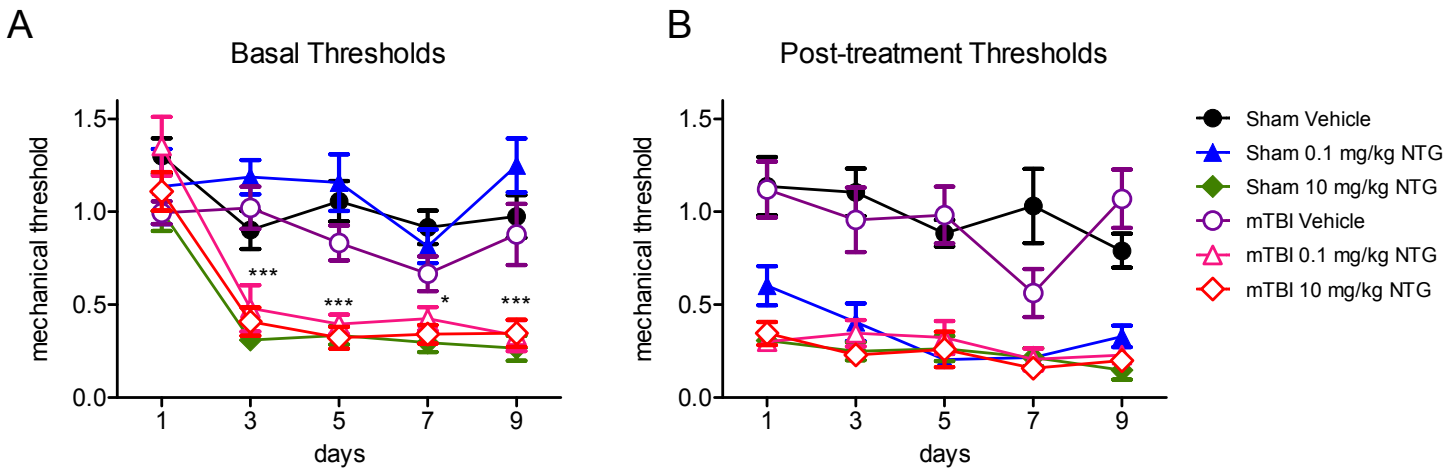

Figure 2: Mice tested 4 weeks post-mTBI show an increased sensitivity to low-dose NTG. Mice were tested every other day for 9 days. Basal responses (A) were determined following which mice were injected with vehicle, low (0.1 mg/kg IP), or high (10 mg/kg IP) doses of NTG, and tested again 2h later (B). We compared the low dose groups using 2-way RM ANOVA with Holm-Sidak post-hoc analysis, factors of injury and time. There was a significant effect of injury, time and interaction ($p<0.001$) for basal responses. For post-treatment responses there was a significant effect of time, but not injury or interaction. n=8/group, *$p<0.05$, ***$p<0.001$.

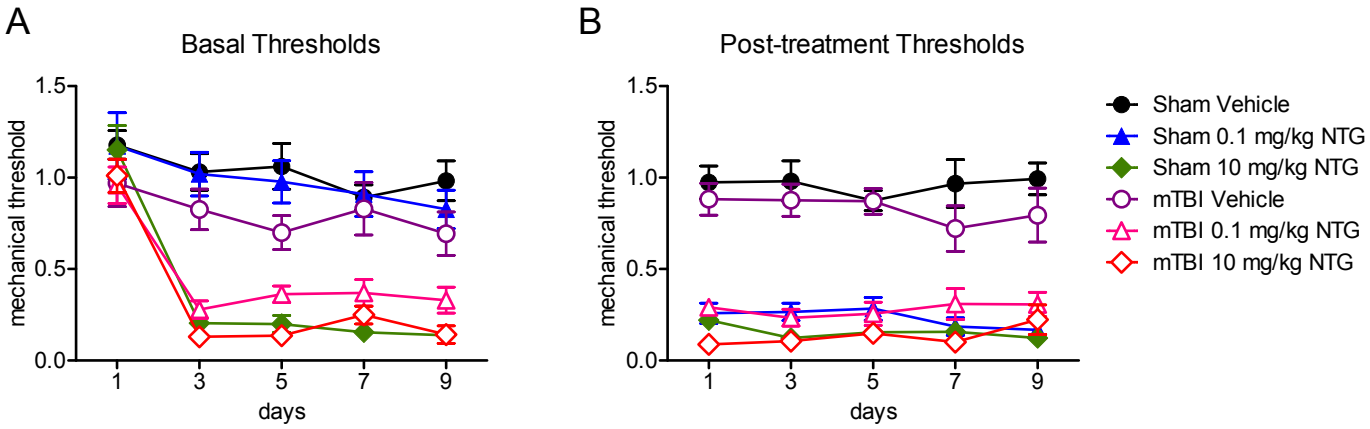

Figure 3: Mice tested 12 weeks post-mTBI show an increased sensitivity to low-dose NTG. Mice were tested every other day for 9 days. Basal responses (A) were determined following which mice were injected with vehicle, low (0.1 mg/kg IP), or high (10 mg/kg IP) doses of NTG, and tested again 2h later (B). We compared the low dose groups using 2-way RM ANOVA with Holm-Sidak post-hoc analysis, factors of injury and time. There was a significant effect of injury and time ($p<0.001$), but no significant interaction for basal responses. For post-treatment responses there was a significant effect of time, but not injury or interaction. n=11-12/group.

Our results indicate that mTBI alone does not alter naïve baseline mechanical paw withdrawal thresholds (day 1 Figures 1A, 2A, & 3A). With regards to migraine-associated pain, we observed that regardless of injury, a high dose of NTG produced both a basal hypersensitivity (Figures 1A, 2A, & 3A) and an acute hyperalgesia (Figures 1B, 2B, & 3B), at all time points tested. However, we found that animals that underwent mTBI before testing were more sensitive to the low dose of NTG, and these animals developed basal hypersensitivity to this dose, unlike sham controls (Figures 1A). This hypersensitivity was observed at all time points post-mTBI (Figures 2A and 3A). These results indicate that mTBI produces an increased susceptibility to chronic migraine.

We next determined if mTBI affected the recovery time from NTG-induced basal hypersensitivity. In both sham and mTBI mice chronic high-dose NTG produced basal hypersensitivity, an effect also observed with low-dose NTG in mTBI mice. In the groups tested 2 weeks post-mTBI, we assessed their basal mechanical thresholds for several days following the final injection of veh/NTG (Figure 4). There was no significant difference in recovery time (ie time for mechanical thresholds to return to naïve baseline levels). These results indicate that mTBI injury does not change the rate of recovery from chronic migraine-associated pain.

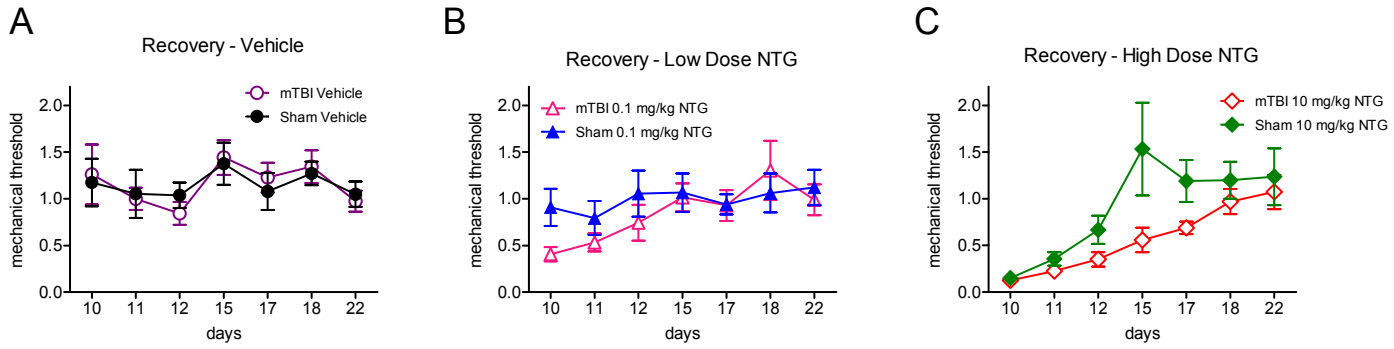

Figure 4: Recovery time from chronic NTG was not affected by mTBI. Mechanical responses were assessed following the final injection of veh/NTG to determine the recovery time from NTG-induced basal hypersensitivity. For clarity the data is expressed as the vehicle groups (A), low dose NTG (B), and high dose NTG (C). n=8-12/group. Regardless of injury, animals recovered from NTG at the same rate.

We have also collected tissue for gene expression studies. These animals were tested as in Figures 1, 2 and 3, but instead of recovery, their tissue was collected 24h after the final vehicle/NTG injection for molecular analysis. We have performed RNA extraction on this tissue, and the qPCR is ongoing.

In order to determine if mTBI would prime animals for chronic migraine pain, we also had two separate groups of mice that underwent sham or mTBI, and we collected their tissue 2 weeks post-injury (ie they did not undergo behavioral testing with NTG). We have examined the gene expression levels for pituitary adenylate cyclase associated polypeptide (PACAP) and calcitonin gene related peptide (CGRP) in the trigeminal ganglia and trigeminal nucleus caudalis from these animals, and we find that mTBI produces a significant increase in levels of both neuropeptides in the trigeminal nucleus (Figure 5). We are currently analyzing other brain regions from these groups. We have also collected samples from sham and mTBI 4 and 12 weeks post-injury.

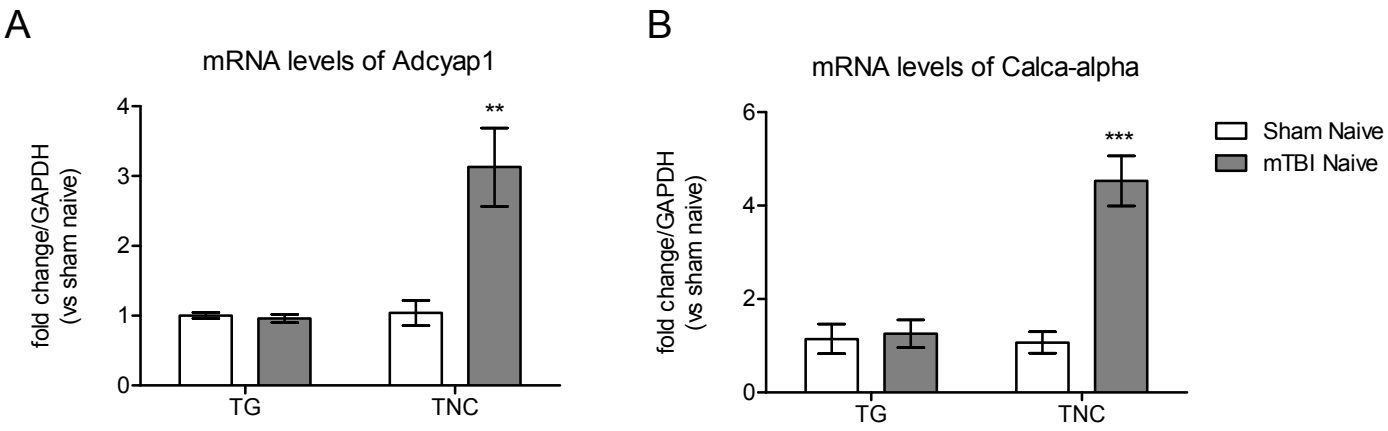

Figure 5: Mild traumatic brain injury upregulates PACAP and CGRP mRNA expression a migraine related brain region. Tissue was collected from animals 2 weeks after sham or mTBI, and expression of PACAP (A, Adcyap1), and CGRP (B, Calca-alpha) were determined using qPCR in the trigeminal ganglia (TG) and trigeminal nucleus caudalis (TNC). Sham vs. naïve animals were compared using a t-test with Bonferroni correction. n=4/group, **p<0.01, ***p<0.001.

2) Specific Objectives –

We have accomplished most of Specific Aim 1, and are about 1/3 of the way through Specific Aim 2. We are on track to complete the goals of this project within the 18 month time frame of the grant.

3) Major Findings –

Our behavioral results show that mTBI results in an increased sensitivity to the migraine trigger NTG, and that this heightened effect is long-lasting. In addition, we highlight a molecular mechanisms that might be responsible for this increased sensitivity. We observe that mTBI produces increased levels of the pro-migraine related peptides CGRP and PACAP in the trigeminal nucleus caudalis, a region with a prominent role in headache. Overall, our results indicate that we have established a mouse model of post-traumatic headache by combining the closed head weight drop model and the chronic NTG migraine model.

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

Nothing to report.

How were the results disseminated to communities of interest?

We have presented our preliminary findings in a few different settings. We have presented posters and talks of this work at internal UIC conferences (University of Illinois at Chicago, Department of Psychiatry Research Extravaganza 2015 and 2016; and Departmental seminar series), as well as locally at a Chicago chapter Society for Neuroscience conference. We will also present our studies in a poster to the headache community during the European Headache and Migraine Trust International Congress (EHMTIC) in September 2016. We are on track to submit a manuscript of this work within the next 6 months.

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

We are continuing to perform gene expression studies on tissue already collected (Specific Aim 2, Subtask 4 and 5). We will present our data at the EHMTIC conference in September, and finalize a manuscript or our work by December 2016.

4. **IMPACT**:

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

We have yet to present our results to the headache and pain community. We will be presenting at a headache meeting in September (EHMTIC), and hope to submit a manuscript for publication in the New Year.

What was the impact on other disciplines?

We have started a collaboration with a MD from the local VA hospital. Dr. Joanne Tobacman is a specialist in brain extracellular matrix and neurological diseases. She will examine tissue from the site of injury from mTBI mice and determine if there are any changes in extracellular matrix proteins following injury.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report.

6. **PRODUCTS**:

Publications, conference papers, and presentations:

Other publications, conference papers, and presentations:

Poster presentations:

- Characterization of a novel model of post-traumatic headache. Research Extravaganza at UIC. University of Illinois at Chicago, Chicago, Illinois, September 2015.
- Characterization of a novel model of post-traumatic headache. Chicago Chapter of Society for Neuroscience. Northwestern University, Chicago, Illinois. April 2016.

The following posters have been accepted for presentation in September 2016:

- Characterization of a novel mouse model of post-traumatic headache. EHMTIC. Glasgow, UK. September 2016.
- A novel mouse model of post-traumatic headache. Research Extravaganza at UIC. University of Illinois at Chicago, Chicago, Illinois. September 2016.

Abstracts are included in the Appendix.

Oral presentations:

- Characterization of a novel model of post-traumatic headache. Department of Psychiatry, University of Illinois at Chicago, Chicago, USA, June 2016. Neuroscience Institute Seminar.
- Characterization of a novel model of post-traumatic headache. Department of Pharmacology, University of Michigan, Ann Arbor, USA, October 2015. Lab exchange.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

The following individuals have worked on this project:

Name:	Amynah Pradhan
Project Role:	Principal Investigator
Nearest person month worked:	3
Contribution to project:	Dr. Pradhan has managed, designed, analyzed, and interpreted the experiments performed in this grant.

Name:	Alycia Tipton
Project Role:	Technician
Nearest person month worked:	5
Contribution to project:	Ms.Tipton has performed behavioral experiments and collected tissue for gene expression studies.

Name:	Huaibo Zhang
Project Role:	Postdoctoral Fellow
Nearest person month worked:	2
Contribution to project:	Dr. Zhang has helped perform gene expression studies, and has trained Ms. Segura in molecular biology techniques specific for this grant.

Name:	Laura Segura
Project Role:	Graduate Student
Nearest person month worked:	6
Contribution to project:	Ms.Segura has performed behavioral experiments and analyzed tissue for gene expression studies.

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

Yes. After April 2016, I am no longer supported on my R00 (DA031243, In vivo implications of agonist selective activation of delta opioid receptor), as that grant has ended. I am now being supported by a R01 that I was awarded 08/01/2016.

My current support is as follows:

PR141746– W81XWH-14-PRMRP-DA - DOD Discovery Award (Pradhan A, PI) – 07/20/2016-01/19-2017
3 calendar months/year for 1.5 years time and effort. Budget = $200,000 total direct costs.
Synergistic mechanisms between traumatic brain injury and migraine.
In this application we propose to examine the effects of a mild traumatic brain injury on a mouse model of chronic migraine, with the aim to uncover synergistic mechanisms between these two pathologies.

1R01DA040688 (Pradhan A, PI) – (08/01/2016-07/31/2021) 4.8 calendar months/year time and effort.
NIH-NIDA $225,000/year direct costs.
The role of delta opioid receptors in trigeminovascular pain.
The goal of this grant is to understand the fundamental role of delta opioid receptors and endogenous opioids in migraine, with a focus on receptors located within the forebrain.

P50AA022538 Center for Alcohol Research in Epigenetics (Pandey S, PI)
Pilot Project 1, 04/01/2015-03/31/2017 0.9 calendar months time and effort
NIH-NIAAA $45,000 direct costs/year (for Pilot Project 1)
Mitochondrial DNA methylation and alcohol withdrawal-induced pain.
In this proposal we will examine the role of mitochondrial epigenetics on pain induced by alcohol withdrawal.

There is no overlap between any of these projects.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

Nothing to report.

9. APPENDICES:

CHARACTERIZATION OF A NOVEL MOUSE MODEL OF POST-TRAUMATIC HEADACHE

L. Segura[1], A.A. Pradhan[1]
[1]*Department of Psychiatry, University of Illinois at Chicago*

Mild traumatic brain injury (mTBI) impacts approximately 1.3 million Americans per year, causing behavioral, cognitive, and emotional deficits. Of the many disabilities resulting from mTBI, post-traumatic headache (PTH) is the most common and long-lasting impairment. Often persisting for up to a year, PTH is most commonly associated with a migraine phenotype. To date, the mechanisms underlying the progression of mTBI to PTH have not been fully elucidated. We aim to develop a novel mouse model that reflects the relationship between mTBI and PTH by combining the closed head weight drop method and the nitroglycerin (NTG) chronic migraine model. NTG is a known human migraine trigger that also produces migraine-associated hyperalgesia in mice. In the mTBI groups, a 30 gram weight impacts the intact crania of anesthetized C57Bl6/J adult male mice. Sham groups undergo anesthesia, but are not impacted. After 2 weeks of recovery, mice are chronically treated with saline, 0.1 mg/kg NTG, or 10 mg/kg NTG over 5 test days. Basal and post-treatment mechanical thresholds are assessed using von Frey hair stimulation. Only the mTBI group developed a progressive and sustained basal hypersensitivity to the low dose of NTG, while the high dose of NTG produced hypersensitivity in both sham and mTBI groups. Both doses of NTG induced comparable acute (post-treatment) hyperalgesia in both groups, 2 hours after injection. Additionally, mTBI groups treated with either a low/high dose of NTG showed a longer recovery time in comparison to their sham counterparts. mTBI appears to produce an increased sensitivity to migraine-associated pain within the NTG model of chronic migraine. Future gene expression studies will explore the role of neuropeptides associated with migraine in this mouse model.

Presented at Chicago Chapter for the Society for Neuroscience April 2016, Chicago IL

CHARACTERIZATION OF A NOVEL MOUSE MODEL OF POST-TRAUMATIC HEADACHE

L. Segura[1], H. Krishnan[1], S.C. Pandey[1], A.A. Pradhan[1]
[1]Department of Psychiatry, University of Illinois at Chicago

Mild traumatic brain injury (mTBI) impacts millions of people worldwide causing behavioral, cognitive, and emotional deficits. Post-traumatic headache (PTH) is the most common and long-lasting impairment observed following mTBI. Often persisting for up to a year, PTH is most frequently associated with migraine characteristics. The mechanisms underlying the progression of mTBI to PTH are not fully understood. The aim of this study was to develop a novel mouse model of PTH, by combining the closed head weight drop mTBI method and the nitroglycerin chronic migraine model. To induce mTBI, a 30 gram weight was dropped onto intact crania of mildly-anesthetized C57Bl6/J male mice. Mechanical responses to chronic-intermittent administration of nitroglycerin was determined 2, 4, and 12 weeks post-mTBI. Low (0.1 mg/kg) and high (10 mg/kg) doses of nitroglycerin were used, as only the high dose has been reported to produce basal hypersensitivity in naïve mice. The mTBI group showed basal hypersensitivity to both low and high doses of nitroglycerin, unlike sham controls which were only sensitive at the high dose. Additionally, following the last injection of nitroglycerin, the time to recover mechanical responses to control levels was significantly longer in the mTBI group compared to shams. mTBI in mice appears to produce increased sensitivity to migraine-associated pain induced by nitroglycerin, and this procedure may be used to model PTH. Gene expression studies are underway to explore changes in CGRP and PACAP associated with mTBI and chronic nitroglycerin.

Accepted for presentation at European Headache and Migraine Trust International Congress September 2016