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

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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 large proportion of individuals with PTH do not have headache resolution during the acute phase and have PTH persistence (PPTH). Optimally, individuals who are at high 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 will address 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

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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 with machine-learning techniques 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

3. Accomplishments

Individual Project #1 - Pre-Clinical Studies

1) Major activities during this reporting period: We have continued to determine the characteristics of repetitive mTBI on the establishment of PPTH and we have begun the characterization of mTBI to potential migraine triggers including exercise. In addition, we have begun testing the efficacy of targeting CGRP receptors in the treatment and/or prevention of PTH and PPTH.

2) Specific objectives: Our goals are to establish if mTBI acts to sensitize to subsequent triggering stimuli to promote 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.

3) Significant results or key outcomes:

The goals of the preclinical studies in this reporting period were to investigate the contributions of CGRP signaling at the CGRP receptor in the establishment of PTH and in the transition to PPTH. We became aware of the fact that erenumab, the clinically available CGRP receptor antibody that will be used in the clinical trial does not have affinity for rodent CGRP receptors, precluding the use of this molecule for our proposed studies. We therefore requested and received a CGRP receptor monoclonal antibody from Amgen that has affinity for rodent CGRP receptors. This experimental antibody, however, was then determined to have affinity for the rat, but not the mouse, CGRP receptor. As our goal was to determine the consequences of CGRP receptor signaling in PTH and PPTH, in parallel with the clinical studies, we undertook a series of studies that were not funded by this DoD award to establish the mTBI model in rats and evaluated the Amgen rat CGRP receptor monoclonal antibody (Lot #172-33-1, Amgen). While these studies are not a part of our DoD proposal, the outcomes of the studies are informative in allowing us to determine how we can complete the experiments that we proposed.

Figure 1 demonstrates that female Sprague-Dawley rats receiving a mTBI from a weight drop developed a period of transient periorbital and hindpaw allodynia that resolved by approximately day 16 post-mTBI, largely replicating our findings in the mouse. We next did a dose-ranging study with the Amgen antibody given at 10 or 30 mg/kg, i.p. delivered 2 hrs after the mTBI; an additional group of rats received a second i.p. dose of the antibody at 30 mg/kg. The timing of the antibody delivery is shown by the green lines in Figure 1. Transient periorbital and hindpaw allodynia induced by the mTBI had resolved by day 20 at which point the animals were exposed to a period of bright light stress (BLS) and assessment of periorbital and hindpaw allodynia was reassessed.

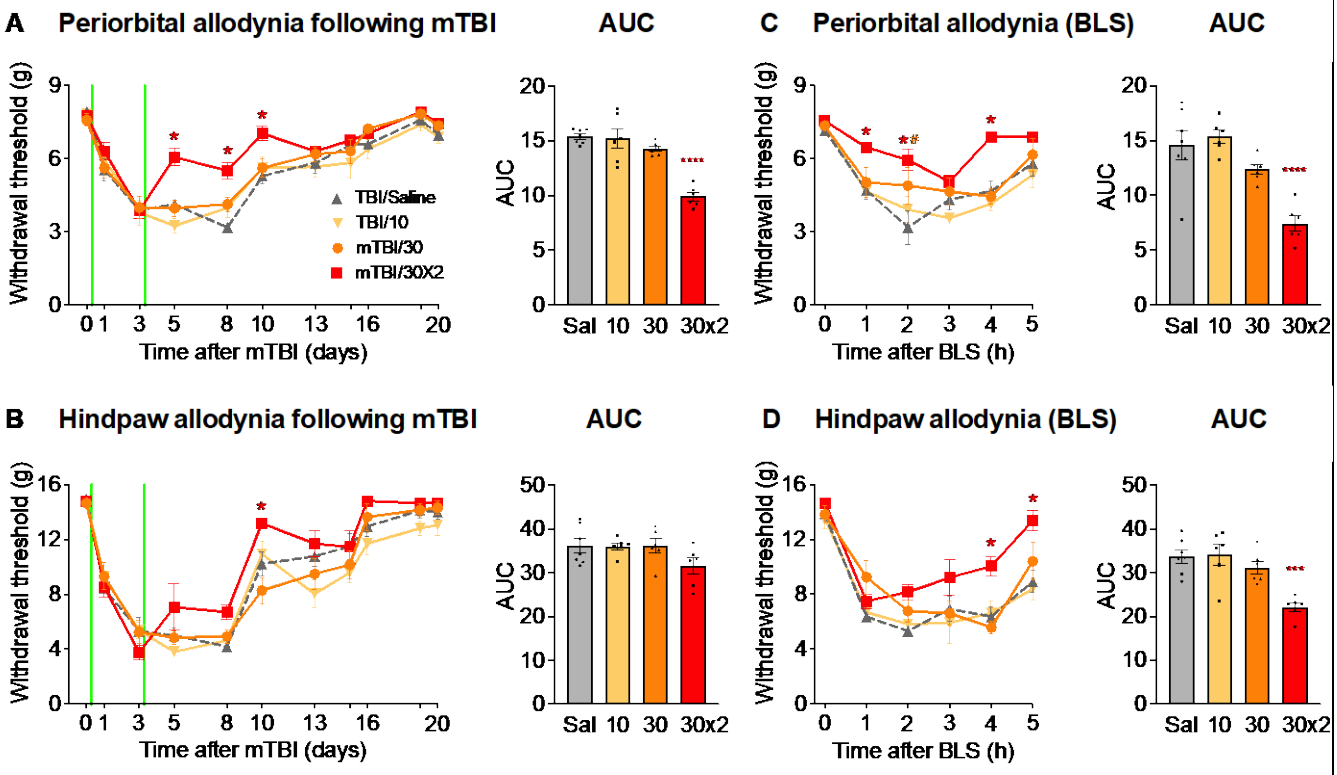


Figure 1. Anti-CGRP mAb (Lot # 17233-1; Amgen) administered to female rats 2 h after mTBI dose dependently inhibits mTBI-induced allodynia and central sensitization revealed by reduced allodynia in response to stress challenge on day 21 after mTBI (pilot studies not funded by the DoD award). Female Sprague Dawley rats that received mTBI injury on day 0 and 2 h later (green line) received intraperitoneal dose of either saline (N=7), or 10 mg/kg (N=6) anti-CGRP mAb, or 30 mg/kg (N=12) anti-CGRP mAb. Periorbital (**A**) and hindpaw (**B**) mechanical allodynia was assessed using calibrated von Frey filaments on indicated days throughout the 20-day period. On day 3, after behavioral testing, six animals from the 30 mg/kg (N=12) anti-CGRP mAb group received an additional dose of 30 mg/kg (N=12) anti-CGRP mAb (green line); this group is designated as mTBI/30X2. Time course of periorbital (A) and hindpaw (B) allodynia is depicted on the left, corresponding analysis of the area under the curve (AUC) over the 20-day period is shown on the right. On day 20 after the behavioral assessment, all animals were exposed to a stress stimulus from bright lights (BLS) for 1 h. Next day (day 21) withdrawal thresholds were assessed, and all rats were again exposed for 1 h to BLS. Time course of periorbital (**C**) and hindpaw (**D**) allodynia was assessed for 5 h following BLS. The time course is shown on the left, corresponding AUC is shown on the right. Statistically significant ($P<0.05$) decrease was observed for the mTBI/30X2 group at times indicated by a red star (*) and for the mTBI/30 group at one time indicated by an orange hashtag (#).

The data from these pilot experiments demonstrated that (a) a mTBI produces periorbital allodynia interpreted as a surrogate output of PTH; (b) periorbital allodynia is accompanied by hindpaw allodynia suggestive of central sensitization; (c) following resolution of PTH, a persistent state can be revealed by a provocative BLS stimulus suggesting long-lasting neural adaptations that are likely important in chronification of PTH into a persistent state, (d) CGRP receptor signaling is critically important in promoting

the acute PTH phase as well as the sensitized state of vulnerability that leads to pain responses from normally subthreshold stimuli in the PPTH phase and (e) that the Amgen antibody shows significant but incomplete effects, especially when evaluating hindpaw allodynia, at the doses tested. The last point is particularly significant as it suggests that a full blockade of CGRP receptor was not achieved at the doses administered and that a greater effect might have been possible with a higher dose of antibody. Unfortunately, this was not possible due to the amount of CGRP receptor antibody that was made available to us by Amgen and the poor potency of the antibody precluded our use of this material for future studies. For that reason, we concluded that the use of the Amgen antibody would not be feasible for successful completion of our DoD experiments. We therefore pursued our mechanistic investigation with administration of olcegepant, a CGRP receptor small molecule antagonist in our originally characterized mouse model (see Figure 2).

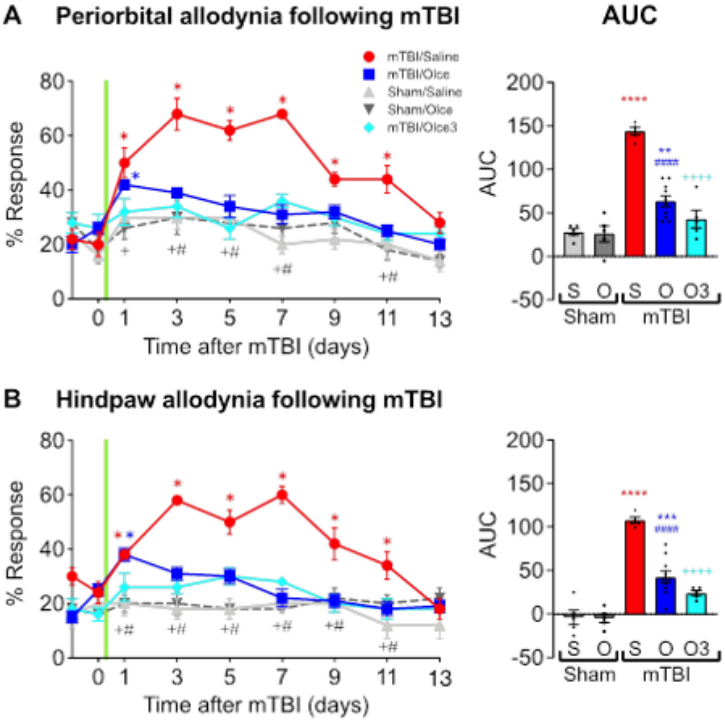


Figure 2. Repeated olcegepant treatment inhibits mTBI-induced allodynia in female mice. Female ICR mice received sham or mTBI injury on day 0, and then 2 h later (green line) were treated with saline or olcegepant either daily at 1 mg/kg dose or every 3 day at 3 mg/kg (Olce3). Periorbital (**A**) and hindpaw (**B**) mechanical allodynia was assessed as percent response to probing with von Frey filaments on indicated days throughout the 13-day period. The time course of periorbital (A) and hindpaw (B) allodynia is shown on the left, corresponding analysis of the area under the curve (AUC) over the 13-day period is shown on the right. Compared to the responses of sham groups, statistically significant ($P<0.05$) increase in % response was observed for the mTBI/saline group (N=5) at times indicated by a red star (*) and for the mTBI/Olcegepant group at one time indicated by a blue hashtag (#). Tactile responses of mTBI/Olce and mTBI/Olce3 groups were reduced compared to mTBI/saline mice at times indicated by black # and + signs, respectively.

While different from the CGRP receptor monoclonal antibody, the mechanism of blocking CGRP signaling at the CGRP receptor remains the same, allowing for conclusions to be relevant to the clinical trial with erenumab. Our data with the mouse mTBI model were consistent with our previous findings as well as those observed in rats. In these experiments, we administered olcegepant at 1 mg/kg daily beginning 2 hr after the mTBI or at 3 mg/kg every 3 days after the mTBI. Blockade of CGRP receptor with olcegepant produced an almost complete block of PTH for the entire time course of evaluation with slightly better effects observed at the higher dose. These data confirm the critical role of the CGRP receptor in this PTH phase. Experiments evaluating the PPTH phase with treatment with olcegepant are currently underway.

In addition, a key milestone of this last review period was development and investigation of the role of repetitive mild traumatic brain injury (rmTBI) on PTH and PPTH outcomes. For rmTBI, animals were briefly anesthetized before being subjected to an identical weight-drop procedure as with single mTBI except it was repeated once a day for 3 days (i.e. 3 total impacts, 1/day for 3 days). As an important first step and consistent with clinical data, we have now validated that like the single mTBI model, induction of repetitive mTBI does not induce skull fractures, seizures or induce any significant mortality or neuropathology. Over this review period we have also made significant progress using this rmTBI model to examine for changes in cortical spreading depression (CSD). Induction of CSD underlies migraine aura and has been shown to activate known migraine pain pathways. Based on our data from a single mTBI induced PPTH model, we had observed a significant increase in the probability of inducing CSD – indicative of the induction of headache promoting neuropathophysiology. Using a similar intrinsic optical signal (IOS) imaging approach, we have now tested if rmTBI similarly alters CSD. Animals were tested 14-28 days after the last mTBI, and animals exposed to rmTBI continue to display a marked increase in the probability of inducing CSD (i.e. a decrease in CSD threshold) following electrical stimulation (1ms pulse width)(Figure 3).

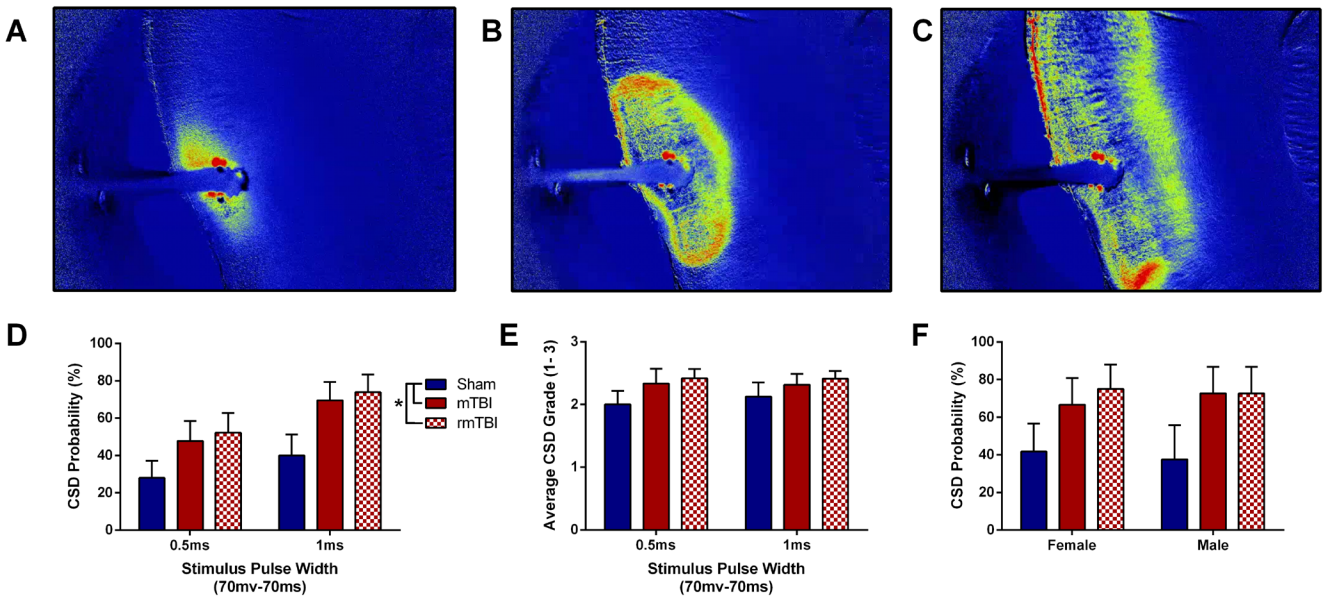


Figure 3. Repetitive mTBI increases the probability of inducing cortical spreading depression (CSD). A-C) Intrinsic optical signal (IOS) imaging of electrical induction (0.5-1.0 ms pulse width, 100Hz, 70mV amplitude, 70ms train duration). Note the propagating wave of IOS increase indicative of CSD. D-E) Bar graphs of CSD induction probability and grading between sham, mTBI and rmTBI following induction by electrical stimulation (0.5 or 1.0ms pulse width). F) Bar chart comparing CSD induction probability across female and male animals.

This increase was not statistically significantly different between single and repetitive mTBI (P=0.83) but both were statistically significantly increased compared with sham (P<0.05). In our initial experiments, electrical stimulation was set using pulse widths of 1ms that produced a near threshold response in sham animals (Fig 3D). However, following single or repetitive mTBI this CSD induction probability reaches ~80%. This introduced the possibility of a “ceiling-effect” when examining between single and repetitive mTBI. To test this, we have also now included a lower stimulation pulse width (i.e. 0.5ms) with an induction probability in sham animals near 25%. This stimulation value ensures adequate “headroom” for potential increases in CSD probability. However, even after adjusting the stimulation value there was no statistical difference between animals subjected to single or repetitive mTBI but, again, both remained elevated over sham animals. We next examined if the severity of CSD was altered using a graded scoring system, but no significant differences were observed between sham, mTBI or rmTBI. Further testing of other CSD properties such as velocity, duration, and peak amplitude are currently under investigation. Finally, we have begun stratified analysis for potential sex differences across changes in CSD probability. As indicated in Figure 3F, no sex differences were observed in mTBI or rmTBI induced increases in CSD probability.

Therefore, the data suggest that subjection to single or repetitive mTBI significantly decreases the threshold for CSD and is indicative of the induction of migraine-like pathophysiology in our PTH model.

4) Other achievements: We have completed our initial characterization of the mouse PPTH model with bright light stress as a second triggering event. We have determined that an initial mTBI produces transient cephalic as well as extra-cephalic allodynia that persists for approximately 14 days in male mice, interpreted as a preclinical surrogate measure of PTH. The presence of extra-cephalic allodynia is indicative of the development of central sensitization. Once the initial transient allodynia has resolved, we have demonstrated that only mice previously exposed to an mTBI event will develop cephalic and extra-cephalic allodynia to a bright light stress stimulus, interpreted as a preclinical surrogate of PPTH. Bright lights were chosen as they produce a psychological stress in mice and can be used in parallel human studies in Project 2.

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 (01/31/21)
Determine if mTBI sensitizes mice to multiple migraine triggers (e.g. exercise)	6-18	Ongoing
Determine the effect of repetitive mTBI on PPTH	12-24	Ongoing
Milestone achieved: established mouse model of migraine trigger induced PPTH	24	Pending
Major Task 3: Anti-CGRP Dosing in Mice		
Complete time course of CGRP blood levels in mouse PTH model	18-30	Ongoing
Determine the dosing of anti-CGRP antibody in mice	24-36	Ongoing
Milestone achieved: established dosing of anti-CGRP mab in mice and time course of elevated CGRP levels post TBI	36	Pending
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	Ongoing
Determine efficacy of anti-CGRP antibody administered at 45 days post TBI in mouse PPTH model	36-48	Pending
Determine the efficacy of anti-CGRP antibody administered late post TBI in mouse PPTH model	36-48	Pending
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 8/31/21, 25 human subjects have been enrolled into this Focused Program. One of these subjects signed consent but then did not participate in the study. A total of 57 study visits have been completed by the remaining 24 subjects. These subjects have completed study questionnaires and interviews at every visit, 57 quantitative sensory tests for determination of cutaneous heat pain thresholds have been completed, and 56 light discomfort threshold tests have been completed.

2) Specific objectives: All research subjects 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, stress and post-traumatic stress disorder, pain catastrophizing, and cognitive function. Data will be 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 and with treatment response in the clinical trial. 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 will be 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 and 16 weeks later will be assessed.

3) Significant results or key outcomes: First research visit data from healthy control subjects and those with acute PTH are shown in the following 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. Cutaneous pain thresholds to heat stimuli

and the impact of a bright light stressor on such thresholds are also demonstrated. Overall, these results demonstrate the substantial burden that is associated with PTH.

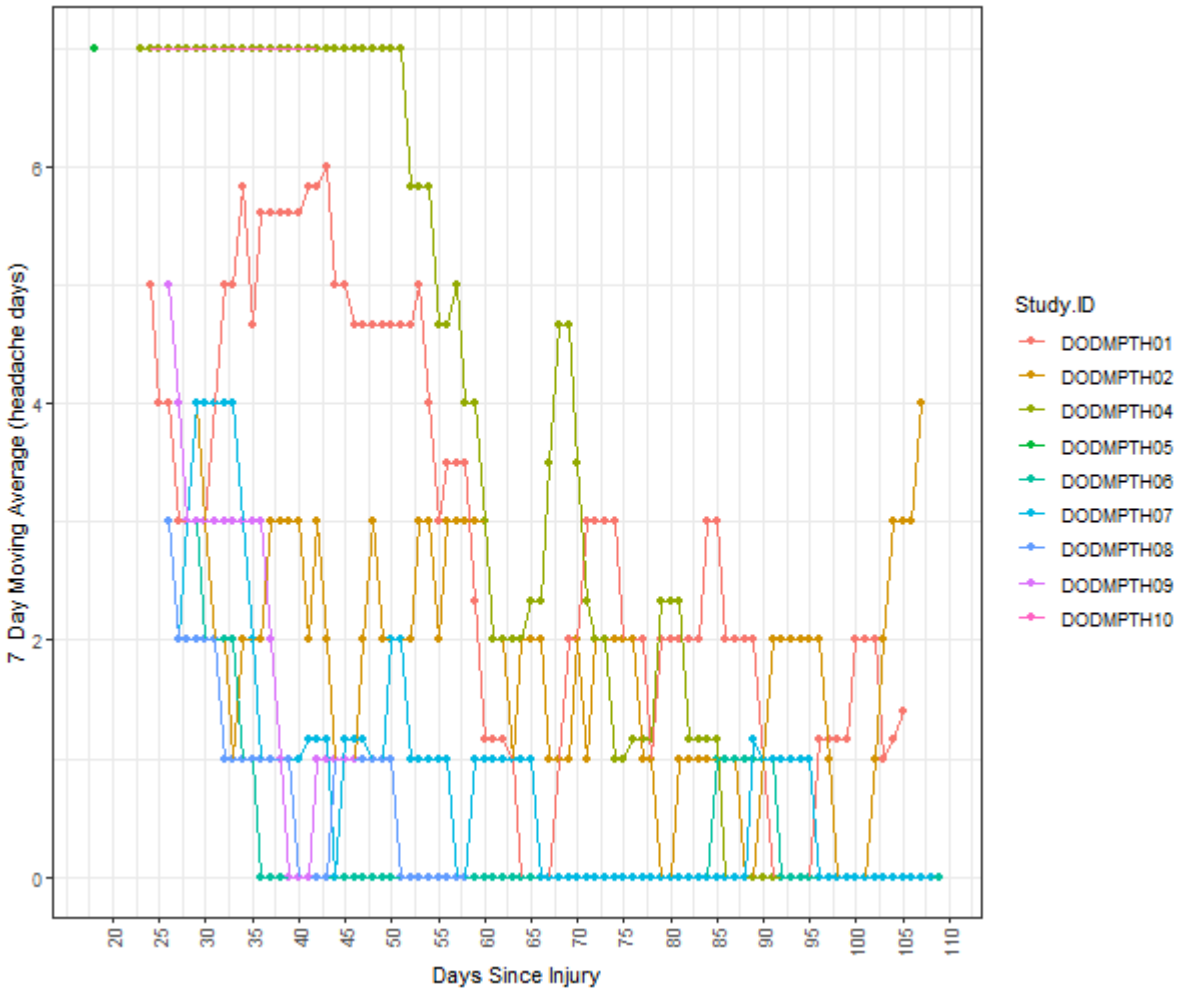
	Healthy Controls			Post-Traumatic Headache		
	N	**Mean (sd)**	**Median [1Q 3Q]**	**N**	**Mean (sd)**	**Median [1Q 3Q]**
Age	16	41 (13.06)	38.6 [29.5 50.2]	9	36.5 (13.25)	37.3 [25.7 41.2]
BDI	16	3.5 (4.31)	2 [0 6.3]	9	11 (8.8)	8 [7 15]
COWAT t-score	16	49.7 (11.76)	49.5 [40.6 59]	9	44.8 (11.05)	41.2 [33.5 53.6]
Hyperacusis	16	5.6 (3.01)	6 [4 7.3]	9	12.1 (13.73)	7 [5 11]
Insomnia	16	4.4 (4.29)	3 [1.8 5.8]	9	14.3 (5.7)	15 [13 17]
PAQ: Photophobia	16	25.8 (2.1)	26 [24.8 27]	9	24.4 (2.19)	25 [23 26]
PCS total score	16	8.8 (10.36)	6.5 [2 10.5]	9	8.9 (8.12)	7 [4 11]
PCS: helplessness	16	2.8 (5)	1 [0 2.5]	9	3.1 (3.59)	2 [1 4]
PCS: magnification	16	1.5 (1.83)	2 [0 2]	9	1.7 (1.73)	1 [0 3]
PCS: rumination	16	4.4 (4.56)	3.5 [1 6]	9	4.1 (4.01)	2 [1 6]
PTSD score	16	0.4 (0.81)	0 [0 1]	9	1.6 (1.51)	1 [0 3]
SCAT total score	16	3.9 (5.92)	1 [0 6.3]	9	30.7 (15.59)	24 [23 34]
SCAT number of symptoms	16	2.7 (3.26)	1[0 5]	9	13 (4.77)	14 [9 17]
STAI: State anxiety	16	27.8 (7.38)	24 [23 33.5]	9	33.3 (8.57)	31 [26 41]
STAI: Trait anxiety	16	31.3 (8.97)	27 [24 37.3]	9	38.8 (15.43)	36 [27 40]
Trails A, z-score	16	0.5 (0.85)	0.8 [0.1 1.1]	9	0.001 (1.81)	0.6 [-0.2 1.3]
Trails B, z-score	16	0.8 (0.51)	0.6 [0.4 1.2]	9	-0.023 (1.91)	0.9 [-0.8 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.

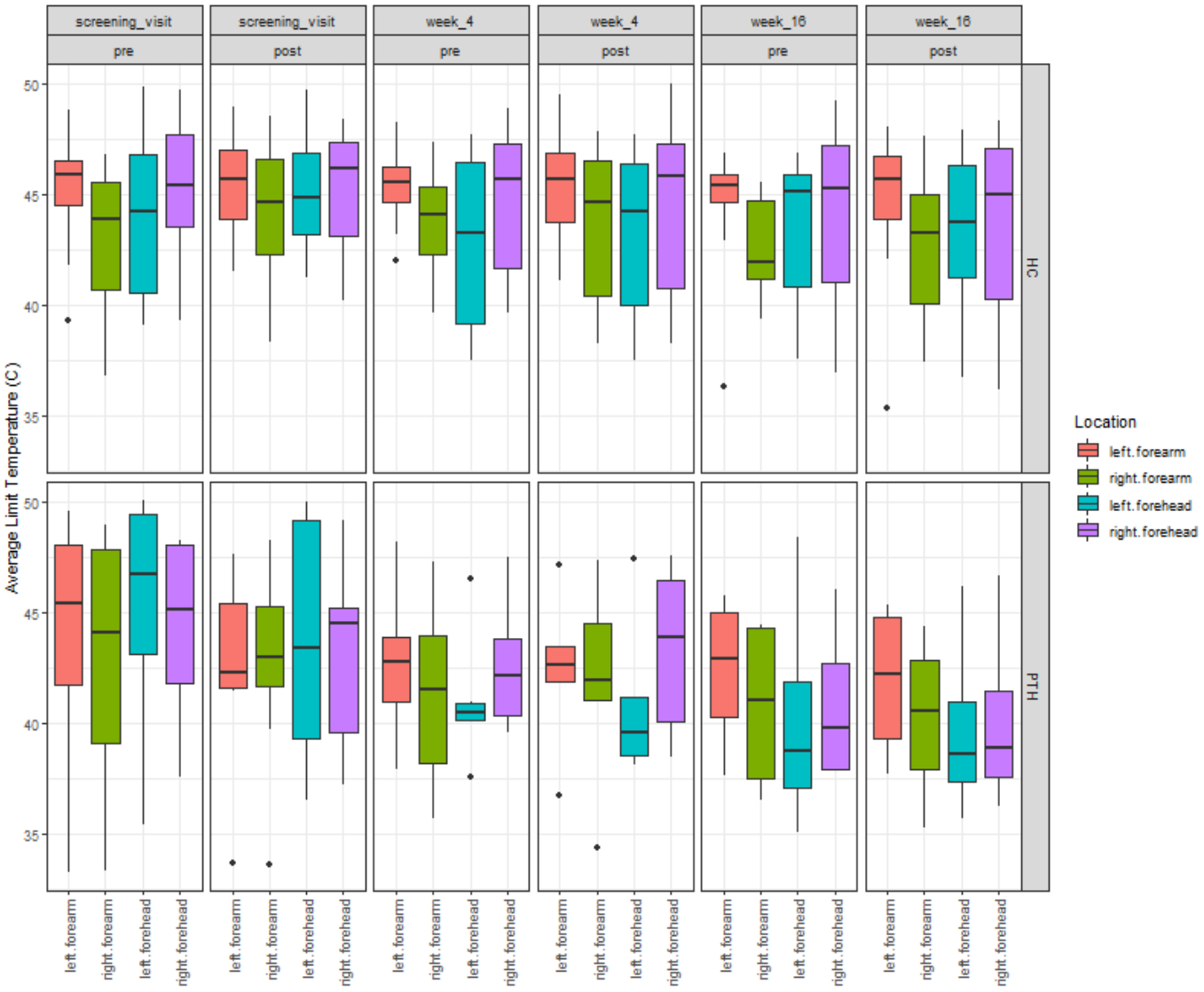
Variable	Response	Healthy Controls	Post-Traumatic Headache
Sex at Birth	Female	10	6
	Male	6	3
Gender	Woman	10	6
	Man	6	3
Race	Asian	0	2
	White/Caucasian	15	6
	Black/African American	0	1
	Other	1	0
Ethnicity	Hispanic	5	0
	Non-Hispanic	11	9
Education	High School Graduate (12th Grade)	1	2
	Some College; No Degree	2	0
	Associate Degree	3	2
	Bachelor's Degree	9	3
	Master's Degree	1	1
	Doctoral Degree	0	1
SCAT: Symptoms Worse with Physical Activity	No	16	4
	Yes	0	5
SCAT: Symptoms worse with Mental Activity	No	16	3
	Yes	0	6

Responses to Categorical variables. SCAT: Sport Concussion Assessment Tool

Headache Frequency Changes: None of the PTH subjects reported headaches prior to their injury. In the first period following enrollment PTH subjects completed 24.3 diary entries/28-day month and reported 12.1 headaches (not imputed). In the second period following enrollment (p2) a mean of 5.5 headache days have been reported with a minimum of zero and maximum of 14 headaches reported. For the six PTH subjects who have begun or completed p2, the number of headaches reported in p2 is 30% of the amount reported in p1.



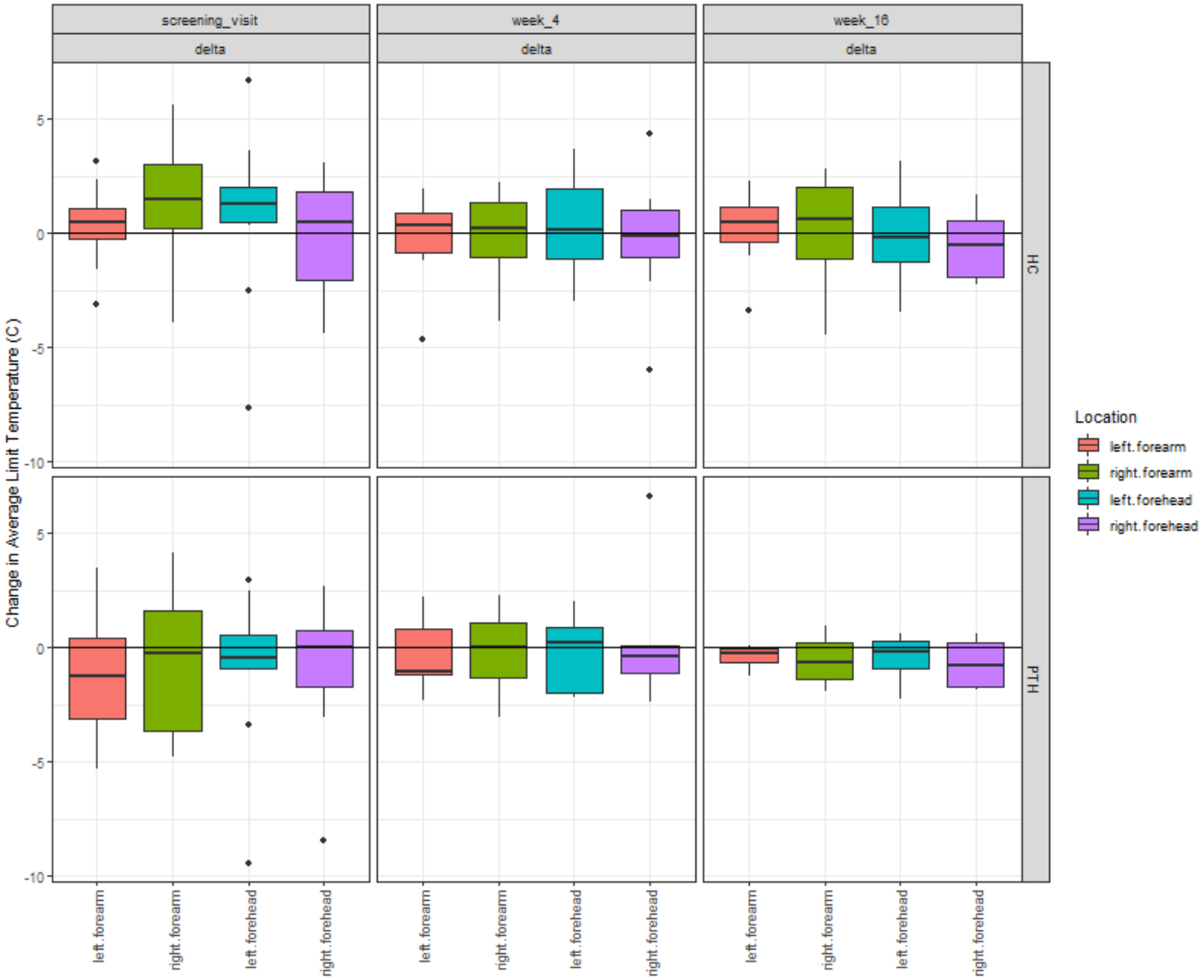
Individual 7 day moving average of diary-reported headache days by days since injury



Average cutaneous pain thresholds at each location, pre- and post-bright light exposure, by time point, and patient group. The figure above shows the pain threshold limits by 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 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=15)	44.6	45.1	0.5	44.1	45	0.9
PTH (n=8)	44.4	43.5	-1	43.7	42.7	-0.9
p-value	0.92	0.25	0.21	0.79	0.10	0.06

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. When comparing healthy controls to those with PTH, there are no significant differences in the baseline pain thresholds or changes that occur following bright light stress. However, 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. It is possible that this could become a significant finding once larger sample sizes are available.



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

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 (25 subjects completed)
Completion of Follow-Up Structured Interviews and Study Questionnaires	7-42	Ongoing (24 subjects completed one or more follow-up visits; 32 follow-up visits completed)
Completion of Initial Quantitative Sensory Testing and Visual Discomfort Threshold Testing	4-36	Ongoing (25 subjects completed)
Completion of Follow-Up Quantitative Sensory Testing and Visual Discomfort Threshold Testing	7-39	Ongoing (24 subjects completed one or more follow-up visits for testing; 32 follow-up visits for testing completed)
Major Task 4: Interim and Final Data Analyses		
Interim Analyses: Clinical Phenotypes, Neurophysiology Test Results	20-24	Pending
Final Analyses: Clinical Phenotypes, Neurophysiology Test Results	39-48	Pending

Individual Project #3: Neuroimaging

1) Major activities during this reporting period: As of 8/31/21, 24 human subjects have completed at least one research brain MRI. A total of 56 MRIs have 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) arterial spin labeling; 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 will identify 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, cerebral perfusion, resting state functional connectivity, and brain activations in response to noxious stimuli.

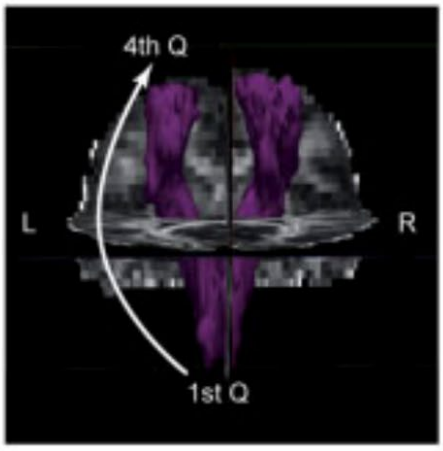
3) Significant results or key outcomes: One subject had a positive finding on imaging and was excluded from further analysis, 1 subject was not able to continue imaging due to claustrophobia, and one subject was excluded due to technical issues at the scanner, leaving a total number of subjects for which baseline images were acquired at n=21 (12 healthy controls and 9 subjects with PTH). Mean age for healthy controls was 38.0 and mean age for subjects with PTH was 34.9. There were no significant differences for age between groups (p=0.58). All T1-weighted images were quality checked, post-processed, and segmented for regional cortical thickness, volume, area, and curvature using the FreeSurfer, 7.1.1. pipeline. Hippocampal, thalamic, amygdala and brainstem areas were additionally segmented for subcortical volume.

Preliminary Results:

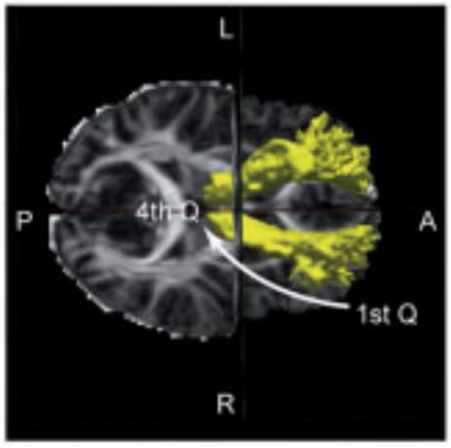
Brain Structure

Volume, Thickness, Area, Curvature, and White Matter Tract Integrity: Although these preliminary results need to be interpreted with caution (since they are preliminary), there were regional group differences that were trending significance or showed significant differences between healthy controls and those with PTH, including measures of brain *volume* (right precentral volume, p=0.064; left paracentral volume, p=.0082), *thickness* (right insula thickness, p=0.055; right lateral occipital thickness, p=0.073), *area* (left isthmus cingulate, p=0.072; left paracentral, p=0.087; left superior parietal, p=0.065), and *curvature* (right temporal banks, 0.018; right paraorbitalis, p=0.015; left mediotemporal, p=0.036). In addition, there were significant group differences in *volume* for the following subcortical regions: medulla, 0.014; pulvinar (averaged for left and right), p=0.032; left whole hippocampal head (p=0.039), and right whole hippocampal head (p=0.05), and trending significant group differences for the right paralaminar nucleus of the amygdala (p= 0.05). Additionally, there were significant group differences or trending significance of fractional anisotropy indicative of tract integrity for the right thalamic radiations (p=0.039) and the right corticospinal tract (p=0.043).

Fibertract differences between patients with post-traumatic headache and healthy controls



Cortico-spinal tract



Anterior thalamic radiations

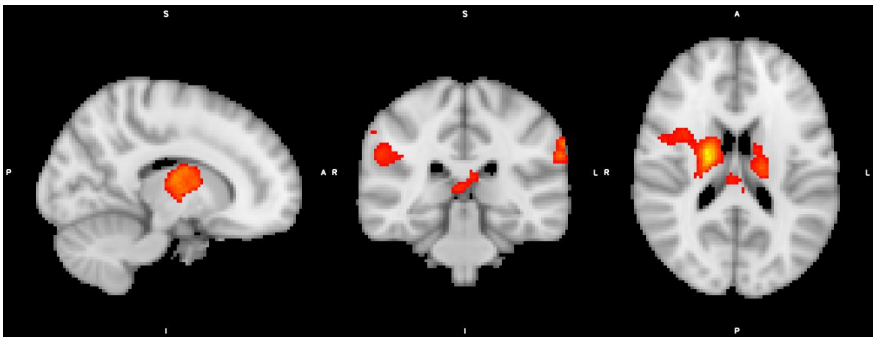
T2*: SPM12 was used to co-register T2* data to T1-weighted images, then was normalized to Montreal Neurological Institute (MNI) space and smoothed with 8 mm full width half-maximum (FWHM) kernel. Voxels within 4 mm of cerebrospinal fluid (CSF) were excluded to avoid signal contamination. Decreased

T2* in PTH compared to HC was observed in left post central, left anterior cingulate, right supplementary motor area, and in the right anterior cingulate ($p<0.001$ volume, cluster volume of 10 voxels or more).

Brain Function

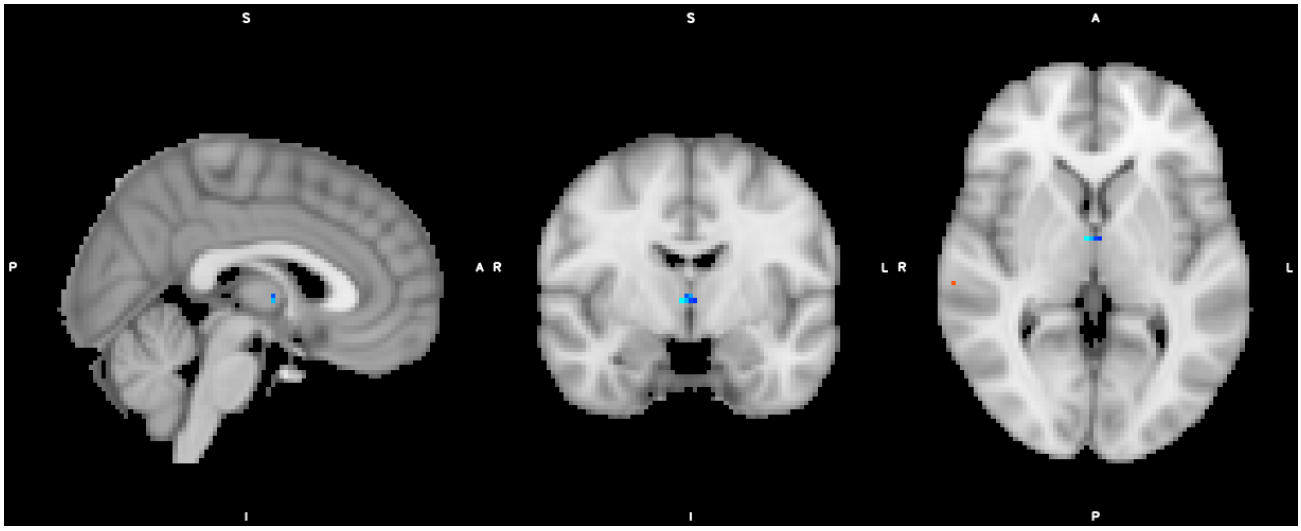
Painful Stimulation: All preprocessing and general linear model (GLM) estimation of brain activation patterns for the event-related portion of the fMRI were performed using SPM12, interfaced with Matlab. Functional images were realigned to the mean volume in the series, motion-corrected, realigned to each individual's structural images, and smoothed using an 8 mm full width half maximum Gaussian kernel. Brain regions activated in response to painful stimuli were identified by generating contrast maps representing brain activations associated with 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 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.

Baseline Time Point (N_{HC} = 14 , N_{PTH} = 9) - Main Effect (F-statistic) of Painful Stimulation: There is a robust main effect in the thalamus, bilateral supramarginal area, cerebellum, and insula ($p < 0.001$ cluster threshold of 10 voxels) as shown below:



Group Differences to Painful Stimulation: Two group t-test of PTH < HC (p<0.001 no cluster volume threshold) indicates that PTH subjects have suppressed activation in the thalamus compared to HC (average fMRI contrast of PTH subjects at cluster center is -0.5% and HC = 1.9%)

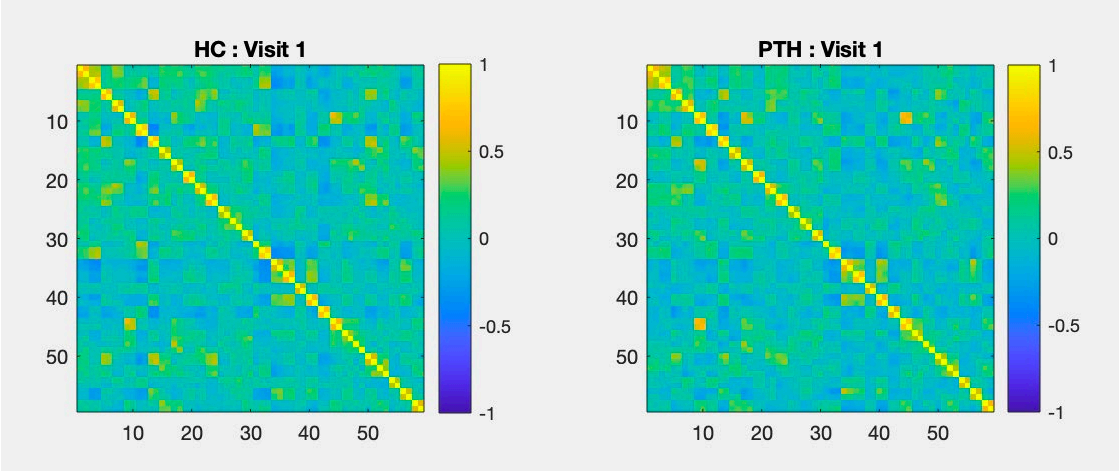
Two group T-test of PTH > HC (P<0.001 no cluster volume threshold) showed PTH subjects had increased activation in right frontal and right temporal regions compared to healthy controls.



Resting State Functional Connectivity: 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 Montreal Neurological Institute 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 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 29 bilateral and one midline region important for pain processing or multisensory integration. The ROIs were defined by spheres with eight mm diameter centered on the MNI coordinates. The Pearson correlation coefficient was reported between regions. Following the correlation analysis, Fisher r-z transforms were calculated for each seed-seed pair and used in t-tests. Connections with absolute correlations values of less than 0.1 for both HC and PTH were omitted from subsequent analysis.

Two-Group T-Test (PTH vs. HC): Differences in resting state functional connectivity between PTH and healthy controls were identified for the following region pairs: right anterior cingulate with left fusiform gyrus, left anterior cingulate with left fusiform gyrus, and left thalamus with left middle occipital ($p<0.005$, uncorrected).



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

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 (24 subjects completed)
Completion of Follow-Up Patient Imaging	7-39	Ongoing (32 follow-up MRIs have been completed, including 18 subjects who completed the 4-week follow-up MRI and 14 subjects who completed the 8-week follow-up MRI)
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	Pending
Final MRI Data Analyses	40-48	Pending

Individual Project #4: Molecular Biomarkers

1) Major activities during this reporting period: As of 8/31/21, 25 human subjects have contributed at least one blood sample. A total of 56 blood samples have been collected yielding the following aliquots to date: 54 whole blood, 292 plasma, and 221 serum aliquots.

2) Specific objectives: We aim to incorporate multi-genomic approaches in attempts to develop 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 persistent PTH so that they may be more intensely managed during the acute phase. b) *Fluid-based Markers for PPTH* – Longitudinal blood collections 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 to erenumab response.

3) Significant results or key outcomes: 21 individual participants have completed APOE genotyping. All DNA samples are currently staged for genome-wide SNP genotyping. We will wait until we have 24 total samples at a minimum to initiate this genotyping assay – we expect that to occur during the next quarter. All ELISA targets are assessed in batches of 23 samples and we have completed ELISA analysis for all ELISA-based targets for 46 total blood samples. The remaining samples (n=2) are currently staged and we are awaiting additional samples to trigger the next series of ELISAs. This means that 96% of biospecimens have completed ELISA analysis. The Meso-Scale Discovery (MSD) based assays require 39 samples at a minimum. We have completed 39 samples in the MSD assay – equivalent to 81% of all available biospecimens. We currently have 9 samples staged for MSD-based analysis. We expect to complete a set of 39 samples on MSD in the coming quarter.

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 (56 blood draw visits from 25 subjects, yielding the following numbers of aliquots: 54 whole blood, 292 plasma, 221 serum)
Specimen Processing and Analyses	4-42	Ongoing APOE genotyping: 100% completed SNP genotyping: all samples staged, waiting for 24 samples to initiate assay ELISA assays: 96% of samples received are complete across all targets. Meso-Scale Discovery: 81% of received samples are complete. 9 samples currently staged to initiate assay
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: 1 subject has been enrolled and randomized in the clinical trial. One patient has received study medication (erenumab or placebo) and 0 patients have completed the clinical trial thus far.

2) Specific objectives: This clinical trial will determine whether intervention with a CGRP receptor monoclonal antibody (erenumab) in a population of patients who have PTH for 35-56 days prevents the further persistence of PTH.

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

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 (1 patient enrolled into clinical trial)
Patient Follow-up Complete	42	Ongoing (1 patient completed one or more follow-up visits in clinical trial; 4 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 continued the multivariate methodological development effort including extension of our published work and development of new algorithms, in parallel to the data collection and processing in the other individual projects. To date, our goal has been to make these algorithms ready for use as soon as more data become available. Our machine learning/deep learning algorithms aim to support advanced multivariate analysis of the datasets derived from each of the other individual projects and to provide integrated analysis of the combined data from those projects. These advanced analyses will contribute to identifying predictors for PPTH, mechanisms of PPTH, and predictors for PTH treatment response.

2) Specific objectives: We were developing several algorithms related to Aim 1-4 of our individual project #6:

   a. Aim 1: extension of our MMI-DDS machine learning pipeline for predictive modeling using multi-faceted dataset. Related to aim 1, we had three efforts: (a) We investigated leveraging between-modality relationships in building the predictive model, which aimed to complement our existing multi-modality predictive modeling pipeline that was based on feature concatenation or ensemble learning. (b) We established a computational pipeline from fMRI for machine learning. fMRI is a 4D dataset with rich information. It is also less standard in terms of what features to extract to train machine learning predictors, compared with structural MR imaging. There are various algorithms in the literature to extract features from fMRI. We integrated several widely used algorithms into a pipeline to efficiently produce multi-faceted complementary features from fMRI. This capability will help us produce complementary, robust feature sets from the fMRI of each patient as soon as more patient data become available. (c) We implemented a new Shapley method (interpretable Artificial Intelligence approach) to connect features between modalities which can be used for feature selection to further improve predictive model performance and model interpretability.

   b. Aim 2: extension of our MMFM model for PPTH sub-classification and predictive modeling for subgroups. Related to aim 2, we developed a new deep learning ensemble approach to identify brain

region activations for PTH patients using T1-weighted MRI images. The brain activations can be used to interrogate the patient heterogeneities (e.g., headache severity) as well as tracking the activation changes over time (Aim 3).

c. Aim 3: Longitudinal models for PPTH mechanism discovery. Related to aim 3, we had two efforts: (a) We implemented functional data analysis for feature extraction from longitudinal data. (b) Recognizing the limitation and challenges from small datasets, we investigated the use of Maximum Mean Discrepancy (MMD) on structural MRI data to construct robust benchmark core by identifying the healthy controls from the data collected from this study and previous studies.

d. Aim 4: Image-genomic analysis. Related to aim 4, we implemented high-dimensional canonical correlation analysis to interrogate image-genomics relationships.

e. Aim 5: Transfer learning between animal and human models. Pending

3) Significant results or key outcomes:

(A) Between-modality relational predictors (Aim 1)

We previously published a machine learning pipeline for integrating multi-modality datasets to build a predictive model. This pipeline, as well as other existing machine learning algorithms in multi-modality data integration, does not consider that the relations between the multiple modalities could be a predictive marker. We aim to augment our multi-modality pipeline by incorporating between-modality relational predictors. We considered an algorithm called regularized general canonical correlation analysis (RGCCA), which derived the maximum canonical correlation between two modalities with high-dimensional features. As proof of concept, we leveraged the dataset from another project that contained 25 healthy controls (HCs) and 16 patients with PTH. RGCCA was applied to the HC group to find the maximum canonical correlation between two modalities, c_HC. The derived coefficients were applied to the PTH group and the between-modality correlation within the PTH group was computed, c_PTH. Since the same coefficients were used to compute the between-modality correlations of the HC and PTH groups, a significant

difference between c_HC and c_PTH would imply that there exists between-modality relational difference between HC and PTH, suggesting the potential of leveraging the relational difference in a predictive model for PTH persistence and treatment response.

The dataset we used to demonstrate the methodology was from another project, which included structural imaging measures and mobile-collected speech data for PTH and HC subjects. From the result summarized in the table below, we can see there is significant between-modality relational difference between PTH and HC. We plan to apply this algorithm to interrogate the relational difference between structural and functional images as well as between imaging and other non-imaging datasets (e.g., biomarker and phenotype/neurophysiology) collected in this DoD project. We plan to further develop capacities to incorporate the between-modality relational predictors into our predictive modeling pipeline to predict PTH persistence and treatment response.

Modalities to correlate with each other	Between-modality canonical correlation derived from PTH, c_PTH	Between-modality canonical correlation from HC by using the same coefficients derived from PTH, c_HC
sMRI area vs sMRI thickness	0.714 (p=0.003)	0.086 (p=0.762)
sMRI area vs speech	0.715 (p=0.003)	0.483 (p=0.068)
sMRI thickness vs speech	0.596 (p=0.019)	-0.185 (p=0.509)

(B). Functional network identification for PTH (Aim 1)

The literature suggested that functional brain networks such as the default mode network (DMN), executive network (EN), dorsolateral attention network (DAN), and salience network (SN) may be disrupted by TBI. Identifying the alterations of these networks might help build predictive models for PPTH and treatment response. Instead of using pre-defined seed ROIs to interrogate the functional connectivity, we used data-driven independent component analysis (ICA) to learn coherent spatial patterns of spontaneous BOLD activity at rest, derive ICs corresponding to the aforementioned networks, and interrogate the alterations in spatial-temporal characteristics of these networks between PTH and HC as well as their correlation with clinical metrics. We tested this algorithm using the rs-fMRI obtained from 15 PTH patients after mild TBI and 21 healthy HCs collected from another project with similar data structure as the DoD project. Several

ICs were found to be related to DMN, EN, DAN, and SN. Autocorrelations in EN and DAN were found to be significantly higher in PTH compared to HC ($p=0.012$ and $p=0.022$, respectively). Zmax of PTH was significantly lower in EN compared to HC ($p=0.010$). No significant differences were found between PTH and HC in DMN and SN. Also, autocorrelations in DAN were found to have significant positive correlations with MIDAS Total Score ($p=0.005$), SCAT # Symptoms ($p=0.004$), and SCAT Total Score ($p=0.004$). Autocorrelation in EN had a significant positive correlation with SCAT # Symptoms ($p=0.008$). Within the SN, autocorrelation had a significant negative correlation with MIDAS Total ($p=0.004$). Within the DMN, Zmax, skew and kurtosis of the IC spatial map had significant negative correlations with the Trails B Z-score ($p < 0.001$). These initial results provided promise of including functional network alterations as predictors for PPTH.

(C). SHAP method to connect features between modalities (Aim 1)

To further investigate the between-modality feature/predictor relationship, we implemented a new Shapley value-based approach (SHAP) on top of a multi-layer perceptron model (a multivariate predictive model) to interpret and examine the underlying relationship between the features from different modalities. Since SHAP modeling enables the interrogations on each subject base, it has the great potential to support studies including ours with a relatively small dataset to discover the feature relationships focusing on each individual subject and avoid potential biases from group-based study. As proof of concept, we leveraged a dataset collected by co-investigator Chong and principal investigator Schwedt that included 22 individuals with PTH. We implemented SHAP to identify salient speech features (total 17 features) responsible for headache intensity changes (derived from month 1). Using the 5 speech features identified by SHAP, prediction on headache intensity in month 3 was significantly improved. The prediction error (measured by Mean Squared Error) was reduced to ~0.3 from ~1. We plan to continue the efforts to investigate the multi-modality data collected from other projects and integrate SHAP with our MMI-DDS machine learning pipeline.

(D). Deep Learning for Neuroimaging Biomarker Discovery for PPTH sub-classification (Aim 2)

To discover neuroimaging biomarkers responsible for PTH, we employed state-of-the-art deep learning technology and developed a robust data-driven approach. First, we found abnormal regions, unique to PTH patients, from structural MRI by harnessing the discriminative power of the deep convolutional neural networks (CNN). We collected an MRI dataset with 521 healthy cohort (HC), 35 migraine patients (MCM), 23 patients with persistent post-traumatic headache (PPTH) and 26 patients with acute PTH. We trained 30 CNN models (3 architectures: ResNet10, ResNet18, and ResNet34; 10 models from each architecture) to categorize the images into 3 groups based on the distinctiveness of these images in voxel space: HC, MCM, PTH*(combined PPTH and PTH). Please note that we combined PPTH with acute PTH during the training process to support later PPTH sub-classification (to be conducted in the next budget period). The models were trained to classify PTH* with 100% accuracy and Area Under Curve (AUC). This overfitting performance is desired to support identification of voxels (a.k.a. activation) contributing to differentiate PTH* vs. other groups. In the current effort, we extracted only the activation voxels that made the 26 PTH images falling into the PTH* category. Since we trained our models for 3 categories (HC, MCM, PTH*), the major abnormalities related to migraine should be filtered out from the voxels we extracted from the PTH* category and most of the left-out voxels should represent only PTH*. As we have trained 30 CNN models, each of the PTH patients will have 30 different predictions. For the final prediction, we averaged all the predictions for each patient which removed the variance in prediction. The activation voxels were then mapped to the MNI space to locate the brain regions of interest (ROIs) as potential imaging biomarkers. Next, we implemented a Logistic Regression model to correlate the activation intensity from these brain ROIs with patient recovery status (defined using headache diary). From our initial experiments, the Logistic Regression model suggested 5 ROIs may be most responsible for the PTH patients being “not recovered”: right frontal pole white matter, left pars orbitalis white matter, right frontal pole gray matter, left temporal pole gray matter, and optic chiasm. The Logistic Regression model also suggested ROIs that were activated in patients who “recovered”. The top 5 such brain regions were: right medial orbitofrontal gray matter, right lateral orbitofrontal gray matter, right isthmus cingulate white matter, right hemisphere parahippocampal gray matter, and left cerebellum white matter. Some of the brain regions might be specific to our small cohort of patients such as left vs right hemisphere. However, the results are interesting in a broader sense given our method can suggest ROIs related to both “recovered” and “not recovered”

outcomes. This will help us in further investigation to not only understand the progression of PTH but also possibly PPTH sub-classification.

(E). Functional data analysis for longitudinal modeling (Aim 3)

This DOD project collects longitudinal datasets which provide an opportunity to leverage the change to build a better predictive model for PTH persistence and treatment response. We aimed to develop a data-driven approach to extract features from longitudinal data to facilitate the predictive modeling. This will complement knowledge-driven feature extraction approaches such as using the change magnitude, statistical summaries like mean, min, max, standard deviation, etc.

We implemented an algorithm called Principal Analysis by Conditional Expectation (PACE), which extracted features from longitudinal data that captured the dominant modes of temporal variability. As proof of concept, we leveraged the dataset from another project and applied PACE to the longitudinal data of speech signals used to track PTH progression. The extracted features, namely functional principal components (FPCs), showed significant correlations with various outcome measures of the PTH patients, such as SCAT, average headache severity and headache days at 3- and 6-months post-enrollment. FPCs extracted from various longitudinal speech features were significant in differentiating the PTH and HC groups, with p values ranging from 0.006-0.046. Furthermore, within the PTH subjects, the correlations of the FPCs with SCAT # symptoms & total score were significant ($p<0.005$) and the correlations with average headache severity were also significant ($p=0.025$). We plan to apply this algorithm to extract features from the longitudinal datasets collected in this DOD project and further incorporate the FPCs as features to augment the capacity of our predictive model.

(F) MMD method to identify representative homogenous controls (Aim 3)

To assist the longitudinal modeling, we investigated data curation and harmonization focusing on the HC group. The goal was to enrich our HC dataset by “borrowing” data available from our previous studies. Note simply combining all HCs from different studies will not work considering the batch effect (acquisition protocols, scanners), noise, etc. Maximum Mean Discrepancy (MMD) was used as a metric to help

identifying representative homogeneous HCs from multiple sources using statistical machine learning. This effort is crucial, especially for longitudinal studies where patient dropout often occurs. As a proof of concept, we leveraged migraine neuroimaging datasets available to us: dataset 1(DS1) has 54 HCs, dataset 2 (DS2) has 42 HCs, dataset 3 (DS3) has 5 migraineurs. Other than neuroimaging, DS3 has migraine days for each subject collected over 2-3 time points. Using MMD, we were able to identify 23 HCs from DS1 and 23 HCs from DS2 serving as the HC core. To account for the individual variability, we derived the relative changes - difference between two time points for each feature (neuroimaging features, migraine days) for each subject. The Pearson correlation on measures from the relative changes was 0.9005 ($p=0.001$). We plan to (1) add this HC core dataset to support this individual project #6 on structural MRI related modeling, and (2) apply this MMD approach to identify HC cores on data from other modalities.

(G) Image-genomics analysis (Aim 4)

This DOD project collects imaging and genomic data for subjects with PTH attributed to mTBI. Correlating image and genomic features will help identify genetic underpinnings of brain alterations, contributing to mechanism and knowledge discovery. Because both imaging and genomic datasets are high-dimensional and have high within-modality feature correlation, univariate correlation analysis for each pair of image and genomic features does not work. We aimed to use machine learning to automatically search for significant image-genomic correlations. We implemented a PCA-CCA algorithm, which worked by first performing PCA to each modality of data (imaging, genomics) for dimension reduction, and then performing CCA on the PCs extracted from each modality to find the maximum canonical correlation between the two modalities, and finally applying a reverse-operator to identify the significant contributing features to the maximum between-modality correlation. As proof of concept, we leveraged the dataset from another project and applied the PCA-CCA algorithm to the structural multiparametric MRI and RNA-seq data for 32 individuals with brain cancer. 300+ image features and 5000+ genes were used in the analysis. This dataset has similar data types as our DOD project, and was used for developing and validating the PCA-CCA algorithm. Using cross validation, the average canonical correlation found between imaging and genomic modalities ranged from 0.79 to 0.89, demonstrating the stability of the algorithm. Across different runs, 30-50 image features and 200-500 genes were found to be significantly contributing the between-

modality correlation. We plan to apply this algorithm to discover imaging-genomics/biomarker relationships in this DOD project.

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	Ongoing
Extension of our MMFM model for PPTH sub-classification and predictive modeling for subgroups (Aim 2)	8-18	Ongoing
Longitudinal models for PPTH mechanism discovery (Aim 3)	12-20	Ongoing
Image-genomics analysis (Aim 4)	18-26	Ongoing
Transfer learning between animal and human models (Aim 5)	20-32	Pending
Major Task 3: Model validation		
Simulation data experiments and model accuracy and validity check[2]	4-40	Pending
Model application on datasets from Projects #1-5, result evaluation & validation[3]	6-48	Pending

Planned Activities During the Next Reporting Period

Major tasks during the next period include:

- Investigating the role of mTBI in promoting PTH and PPTH
- Investigating the efficacy of treatment with anti-CGRP antibody for PTH and PPTH
- Continuing current human subject recruitment efforts and further extending our human subject recruitment campaign.
- Enrollment of additional human subjects who will participate with:
    - Structured interviews
    - Study questionnaires
    - Quantitative sensory testing with heat and bright light stimuli
    - Brain MR imaging
    - Biospecimen collection
    - Enrollment into the clinical trial
- MR imaging data processing and quality control assurance
- Biospecimen processing and quality control assurance
- Test and validate the machine learning algorithms developed in previous reporting periods on the continuously accumulated datasets, making adjustment and improvement on the algorithms
- Deep learning framework for incorporating multi-modal data for predictive modeling
- Interpretable models to analyze the decision of the deep learning framework

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 phase II randomized placebo-controlled 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 within prior progress reports, the process of obtaining DOD HRPO and ACURO approvals took much longer than anticipated, delaying much of the research work. The human protocol was submitted to the DOD HRPO on 11/16/19 and full approval was not obtained until 11/24/2020. After obtaining Mayo Clinic and University of Arizona IACUC approvals, documents were submitted to the DOD ACURO on 1/9/2020. DOD ACURO approval for the Mayo Clinic pre-clinical studies was received on 3/17/20, but approval for the University of Arizona was not received until 10/9/2020. Permission to conduct face-to-face visits at the Phoenix VA was not granted until 2/23/21. We are focused on trying to catch up with enrollment of human subjects and the animal studies, but the late HRPO and ACURO approvals might delay our timelines for completing the research.

The COVID-19 pandemic has negatively impacted the enrollment of human subjects. The pandemic causes a slowing of clinical and research activities, the extent to which fluctuates in relation with COVID prevalence in the Phoenix region. Overall, patients have been less interested in seeking in-person care in the outpatient neurology clinics, there have been fewer individuals presenting to the emergency department with concussion, and there has been hesitancy about coming to the clinic for research studies. There may have also been a decline in the incidence of concussion, due to fewer individuals participating in sports and less frequent motor vehicle use. We continue to work diligently to enroll patients into this research. Our recruitment efforts have been significant, including:

- Mayo Clinic enrollment strategies include:
    - Searching Mayo Clinic Emergency Department patient lists three times a week.
    - Using “Slicer/Dicer” program within the electronic medical record to search for candidates at all Mayo AZ sites.
    - Advertising the study on Mayo Clinic social media including Facebook and Twitter

- The study is registered with and listed on Research Match and Mayo Clinic Clinical Trials websites
- Mayo Clinic classified advertisements about the study
- Information about the study on Mayo Clinic electronic signs, some targeting Mayo employees and others targeting Mayo patients. This has included e-signs at the Mayo Hospital, Scottsdale Clinic, Family Medicine Clinics at Thunderbird, Arrowhead, San Tan Valley, and Phoenix.
- Frequent engagement with Individual Project lead investigators about recruitment efforts
- Personal visit and presentation to Mayo Clinic San Tan Primary Care Clinicians and Nursing Staff
- Frequent reminder e-mails to Mayo Clinic concussion and headache specialists reminding them to identify patients for the study as they are seen in clinical practice
- Outreach to community chiropractors.
- Recruitment materials at external sites (e.g. gyms, coffee houses)
- A patient recruitment letter was approved by the Mayo Clinic Institutional Review Board. The clinical trial principal investigator sent the recruitment letter to the following:
    - Maricopa County emergency departments including Banner hospital system and Mayo Clinic Hospital
    - Arizona College of Emergency Physicians
    - Arizona Athletic Trainers Association
    - Director of Barrow Neurological Institute Concussion Program
    - Arizona Interscholastic Association Sports Medicine Advisory Committee
    - ASU Student Health Center physician director
    - ASU club sports lead physician
    - Mayo Clinic Return to Play Clinic
    - Arizona College of Nursing

  This letter highlighted the opportunity for patients who sustained a concussion to participate in a clinical treatment trial and provided the research coordinator's name and contact information.

- May 2021 - Dr Dodick gave a Grand Rounds lecture to Mayo Clinic Emergency Department providers and presented the study and recruitment flyer.
- July 2021 - Dr Dodick gave a Grand Rounds lecture to Honor Health/John C Lincoln ED Physicians and he introduced the research study
- Outreach to concussion and headache clinicians at other facilities in the Phoenix region, requesting collaboration and identification of patients for the study.
- Continued outreach to Luke Air Force Base regarding identification of patients who might be interested in the study.

- Phoenix VA Healthcare System enrollment strategies include chart review of Emergency Department patients and medical records review of pertinent diagnosis codes; referral from the Emergency Department, Primary Care, and Specialty Care clinics; advertising flyers; electronic advertising, for example, digital billboards in Medical Center elevators and Facebook posts; and, details about the study placed on the VA phone system “on hold” message. To date, more than 1500 subjects have been pre-screened for enrollment, and none have been successfully enrolled. Challenges include capturing potential subjects very early post-onset and frequent ineligibility due to comorbidities such as mental health diagnoses and/or substance abuse. Additional challenges for recruitment were due to the COVID pandemic occurring in early 2020 through early 2021. VA sites were not authorized to conduct in person visits due the pandemic therefore enrollment opportunities suffered. Usual enrollment activities like making our flyers available in the Primary Care clinics and having Research Outreach activities were also halted for COVID-related safety reasons.

6. Products

We have published the following manuscripts about PTH/TBI since the start of this research (related to the topic of this research, although not funded by this grant):

- Kopruszinski CM, Turnes JM, Swiokla J, Weinstein TJ, Schwedt TJ, Dodick DW, Anderson T, Navratilova E, Porreca F. CGRP monoclonal antibody prevents the loss of diffuse noxious inhibitory controls (DNIC) in a mouse model of post-traumatic headache. *Cephalalgia* 2021;41:749-759.

- Chong CD, Berisha V, Ross K, Kahn M, Dumkrieger G, Schwedt TJ. Distinguishing persistent post-traumatic headache from migraine: classification based on clinical symptoms and brain structural MRI data. *Cephalalgia* 2021;41:943-955.

- Chong CD, Zhang J, Li J, Wu T, Dumkrieger G, Nikolova S, Ross K, Stegmann G, Liss J, Schwedt TJ, Jayasuriya S, Berisha V. Altered speech patterns in subjects with post-traumatic headache due to mild traumatic brain injury. *J Headache Pain* 2021;22:82.

- Ishii R, Schwedt TJ, Trivedi M, Dumkrieger G, Cortez MM, Brennan KC, Digre K, Dodick DW. Mild traumatic brain injury affects the features of migraine. *J Headache Pain* 2021;22:80.

- Pena A, Dumkrieger G, Berisha V, Ross K, Chong CD, Schwedt TJ. Headache characteristics and psychological factors associated with functional impairment in individuals with persistent posttraumatic headache. *Pain Med* 2021;22:670-676.

- Schwedt TJ. Post-traumatic headache due to mild traumatic brain injury: current knowledge and future directions. *Cephalalgia* 2021;41:464-471.

- Howard L, Schwedt TJ. Posttraumatic headache: recent progress. *Curr Opin Neurol* 2020;33:316-322.

- Kim SK, Chong CD, Dumkrieger G, Ross K, Berisha V, Schwedt TJ. Clinical correlates of insomnia in patients with persistent post-traumatic headache compared with migraine. *J Headache Pain* 2020;21:33.

- Hanna JJ, Chong CD, Dumkrieger G, Ross K, Schwedt TJ. Sensory hypersensitivities in those with persistent post-traumatic headache versus migraine. *Cephalalgia Reports* 2020;3:1-7.

- Navratilova E, Rau J, Oyarzo J, Tien J, Mackenzie K, Stratton J, Remeniuk B, Schwedt T, Anderson T, Dodick D, Porreca F. CGRP-dependent and independent mechanisms of acute and persistent post-traumatic headache following mild traumatic brain injury in mice. *Cephalalgia* 2019;38:1762-1775.

Conference abstracts/presentations related to PTH/TBI:

- Dumkrieger G, Chong CD, Ross KB, Berisha V, Schwedt TJ. Differentiating between migraine and post-traumatic headache with a machine learning classifier. American Headache Society Annual Scientific Meeting 2021.

- Ishii R, Dodick DW, Trivedi M, Dumkrieger G, Schwedt TJ. A history of mild traumatic brain injury is associated with disability and severity of migraine. American Headache Society Annual Scientific Meeting 2021.

- Gaw N, Dumkrieger G, Schwedt TJ, Wu T, Berisha V, Nikolova S, Ross K, Li J, Chong CD. Alteration of dorsolateral attention functional brain network in post-traumatic headache. Poster presentation, Accepted by 2021 Military Health System Research Symposium.

- Gaw N, Schwedt TJ, Dumkrieger G, Wu T, Berisha V, Nikolova S, Ross K, Li J, and Chong CD. Functional brain network alterations in post-traumatic headache: Relationship between the dorsolateral attention network and patient symptoms. The 2nd Annual HEAL Initiative Investigator Meeting (2021, May).

- Gaw N, Chong C, Schwedt T, Berisha V, Wu T, Ross K, Dumkrieger G, Zhang J, Nikolova S, and Li J. Multi-modal predictive model for persistent post-traumatic headache. Accepted for presentation at The Institute for Operations Research and the Management Sciences Annual Meeting, Anaheim, CA (2021, October).

- Shah J, Chong C, Schwedt T, Berisha V, Li J, Ross K, Dumkrieger G, Zhang J, Gaw N, Nikolova S, and Wu T. Interpreting deep learning model predictions using Shapley values. Accepted for presentation at The Institute for Operations Research and the Management Sciences Annual Meeting, Anaheim, CA (2021, October).

- Starling AJ, Jarvis N, Schwedt TJ. Cutaneous heat pain thresholds in posttraumatic headache attributed to mild traumatic brain injury. American Headache Society Annual Scientific Meeting 2019.

We have delivered/will be delivering the following lectures related to the topic of this focused program:

- Schwedt TJ. Post-traumatic headache: sensory testing and imaging. International Headache Congress 2021.

- Schwedt TJ. Imaging migraine and post-traumatic headache. Headache Cooperative of New England Annual Meeting. April 2021.

- Schwedt TJ. Research imaging of migraine and post-traumatic headache. Mayo Clinic Neuroscience Conference. March 2021.

7. Participants & Other Collaborating Organizations

Site 1: Mayo Clinic

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Overall PI: Todd J. Schwedt, MD

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Individual Project Leader: Trent Anderson, PhD

Site 3: Arizona State University

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Individual Project Leader: Matt Huentelman, PhD

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Individual Project Leader: Katherine Ross, PhD

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Individual Project Leader: Jing Li, PhD

8. Special Reporting Requirements

Not applicable

Appendices

None