AWARD NUMBER: W81XWH-19-1-0534

TITLE: A Multidisciplinary Translational Approach to Investigate the Mechanisms, Predictors, and Prevention of Persistent Post-Traumatic Headache

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REPORT DATE: OCTOBER 2023

TYPE OF REPORT: Annual Progress Report

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

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2023	Annual Progress Report	1SEPT2022 - 31AUG2023

4. TITLE AND SUBTITLE:
A Multidisciplinary Translational Approach to Investigate the Mechanisms, Predictors, and Prevention of Persistent Post-Traumatic Headache

5a. CONTRACT NUMBER
W81XWH-19-1-0534

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

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)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Annually in the US there are ≈2.8 million TBI-related ED visits, hospitalizations, and deaths and over 2 million individuals are diagnosed with mild TBI (mTBI). From 2000 to 2016, 360,000 US armed service members were diagnosed with TBIs, of which over 80% were mTBIs. Post-traumatic headache (PTH) is the most common symptom following mTBI. Although some PTHs resolve within the first few days, a substantial proportion of individuals with PTH do not have headache resolution during the acute phase and have PTH persistence (PPTH). Optimally, individuals who are at elevated risk for PPTH would be identified and treated during the acute stage of PTH, prior to and with the intent of preventing PTH persistence. This Focused Program is addressing this area of need by investigating mechanisms for PTH persistence, biomarkers that predict an increased risk for PPTH, and methods of preventing the development of PPTH. This Focused Program consists of six synergistic, non-interdependent, individual projects that address the overarching goal via use of PTH animal models, human investigations of individuals with PTH via in-depth phenotyping, neurophysiology testing, imaging brain structure and function, and via human and animal molecular and genetic biomarker identification. Finally, this Focused Program includes a phase II clinical trial of a CGRP receptor monoclonal antibody administered during the acute stage of PTH with the intent of preventing PTH persistence.

15. SUBJECT TERMS

post-traumatic headache, traumatic brain injury, concussion, calcitonin gene related peptide, magnetic resonance imaging, quantitative sensory testing, genetics, biomarkers

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
U	U	U	UU	54	**PHONE NUMBER** *(include area code)*

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1. Introduction

The overarching goal of this Focused Program is to identify mechanisms and predictors for the transition from acute post-traumatic headache (PTH) to persistent PTH (PPTH) and methods to prevent this transition. This Focused Program consists of six synergistic, non-interdependent, individual projects that address this overarching goal via use of animal models of PTH due to mild traumatic brain injury (mTBI), human investigations of individuals with PTH via in-depth phenotyping, neurophysiology testing including quantitative sensory testing and visual discomfort threshold testing, magnetic resonance imaging of brain structure and function, and via human and animal molecular and genetic biomarker identification. This Focused Program also includes a phase II clinical trial of a calcitonin gene-related peptide (CGRP) receptor monoclonal antibody administered during the acute stage of PTH with the intent of preventing PTH persistence. Advanced multivariate modeling methods will be utilized to identify the optimal combination of data from the individual projects for predicting PTH persistence and for predicting treatment response to the CGRP receptor monoclonal antibody.

2. Keywords

Post-Traumatic Headache, Traumatic Brain Injury, Concussion, Calcitonin Gene-Related Peptide, Magnetic Resonance Imaging, Quantitative Sensory Testing, Genetics, Biomarkers

3. Accomplishments

Individual Project #1 - Pre-Clinical Studies

1) *Major activities during this reporting period*: Over the course of the last funding period our focus has been two-fold. First, we extended the findings of our studies across both sexes of animals and examined for potential sex-dependent differences in PTH related outcomes. Second, we examined if sex differences extended to the efficacy of anti-CGRP treatment on PTH outcomes. This included the direct testing of how timing of intervention (early or delayed treatment after a mTBI) influenced the efficacy of treatment. These findings build on our previously detailed results establishing the development of mTBI induced pain behaviors across the acute and persistent phases of PTH. We have continued to demonstrate the development of tactile allodynia in both periorbital and hindpaw regions -

indicative of the development of central sensitization. The persistent PTH (PPTH) phase animals display development of provocable allodynia after brief exposure to stress (i.e. bright light stress) and long-lasting decreases to the threshold of induction of cortical spreading depression (CSD). Treatment with the anti-CGRP receptor antagonist olcegepant after mTBI significantly reduced both acute and persistent PTH pain behavior outcomes. While the efficacy of this treatment was superior when delivered early after a mTBI, we now demonstrate olcegepant remains effective even when treatment is delayed until the PPTH phase. In combination with additional specific directed experiments outlined below, we have now fully completed Major Tasks 1-3 with significant progress across the final remaining Major Task 4. In this next reporting period our focus is on completion of Major Task #4 with emphasis on continued determination of the therapeutic window of efficacy of olcegepant treatment across PTH outcomes induced by single and repetitive mTBI.

2) *Specific objectives*: Our goal is to establish if mTBI acts to sensitize to subsequent triggering stimuli to promote acute PTH and/or PPTH. This is analogous to "two-hit" hyperalgesic priming, where in this case, the first mTBI acts as the sensitizing stimulus. The second stimulus is either a second mTBI or an innocuous stimulus, both of which could promote increased persistence of PTH compared to a single mTBI event.

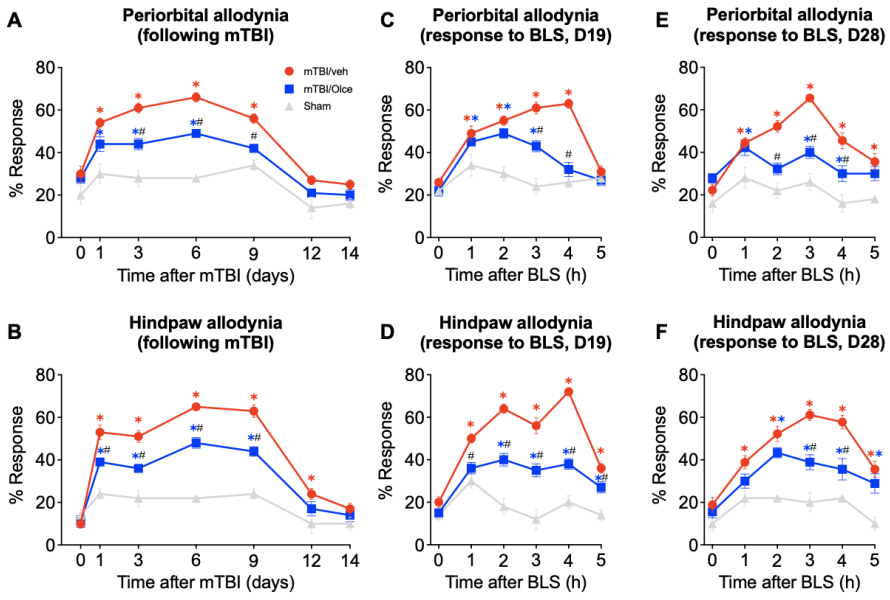


Figure 1. Early treatment with the anti-CGRP receptor antagonist olcegepant significantly reduces mTBI induced Acute and Persistent Post-Traumatic Headache (PPTH) allodynia in mice. Mice were subjected to a single mTBI and pain-relevant behavioral measures monitored. Animals were assessed for changes in periorbital allodynia or hindpaw allodynia in the acute PTH (i.e. >14 days after mTBI) or persistent PTH phase. For clarity, data from only male mice are presented here however similar results were observed in female animals.

3) *Significant results or key outcomes*: To examine for sex-dependent differences in our studies we have now completed testing all major PTH outcomes and anti-CGRP treatment efficacy in both male and female animals. First, we continued examination of the efficacy of early intervention with the anti-CGRP receptor antagonist olcegepant. Olcegepant was delivered to animals at 3mg/kg starting at 2 hours after a mTBI. Due to the rapid clearance of olcegepant from animals and to ensure optimal dosing, supplemental doses of olcegepant were delivered every 3 days across the acute PTH phase (total of 5 doses). In both male and female animals, treatment with olcegepant significantly reduced allodynia across both the acute and persistent phases of PTH. Results for male animals are displayed in Figure 1. We next examined the efficacy of olcegepant at inducing analgesia in male and female animals across the phases of PTH. Specifically, we compared normalized area under the curve (AUC) for mTBI/olcegepant / average normalized AUC for mTBI/vehicle * 100%. As detailed in Figure 2, a clear sex-dependency was observed with increased analgesic efficacy of olcegepant in female animals. We believe this is an important finding that suggest

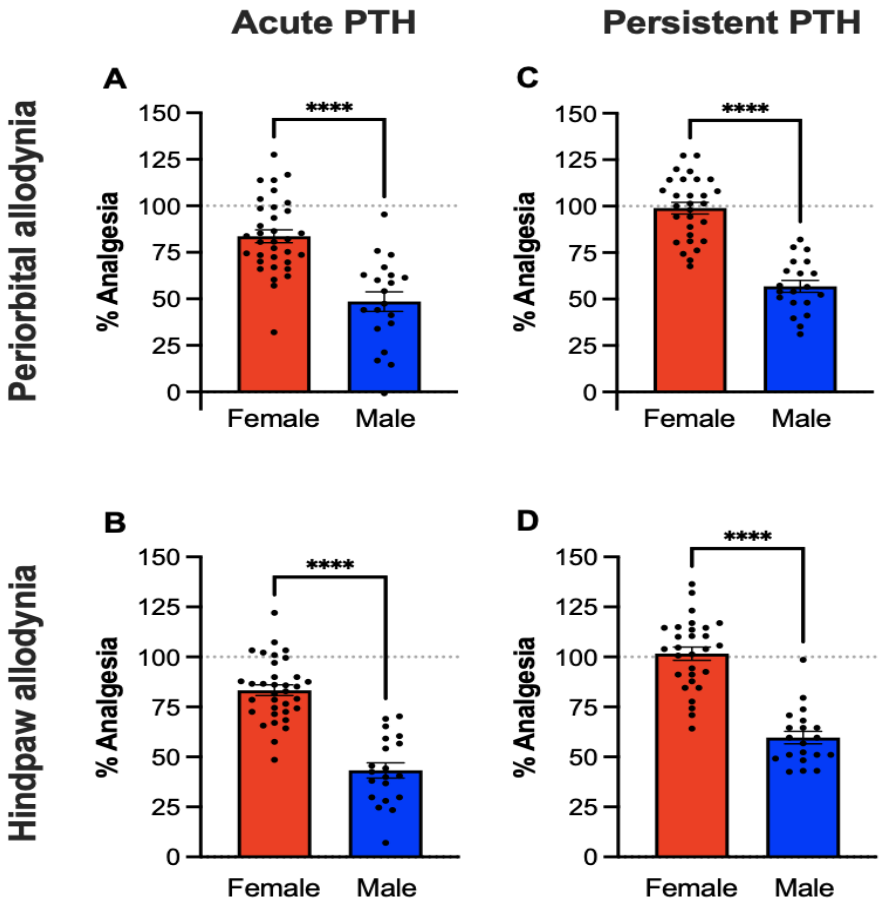


Figure 2. Treatment with the anti-CGRP receptor antagonist olcegepant demonstrates sex-dependency with increased efficacy in female animals. Male and female animals were subjected to a mTBI and then treated with olcegepant beginning early after the mTBI (i.e. 2hrs) and continued across the acute PTH phase (3mg/kg, every 3 days). Periorbital and hindpaw allodynia were measured across the acute PTH phase. The efficacy of olcegepant at inducing analgesia after mTBI was determined by calculating the percent change in the area under the allodynia curve with treatment compared to vehicle. Average data for male and female are presented for both periorbital and hindpaw allodynia.

the efficacy of anti-CGRP treatment may be sex-dependent with potential expectations of enhanced efficacy in female patients.

We next examined if the efficacy of olcegepant at reducing mTBI induced allodynia continued when treatment was delayed following the mTBI until persistent PTH (PPTH) had developed. Specifically, olcegepant (3mg/kg) was administered to male or female mice 14 or 28 days after the mTBI. Olcegepant continued to significantly reduce bright light stress (BLS) induced allodynia when administered at either time-point in both sexes (Figure 3). However, it is of note that the overall efficacy of olcegepant on PPTH allodynia was reduced when compared with our previous results using an "early" strategy with olcegepant administration in the acute PTH phase. The effect of delayed olcegepant treatment was most pronounced when comparing the overall allodynic burden (i.e. area under the curve) and examining the percent analgesia similar to Figure 2. These results suggest that anti-CGRP treatment reduces PTH allodynia across a wider window of therapeutic opportunity, but that efficacy is enhanced by early intervention after mTBI. We believe these results may be important considerations in informing clinical trial design and treatment guidelines for PTH.

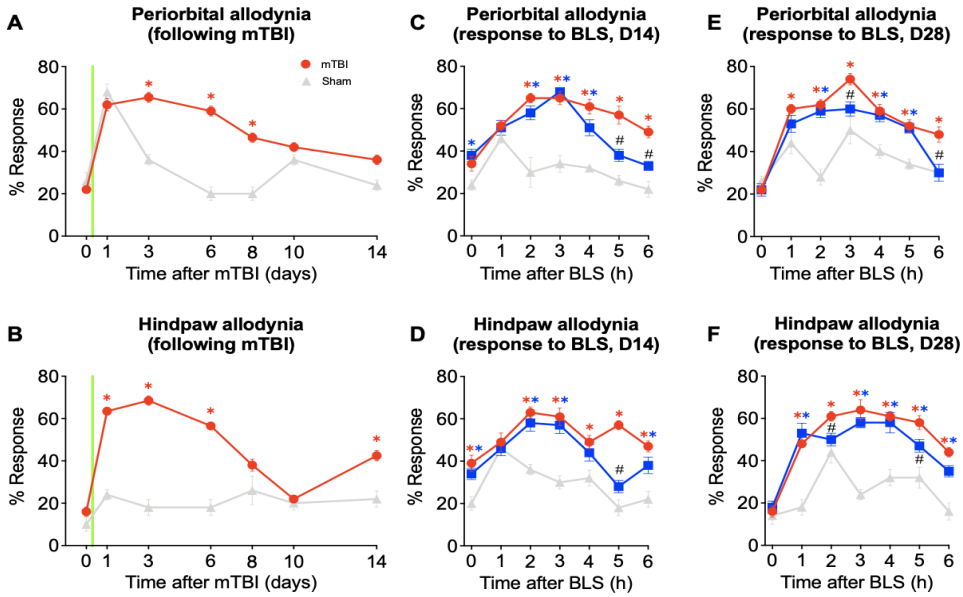


Figure 3. Delayed treatment with the anti-CGRP receptor antagonist olcegepant significantly reduces stress induced persistent Post-Traumatic Headache (PPTH) allodynia in female mice. Female mice were subjected to a single mTBI (green bar) and pain-relevant behavioral measures monitored. Animals were assessed for changes in periorbital allodynia (A) or hindpaw allodynia (B) in the acute PTH (i.e. >14 days after mTBI) or persistent PTH phase. As previously reported, animals developed allodynia in the acute PTH phase (A and B) that could be re-instated in the persistent PTH phase by brief exposure to bright light stress (BLS)(C-F). Sham injury responses are displayed in grey and mTBI responses in red. In the persistent PTH phase, mice received delayed treatment with the anti-CGRP receptor antagonist olcegepant (3mg/kg) by administration on day 14 post-mTBI (C and D) or on day 28 post-mTBI (E and F). Animals were then exposed to BLS and periorbital and hindpaw responses recorded (response to treatment indicated by blue line). Note the small but significant effect of delayed treatment with olcegepant on reducing PPTH allodynia. *This figure was previously reported

As outlined in Major Task #2, a specific goal of this project was to determine if mTBI sensitizes animals to multiple migraine triggers. Specifically, in addition to the demonstrated ability of bright light stress (BLS) to induce allodynia in the PPTH phase we planned testing of exercise, another commonly reported migraine trigger. However, in conducting these experiments we noted that in mice the act of changing cages alone induced allodynia – but only in animals previously subjected to a mTBI. Cage changing is a known and previously demonstrated stress to animals and as such we believe acts similar to BLS in inducing allodynia in the PPTH phase. This finding prevented further testing of sensitivity to exercise as a trigger in the PPTH phase but demonstrates the reproducibility of our PTH model and sensitization to other forms of stress. Data from these studies are presented in Figure 4. Detailed in Major Aim 3 we also had planned examination of the time course of CGRP changes in our animal PTH model. However, despite our extended efforts the commercial CGRP kits tested failed to provide sufficient accuracy, sensitivity and reproducibility to allow further testing.

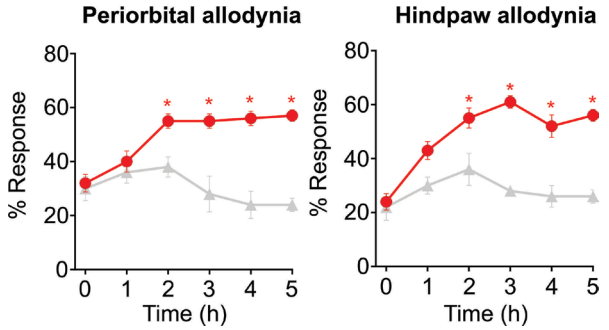


Figure 4. mTBI sensitizes animals to cage changing stress. Animals were subjected to sham (grey) or mTBI (red) and pain relevant behaviors monitored. At 14 days after the mTBI animals were subjected to cage changing stress and periorbital (top) or hindpaw (bottom) allodynia measured.

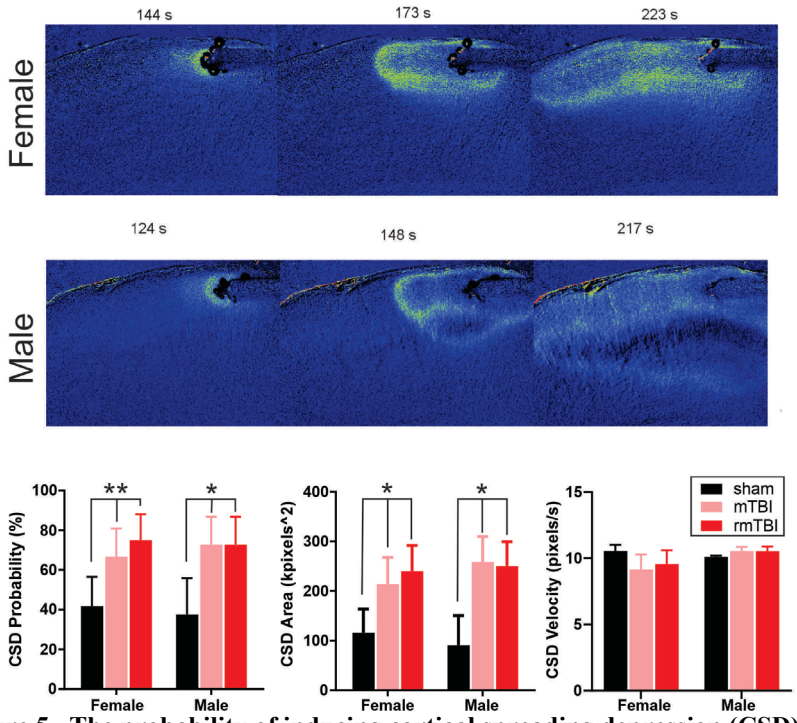


Figure 5. The probability of inducing cortical spreading depression (CSD) is significantly increased in the PPTH phase across both male and female animals. (Top) Intrinsic optical signal (IOS) imaging of CSD in male or female mice 14 days after being subjected to a mTBI. (Bottom) Bar graphs of average changes in CSD properties (induction probability, area of activation and velocity) between sham, single mTBI (mTBI) and repetitive mTBI (rmTBI, 1 impact/day for 3 days). Note while CSD probability is increased after mTBI or rmTBI no significant sex-dependent differences were observed.

In continued testing of sex differences, we also examined for changes in sensitivity in threshold

and properties of cortical spreading depression (CSD) following a mTBI. CSD underlies the induction of migraine aura, can induce headache pain and altered CSD properties are indicative of the development of headache pathophysiology. As previously reported, following a mTBI animals display a long-lasting period where the threshold for induction of CSD is reduced (i.e. as determined by an increase in the probability of CSD induction to a near threshold stimulus). In this reporting period we carefully analyzed for sex-dependent differences in CSD properties (probability of induction, area of activation and velocity) after either a single or repetitive (i.e. 3x mTBI, 1/day) mTBI. As detailed in Figure 5, while the probability of induction of CSD was significantly increased in mTBI animals this increase was not statistically different between male and female animals ($p>0.05$). Similarly, no statistically significant changes to other CSD properties were observed in mTBI compared to sham animals. Finally, we have now also compiled preliminary data on the efficacy of olcegepant treatment in reducing PTH related changes in CSD induction probability. As in our behavioral experiments, early olcegepant treatment (3mg/kg) began 2 hours after the mTBI and continued once every 3 days throughout the acute PTH phase (i.e. day 1-14 post-mTBI). Male animals were then prepared for IOS imaging and CSD induction probability calculated as previously described. While a small trend to reduced CSD induction probability in olcegepant treated animals was observed it failed to reach statistical significance (Figure 6).

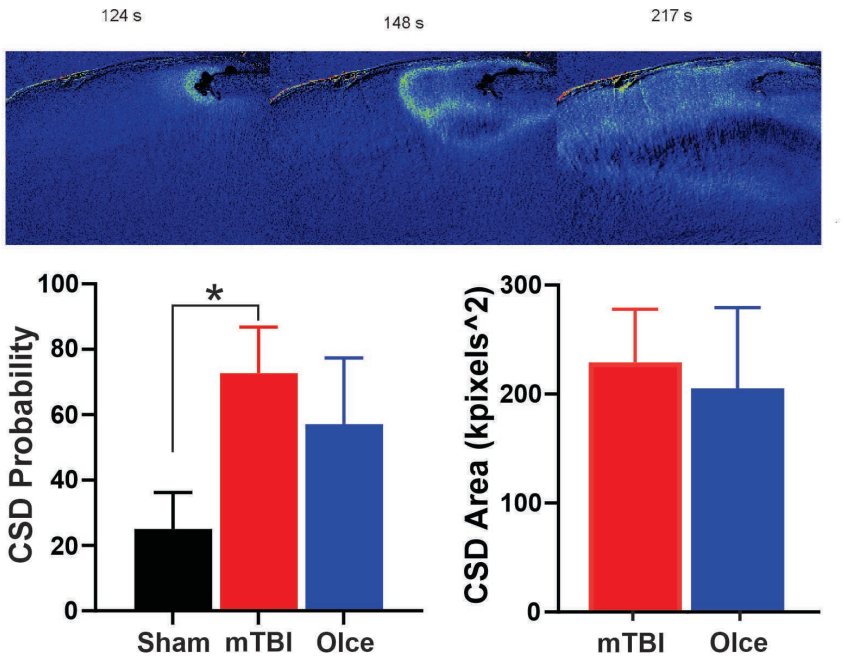


Figure 6. Treatment with the anti-CGRP receptor antagonist fails to reduce mTBI induced increase in CSD induction probability. (Top) Intrinsic optical signal (IOS) imaging of CSD across initiation and lateral cortical spread. Image series is from an olcegepant treated mTBI animal. (Bottom) Bar graphs of changes in CSD induction probability and area of activation across sham, mTBI vehicle (mTBI) or mTBI olcegepant treated (Olce) male animals.

Over the next reporting period we will finalize completion of Major Task #4 focused on the efficacy of delayed anti-CGRP treatment while continuing replication of the key findings including emergence of potential sex-dependent outcomes and treatment efficacy.

4) *Other achievements*: We have recently re-submitted a collaborative manuscript for publication entitled "Identification of *brain areas in mice with peak neural activity across the acute and persistent phases of post-traumatic headache*." While this neural circuit study is not directly part or funded by this DOD study it is a direct extension of results produced herein. This study details the unique neural circuit driving the development of acute and persistent PTH allodynia and is highly relevant to the central questions of this proposal including understanding the underlying cause of PTH and identifying key areas for intervention.

    - Rudolph M, Kopruszinski C, Wu C, Navratilova E, Schwedt T, Dodick D, Porreca F, Anderson TR. Identification of brain areas in mice with peak neural activity across the acute and persistent phases of post-traumatic headache. (submitted)

Individual Project #1: Pre-Clinical Studies	Timeline (Months)	Completion Status
Major Task 1: Animal Study Approvals		
Approval at Mayo Clinic	1-3	Completed (12/30/19)
Approval at University of Arizona	1-3	Completed (2/6/20)
DOD Approval	1-3	Completed (10/9/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (10/9/20)
Major Task 2: Mouse Model of PPTH		
Complete characterization of mouse PPTH model	3-12	Completed (1/31/21)
Determine if mTBI sensitizes mice to multiple migraine triggers (e.g. exercise)	6-18	Completed (8/31/2023)
Determine the effect of repetitive mTBI on PPTH	12-24	Completed (8/31/2023)
Milestone achieved: established mouse model of migraine trigger induced PPTH	24	Completed (8/31/2023)
Major Task 3: Anti-CGRP Dosing in Mice		
Complete time course of CGRP blood levels in mouse PTH model	18-30	Completed (see below)
Determine the dosing of anti-CGRP antibody in mice	24-36	Completed (8/31/2023)
Milestone achieved: established dosing of anti-CGRP mAb in mice and time course of elevated CGRP levels post TBI	36	Completed (8/31/2023)
Major Task 4: Efficacy of Anti-CGRP in Mouse Model of PTH		
Determine the efficacy of anti-CGRP antibody administered early post TBI in mouse PPTH model	24-36	Completed (8/31/2023)
Determine efficacy of anti-CGRP antibody administered at 45 days post TBI in mouse PPTH model	36-48	Ongoing
Determine the efficacy of anti-CGRP antibody administered late post TBI in mouse PPTH model	36-48	Ongoing
Milestone achieved: determination of the efficacy of anti-CGRP antibody in mouse PPTH model	48	Pending

Individual Project #2: Phenotyping and Neurophysiology

1) *Major activities during this reporting period*: As of 8/31/23, 69 human subjects have been enrolled (i.e., signed consent form) into this Focused Program. Six of these subjects signed consent but then did not participate in the study and two completed some of the initial study procedures and then stopped participating due to MRI issues (claustrophobia, abnormal brain MRI). A total of 166 study visits have been completed by 63 subjects. These subjects have completed study questionnaires and interviews, 135 quantitative sensory tests for determination of cutaneous heat pain thresholds, and 133 light discomfort threshold tests.

2) *Specific objectives*: Research participants have detailed phenotyping at the time of enrollment, 4 weeks later, and 16 weeks after enrollment using a set of questionnaires that collect information on demographics, TBI characteristics and symptoms, headache characteristics, sensory hypersensitivities, autonomic dysfunction, anxiety, depression, sleep, disability, post-traumatic stress disorder, pain catastrophizing, and cognitive function. Data are used for in-depth clinical description of our patient population, assessment of PTH outcomes, and for univariate and multivariate analyses to quantify the associations of the phenotype variables with headache persistence. All research participants with PTH maintain a daily headache and symptom diary to provide data on headaches, treatment, and presence and severity of associated symptoms. Data from the headache and symptom diary are used to assess changes over time, to determine who has headache persistence vs. resolution, and to track response to treatment for those in the clinical trial. Quantitative sensory testing is used to determine heat pain thresholds and temperatures required to elicit moderately intense pain. The effect of a bright light stressor on cutaneous heat pain thresholds are measured. Change in heat pain thresholds at baseline vs. 4 weeks and 16 weeks later are assessed.

3) *Significant results or key outcomes*: First research visit (i.e., baseline) data from healthy control subjects and those with acute PTH are shown in the following two tables. The tables summarize patient demographics and scores from questionnaires that asses post-TBI symptoms, anxiety, depression, cognitive function, insomnia, photophobia, pain catastrophizing, and post-traumatic stress. Overall, these

results demonstrate the substantial burden that is associated with PTH due to post-mTBI symptoms, visual and auditory hypersensitivities, sleep issues, and psychological sequelae.

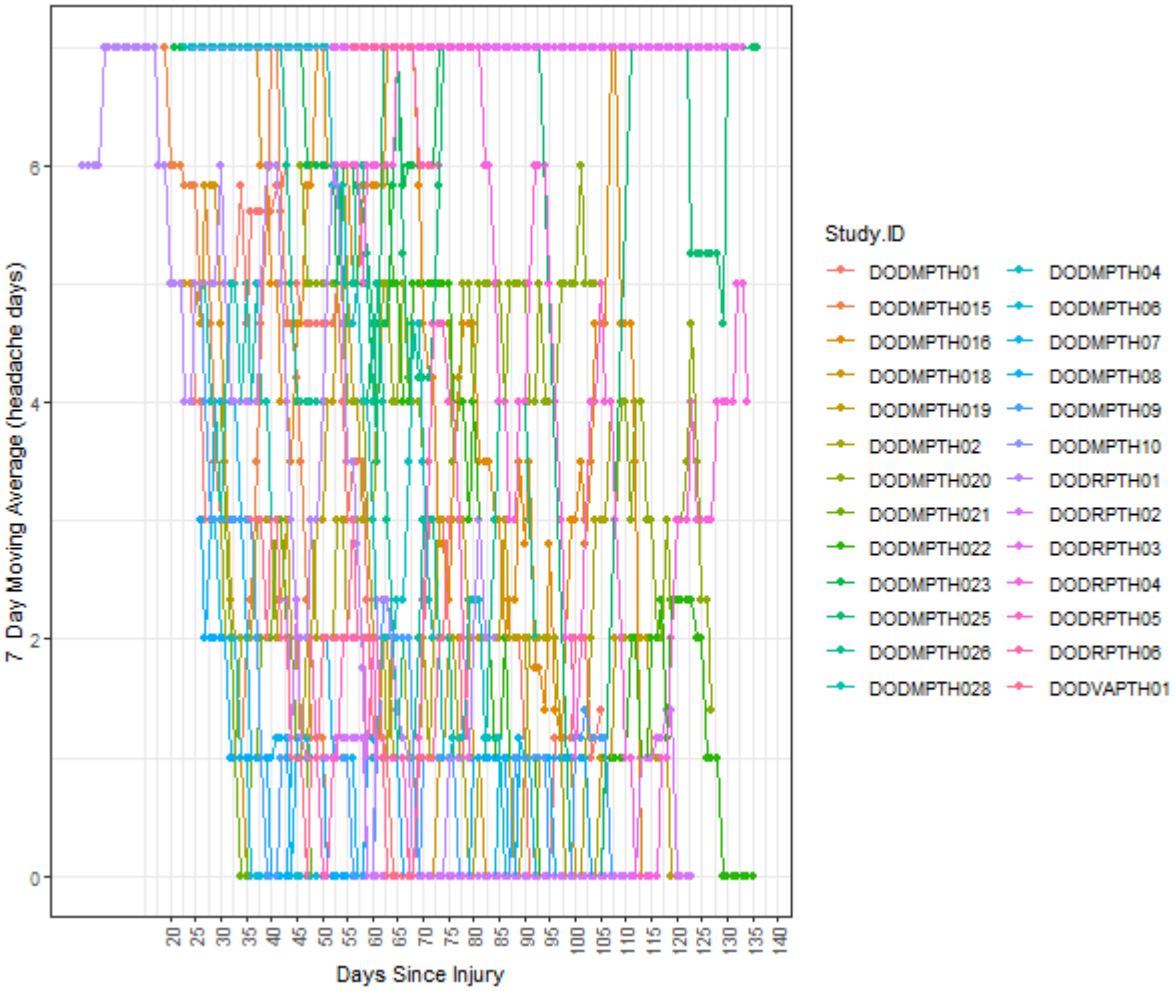
	Healthy Controls			Post-Traumatic Headache		
	N	**Mean (sd)**	**Median [1Q 3Q]**	**N**	**Mean (sd)**	**Median [1Q 3Q]**
Age	30	39.3 (15.82)	37.3 [26.7 48.8]	31	39.4 (16.69)	35.5 [24.9 55.3]
BDI	30	3.4 (3.61)	2 [0.2 5.8]	31	12.1 (8)	10 [7 15]
COWAT t-score	30	46.1 (12.01)	44.9 [36.1 57]	29	46 (12.68)	43.9 [33.5 55]
Hyperacusis	30	5.7 (3.23)	6 [3.2 7.8]	31	17.5 (10.48)	19 [7.5 24]
Insomnia	30	4.7 (4.02)	3.5 [2 7.8]	31	12.5 (5.76)	13 [8.5 17]
PAQ: Photophobia	30	0.9 (1.23)	0.5 [0 1]	31	3 (2.24)	3 [1 4]
PCS total score	30	8.8 (9.88)	4.5 [2 11.8]	31	12.3 (9.11)	11 [6.5 18.5]
PCS: helplessness	30	3.3 (4.89)	1 [0 4]	31	5 (4.67)	3 [2 8.5]
PCS: magnification	30	1.2 (1.57)	1 [0 2]	31	2.4 (1.8)	2 [1 3]
PCS: rumination	30	4.3 (4.37)	3 [1 6]	31	4.8 (3.54)	4 [1.5 7]
PTSD score	30	0.3 (0.8)	0 [0 0]	31	1.4 (1.5)	1 [0 2.5]
SCAT number of symptoms	30	1.9 (2.65)	1 [0 3]	31	13 (5.34)	14 [9 17.5]
SCAT total score	30	2.7 (4.63)	1 [0 3.8]	31	28.1 (15.52)	27 [18.5 36.5]
STAI: State anxiety	30	26.4 (7.19)	23 [21 31]	31	35.9 (11.43)	36 [25 44]
STAI: Trait anxiety	30	31 (9.2)	28 [24 36]	31	40.5 (14.02)	38 [29 47.5]
Trails A, z-score	30	0.4 (0.77)	0.5 [-0.2 1]	29	0.3 (1.23)	0.5 [-0.3 1.1]
Trails B, z-score	30	0.6 (0.58)	0.4 [0.3 1]	29	0.5 (1.27)	0.8 [0.3 1.1]

Total scores unless otherwise specified. N = sample size; sd = standard deviation; BDI: Beck Depression Inventory, COWAT = controlled oral word association test; PAQ: Photosensitivity Questionnaire, PCS: Pain Catastrophizing Score, SCAT = sport concussion assessment tool; STAI: State Trait Anxiety Inventory.

Variable	Response	Healthy Controls	Post-Traumatic Headache
Sex at Birth	Female	21	18
	Male	9	13
Gender	Woman	21	19
	Man	9	12
Race	Asian	0	2
	Black/African American	1	1
	White/Caucasian	28	28
	Other	1	0
Ethnicity	Hispanic	6	4
	Non-Hispanic	24	27
Education	High School Graduate (12th Grade)	1	2
	GED or equivalent	0	1
	Some College; no Degree	8	5
	Associate Degree	5	3
	Bachelor's Degree	12	13
	Master's Degree	2	3
	Doctoral Degree (MD, PhD, DDS, DVM)	2	4
SCAT: Symptoms Worse with Physical Activity	No	30	10
	Yes	0	21
SCAT: Symptoms worse with Mental Activity	No	30	5
	Yes	0	26

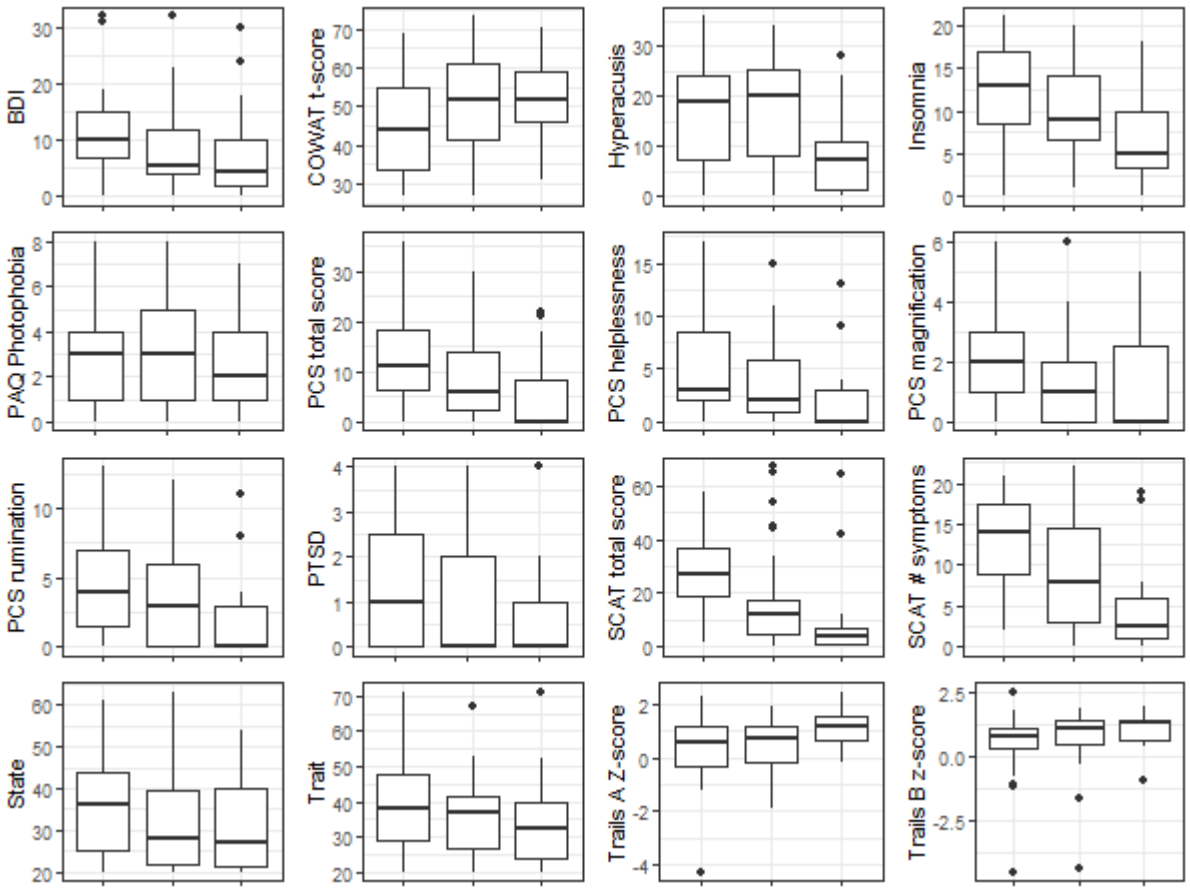
SCAT: Sport Concussion Assessment Tool

Headache Frequency Changes: Twenty-nine PTH participants have completed the first 28-day diary completion period with adequate data. In the first period following enrollment, PTH participants completed 27 diary entries/28-day month and reported 16.0 days with headache. In the second period following enrollment (p2), a mean of 10.4 headache days have been reported with a minimum of zero and maximum of 28 headache days reported. For the PTH participants who have completed p2, the number of headaches reported in p2 is 62.3% of the amount reported in p1.



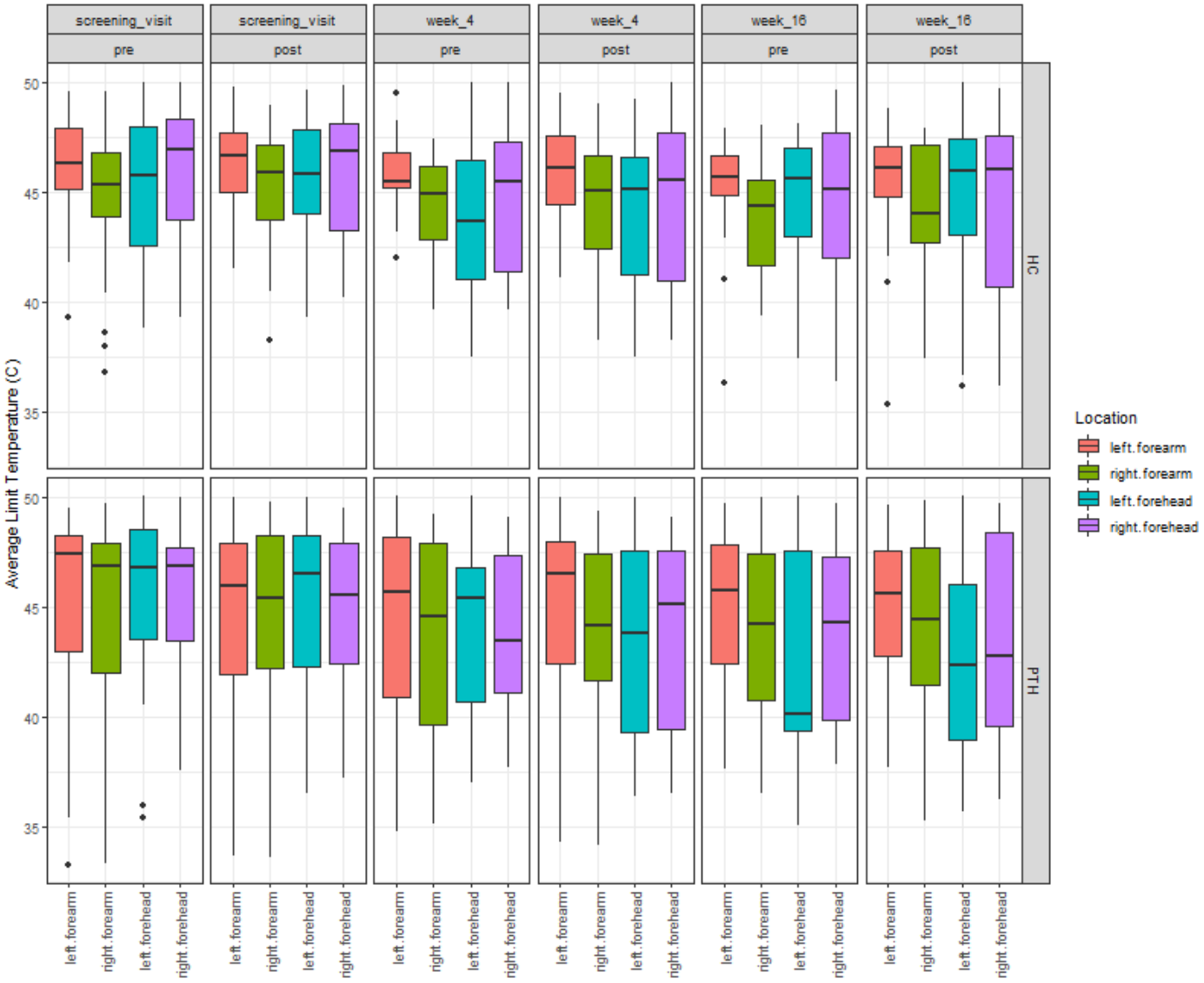
Individual 7 day moving average of diary-reported headache days by days since injury.

Longitudinal Changes in Questionnaire and Cognitive Assessment Scores: Amongst those with PTH, baseline, 4-week, and 16-week scores for depression, anxiety, hyperacusis, insomnia, pain catastrophizing, post-traumatic stress disorder, post-concussion symptoms according to the SCAT, and cognitive test performance are demonstrated in the figure below. Overall, there is a lessening of symptoms and improvement in performance over time.



Questionnaire and Cognitive Assessment Scores for those with PTH at each of their three research visits. BDI = Beck Depression Inventory; COWAT = controlled oral word association test; PAQ = photosensitivity assessment questionnaire; PCS = pain catastrophizing scale; PTSD = post-traumatic stress disorder; SCAT = sport concussion assessment tool symptom checklist; State = state anxiety; Trait = trait anxiety.

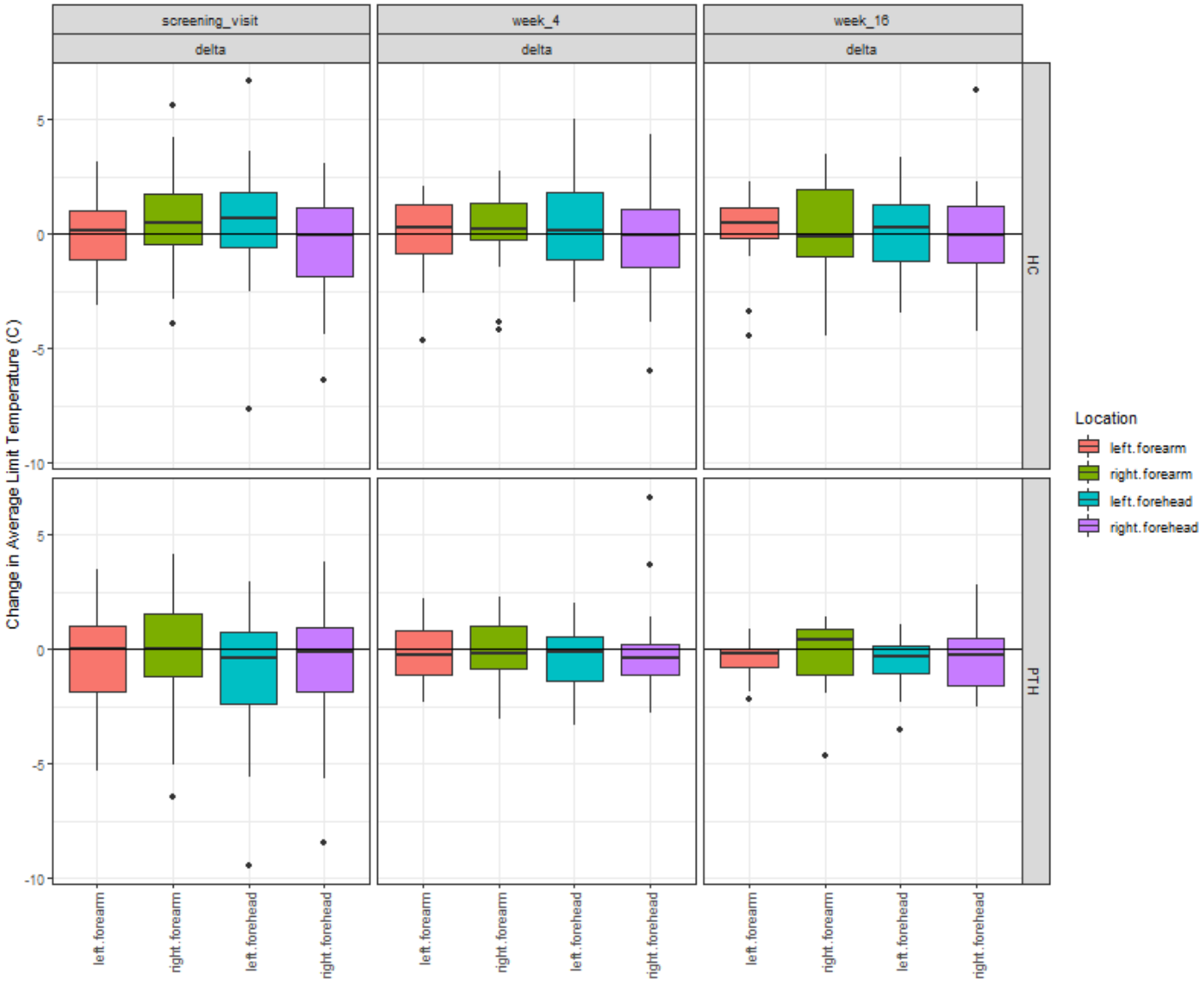
Cutaneous Pain Thresholds: Heat pain thresholds on the skin of the forehead and forearms are measured using the method of limits at each visit. Pain thresholds are measured prior to and following exposure to bright light.



Average cutaneous pain thresholds at each body location, pre- and post-bright light exposure, by time point, and patient group. The figure above shows the pain thresholds by body site, pre- and post-light exposure over time for the healthy control and PTH groups. PTH thresholds are similar to those of the healthy control group in the screening visit, but they trend lower as time passes (suggesting development of sensitization) while the thresholds of the healthy control group remain approximately the same.

	Baseline Average Pain Threshold Forehead (°C)	Post-Light Stressor Average Pain Threshold Forehead (°C)	Change from Baseline in Average Pain Threshold Forehead (°C)	Baseline Average Pain Threshold Forearm (°C)	Post-Light Stressor Average Pain Threshold Forearm (°C)	Change from Baseline in Average Pain Threshold Forearm (°C)
Healthy Control (n=26)	45.7	45.7	0.057	45.5	45.9	0.42
PTH (n=21)	45.7	44.9	-0.76	45.0	44.6	-0.41
p-value	0.97	0.37	0.21	0.64	0.17	0.12

Cutaneous Pain Thresholds Prior to and Following a Bright Light Stressor. The table shows heat pain thresholds at the forearm and forehead in healthy controls and in those with PTH prior to and following visual stress with bright light at the baseline timepoint. When comparing healthy controls to those with PTH, there is a difference in the change in forearm and forehead pain threshold pre- and post-light exposure between the PTH and healthy control groups (non-significant p-value). It is notable that pain thresholds are reduced at the forearm and forehead following bright light stress in those with PTH, but not in healthy controls.



Cutaneous Pain Thresholds Prior to and Following a Bright Light Stressor: This chart shows the change in heat thresholds after bright light exposure by body location over time. The post-light temperature limits tend to be unchanged or slightly higher than the pre-light temperature limits for the healthy control group. The opposite is true for the PTH group, meaning that the PTH group becomes more sensitive to cutaneous stimulation after the light exposure.

4) *Other achievements*: N/A

Individual Project #2: Clinical Phenotyping and Neurophysiology	Timeline (Months)	Completion Status
Major Task 1: Human Study Approvals		
IRB approval at Mayo Clinic	1-3	Completed (5/15/20)
DOD HRPO Approval	1-3	Completed (11/24/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (11/24/20)
Major Task 2: Case Report Form and Database Development		
Development of case report forms	1	Completed (month 1)
Development of study database	1	Completed (month 1)
Major Task 3: Clinical Phenotyping and Neurophysiology Testing		
Completion of Initial Structured Interviews and Study Questionnaires	4-36	Ongoing (63 participants completed)
Completion of Follow-Up Structured Interviews and Study Questionnaires	7-42	Ongoing (52 participants completed one or more follow-up visits; 104 follow-up visits completed)
Completion of Initial Quantitative Sensory Testing and Visual Discomfort Threshold Testing	4-36	Ongoing (55 participants completed)
Completion of Follow-Up Quantitative Sensory Testing and Visual Discomfort Threshold Testing	7-39	Ongoing (46 participants completed one or more follow-up visits for testing; 80 follow-up visits for testing completed)
Major Task 4: Interim and Final Data Analyses		
Interim Analyses: Clinical Phenotypes, Neurophysiology Test Results	20-24	Ongoing
Final Analyses: Clinical Phenotypes, Neurophysiology Test Results	39-48	Pending

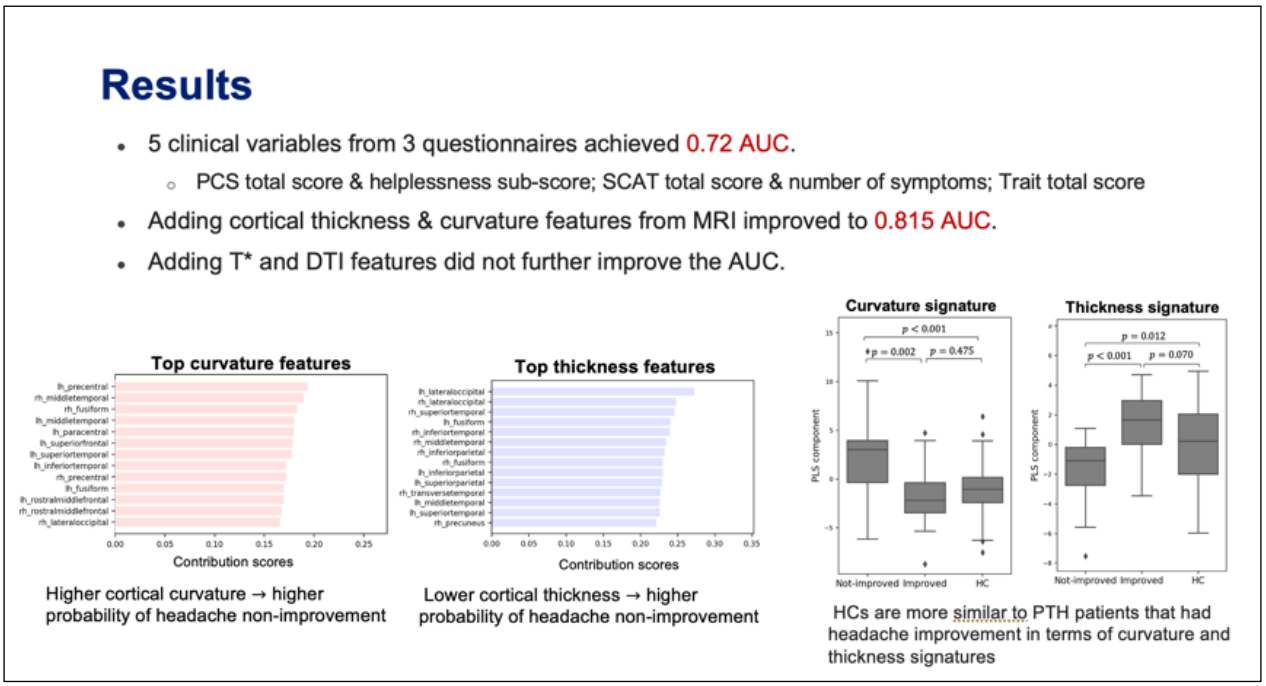
Individual Project #3: Neuroimaging

1) *Major activities during this reporting period:* As of 8/31/23, 54 human subjects have completed baseline brain MRI. A total of 138 MRIs has been completed. The prospective, longitudinal MRI protocol includes structural and functional imaging, using the following sequences: a) high-resolution T1-weighted image; b) T2-weighted image; c) diffusion tensor imaging; d) T2*; e) resting state blood oxygenation level dependent (BOLD); f) event-related BOLD in response to noxious heat applied to the skin.

2) *Specific objectives:* Structural and functional brain MRI data will be analyzed with a goal of identifying imaging: a) predictors of PPTH; b) changes that occur within individuals as they have headache persistence or resolution; c) predictors of treatment response to a CGRP monoclonal antibody. Imaging data will allow for determination of brain regional volumes, cortical thickness, cortical surface area, white matter tract integrity, iron deposition, resting state functional connectivity, and brain activations in response to noxious stimuli.

3) *Significant results or key outcomes:* All T1-weighted images were quality checked, post-processed, and segmented for regional cortical thickness, volume, area, and curvature using the most recently available FreeSurfer, 7.2. pipeline. Hippocampal, thalamic, amygdala and brainstem areas were additionally segmented for subcortical volume. DTI imaging of all available time-points has been post-processed using FreeSurfer, 7.2 (Tracula pipeline) to assess tract-based metrics for 42 white matter pathways. T2* signal has been investigated.

An analysis to assess the accuracy for predicting headache improvement/non-improvement using baseline measures collected at the first time-point (0-59 days post-TBI) showed that an accuracy of 0.72 AUC was achieved using only three questionnaires from our comprehensive questionnaire battery. Adding T1-weighted measures of cortical curvature and thickness measures as features improved the classification accuracy to 0.815 AUC for predicting headache improvement. These data were presented as an oral presentation at the American Headache Society Annual Scientific Meeting in June 2023.



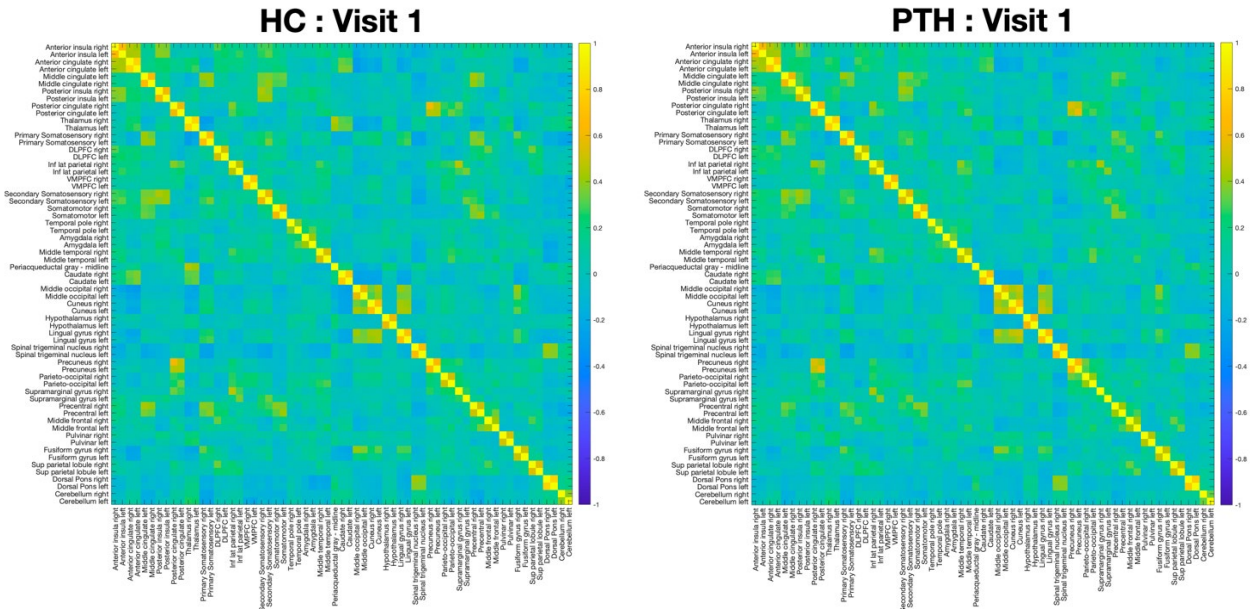
An analysis to assess the accuracy for predicting headache improvement/non-improvement at 3-4 months post mTBI using five variables from three clinical baseline questionnaires and the top 10 most important variables from the daily headache diary yielded an accuracy of 76% for predicting headache improvement based on 4-weeks of diary data and an accuracy of 83% using 7-weeks of headache diary data.

Model / Time length of diary data	All variables	All variables (slope only)	Top 10	Top 10 (slope only)	Top 5	Top 5 (slope only)
+ 3wk	0.578	0.656	0.607	**0.677**	0.594	0.649
+ 4wk	0.684	0.737	0.728	**0.762**	0.709	0.743
+ 5wk (N=52)	0.715	0.715	0.715	**0.739**	0.735	0.744
+ 6wk (N=52)	0.759	0.752	0.770	**0.780**	0.776	0.774
+ 7wk (N=52)	0.781	0.811	0.800	**0.835**	0.830	0.820
+ 8wk (N=51)	0.733	0.754	0.756	**0.799**	0.754	0.768

Resting state functional connectivity analyses were updated for this progress report. A region of interest approach (ROI) was used to interrogate functional connectivity patterns. The ROIs for this study were selected based on our previous findings and included 31 bilateral and one midline region important for pain processing or multisensory integration. The ROIs were defined by spheres with eight mm diameter centered on MNI coordinates. The Pearson correlation coefficient was reported between regions. Following the correlation analysis, Fisher r-z transforms were calculated for each ROI-ROI pair and used in the t-tests.

PTH vs. HC (Two-Group T-Test): Resting state functional connectivity in those with PTH (N=20) compared to healthy controls (N=24) at research visit one, with $p<0.005$, uncorrected.

Baseline: PTH vs HC		Correlation Coefficient		
Region 1	Region 2	HC Visit 1	PTH Visit 1	p-value
Thalamus right	Periacqueductal gray - midline	0.45	0.21	0.0001
Thalamus left	Periacqueductal gray - midline	0.42	0.23	0.001
Periacqueductal gray - midline	Fusiform gyrus left	-0.14	0.00	0.001
Temporal pole left	Cerebellum right	-0.04	0.11	0.003
Middle frontal left	Cerebellum left	-0.15	0.00	0.004
VMPFC left	Secondary Somatosensory left	0.16	-0.02	0.004



Correlation coefficients amongst the seed ROIs in healthy controls (HC) and those with post-traumatic headache (PTH). Data collected during baseline visit.

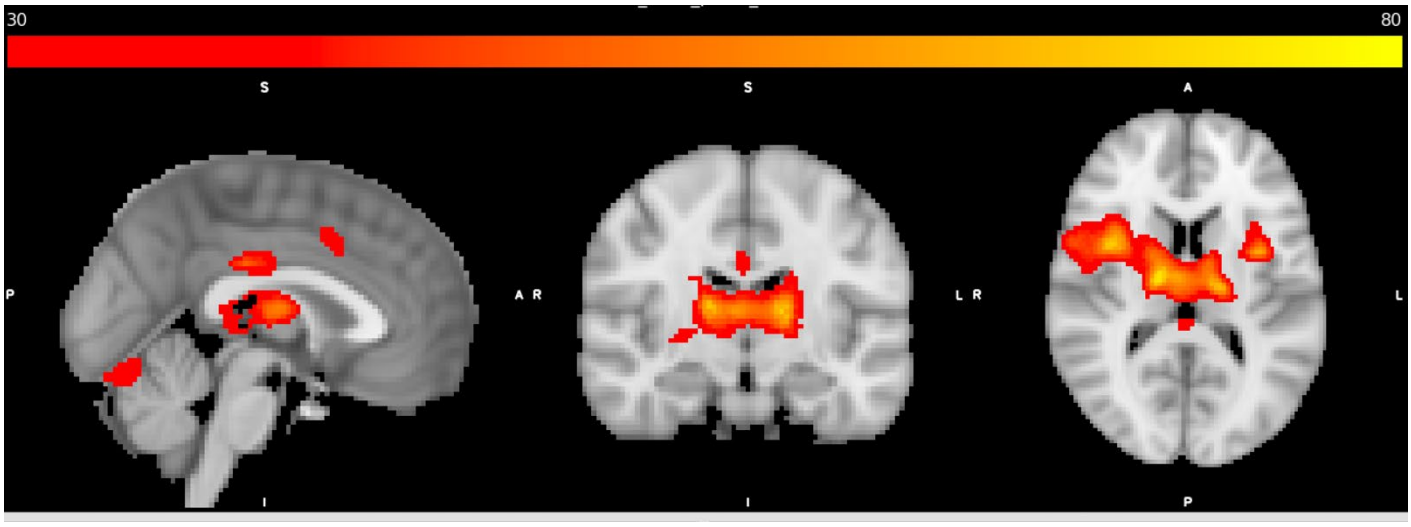
PTH Research Visit 1 vs. Research Visit 2 (Paired Two-Group T-Test, N=20): Changes in resting state functional connectivity strength amongst those with PTH from visit one to visit two are listed in the table below ($p<0.005$, uncorrected).

		Correlation Coefficient		
Region 1	Region 2	PTH visit 1	PTH Visit 2	p-value
Periacqueductal gray - midline	Sup parietal lobule right	0.06	-0.06	0.0003
Posterior cingulate right	Secondary Somatosensory left	-0.07	0.05	0.0006
Primary Somatosensory right	Sup parietal lobule right	0.06	0.21	0.0006
Primary Somatosensory left	Sup parietal lobule right	-0.01	0.16	0.0007
DLPFC left	Temporal pole left	0.00	-0.12	0.003
VMPFC right	Middle temporal right	0.12	0.25	0.003
Posterior insula left	VMPFC left	0.00	0.13	0.003
Middle occipital left	Spinal trigeminal nucleus right	-0.08	0.03	0.003
Primary Somatosensory right	Middle occipital left	0.16	0.02	0.004
VMPFC left	Secondary Somatosensory left	-0.06	0.08	0.005

Pain Stimulation fMRI: All preprocessing and general linear model (GLM) estimation of brain activation patterns for the event-related portion of the fMRI were performed using SPM12, interfaced with MATLAB.

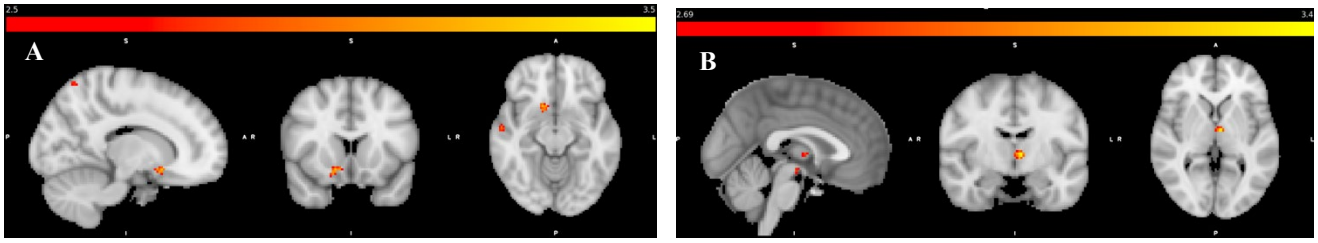
Functional images were realigned to the mean volume in the series, motion-corrected, realigned to each individual's structural images, and smoothed using an 8 mm full width half maximum (FWHM) Gaussian kernel. Brain regions activated in response to painful stimuli were identified by generating contrast maps representing brain activations associated with moderate intensity painful stimuli preceded by auditory cue vs. auditory cue with no painful stimuli. The BOLD signal was modeled by the stimulus onset with duration equal to 12.5 seconds (5 MRI frames). The contrast maps were normalized to standard stereotaxic space (MNI template) using the high resolution co-registered anatomical T1-weighted image. A main effects analysis identifies brain regions activated in subjects and a two-sample analysis identifies regions differentially activated when comparing subject cohorts. Cluster threshold correction and multiple comparisons correction were utilized.

Main Effect (F-statistic) of Painful Stimulation, Baseline Time Point (N_{HC} = 28 , N_{PTH} = 23): There is a robust main effect in the bilateral thalamus, putamen, supramarginal gyrus, cerebellum, and insula ($p < 0.05$ FWE corrected) as shown below. These are brain regions commonly identified to be active in response to pain stimuli applied to the left forearm, thereby demonstrating the quality of our event-related paradigm.



PTH vs. Healthy Controls – Pain Stimulation (N_{HC} = 28, N_{PTH} = 23): Two group T-test showed A) greater activation in response to painful stimulation in bilateral parietal, right temporal, occipital, left cuneus, and

right ventral striatum amongst those with PTH compared to HC and B) reduced activation in PTH compared to HC in left thalamus and bilateral red nucleus (p<0.005 with 10 voxel cluster volume threshold).



4) *Other achievements*: N/A

Individual Project #3: Human Neuroimaging	**Timeline (Months)**	**Completion Status**
Major Task 1: Human Study Approvals		
IRB approval at Mayo Clinic	1-3	Completed (5/15/20)
DOD HRPO Approval	1-3	Completed (11/24/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (11/24/20)
Major Task 2: Magnetic Resonance Imaging and Data Processing		
Completion of Initial Patient Imaging	4-36	Ongoing (54 participants completed)
Completion of Follow-Up Patient Imaging	7-39	Ongoing (84 follow-up MRIs have been completed)
MRI Data Processing	4-40	Ongoing
Major Task 3: Data Analyses		
MRI Data Analyses for Quality Control	4-39	Ongoing
Interim MRI Data Analyses	20-24	Ongoing
Final MRI Data Analyses	40-48	Pending

Individual Project #4: Molecular Biomarkers

1) *Major activities during this reporting period:* As of 8/31/23, 55 human subjects have contributed at least one blood sample. A total of 140 blood samples have been collected yielding the following aliquots to date: 137 whole blood, 687 plasma, and 542 serum.

2) *Specific objectives:* We aim to incorporate multi-genomic approaches in attempts to identify biomarkers of clinical relevance to PTH persistence as follows: a) *Genetic Risk Markers for PTH Persistence* – Genomic data will be used to identify common and rare genetic variants that may be enriched in the PPTH cohort. The goal is to identify individuals who may be at elevated risk for PPTH so that they may be more intensely managed during the acute phase. b) *Fluid-based Markers for PPTH* – Longitudinal blood specimens will be analyzed for protein biomarkers that indicate an elevated risk for PPTH. c) *Responder / Non-Responder to Erenumab* – We will analyze longitudinal blood samples with the goal of identifying biomarkers that correlate with response to erenumab. Additionally, the genotyping data will be leveraged with the goal of identifying a genetic basis for erenumab response.

3) *Significant results or key outcomes:* 53 individual participants have completed APOE genotyping. All DNA samples are currently staged for genome-wide SNP genotyping. All ELISA targets are assessed in batches of 23 samples, and we have completed ELISA analysis for all ELISA-based targets for 115 total blood samples – equivalent to 88% of all available biospecimens. The remaining 15 available specimens will be staged for the next ELISA batch. The Meso-Scale Discovery (MSD) based assays require 39 samples at a minimum. We have completed 117 samples in the MSD assay – equivalent to 90% of all available biospecimens. The remaining 13 samples will be staged for the next MSD batch.

4) *Other achievements:* N/A

Individual Project #4: Molecular Biomarkers	Timeline (Months)	Completion Status
Major Task 1: Human Study Approvals		
IRB approval at Mayo Clinic	1-3	Completed (5/15/20)
TGen approvals	1-3	Completed
DOD HRPO Approval	1-3	Completed (11/24/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (11/24/20)
Major Task 2: Establishment of Biospecimen Collection, Storage, and Transfer Procedures		
Mayo Clinic	1	Completed (month 1)
TGen	1	Completed (month 1)
Major Task 3: Specimen Collection and Processing		
Specimen Collection	4-42	Ongoing (140 blood draw visits from 55 participants)
Specimen Processing and Analyses	4-42	Ongoing APOE genotyping: 100% completed. SNP genotyping: 100% completed. ELISA assays: 100% of samples received are complete across all targets. Meso-Scale Discovery: 100% of received samples are completed.
Major Task 4: Data Analyses		
Biospecimen Analyte Quality Control	4-42	Ongoing
Next Generation Sequencing, Array, and ELISA Quality Control	4-42	Ongoing
Multi-Omic Biomarker Identification and Association Analyses	4-42	Ongoing

Individual Project #5: Clinical Trial

1) *Major activities during this reporting period:* Six participants have been enrolled and randomized in the clinical trial. Six participants have received study medication (erenumab or placebo) and three participants have completed the clinical trial.

2) *Specific objectives:* This clinical trial will provide insights into whether intervention with a CGRP receptor monoclonal antibody (erenumab) in a population of participants who have acute PTH prevents PTH persistence.

3) *Significant results or key outcomes*: none during this reporting period.

4) *Other achievements:* N/A

Individual Project #5: Clinical Trial	**Timeline (Months)**	**Completion Status**
Major Task 1: Human Study Approvals		
IRB approval at Mayo Clinic	1-3	Completed (5/15/20)
DOD HRPO Approval	1-3	Completed (11/24/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (11/24/20)
Major Task 2: Investigational Drug Exemption		
IND Application Submission	1	Completed (Exemption Granted)
IND Application Approval	2	Completed (Exemption Granted)
Major Task 3: Major Task 4: Clinical trial reporting structure		
Establish Clinical Trial Database, Case Report Forms and Randomization Scheme	1-2	Completed (month 1)
Major Task 4: Patient Enrollment		
Patient Enrollment into Clinical Trial	4-36	Ongoing (6 participants enrolled into clinical trial)
Patient Follow-up Complete	42	Ongoing (6 participants completed one or more follow-up visits in clinical trial; 30 total follow-up visits completed)
Major Task 5: Data Analyses and Reporting		
Analyses of Clinical Trial Data	42-45	Pending
Clinical Trial Outcome Reporting	45-48	Pending

Individual Project #6: Multivariate Modeling

1) *Major activities during this reporting period:* We focused on the evaluation and validation of developed multivariate models. As the data collection and processing in the other individual projects progressed, we started data fusion - integrating data from different sources in model development.

2) *Specific objectives:* We aim to develop and validate machine learning (ML) and deep learning (DL) models to support advanced multivariate analysis of the dataset generated from each of the other individual projects including: (a) expansion of our developed MMI-DDS machine learning pipeline to predict PTH persistence using neuroimaging, phenotype, neurophysiology, and molecular/genetic biomarker data; (b) delineation of patient heterogeneity in the development of PTH persistence; (c) longitudinal analysis to understand PTH mechanisms; (d) image-genomics analysis to understand the association between imaging and genomic markers of PTH persistence; and (e) transfer learning between animal and human models.

3) Significant results or key outcomes:

(A) Unsupervised DL model development for headache detection from an unannotated mixed dataset

Rationale: We built a novel unsupervised DL model, Brainomaly, for headache detection. This novel headache detection method leverages our validation metric utilizing imperfect annotation and improved image-to-image translation technique achieving greater headache detection. We tested the model's performance for separating patients with migraine, post-traumatic headache (PTH), or persistent post-traumatic headache (PPTH) from healthy individuals. Brainomaly learns from large unannotated datasets, as large, annotated MRI datasets from those with headaches are rare.

Model: Brainomaly is a GAN-based image-to-image translation method. It is trained (Figure IP6-1) to remove the neurologic diseases from any (either from individuals with neurologic diseases or healthy subjects) T1-weighted MR input brain image and generate T1-weighted MR image of the corresponding healthy brain using (1) a set of "unannotated mixed brain MRIs" containing T1-weighted brain MRIs from individuals with neurologic diseases and healthy subjects and (2) another set containing T1-weighted

brain MRIs only from healthy subjects. Once trained, the generator turns any brain MRI into the MRI of the corresponding healthy brain. Please note, if the input MRI is already of a healthy brain, then the generator is expected to keep the output unchanged from the input. Hence, subtracting the generated MRI of the healthy brain from its input would reveal structural changes if the input MRI were of an abnormal brain. We use the average value of the resultant difference map as the disease detection score, where higher values indicate a higher likelihood that the brain MRI is from someone with neurologic disease. Brainomaly learns to generate an additive map that indicates how much each voxel in the input brain MRI should be changed to make the brain look healthy. The final MRI of a healthy brain is generated by performing tanh activation on the voxel-wise addition of the input image and the additive map. The use of additive maps alleviates the requirement of cycle-consistency loss as Brainomaly does not generate the image directly, improving image-to-image translation, thus, disease detection.

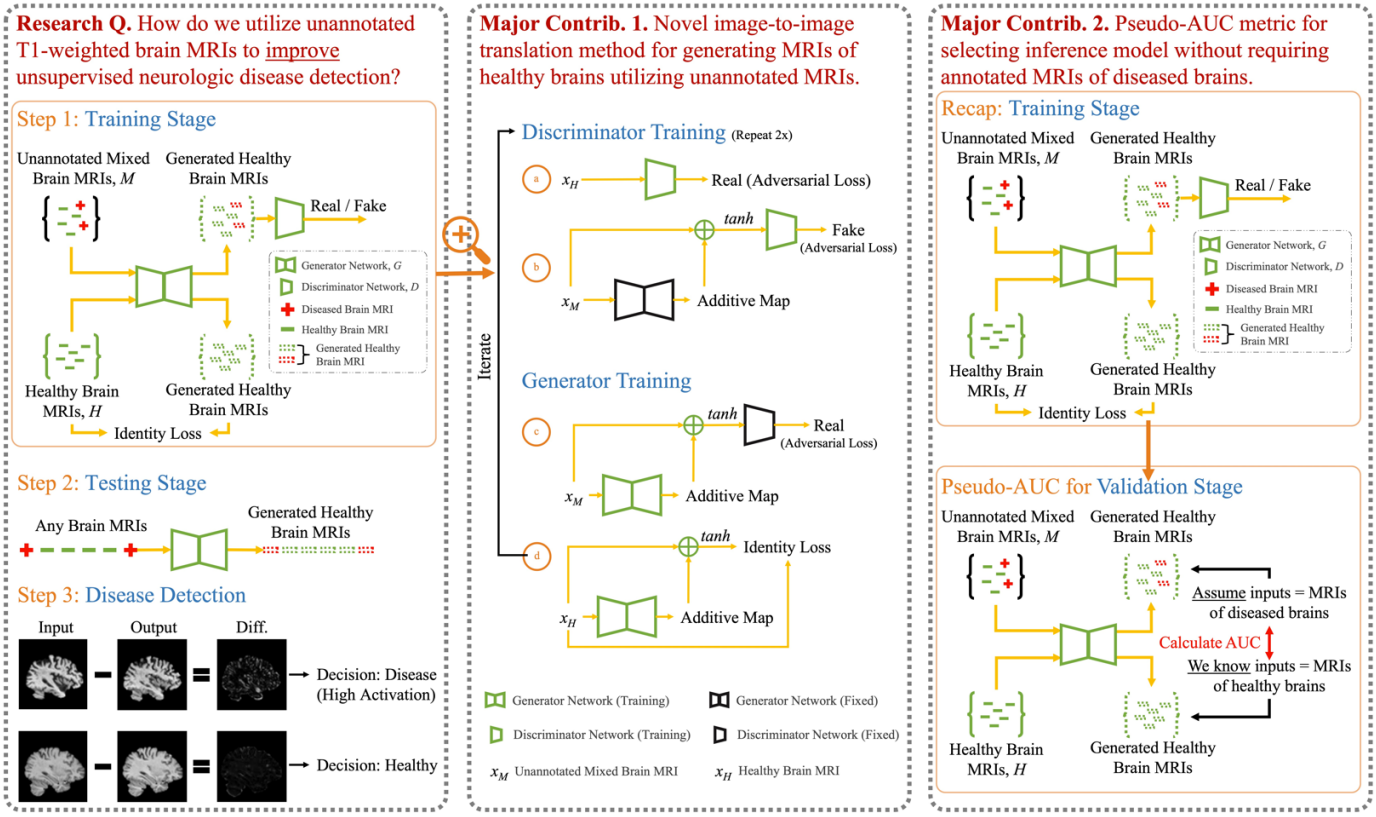


Figure IP6-1. Overview of the Brainomaly method and its major innovation.

Brainomaly learns from the given T1-weighted brain MRIs by iteratively going over the entire dataset. Therefore, it produces a model after a fixed set of iterations. In a supervised learning setting, these models would be evaluated on a small validation dataset, and the best-performing model would be selected for inference. Therefore, we use our proposed AUC_p metric for model selection. To calculate

AUC_p, we first generate the *disease detection scores* for each model. To get these scores, we translate all the given brain MRIs to MRIs of healthy brains using a trained generator model of Brainomaly. Then, we subtract the generated MRIs of healthy brains from their corresponding input MRIs. If the brain in the input MRI is diseased (*i.e.,* abnormal), the resultant difference map would reveal structural changes. For an input MRI of a healthy brain, the difference map should reveal less or no structural changes. We call the voxels showing the structural changes *activations*. The average of all the activations in the difference map of an input MRI is its *disease detection score*, where a higher score indicates a higher likelihood of the input brain being diseased. As we already know that set *H* (see Figure) contains only healthy-brain MRIs, we assume the labels for MRIs in the unannotated mixed brain MRI set, *M* (see Figure), to be diseased brains. Then, we use these *imperfect* annotations as ground truths along with the *disease detection scores* in the traditional AUC calculation resulting in AUC_p scores. Once the AUC_p scores are available for all the models, we select a model with the highest AUC_p score for inference.

Results: Our dataset contained MRIs of 48 acute post-traumatic headaches (APTH) patients, 49 persistent post-traumatic headaches (PPTH) patients, and 532 healthy individuals (HC). In addition, we added 96 migraine patients to support on-going subject heterogeneity interrogations. Among these, MRIs of 428 healthy individuals were collected from the publicly available IXI dataset. For our experiments, we randomly selected 232 MRIs of healthy individuals for the known healthy set. Our unannotated mixed set contained 48 migraine, 24 APTH, 24 PPTH, and 150 HCs. We tested our models on the rest of 48 migraine, 24 APTH, 25 PPTH, and 150 HCs.

Brainomaly outperforms the existing methods in headache detection by a large margin. It achieved an average headache detection AUC of 89.60. Using our proposed AUC_p model selection metric, the second-best method, HealthyGAN, improved its AUC from 76.95 to 80.88, but it still fell behind Brainomaly's performance. Other baseline methods like ALAD, Ganomaly, and DDAD achieved even poorer AUCs of 0.6955, 0.6913, and 0.6280, respectively. f-AnoGAN and ALOOC just failed in this task.

Methods	Headache Detection AUC
f-AnoGAN	40.71
ALOOC	48.05
DDAD	62.80
Ganomaly	69.13
ALAD	69.55
HealthyGAN	76.95
HealthyGAN + AUCp	80.88
Brainomaly (proposed method)	**89.60**

Table IP6-1. Summary of headache detection results.

Conclusion: Our experiment yielded promising results showing the effectiveness of the proposed Brainomaly's improved image-to-image translation and AUC_p metric for model selection.

(B) *Biomarker: MRI signatures of brain age as a potential biomarker to understand PTH persistence and patient heterogeneity.*

Rationale: We hypothesized that the difference between the biological ages captured by bio-signatures from brain MRI and chronological ages, Δ_{age}, may act as a surrogate neuroimaging biomarker to support acute PTH (APTH) investigations.

Method: We trained a deep learning based ResNet-18 model using a novel ORdinal Distance Encoded Regularization (ORDER) loss on a healthy cohort (HC) of 7,377 individuals (public datasets: IXI, ICBM, ABIDE, OASIS, NACC) to predict biological age given an input T1-weighted brain MRI image. Our model outperforms other state-of-art models with mean absolute error (MAE)=2.56 on the held-out test set. To investigate if acute PTH subjects develop accelerated aging signatures we applied this trained model to a HC cohort (N=77, age=38.4) collected from our prior studies, DS1 and DS2 (both available from team's previous projects), and an APTH cohort (N=71, age=39.3) collected from DS1, DS2 and DS3 (this project).

Results: Applying this model independently to HC and APTH cohorts, the predicted Δ_{age} (predicted biological age – actual chronological age) were -1.4 and 1.3, respectively. This indicates that APTH subjects may have structural changes similar to accelerated aging which can be used as a biomarker for

better diagnosis and early detection. Moreover, Δ_{age} for APTH (=1.2) was much close to subjects who were diagnosed with mild cognitive impairment at baseline and converted to Alzheimer's disease in longitudinal follow-ups (Δ_{age}=1.2) collected from ADNI in a separate study. Considering that different data sources may have heterogeneity due to different scanner and imaging protocols; we compare Δ_{age} values for each source separately. For DS1, Δ_{age} for HC (N=40, age=38.5) and APTH (N=37, age=42.4) was -0.6 and 0.5 respectively; for DS2, Δ_{age} for HC (N=26, age=38.3) and APTH (N=24, age=38.3) was -2.2 and 4.2; whereas DS3 Δ_{age} for APTH (N=10, age=37.3) was -0.9. The model was able to accurately differentiate between HC and APTH for DS2 with matching age distributions. Whereas for DS1 the difference in Δ_{age} between groups is relatively small. Since Δ_{age} is dependent on actual age and APTH subjects in DS1 are older than HC, adjustment methods to Δ_{age} depending on actual age will be included in future studies. We also note that Δ_{age} for DS3 is relatively low, however that dataset size is small, and we need more efforts to explore this cohort further.

Conclusion: Mean Δ_{age} from PTH is larger than mean Δ_{age} from HC. This suggests there might be an imaging signature related to brain age that deep learning can capture from MRI. We plan to explore how to further adjust Δ_{age} depending on actual age making it a reliable biomarker. Future analyses will test whether this age signature is a predictor for PTH persistence.

(C) *Classification using event-based fMRI for differentiating PTH and HC*

Rationale: We preliminarily explored event-based fMRI data aiming to identify features that are informative for predicting PTH improvement vs. without improvement. While awaiting sufficient sample sizes and outcome data for prediction, we aimed to differentiate PTH and HC. These results should be informative to the prediction of improvement status of PTH patients.

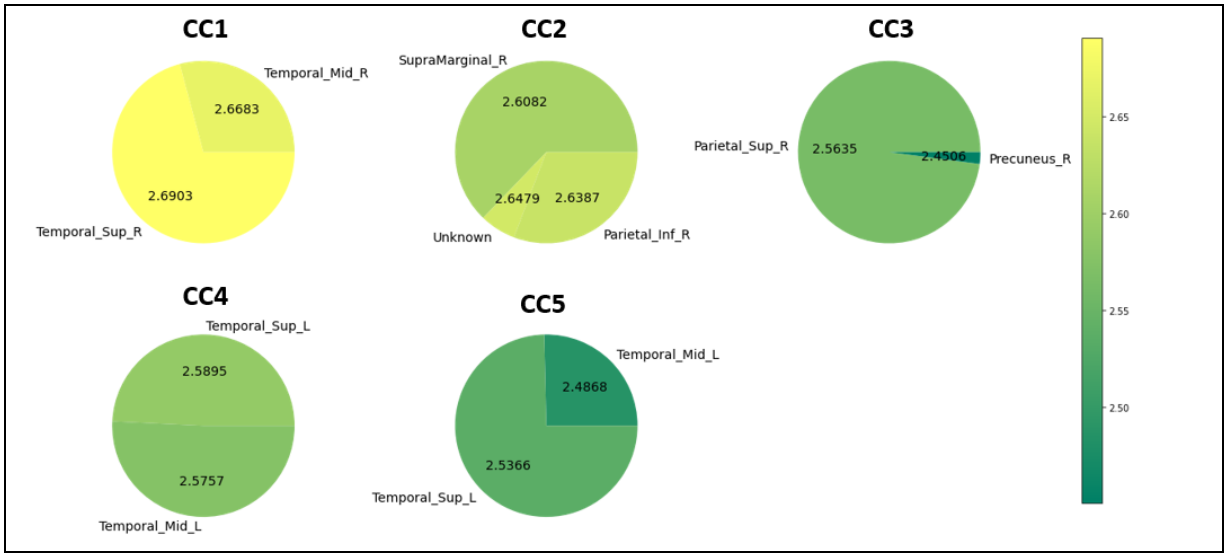
Method: We used 25 HC subjects and 18 PTH patients. Among the PTH patients, 9 are improved and 5 non-improved at three months. Event-based fMRI were collected from an experiment under Pain, NoPain (i.e., non-painful heat stimulation), and NoStim conditions. Analysis was performed to compare Pain vs NoPain and Pain vs NoStim between HC and PTH. One sided t-test was used to identify significant

voxels that formed clusters. The average contrast coefficients within each cluster were used to build multivariate classification models to differentiate PTH and HC based on leave-one-subject-out cross validation. The same clusters were also used to compare PTH with and without improvement.

Results: At a 0.01 significance level and a cluster size of at least 20 voxels, clusters were found with PTH>HC in the contrast coefficients, under the Pain vs NoPain and Pain vs NoStim conditions. When using the clusters found under the Pain vs NoPain condition to classify PTH and HC, the classification accuracy is low. The same thing happened when using the clusters found under the Pain vs NoStim condition. Therefore, we took an alternative approach and identified “common clusters (CCs)” defined as the overlapping portion between a cluster found under Pain vs NoPain and a cluster found under Pain vs NoStim. Five CCs were found. Their detailed information is shown in the table below.

	Contrast	Number of voxels in the cluster	Peak stat in the cluster	Coordinates of the peak stat	Number of voxels in the shared portion of clusters found in two contrasts
Common Cluster 1	Pain vs NoPain	430	3.2798	(58, -8, -12)	216
	Pain vs NoStim	252	3.1571	(56, -10, -10)	
Common Cluster 2	Pain vs NoPain	80	2.7216	(62, -46, 42)	78
	Pain vs NoStim	292	3.1912	(60, -44, 48)	
Common Cluster 3	Pain vs NoPain	124	2.9283	(18, -64, 62)	96
	Pain vs NoStim	133	2.8692	(18, -64, 66)	
Common Cluster 4	Pain vs NoPain	181	2.9105	(-56, -6, -10)	132
	Pain vs NoStim	246	3.0862	(-58, -6, -10)	
Common Cluster 5	Pain vs NoPain	192	2.8387	(-60, -46, 16)	67
	Pain vs NoStim	132	2.7113	(-54, -42, 12)	

Furthermore, to interpret each CC, we computed the percentages of voxels in each CC that overlap with each brain region using Automated Anatomical Labeling (AAL). The results are shown as follows:



CC1 is located in the right hemisphere, overlapping with middle and superior temporal lobe. CC4 and CC5 overlapped with the same AAL regions but in the left hemisphere. CC2 is located in the right hemisphere, overlapping with supramarginal and inferior parietal regions. CC3 is also located in the right hemisphere, overlapping primarily with superior parietal with a small contribution from precuneus.

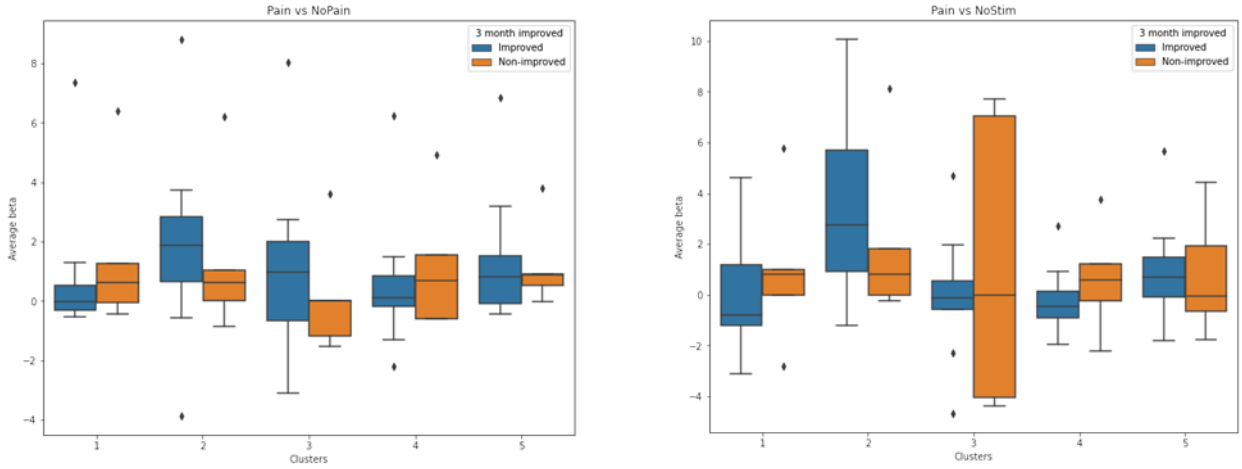
Next, we examined the reproducibility of the CCs by repeating the aforementioned analysis on subsets of the subjects (i.e., under the leave-one-subject out scheme). We computed the Intersection over Union (IOU) index that measures the overlapping proportion between a CC found in the full-subject analysis and a CC found in the leave-one-subject-out analysis. The mean ± std for the IOU Of each CC is:

		CC1	CC2	CC3	CC4	CC5
IOU mean ± std	Pain vs NoPain	0.9606 ± 0.0472	0.7483 ± 0.2773	0.8522 ± 0.1402	0.7746 ± 0.2795	0.8311 ± 0.1759
	Pain vs NoStim	0.7984 ± 0.2082	0.9400 ± 0.0673	0.8641 ± 0.1711	0.8950 ± 0.1582	0.7378 ± 0.3224

Using the CCs, the logistic regression and random forest classifiers yielded classification AUC of 0.8126 and 0.7979, respectively.

Finally, we plotted the bar charts for comparing improved vs non-improved PTH based on the CCs. The current sample sizes (9 and 5) are too small to yield statistically significant results. However, we can

observe that the trends are similar between Pain vs NoPain (left) and Pain vs NoStim (right) in terms of comparing the improved and non-improved groups.



Discussion: When examining the contrast maps under the Pain vs NoPain, and Pain vs NoStim conditions, significant clusters can be found with PTH>HC, but not the other way around. This implies more pain-induced activation in these clusters in those with PTH compared to HC. No significant clusters were found under the NoPain vs NoStim condition, which was expected. Furthermore, using the clusters found under each of Pain vs NoPain and Pain vs NoStim conditions separately, the classification accuracy for differentiating PTH and HC was low. In comparison, when using the CCs, which were clusters that existed under both conditions, the classification accuracy was significantly improved. This is likely because the CCs were less susceptible to noise, i.e., the clusters found under each condition can cross-validate each other resulting in the CCs.

Conclusion: Event-based fMRI data contains discriminant information to differentiate PTH and HC. Future research will explore if the identified CCs can be used to differentiate or predict PTH improvement vs. no improvement at three months.

(D) Machine learning (ML) models for integrating multi-modal datasets to predict PPTH

Rationale: We comprehensively evaluated our previously developed ML pipeline for integrating multi-modal datasets to predict PPTH, including pre-injury headache history, PTH features, clinical questionnaires, structural imaging including T1-weighted MRI, T2*, and DTI fibertract data. We reported the best prediction accuracy and the involved data modalities to reach that accuracy, as well as reported

the modalities that did not significantly contribut to the prediction with other modalities included. This was to help trim down the large datasets needed for each patient to predict PPTH individually and accurately.

Method: Our dataset included 48 PTH subjects and 78 healthy controls (HCs) (some of whom were enrolled in this DOD Focused Program). The pre-injury headache history included pre-injury headache frequency and existence of pre-morbid migraine. The PTH features included migraine-like headaches and whether headaches have been continuous since onset. The clinical dataset included five variables extracted from three questionnaires (Sports Concussion Assessment Tool-symptom checklist, Pain Catastrophizing Scale, and the Trait Anxiety Inventory Scale) that were selected based on our prior work demonstrating their value in predicting PTH improvement. The T1-weighted MRI dataset included 68 thickness variables and 68 mean curvature variables extracted from structural MRI which were found to be predictive for headache improvement based on our prior work. The T2* dataset included 63 variables. The DTI fibertract data included fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) measures from 42 regions of interest, totaling 168 variables. Since MRI measures are known to be affected by sex and age, we used confound regression based on HC data to account for potential confounding effects from sex and age in using the MRI measures for classification. Then, Partial Least Squares (PLS) was used for dimension reduction. Logistic regression was used to predict improvement vs non-improvement of PTH subjects using the PLS components and three covariates (age, sex, and time between injury and enrollment) as predictors. The PLS and logistic regression classifier were trained with leave-one-out cross-validation scheme.

Results: Among the imaging modalities, T1-weighed MRI yielded the best CV AUC (0.801) when combined with the five clinical variables compared to T2* (0.624) and DTI (0.628) combined with clinical variables. Thus, we use clinical + T1 as the baseline model and see if its prediction accuracy can be improved adding other data modalities. Results in Table 1 showed that adding the two headache features improved the CV AUC to 0.817, but additionally including pre-injury headache features did not improve the classification performance. Including T2* and DTI also did not add predictive value to the baseline model despite using T2* and DTI alone had moderate prediction accuracy.

Table 1.

Model	CV AUC (N=48)
Baseline: Clinical (five variables) + T1 (thickness & curvature)	**0.801**
Baseline + headache features	**0.817**
Baseline + headache and pre-injury headache features	**0.796**
Baseline + T2*	0.791
Baseline + DTI	0.771

Conclusion: This work showed that a model including clinical + T1 + headache features was best performing (CV AUC 0.817) among the multi-modal combinations of these clinical variables and imaging measures (T1, T2*, DTI).

(E) ML models using fMRI data to predict PPTH

Rationale: This project also collected functional MRI datasets. We evaluated the prediction accuracy of resting state fMRI, with plans to incorporate this modality into the multi-modal prediction pipeline upon demonstrating the value of fMRI.

Method: Our dataset included 48 PTH subjects and 78 healthy controls (HCs). Resting state functional MRI data were pre-processed using standard procedures including the following steps: slice-time correction, motion correction, re-alignment, skull and non-brain tissue removal, spatial smoothing, and alignment to an average MNI template. Further post-processing steps included band-pass filtering (0.01-0.1 HZ) and removal of variance related to framewise displacement, white matter signal, and cerebrospinal fluid signal. A total of 63 regions of interest (ROI) including 31 bilateral and one midline regions were considered. Functional connectivity between each ROI-ROI pair was measured by Pearson correlation coefficient. Fisher's r-z transform were applied to normalize the correlations. Connections with absolute z values of less than 0.05 were considered not functionally connected and set to zero. This process extracted a connectivity matrix of dimension 63 by 63, which is then flattened into a 1953-dimensional feature vector. The lasso model was used to train a classification model to predict headache improvement vs no improvement using the feature vector and three covariates (age, sex, time between injury and enrollment). Since features are high-dimensional, the lasso model used L1 regularization to shrink the coefficients and only retain the most predictive features. The classification model was trained

via leave-one-out cross-validation (LOOCV) scheme. The sparsity hyperparameter in the L1 regularizer was tuned via grid search within the internal [0.1,0.15,0.2,…,0.9] using internal LOOCV, i.e., within each outer LOOCV iteration, 47 patients were used in training and one patient was left-out as the test patient, the 47 training patients were used to run an internal LOOCV to tune hyperparameters.

Results: The best lasso model achieved 0.811 CV AUC for predicting headache improvement vs no improvement for PTH patients. Figure 1 visualizes the magnitude of estimated regression coefficients of each ROI-ROI pair averaged across the CV. The most predictive connections included left precuneus with right middle frontal, right posterior cingulate with left anterior insula and with right lingual gyrus, right inferior lateral parietal with left temporal pole and with spinal trigeminal nucleus, right anterior cingulate with left pulvinar, right precuneus with left caudate, left temporal pole, and left inferior lateral parietal. Age, sex, and time between injury and enrollment were not selected as predictive features by the model. It is worth noting that if two features are highly correlated, L1-regularization only retains one of them to minimize the number of selected features. Thus, this model only captures a subset of features that is predictive of headache improvement. To further validate the model, we applied the model to generate predictions of headache improvement for HCs. Predictions for HCs were generated within each LOOCV iteration and averaged across the iterations. The comparison of predictions generated for HC vs PTH improved vs PTH not improved is shown Figure 2. The model predictions for HC were not significantly different from those for PTH patients who had headache improvement ($p=0.149$, two-sided t-test). The predictions for PTH patients who did not have headache improvement was significantly lower than PTH patients who had headache improvement ($p<0.001$) and HC ($p<0.001$).

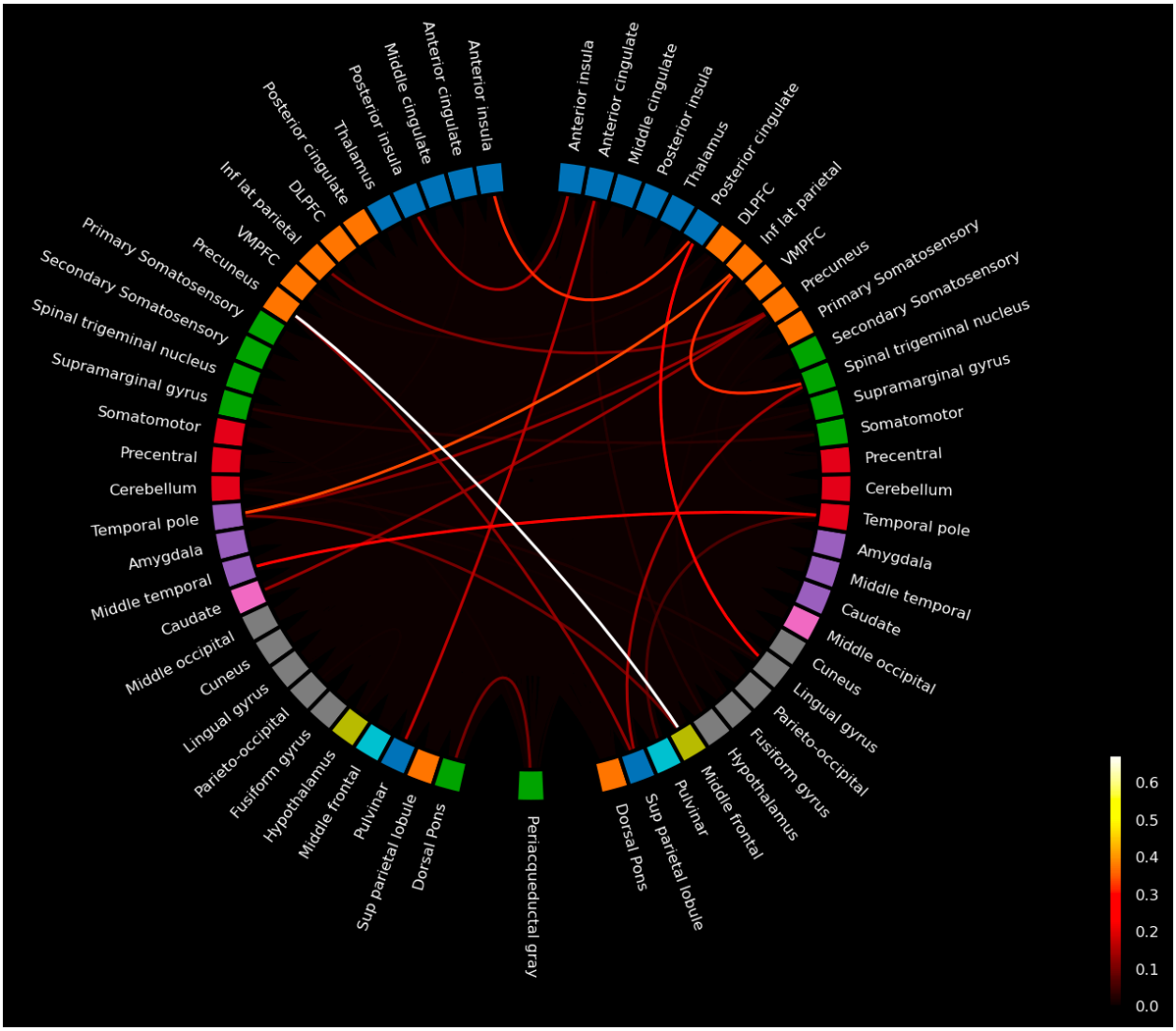


Figure 1. Estimated regression coefficients of each functional connectivity pair in the classification model. Regions from the same functional subnetwork are colored the same.

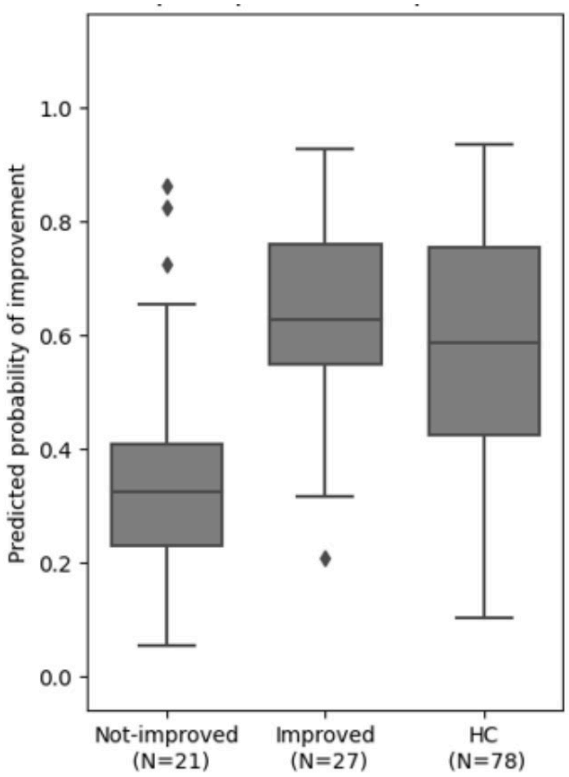


Figure 2. Group comparison of model predictions for HC, PTH improved, and PTH not-improved subgroups.

Conclusion: This work showed that fMRI has good utility for predicting headache improvement for patients with PTH after mTBI. The connections from regions such as precuneus, middle frontal, posterior cingulate, anterior cingulate, anterior insula, inferior lateral parietal, temporal pole, and spinal trigeminal nucleus regions contributed the most to the prediction of headache improvement.

Individual Project #6: Multivariate Modeling	Timeline (Months)	Completion Status
Major Task 1: Animal and Human Study Approvals		
Approval at Arizona State University	1-3	Completed
DOD HRPO Approval	1-3	Completed (11/24/20)
Milestone achieved: All approvals obtained; renewed annually	3	Completed (11/24/20)
Major Task 2: Statistical and machine learning model development[1]		
Extension of our MMI-DDS machine learning pipeline for predictive modeling using multi-faceted datasets (Aim 1)	4-15	Completed
Extension of our MMFM model for PPTH sub-classification and predictive modeling for subgroups (Aim 2)	8-18	Completed
Longitudinal models for PPTH mechanism discovery (Aim 3)	12-20	Completed
Image-genomics analysis (Aim 4)	18-26	Ongoing
Transfer learning between animal and human models (Aim 5)	20-32	Ongoing
Major Task 3: Model validation		
Simulation data experiments and model accuracy and validity check	4-40	Completed
Model application on datasets from Projects #1-5, result evaluation & validation	6-48	Ongoing

Planned Activities During the Next Reporting Period

Pre-Clinical Studies: Our characterization of single and repetitive mTBI as a sensitizing stimulus to otherwise normally innocuous stimuli will continue. In this next reporting period emphasis will be towards continued testing of doses and time-points for efficacious anti-CGRP treatment with olcegepant across our experimental outcomes (e.g., allodynia, cortical spreading depression) across both sexes of animals (Pre-clinical Major Aim 4). Results from our work across several aims/sub-aims are now near completion. Detailed analysis is underway and will continue over this reporting period in preparation for submitting articles for publication.

Human Studies: We will continue to enroll participants with PTH and healthy controls into the study. Participants will complete structured interviews, study questionnaires, quantitative sensory testing, visual discomfort threshold testing, brain MRI, and blood sampling. Those participants who qualify will be offered enrollment into the clinical trial. Mayo Clinic in Rochester, MN joined the study during this reporting period and is actively enrolling participants. We are working through the process of adding Mayo Clinic in Jacksonville, FL as another enrolling site. We will continue to work on increasing our rate of patient accrual into the study.

Multivariate Modeling: We will continue the efforts on machine learning and deep learning model evaluation and validation. In addition to progressively testing the performance of the models as more data are collected and processed by other individual projects, we will explore the incorporation of public data (e.g., neuroimaging from healthy subjects) to improve the robustness of our models. In addition, we plan to interrogate patient heterogeneity, specifically, we will develop a semi-supervised method that will identify MRIs of PTH patients from a mixture of headache patients' MRIs. We will evaluate the utility of combining datasets from clinical questionnaires, structural MRI, and functional MRI for predicting headache improvement, and evaluate the overlapping information and specific information contained in these data modalities.

4. Impact

The short-term impact from this research includes identification of mechanism(s) underlying PTH persistence from animal and human studies, potential methods to prevent PTH persistence, identification of clinical, imaging, molecular and genetic predictors for PTH persistence, and a clinical trial testing a CGRP monoclonal antibody for treatment of acute PTH and prevention of PTH persistence. The long-term impact from this research includes the ability to identify who is likely to develop PPTH and an individualized medicine approach of preventing PPTH by intervening with treatment early after onset of PTH in those individuals who are in need of such therapy (i.e. those likely to develop PPTH and those likely to respond to the treatment). This approach would substantially reduce the burden from PPTH amongst civilian and military populations.

5. Changes/Problems

As reported in prior progress reports, obtaining DOD HRPO and ACURO approvals took much longer than anticipated, delaying initiation of the research. Despite trying to catch up, the late HRPO and ACURO approvals have contributed to delays in our timelines for completing the research. The COVID-19 pandemic negatively impacted the ability to enroll human participants into this Focused Program, resulting in further delays with human enrollment.

Our recruitment efforts have been significant as detailed in prior progress reports. Changes to participant eligibility criteria increased the pool of individuals who qualify for these studies. This has resulted in an increase in participant enrollment. We have added Mayo Clinic in Rochester, Minnesota as an enrolling site and they have enrolled six participants into this study.

Phoenix VA Healthcare System enrollment strategies include chart review of daily Emergency Department and specialty clinic patients and medical records review of pertinent diagnosis codes; advertising flyers; electronic advertising, for example, digital billboards in Medical Center elevators and Facebook posts; and details about the study are placed on the VA phone system “on hold” message. Recently the VA has also created a new newsletter called “Veteran’s News Now” that allows the Public Affairs office to incorporate

the advertising flyer in the newsletter and send directly to Veterans' email. During this annual reporting period, numerous charts have been pre-screened for enrollment, and 28 Informed Consent packets were sent to potential subjects. Three of them were returned as "not deliverable," and four "opt out" postcards were received back (not interested-no further contact). Of the remaining 21 potential participants, six were found to have recent diagnoses of either suicidal intent or recurrent major depression. The remaining 15 were followed up on, but only 1 enrolled. Others either were not interested, couldn't take off work, or had various other reasons for declining to participate. To date, only two participants have been successfully enrolled. Ongoing challenges have also included capturing potential participants early post-onset of PTH and frequent ineligibility due to comorbidities such as mental health diagnoses and/or substance abuse. In addition, many of the potential participants are younger and are employed, which makes it difficult for them to come in for the appointments, even though we try to accommodate their scheduling requests as best as we can.

Mayo Clinic Minnesota has enrolled six research participants thus far. Fifteen individuals who appeared to meet all eligibility criteria have been identified. Five of these fifteen individuals never responded to the recruitment communications, four actively declined to participate, and six were enrolled. Mayo Clinic Rochester's site has developed a system to identify potential candidates through the continuum of care, including from visit to Emergency Department (ED), through hospital admission, and in the outpatient setting. At the start of 2023, site study staff informed PM&R and Sports Medicine clinicians who see patients with mild TBI in different practice settings at Mayo Clinic Rochester Campus regarding the commencement of recruitment to identify clinicians who would be willing to refer potential subjects to the study. To maintain proactive recruitment, site study staff review outpatient and inpatient consult schedules/notes on a daily basis with PM&R Brain Clinic (an outpatient interdisciplinary clinic) and Sports Medicine outpatient clinicians for potential study candidates. In addition, study staff communicate regularly with a RN in the brain clinic who makes phone calls to patients with mild TBI discharged from the acute hospital who are not scheduled for outpatient appointments regarding symptoms post hospital discharge. Following identification or referral, if a subject appears to be eligible on pre-screening review of medical record, then study staff attempt to make initial contact with potential participants by phone call. Additionally, Rochester has recently started

screening the site's ED case reports to attempt to identify patients with mild TBI that may not have been immediately referred or consulted by PM&R clinicians.

6. Products (for this reporting period)

Products that include data from this Focused Program Research

Published Manuscripts

- Rahman Siddiquee MM, Shah J, Wu T, Chong C, Schwedt TJ, Dumkrieger G, Nikolova S, Li B. Brainomaly: unsupervised neurologic disease detection utilizing unannotated T1-weighted brain MR images. *Proceedings of the IEEE/CVF Winter Conference on Applications of Computer Vision (WACV)*, 2024.

- Mao L, Li J, Schwedt TJ, Berisha V, Nikjou D, Wu T, Dumkrieger GM, Ross KB, Chong CD. Questionnaire and structural imaging data accurately predict headache improvement in patients with acute post-traumatic headache attributed to mild traumatic brain injury. *Cephalalgia* 2023;43.

- Siddiquee MMR, Shah J, Chong CD, Nikolova S, Dumkrieger G, Li B, Wu T, Schwedt TJ. Headache classification and automatic biomarker extraction from structural MRIs using deep learning. *Brain Communications* 2023;5:fcac311.

- Chong CD, Nikolova S, Dumkrieger G, Wu T, Berisha V, Li J, Ross K, Schwedt TJ. Thalamic subfield iron accumulation after mild traumatic brain injury as a marker of future post-traumatic headache severity. *Headache* 2023;63:156-164.

- Mao L, Dumkrieger G, Ku D, Ross K, Berisha V, Wu T, Schwedt TJ, Li J, Chong CD. Developing multivariable models for predicting headache improvement in patients with acute post-traumatic headache attributed to mild traumatic brain injury: a preliminary study. *Headache* 2023;63:136-145.

- Siddiquee MMR, Shah J, Wu T, Chong C, Schwedt T, Li B. HealthyGAN: learning from unannotated medical images to detect anomalies associated with human disease. In *International Workshop on Simulation and Synthesis in Medical Imaging* (pp. 43-54). Springer, Cham

Manuscripts that are under review for publication:

- Siddiquee MMR, Shah J, Wu T, Chong C, Schwedt TJ, Dumkrieger G, Nikolova S, Li B. Brainomaly: unsupervised neurologic disease detection utilizing unannotated T1-weighted brain MR images. arXiv preprint, 2023.

Oral presentations:

- Chong CD and Li J. Biomarker Signature to Predict the Persistence of Post-traumatic Headache. NIH 4th Annual Investigator Meeting, Bethesda, Maryland

- Li J. Mao L, Ku D, Schwedt TJ, Berisha V, Wu T, Dumkrieger G, Ross K, Chong CD. Predicting Headache Improvement in Patients with Acute Post-traumatic Headache Attributed to Mild

traumatic Brain Injury Using Imaging, Clinical, and Speech Data: a Multi-Modality Machine-Learning Study. American Headache Society Annual Scientific Meeting. June 2023. Austin, Texas.

Poster presentations:

- Li J, Mao L, Dumkrieger G, Nikjou D, Ross K, Berisha V, Wu T, Schwedt TJ, Chong CD. A machine learning model including questionnaire and structural imaging data predicts headache improvements in patients with acute post-traumatic headache attributed to mild traumatic brain injury. American Academy of Neurology Annual Scientific Meeting 2023. Boston, MA.

- Nikolova S, Schwedt TJ, Zhou Y, Chong BW, Dumkrieger G, Chong C. A novel approach to metabolic imaging of migraine and post-traumatic headache using full brain 3D-MSRI. ISMRM & ISMRT 2023 Annual Meeting. Toronto, ON.

- Siddiquee MMR, Wu T, Chong C, Schwedt TJ, Nikolova S, Dumkrieger G, Li B. Brainomaly: unsupervised headache detection using unannotated T1-weighted brain MRIs. International Symposium on Biomedical Imaging 2023. Cartagena de Indias, Colombia.

Lectures:

- Schwedt TJ. Post-traumatic headache. Uniformed Services University of the Health Sciences. Center for Neuroscience and Regenerative Medicine. May 2023.

- Schwedt TJ. Post-traumatic headache: diagnosis, differentiation, and prognostic modeling. University of California Los Angeles (UCLA) Neurology Grand Rounds. May 2023.

- Schwedt TJ. Post-traumatic headache: diagnosis, imaging evidence, and prognostic modeling. Barrow Neurological Institute Neurology Grand Rounds. January 2023. Phoenix, Arizona.

- Schwedt TJ. Post-traumatic headache: insights from sensory testing and research imaging. European Headache Congress. December 2022. Vienna, Austria.

<u>Topic-related products that do not include data from this Focused Program Research</u>

Published Manuscripts:

- Martindale C, Presson AP, Schwedt TJ, Brennan KC, Cortez MM. Sensory hypersensitivities are associated with post-traumatic headache-related disability. Headache 2023;online ahead of print.

- Ashina H, Dodick DW, Barber J, Temkin NR, Chong CD, Adler JS, Stein KS, Schwedt TJ, Manley GT; TRACK-TBI Investigators. Prevalence and risk factors for post-traumatic headache in civilian patients after mild traumatic brain injury: a TRACK-TBI study. Mayo Clin Proc 2023;online ahead of print.

- Dumkrieger G, Chong CD, Berisha V, Ross K, Schwedt TJ. The value of brain MRI functional connectivity data in a machine-learning classifier for distinguishing migraine from persistent post-traumatic headache. *Front Pain Res* 2023;3:1012831.

- Navratilova E, Oyarzo J, Anderson T, Broide RS, Subramaniam SR, Vazquez-Cintron EJ, Brin MF, Schwedt TJ, Dodick DW, Porreca F. Preclinical assessment of onabotulinumtoxinA for the treatment of mild traumatic brain injury-related acute and persistent post-traumatic headache. Cephalalgia 2022;42:1194-1206.

Manuscripts that are under review for publication:

- Yoon H, Schwedt TJ, Chong CD, Olatunde O, Wu T. Harmonizing Healthy Cohorts to Support Multicenter Studies on Migraine Classification using Brain MRI data. medRxiv preprint, 2023.

- Rudolph M, Kopruszinski C, Wu C, Navratilova E, Schwedt T, Dodick D, Porreca F, Anderson TR. Mapping brain areas activated in mice across the acute and persistent phases of post-traumatic headache.

7. Participants & Other Collaborating Organizations

Site 1: Mayo Clinic Arizona

5777 E Mayo Boulevard; Phoenix, AZ 85054.

Overall PI: Todd J. Schwedt, MD

Individual Project Leaders: Catherine D. Chong, PhD, Amaal Starling, MD, Frank Porreca, PhD

Site 2: University of Arizona College of Medicine - Phoenix

550 E. Van Buren St; Phoenix, AZ 85004

Individual Project Leader: Trent Anderson, PhD

Site 3: Arizona State University

300 E. University Drive; Tempe, AZ 85281

Individual Project Leaders: Teresa Wu, PhD

Site 4: Translational Genomics Research Institute (TGen)

445 N Fifth St; Phoenix, AZ 85004

Individual Project Leader: Matt Huentelman, PhD

Wet Laboratory Technical Expertise: Francis Taguinod, MS

Bioinformatician: Ignazio Piras, PhD

Site 5: Phoenix Veterans Administration Health Care System

650 East Indian School Road; Phoenix, AZ 85012

Individual Project Leader: Katherine Ross, PhD

Site 6: Georgia Tech

755 Ferst Drive NW, Atlanta, GA 30332

Individual Project Leader: Jing Li, PhD

Site 7: Mayo Clinic Minnesota

200 First St. SW, Rochester, MN 55905

Individual Project Leader: Dimitry Esterov, DO

Name: Todd J. Schwedt, MD
Project Role: Focused Program PI
Researcher Identifier (e.g. ORCID ID): ORCID ID 0000-0002-7780-7086
Nearest person month worked: 4
Contribution to Project: Schwedt has continued to oversee the entire Focused Program. He is also serving as co-PI for the clinical phenotyping and neurophysiology individual project and the neuroimaging individual project and as the PI for the clinical trial.

Name: Frank Porreca, PhD
Project Role: Pre-Clinical Studies Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-9430-1098
Nearest person month worked: 2
Contribution to Project: Dr. Porreca has overseen the preclinical experiments modeling mTBI-induced PTH and the development of PPTH.

Name: Trent Anderson, PhD
Project Role: Pre-Clinical Studies Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-3541-7741
Nearest person month worked: 1
Contribution to Project: Dr. Anderson has worked with Dr. Porreca on the preclinical experiments modeling mTBI-induced PTH and the development of PPTH.

Name: George Li, PhD
Project Role: Pre-Clinical Studies Individual Project (post-doctoral fellow)
Researcher Identifier (e.g. ORCID ID): 0000-0003-0033-3895
Nearest person month worked: 9

Contribution to Project: Dr. Li is a post-doctoral fellow in Dr. Anderson's laboratory conducting pre-clinical experiments into the pathophysiology and treatment of mTBI-induced PTH and PPTH. He is involved in animal experimentation, data analysis and interpretation and manuscript preparation.

Name: Amaal Starling, MD
Project Role: Clinical Phenotyping and Neurophysiology Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-1334-1157
Nearest person month worked: 2
Contribution to Project: Dr. Starling is serving as co-PI for the Clinical Phenotyping and Neurophysiology Individual Project.

Name: Catherine Chong, PhD
Project Role: Neuroimaging Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-0702-1600
Nearest person month worked: 2
Contribution to Project: Dr. Chong is the Co-PI of the Neuroimaging Individual Project.

Name: Matt Huentelman, PhD
Project Role: Molecular Biomarkers Individual Project PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-7390-9918
Nearest person month worked: 1
Contribution to Project: Molecular Biomarker Project management and oversight.

Name: Ignazio Piras, PhD
Project Role: Molecular Biomarkers, bioinformatician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Molecular Biomarker Project data analysis

Name: Francis Taguinod
Project Role: Molecular Biomarkers, wet laboratory technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Molecular Biomarker Project wet laboratory assay

Name: Teresa Wu, PhD
Project Role: Multivariate Modeling Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-0113-0654
Nearest person month worked: 1
Contribution to Project: Dr. Wu supervises deep learning model development, data curation and harmonization in individual project #6.

Name: Md Mahfuzur Rahman Siddiquee
Project Role: Multivariate Modeling Individual Project Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to Project: Siddiquee is a PhD student in Dr. Wu's lab, and he contributes to the development of deep learning ensembles to identify brain activation maps to (1) discover and confirm neuroimaging biomarkers; (2) track activation changes over time in responding to treatment.

Name: Jay Shah

Project Role: Multivariate Modeling Individual Project Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Shah is a PhD student in Dr. Wu's lab, and he contributes to the development of the deep learning pipeline as an age model to explore the potential of predicted age from neuroimaging as surrogate biomarker to assist PPTH investigation.

Name: Jing Li, PhD
Project Role: Multivariate Modeling Individual Project Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-7028-3681
Nearest person month worked: 1
Contribution to Project: Dr. Li supervises the development of machine learning algorithms in individual project #6.

Name: Lingchao Mao
Project Role: Multivariate Modeling Individual Project Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Mao is a PhD student in Dr. Li's lab, and she contributes to the development of machine learning algorithms and preliminary analysis.

Name: Dohyun Ku
Project Role: Multivariate Modeling Individual Project Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to Project: Ku is a PhD student in Dr. Li's lab, and he contributes to the development of machine learning algorithms and preliminary analysis.

Name: Katherine Ross, PhD
Project Role: Phoenix VA PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-2715-2621
Nearest person month worked: 1
Contribution to Project: Ross is PI for Phoenix VA and oversees all Research protocol activities i.e., identifies participants, reviews medical records, Reviews submissions to IRB documentation as required.

Name: Dani Smith
Project Role: Research Coordinator at Mayo Clinic Arizona
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 5
Contribution to Project: Smith recruits participants, reviews medical records, conducts study visits, and performs data entry.

Name: Teri Radam
Project Role: Research Coordinator at Mayo Clinic Arizona
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to Project: Radam recruited participants, reviewed medical records, conducted study visits, and performed data entry. Radam retired from Mayo Clinic and thus no longer works on this study.

Name: Simona Nikolova, PhD
Project Role: Research Associate

Researcher Identifier (e.g. ORCID ID): 0000-0003-0987-4567
Nearest person month worked: 6
Contribution to Project: Magnetic resonance imaging data processing and analyses.

Name: Michael Leonard
Project Role: Program Manager and Research Coordinator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 5
Contribution to Project: Leonard has scheduled research meetings, collected regulatory documents, tracked milestones due, created and managed recruitment materials, and assisted with scheduling and completing research study visits.

Name: Shannon Harris
Project Role: Research Coordinator at Phoenix VA
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Harris recruits participants, reviews medical records, conducts study visits, submits IRB documentation as required.

Name: Janice Oyarzo
Project Role: Pre-Clinical Studies Research Technologist
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6
Contribution to Project: Ms. Oyarzo has performed the preclinical experiments modeling mTBI-induced PTH and the development of PPTH.

Name: Gina Dumkrieger, PhD
Project Role: Principal Data Scientist
Research Identifier (e.g. ORCID ID): 0000-0001-9519-5370
Nearest person month worked: 1
Contribution to Project: Dumkrieger created and manages the study database and performs preliminary analyses on the phenotyping and neurophysiology data.

Name: Edita Navratilova, PhD
Project Role: Pre-Clinical Studies Scientist
Research Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3
Contribution to Project: Navratilova is working on the preclinical experiments modeling mTBI-induced PTH and the development of PPTH.

Name: Dmitry Esterov, DO
Project Role: Site investigator at Mayo Clinic in Rochester, MN.
Research Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Dr. Esterov is responsible for all research activities at Mayo Clinic in Rochester, MN.

Name: Zachary Pohlkamp
Project Role: Research Coordinator
Research Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Pohlkamp is the research coordinator at Mayo Clinic in Rochester, MN.

8. Special Reporting Requirements

Not applicable

Appendices

None