AWARD NUMBER: W81XWH-21-2-0457

TITLE: Role of the Endocannabinoid Selective COX-2 Inhibition in Post-Traumatic Headache Associated with Repetitive Mild Traumatic Brain Injury

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REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	01Sep2022-31Aug2023

4. TITLE AND SUBTITLE

Role of the Endocannabinoid Selective COX-2 Inhibition in Post-Traumatic Headache Associated with Repetitive Mild Traumatic Brain Injury

5a. CONTRACT NUMBER
W81XWH-21-2-0457

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER
312512-1.00-66719

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRDC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
PR201992

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Posttraumatic headache (PTH) is one of the most common and debilitating sequelae attributed to traumatic brain injury (TBI). In a substantial number of patients, PTH can become persistent and significantly impair the patients' quality of life. Most TBI cases exhibit migraine and tension type headache phenotypes, suggesting that PTH may share some common pathophysiology with the primary headache disorders. Although modulation of the endocannabinoid system suppresses various types of pain that include migraine and studies from us and others point to its therapeutic potential in the TBI animal models, the role of endocannabinoids in the treatment of PTH has not been explored. Using our recently developed PTH mouse model induced by closed-head impact model of engineered rotational acceleration (CHIMERA), we found that pharmacological inhibition and genetic deletion of monoacylglycerol lipase (MAGL) that degrades the endocannabinoid 2-arachidonoyl glycerol (2-AG) reduced neuroinflammation, calcitonin gene related peptide (CGRP) production and periorbital allodynia, whereas inhibition or deletion of the anandamide (AEA) hydrolytic enzyme, fatty acid amide hydrolase (FAAH) failed to reduce headache and even exacerbated inflammatory response in the trigeminal system. Interestingly, periorbital allodynia in the FAAH knockout mice following repetitive mild TBI (mTBI) is alleviated by inhibition of the transient receptor potential vanilloid 1 (TRPV1), a nonselective cation channel mediating pain initiation and progression. Consistently, arachidonoyl serotonin (AA-5-HT), a dual inhibitor of FAAH and TRPV1 almost completely alleviated TBI-induced inflammatory response and nociception in the FAAH wild type mice. These results suggest that blockade of TRPV1 activity is able to enhance the antinociceptive effect of the endogenous AEA.

15. SUBJECT TERMS None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Posttraumatic headache (PTH) is a common and debilitating sequela attributed to traumatic brain injury (TBI). In a substantial number of patients, PTH can become persistent and significantly impair the patients' quality of life. Most TBI cases exhibit migraine and tension type headache phenotypes, suggesting that PTH may share some common pathophysiology with the primary headache disorders. Recent studies from our group and others have demonstrated that selective inhibition of either monoacylglycerol lipase (MAGL) or fatty acid amide hydrolysis (FAAH) to increase the brain levels of 2-AG or AEA reduces neuroinflammation and restores motor and cognitive function in the animal models of repetitive mild traumatic brain injury (mTBI). However, it has not been investigated whether modulation of the endocannabinoid system can alleviate posttraumatic headache (PTH), a common and disabling symptom associated with TBI. Given that endocannabinoid deficiency has long been proposed to contribute to the pathogenic mechanisms of migraine and modulation of the endocannabinoid system suppresses various types of pain, we hypothesized that augmentation of endocannabinoids AEA and 2-AG should be effective in combating PTH. Using a PTH mouse model induced by closed-head impact model of engineered rotational acceleration (CHIMERA), we found that pharmacological inhibition and genetic deletion of monoacylglycerol lipase (MAGL) that degrades the endocannabinoid 2-arachidonoyl glycerol (2-AG) reduced neuroinflammation, calcitonin gene related peptide (CGRP) production and periorbital allodynia. Surprisingly, inhibition or deletion of the anandamide (AEA) hydrolytic enzyme, fatty acid amide hydrolase (FAAH) failed to reduce headache and even exacerbated inflammatory response in the trigeminal system. These results suggested that AEA and 2-AG can differentially modulate the headache pain initiation and progression. Given that AEA is also an activator of the transient receptor potential vanilloid 1 (TRPV1), a nonselective cation channel mediating inflammation and pain, we anticipated that blockade of TRPV1 activation might be required for the suppressive effect of the FAAH inhibitors on headache like pain. Indeed, we found that dual inhibition of FAAH and TRPV1 has potent anti-inflammatory and anti-nociceptive effects. The underlying mechanisms are currently under investigation.

2. KEYWORDS:

Mild traumatic brain injury, post-traumatic headache, endocannabinoid, monoacylglycerol lipase, fatty acid amide hydrolase, 2-AG, AEA, trigeminal ganglion, trigeminal nucleus caudalis, TRPV1, CGRP, CHIMERA, mice.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

To determine the role of augmentation of endocannabinoids in the treatment of PTH attributed to mTBI, three specific aims were proposed:

1) To characterize the expression of the endocannabinoid signaling components in the trigeminal pain pathway (including trigeminal ganglion, trigeminal nucleus caudalis, and thalamic relay ventral posterior medialis nucleus) at various time points following repetitive mTBI.

2) To determine the therapeutic effect and the underlying mechanisms of a novel SSCI, LM-4131 in post-traumatic headache following repetitive mTBI.

3) To determine the potential synergy between SSCI and the selective MAGL or FAAH inhibitors in post-traumatic headache following repetitive mTBI.

What was accomplished under these goals?

In this funding period, we have shown that inhibition or deletion of the 2-AG hydrolytic enzyme MAGL aleviates both acute post-traumatic headache as well as the transition to persistent post-traumatic headache due to the reduction of inflammatory cells accumulation and CGRP production in the trigeminal system. Surprisingly, selective inhibition or targeted gene deletion of FAAH to elevate AEA in the trigeminal system did not block inflammation and had no effect on periorbital cutaneous pain sensitivity associated with repetitive

mTBI. Given that TRPV1 is an important player to mediate inflammation and nociception, and its expression in the trigeminal ganglion is greatly increased after TBI, we anticipated that activation of TRPV1 by AEA might dampen the headache pain suppressive effect of the FAAH inhibitors. Consistent with this hypothesis, we showed that blockade of TRPV1 significantly reduced inflammation and periorbital allodynia in the FAAH knockout mice and dual inhibition of FAAH and TRPV1 has potent therapeutic effect on PTH attributed to repetitive mTBI. These results suggested that augmentation of AEA and 2-AG might have distinct effects on the initiation and development of PTH and inhibition of TRPV1 activity is crucial for the therapeutic efficacy of the FAAH inhibitors.

1) Inhibition of MAGL suppressed periorbital mechanical allodynia, CGRP receptor expression in the trigeminal ganglion and glial cell accumulation in the trigeminal nucleus caudalis of mice with repetitive mTBI induced by CHIMERA

In this study, we used the recently developed surgical-free CHIMERA mouse model (13) to study PTH associated with repetitive mTBI. After anesthesia with isoflurane, 10 weeks old, male C57BL/6 mice were restrained in the supine position on the CHIMERA device such that their heads were free to move and rested at an angle of approximately 32° such that the frontal and parietal bones lie flat over the hole in the head plate. An impact with 0.7J impact energy was delivered. The piston impacted the center of the scalp in the location aligned with bregma on the skull. Mice received one impact per day for 4 consecutive days. Sham-operated mice receive anesthesia and restraint, but no impact. The FAAH inhibitor PF04457845 (5 mg/kg) and the MAGL inhibitor MJN110 (2.5 mg/kg) were administered at 1h after each impact and then once daily for a total of 7 days. Mechanical allodynia in the periorbital region was examined using the von Frey monofilaments. Periorbital allodynia was observed at 7 days after the last impact, and the increased pain sensitivity was significantly reduced in the MJN110 treated animals. There was a tendency of lowered mechanical sensitivity in the PF04457845 treatment group, but no statistical significance was achieved (Fig. 1A, N=10/group, Mean ± SEM). To determine the therapeutic mechanisms of MJN110, the mRNA levels of GFAP and the CGRP receptors, calcitonin receptor like receptor 1 (Calcr1) and receptor activity modifying protein 1 (Ramp1), were examined and shown to be elevated in the TBI/vehicle group, and reduced by MJN110 treatment (Fig. 1B). Similarly, the increased expression of CD68 microglia, triggering receptor expressed on myeloid cells 2 (Trem2) and interleukin 6 (IL-6) in the TBI/vehicle group was also attenuated in the MJN110 treated groups. Consistently, the increased expression of astrocytes, microglia (Fig. 1C and E) and CGRP in the trigeminal nucleus caudalis (TNC) (Fig. 1D and F) was also dramatically reduced by MJN110 treatment.

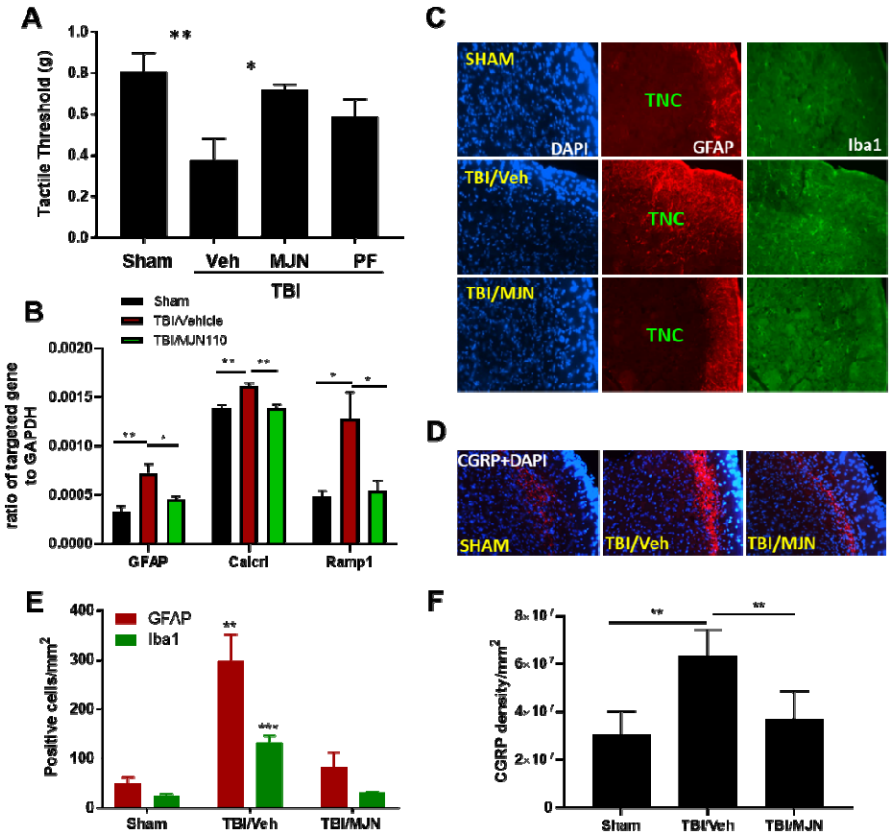

Figure 1. Effects of MJN110 on periorbital allodynia, CGRP receptor expression and glial cells accumulation in trigeminal system at 7 days post-TBI. Treatment with MJN110 (MJN, 2.5 mg/kg), but not PF04457845 (PF, 5 mg/kg) significantly reduced periorbital allodynia (A. * p<0.05 and **p<0.01; N=10 animals/group). The increased expression of GFAP, Calcr1 and Ramp1 in trigeminal ganglion was significantly reduced by MJN treatment (B, * p<0.05 and **p<0.01; N=5/group). The accumulation of astrocytes and microglia labelled by GFAP and Iba1 and the increased expression of CGRP in trigeminal nucleus caudalis (TNC) was also dramatically reduced by MJN110 treatment (C and D). The total number of GFAP and Iba1 positive cells in TNC area was shown in E (*p<0.01 and ***p<0.001; N=5 animals/group) and the intensity of CGRP staining in TNC is shown in F (**p<0.01; N=5 animals/group).

2) Targeted gene deletion of MAGL, but not FAAH suppressed TBI associated periorbital allodynia and inflammation in the trigeminal system

To further determine the role of inhibition of 2-AG and AEA hydrolysis in the treatment of PTH, repetitive mTBI was also induced in the MAGL and FAAH knockout mice. Consistent with the results observed using the pharmacological inhibitor of MAGL, both male and female mice with genetic deletion of MAGL was resistant to periorbital allodynia at 7 days post-TBI (Fig. 2A). The expression of macrophages (Iba1 expressing cells) and CGRP in TG was significantly increased in the wild type ($MAGL^{+/+}$) mice, but dramatically reduced in the MAGL knockout ($MAGL^{-/-}$) mice (Fig. 2B). On the contrary, the mRNA expression of astrocytes, microglia/macrophages detected by GFAP and AIF1 marker genes in TNC was further increased in the FAAH knockout (KO) mice compared with the FAAH wild type (WT) mice, and the increased expression of Trem2 and C1q in the FAAH WT mice was further elevated in the FAAH KO mice (Fig. 2C). Since AEA can directly activate TRPV1 and the FAAH KO mice exhibited proinflammatory phenotype via TRPV1 activation, we assumed that the pain like behavior in the FAAH WT and KO mice was reduced by the selective TRPV1 antagonist. Indeed, treatment with the TRPV1 antagonist SB366791 (1 mg/kg) significantly attenuated periorbital allodynia in the FAAH KO mice, but less so in the WT mice after repetitive mTBI (Fig. 2D).

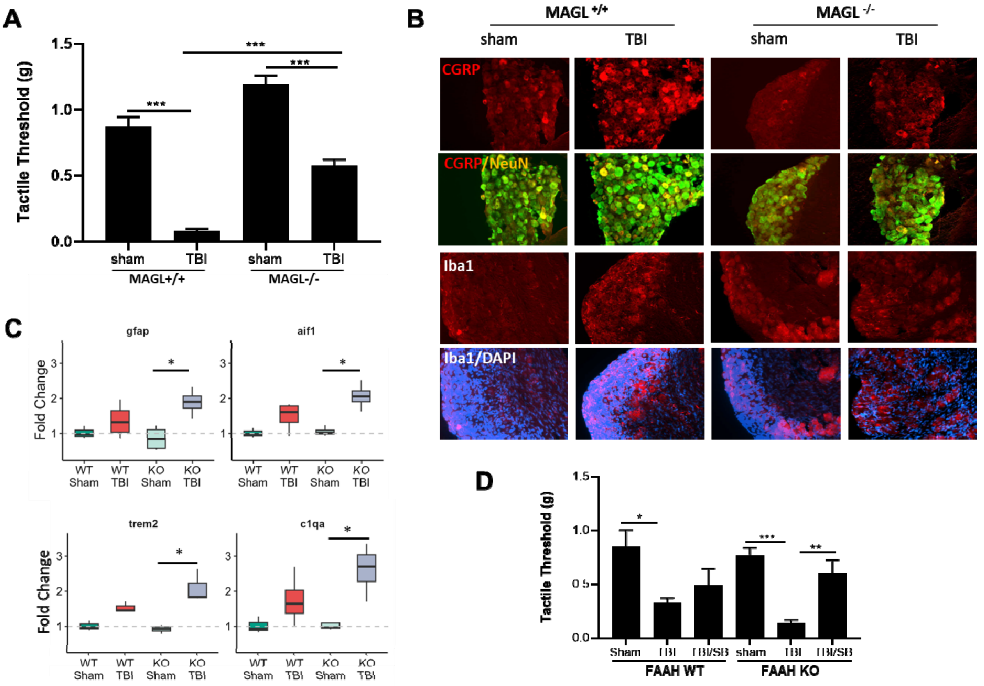

Figure 2. Knockout of MAGL, but not FAAH attenuated TBI induced inflammation and cephalic allodynia. Mice with MAGL-/- exhibited reduced periorbital allodynia (A), macrophage accumulation and CGRP expression in TG (B) at 7 days post-TBI. FAAH-/- mice showed enhanced inflammatory gene expression (C) and the peritoneal allodynia in the FAAH-/- mice was significantly reduced by the TRPV1 antagonist SB366791 (SB, 1 mg/kg) (D). *p<0.05, **p<0.01, ***p<0.001 (N=5-8/group)

3) Glial cell accumulation and increased expression of CGRP in TG and TNC of the FAAH knockout TBI mice were significantly attenuated by blockade of TRPV1

Consistent with the suppressive effect of the TRPV1 antagonist SB366791 on TBI-induced periorbital allodynia in the FAAH KO mice, daily treatment with SB366791 (1 mg/kg) for 7 days also significantly attenuated the increased expression of Iba1 expressing macrophages and CGRP in TG and the accumulation of microglia, astrocytes and CGRP in TNC at 7 days post-repetitive mTBI, whereas the TBI induced glial cell accumulation and CGRP expression in the FAAH wild type mice were less affected by SB366791 treatment (Fig. 3). There results suggest that blockade of both FAAH and TRPV1 should be more ideal to exert potent anti-nociceptive effects.

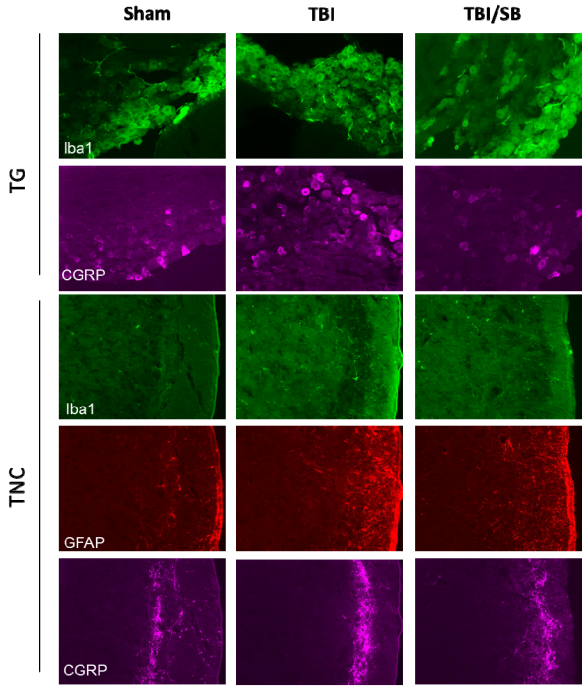

Figure 3. Blockade of TRPV1 suppressed TBI-induced glial activation and CGRP expression in the trigeminal system of the FAAH knockout mice. Treatment with SB366791 (SB, 1 mg/kg), significantly reduced Iba1 and CGRP expression in TG, Iba1, GFAP and CGRP expression in TNC of the FAAH knockout mice at 7 days after repetitive mTBI.

4) Treatment with AA-5-HT alleviated cephalic allodynia and enhanced the expression of anti-inflammatory molecules in trigeminal ganglion

To determine whether simultaneous blockade of the FAAH and TRPV1 is more effective to alleviate PTH, TBI mice was treated with the FAAH inhibitor PF04457845 (5 mg/kg), the TRPV1 antagonist SB366791 (1 mg/kg) alone or their combination. Since AA-5-HT is a dual inhibitor of both FAAH and TRPV1 (67), TBI animals were also treated with AA-5-HT at 2.5 mg/kg. Periorbital allodynia at 7 days post-TBI was partially attenuated by SB366791 or its combination with PF04457845. Noticeably, the periorbital allodynia was completely alleviated by AA-5-HT (Fig. 4A). At 14 days post-TBI, AA-5-HT reduced the increased expression of FAAH, accumulation of macrophages (Iba1 immunoreactive cells) and upregulated the expression of anti-inflammatory maker proteins arginase 1 (Arg1), CD206, the ratio of CD206 versus the proinflammatory CD16/32. The expression of TRPV1 was increased in the TBI mice and not affected by 5-HT treatment (Fig. 4B).

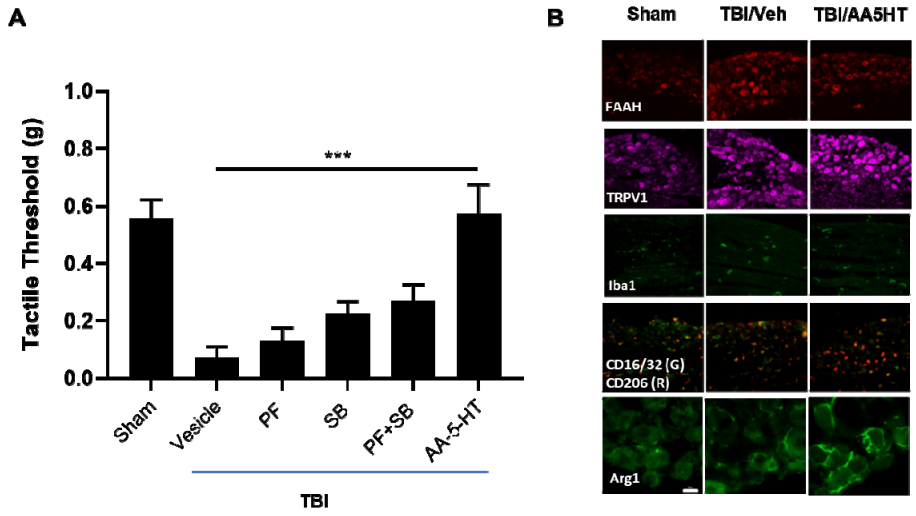

Figure 4. Treatment with AA-5-HT reduced periorbital allodynia and increased the expression of anti-inflammatory proteins in TG 14 days post-TBI. Periorbital mechanical allodynia in TBI mice was completely blocked by AA-5-HT treatment (A). AA-5-HT reduced the increased expression of FAAH and Iba1, but not TRPV1, it also elevated the expression of the anti-inflammatory M2 marker proteins, CD206 and Arginase 1 in TG at 14 days post-TBI (B). ***p<0.001 (N=6/group)

5) Treatment with AA-5-HT reduced the expression of proinflammatory cytokines and CGRP in the TBI mouse trigeminal nucleus caudalis

The mRNA expression of Gfap, Aif1, Trem2 and C1q in TNC was examined at 7 days post-TBI. The increased mRNA expression of Gfap, Aif1, Trem2 and C1q in TNC of the TBI/vehicle group seemed to be elevated in the PF04457845 treatment group, but all were significantly reduced in the AA-5-HT treated animals (Fig. 5A). The immunoreactivity of CGRP, Iba1 and GFAP was also enhanced in TNC and attenuated in the AA-5-HT treated animals (Fig. 5B). These results indicated that AA-5-HT is an effective antinociceptive agent for the treatment of PTH attributed to repetitive mTBI.

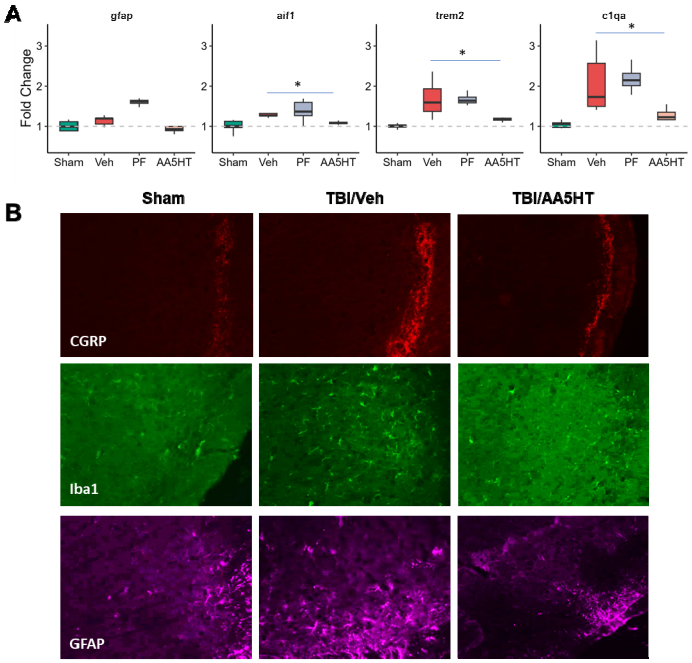

Figure 5. Treatment with AA-5-HT reduced inflammatory gene expression and glial cell accumulation in TBI mouse trigeminal nucleus caudalis. The increased mRNA expression of Gfap, Iba1, Trem2 and C1qa in the TBI mouse trigeminal nucleus caudalis (TNC) was further elevated by PF04457845 (PF) treatment, but completely ablated by AA-5-HT treatment (A). The increased expression of CGRP, Iba1 and GFAP expression in TNC was upregulated in TBI mice and reduced by AA-5-HT treatment (B). (*p<0.05, N=5-6/group).

6) Altered expression of inflammatory and cannabinoid signaling components genes in the TBI mouse periaqueductal gray

It has been known that periaqueductal gray (PAG) is a core region involving in the descending pain control pathway, and augmentation of endocannabinoids in PAG exerts antinociceptive effects. To examine the molecular changes that might impact the descending pain modulation, PAG was dissected at 7 days post-TBI and the expression of several inflammatory and cannabinoid signaling components genes were examined. As shown in Figure 6, cd68, the marker for reactive microglia/macrophages is significantly increased, and the expression of gfap, aif1, c1qa and trem2 seemed to

be also elevated, although the significant differences of these genes were not reached between the sham and TBI animals. Among the metabolic enzymes for endocannabinoids 2-AG and AEA, our results showed that the expression of MAGL, but not FAAH was significantly elevated, suggesting that the endogenous levels of 2-AG, but not AEA in the PAG might be reduced after repetitive mTBI. The increased expression of CB2 receptor is likely correlated with the accumulation of inflammatory cells in the region. Distinct from the increased TRPV1 expression in TG, the expression of TRPV1 in PAG was significantly reduced, consistent with the notion that activation of TRPV1 in peripheral and central pain control pathways possesses an opposite effect, and the reduced expression of TRPV1 in PAG might facilitate pain development. Among the vesicular glutamate and GABA transporters, we found that the expression of vglut3 was reduced, while the expression of vgat was greatly increased, suggesting that the unbalanced excitatory and inhibitory neurotransmission might contribute to the impaired inhibitory control of the descending pain modulatory pathway.

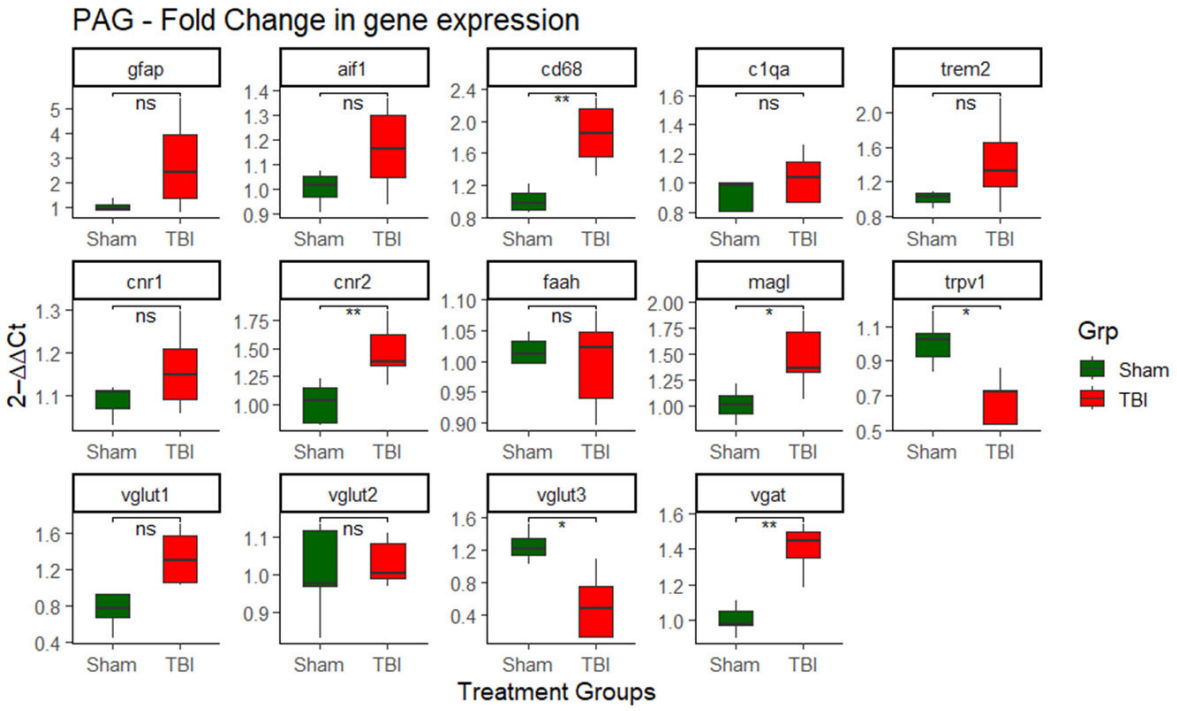

Figure 6. Altered expression of inflammatory, cannabinoid signaling components and vesicular glutamate and GABA transporters in the TBI mouse PAG. At 7 days post-TBI, the expression of cd68, magl and vgat was significantly increased and the expression of trpv1 and vglut3 was significantly decreased. *p<0.05, **p<0.01 (n=5-6/group).

7) Treatment with the substrate-selective COX-2 inhibitor LM-4131 attenuated periorbital allodynia and meningeal mast cell degranulation in mice with repetitive mTBI

In addition to the hydrolytic pathway, the endocannabinoids AEA and 2-AG can be oxygenated by COX-2 to generate inflammatory prostaglandin ethanolamines and prostaglandin glyceryl esters causing inflammatory and neuropathic pain. We have recently shown that the use of substrate-selective COX-2 inhibitor suppresses neuropathic pain induced by chronic sciatic nerve injury. To determine its effect on PTH, the repetitive TBI mice were treated with LM-4131 at 10 mg/kg for 7 days and periorbital allodynia was examined using von Frey test at 7 days post-TBI. The periorbital cutaneous pain sensitivity was greatly enhanced in TBI mice and significantly reduced by LM-4131 treatment (Fig. 7A). Given that mast cell degranulation in meninges might contribute to the pathogenesis of PTH, we also examined the effect of LM-4131 on mast cell degranulation. At 7 days post-TBI, perfused

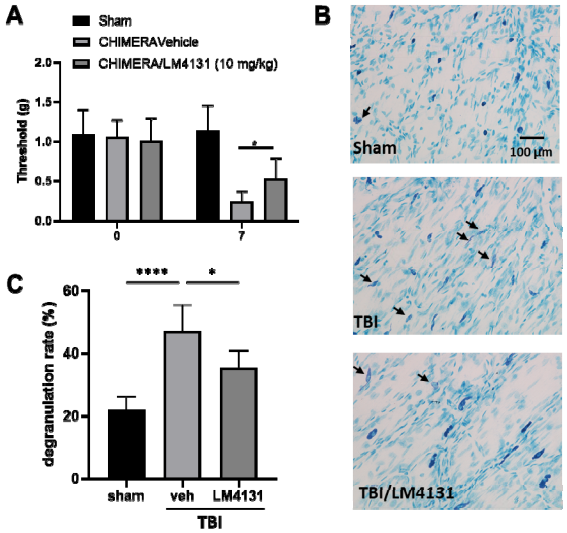

Figure 7. Treatment with LM-4131 attenuated periorbital allodynia and meninge mast cell degranulation in mice with repetitive mTBI. Treatment with LM-4131 significantly reduced TBI-induced periorbital allodynia (A) and mast cell degranulation (indicated by arrow) in meninges (B,C). *p<0.05, ****p<0.0001 (n=6).

meninges were removed from animal cranial bone and mounted on a glass slide. After they were completely dried, toluidine blue (VitroVivo Biotech, MD) was applied to stain mast cells according to the manufacturer's protocol with minor modification. Mast cell degranulation as indicated by faintly stained cells or cells with extensively secreted granules was increased by more than 2-fold in TBI mouse meninges and significantly reduced in the LM-4131 treated animals (Fig. 7B, C). This result suggests that the head pain suppressive effect of LM-4131 might be attributed to its inhibition on mast cell degranulation.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Some results have been presented in the 2023 National Capital Area TBI Research Symposium and will be presented in the annual meeting of Society for Neuroscience, November, 2023 in Washington DC. Two manuscripts were published in the peer reviewed journals, and one manuscript is currently in preparation.

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

1) We will determine whether delayed treatment with the inhibitors of MAGL or dual inhibition of FAAH and TRPV1 can prevent the transition of PTH from acute to the chronic stage triggered by CGRP and bright-light stress at 30 and 60 days post-TBI.

2) We will use RNAscope in combination with immunohistochemistry to assess the cellular expression of MAGL, FAAH and TRPV1 in the trigeminal system at different time points post-TBI.

3) To investigate the role of the substrate-selective COX-2 inhibitor LM-4131 and its combination with FAAH inhibitor in the treatment of PTH in this TBI model system.

4) To determine the molecular changes in TG, TNC and PAG at different time points post-TBI using RNAseq and Mass Spectrometry.

4. IMPACT:

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

Although augmentation of endocannabinoids AEA and 2-AG by inhibition of their hydrolytic enzymes has been shown to exert protective effects in inflammatory and neuropathic pain, migraine and traumatic brain injury and its associated neurodegenerative diseases, the effect of inhibition of the AEA and 2-AG hydrolytic enzymes on the initiation and development of PTH is unknown. Using both pharmacological and genetic approaches, we found that augmentation of endogenous levels of 2-AG alleviated PTH by blocking inflammation and neurogenic CGRP production. However, augmentation of endogenous levels of AEA might exaggerate the inflammation and pain associated with TBI due to its activation of TRPV1 and possibly the other TRP channels. These results suggest that development of selective MAGL inhibitors or the dual inhibitors of FAAH and TRPV1 might be therapeutically useful for the treatment of PTH attributed to traumatic brain injury.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS:

Contrary to our hypothesis, inhibition of AEA degradation *per se* is unable to alleviate PTH likely owing to its alternative activation of TRPV1, thus, we tested the effect of TRPV1 antagonist in the FAAH knockout mice following repetitive mTBI and showed that the TRPV1 antagonist had an antinociceptive effect. Furthermore, we found that dual inhibition of FAAH and TRPV1 seemed to be more effective for the treatment of PTH.

It is noted that the small amount of tissues available in TG, TNC and other brain regions seem to be a limiting factor for performing multiple assays. Therefore, more animals might be required for detailed analysis.

Actual or anticipated problems or delays and action 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 of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

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

1. **Appendix I**: Wen J, Tanaka M, Nagarajan, G, Sackett S and Zhang Y. Inhibition of 2-AG degradation ameliorates posttraumatic headache associated with mild traumatic brain injury. Presented in The National Capital Area TBI Research Symposium, March 9-10, 2023, College Park, Maryland.

2. **Appendix II:** Wen J, Tanaka M, Nagarajan, G and Zhang Y. Modulation of TRPV1 activity by endocannabinoid anandamide in the development and treatment of posttraumatic headache. Will be presented in Annual Meeting for the Society for Neuroscience, November 11-15, 2023, Washington DC.

3. **Appendix III**: Nagarajan G, Wen J, Tanaka M and Zhang Y. Dual fatty acid amide hydrolase and TRPV1 inhibitor reduces inflammation and post-traumatic headache like symptoms induced by repetitive traumatic brain injury. Presented in The National Capital Area TBI Research Symposium, March 9-10, 2023, College Park, Maryland.

4. Wen J, Sackett S, Tanaka M and Zhang Y. Therapeutic Effects of Combined Treatment with the AEA Hydrolysis Inhibitor PF04457845 and the Substrate Selective COX-2 Inhibitor LM4131 in the Mouse Model of Neuropathic Pain. Cells 2023; 12(9), 1275.

5. Tanaka M and Zhang Y. Preclinical Studies of Posttraumatic Headache and the Potential Therapeutics. Cells 2023; 12, 155.

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Nothing to report.

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patents applications, and/or licenses
Nothing to report.

Other products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Yumin Zhang
Project Role	PI
Nearest person month worked	3.6
Contribution to Project	Oversees entire project
Funding Support	Full time federal employee

Name	Joseph McCabe
Project Role	Co-Investigator
Nearest person month worked	1.2
Contribution to Project	Assisting in TBI modeling and behavioral experiments
Funding Support	Full time federal employee

Name	Irwin Lucki
Project Role	Co-Investigator
Nearest person month worked	.60
Contribution to Project	Oversees rat behavioral core at USUHS
Funding Support	Full time federal employee

Name	Sean Moran
Project Role	Collaborator
Nearest person month worked	.60
Contribution to Project	Assist in measurements within mouse brain tissues
Funding Support	Full time federal employee

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.

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS: See attached

9. APPENDICES: See attached