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Abstracts

1 Cannabinoids and Migraine

Erik Zorrilla, Adisa Kuburas, Andrew Russo, PhD

Introduction:

Migraine is a condition that affects around 10% of the world population, and over 40 million people in the US. One of the triggers for migraine is calcitonin gene-related peptide (CGRP). It has been observed that migraine patients given CGRP experience a delayed migraine-like headache, whereas control subjects only experience a mild headache. Elevated CGRP levels have been found in migraine, and medications such as CGRP monoclonal antibodies have been developed to treat migraine. However, these medications are effective in only around 50% of patients. Therefore, there is a need for alternative therapies for the large percentage of migraine patients who do not benefit from these medications.

Methods:

To determine the ability of CBD:THC to reduce migraine-like phenotypes caused by peripheral (CGRP and SNP) and central (CGRP) triggers, we measured the light aversion using the light dark assay. The potential adverse effects of the different combinations of CBD:THC in mice were determined using the following assays: anxiety (light dark and open field), motor function (rotarod), spatial memory (Y-maze), depression (tail suspension).

Results:

Light Aversion (Peripheral administration of CGRP and SNP): Using the light dark assay, we measured the efficacy of various cannabinoid ratios on light aversion. The cannabinoids were peripherally injected 30 minutes prior to peripherally administering either the known migraine triggers CGRP (0.1 mg/kg) or SNP (2.5 mg/kg). Our results demonstrate that pre-treatment with the cannabinoid ratio of 100:1 (100 mg/kg CBD, 1mg/kg THC) rescue light aversion caused by CGRP and SNP.

Light Aversion (Central Administration of CGRP): In this experiment, CBD:THC 100:1 was injected 60 minutes before testing and CGRP was injected 30 minutes before placing mice in the testing chamber in dim light. We report that, mice injected without CBD:THC 100:1 and with CGRP spent significantly less time in the light than those injected with just vehicle treatments during the 30-minute testing period. Interestingly, pretreatment with the 100:1 CBD:THC ratio was able to rescue light aversion caused by centrally administered CGRP.

Testing the adverse effects of peripheral cannabinoids: To address possible safety concerns, we performed a battery of behavior assays. In the light aversion and open field assays, we conclude that no anxiety adverse effects are produced. In the rotarod assay we report that none of the cannabinoid ratios tested produced any negative effects in motor function. To test for any memory impairments, we used the y-maze assay and concluded that none of the cannabinoid ratios produced any adverse effects on memory. In the tall suspension assay which test for depression like symptoms we report no adverse effects. We conclude that none of the cannabinoid ratios tested produce any adverse effects in the assays mentioned.

Conclusion:

Data from our lab indicate that a 100:1 ratio of CBD:THC is effective for treating light aversion (photophobia) from peripheral administration of CGRP and the nitric oxide donor SNP. We also report similar findings where pretreatment with the 100:1 ratio alleviates light aversion caused by central administration of CGRP while minimizing adverse effects. This early research suggests the potential of cannabis-based treatments for peripheral and central mechanisms of migraine pathogenesis.

2 Chemogenetic Inhibition of the Posterior Thalamus: Effects on Migraine-like Phenotypes in Mice

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Migraine is a complex neurological disorder with a key feature of pain and is often accompanied by disabling sensory abnormalities. The mechanisms underlying migraine are not fully elucidated and an unmet clinical need persists for a majority of migraine patients. Sensory abnormalities accompanying migraine include photophobia (light sensitivity) and allodynia (perception of pain to non-noxious stimuli). We aimed to identify central brain regions contributing to migraine sensory sensitivity using mouse behavior models. Our focus was on the posterior thalamic nuclei (PoT), a group of nuclei implicated as a sensory integration center in migraine, using the neuropeptide CGRP to induce a migraine-like state. Previous studies demonstrated the sufficiency of the PoT in inducing migraine-like phenotypes, we asked whether the PoT is necessary for photophobia and allodynia. We undertook a chemogenetic approach utilizing inhibitory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). We used a light dark assay to measure light aversion as a surrogate for photophobia, and the plantar von Frey assay to measure hindpaw touch hypersensitivity as a surrogate for extracephalic allodynia. The mice were split into two viral injection groups: an experimental virus consisting of an AAV vector carrying the inhibitory DREADD hM4Di (pAAV5-*CaMKIIa-hM4Di-mCherry*) or a control virus with the *mCherry* gene only (pAAV5-*CaMKIIa-mCherry*). The virus was injected bilaterally into the PoT. The mice are tested in the light dark assay (to measure photophobia) and the plantar von Frey assay (to measure hindpaw touch sensitivity), with Compound 21 as the designer drug to activate the designer receptor. Here we demonstrate a partial rescue of CGRP-induced touch hypersensitivity following DREADD activation with C21, without undesirable off target effects. We also demonstrate a partial rescue of a light aversive phenotype following DREADD activation without off target effects. In summary, these data suggest that the PoT is necessary for both light aversive behavior and touch hypersensitivity in mice.

3 Exploring the Role of Descending Dopamine Pain Modulation in the Transition to Chronic Pain

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Changes in the central nervous system and descending pain modulation may underlie the transition to chronic pain, but our understanding of these changes is insufficient to predict and prevent the transition to chronic pain. The descending dopamine pain modulation system originates in the A11 nucleus of the hypothalamus and projects ipsilaterally to all levels of the spinal cord. A faciliatory role has been suggested for spinal dopamine in animals vulnerable to chronic pain from a prior inflammatory insult. We hypothesized that A11 neurons become active following peripheral insult to facilitate the transition to chronic pain via D1-like receptor mediated phosphorylation of NMDA receptors. Adult male and female C57BL6/J mice were used in this study. Pain was induced by two intramuscular injections (pH 4.0) five days apart in the left gastrocnemius muscle. To assess pain, muscle withdrawal thresholds (MWT) and paw sensitivity (vF) were assessed before and after induction of the model. We collected tissue or performed pharmacological manipulation at or near the time of the transition to chronic pain, following the second intramuscular injection, to test the role of A11 activity, D1-like receptors, and NMDA receptors in the transition to chronic pain. We found that in our model, D1-like receptor activity is necessary for the transition to chronic pain in male but not female mice, but D1-like receptor mRNA expression is not elevated following the transition to chronic pain. This suggests D1-like receptors may facilitate the transition to chronic pain in males via downstream signaling pathways. Funding: NIH AR073187, T32GM144636.

4 Decreased Transcriptional Activity and Pain in a Marbach-Schaaf Neurodevelopmental Syndrome *PRKAR1B* Mutation

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While neurodevelopmental disorders (**NDDs**) result from improper development of neurons in the central nervous system, our understanding of the underlying mechanisms that cause this developmental abnormality is limited. NDDs appear to stem from *de novo* mutations, as patients with NDDs typically have mutations in proteins such as kinases and transcription regulators. Thus, there is a ***critical need*** to understand the role *de novo* mutations play in NDDs to develop effective treatments for these disorders. Recent studies have identified a novel NDD called Marbach-Schaaf Neurodevelopmental Syndrome (**MASNS**) associated with the *de novo* mutations Q167L, E196K, and R335W in *PRKAR1B*. *PRKAR1B* encodes the regulatory R1β subunit of protein kinase A (**PKA**), a subunit that is primarily expressed in the brain. Children with MASNS demonstrate global developmental delay, speech delay, and delays in the development of gross and fine motor skills. R335W is the most common R1β mutation in children with MASNS and is associated with *reduced pain sensitivity*. These findings suggest that PKA plays a crucial role in the pathogenesis of MASNS. The long-term goal of our research is to develop treatments for MASNS. *Our central hypothesis is that the R335W R1β mutation reduces pain sensitivity by impairing cAMP-mediated activation of PKA*. Data from our lab suggest that the R335W mutation is dominant-negative, as reporter assays show that R335W R1β severely blunts PKA-mediated transcriptional activity in primary hippocampal and cortical neuronal cultures. Moreover, preliminary data show that pain (assessed by the Hargreaves and Von Frey assays) is reduced in a mouse model of R335W. We will continue to elucidate the effects of R335W R1β on the PKA signaling pathway and how it contributes to reduced pain sensitivity in MASNS.

5 Development of a Mouse Model for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex clinical condition characterized by overwhelming fatigue and associated symptoms like pain lasting greater than 6 months. ME/CFS is often initiated after an illness, exacerbated by stress, and associated with immune system changes. The purpose of this study was to develop a clinically relevant mouse model of ME/CFS. We hypothesized that pairing stress with an illness-like immune stimulation would generate long-term fatigue and pain, and thus tested effects of combining one-day of acute restraint stress with the immune activator lipopolysaccharide (LPS, i.p.). Fatigue was assessed using voluntary wheel running (RW) and open field testing. For pain, we assessed mechanical paw sensitivity and muscle withdrawal thresholds. Immune cell phenotype was assessed using spectral flow cytometry. C57BL6 mice were divided into 4 groups: (1) stress+LPS, (2) stress+saline, (3) stress+saline+1-day no-RW, (4) no intervention. The stress+LPS group showed a short duration decrease in RW ($p<0.001$), open field activity ($p<0.001$), and muscle withdrawal threshold ($p=0.005$). Surprisingly, the stress and saline group ran significantly less over the 12-day period showing a long-lasting decrease in RW when compared to the other three groups ($p=0.002$) without changes in open field or pain behaviors long-term (day 10). The stress+LPS group showed alterations in immune phenotypes: increased CD4+ T-cells ($p<0.001$), decreased CD8+ T-cells ($p=0.007$), and decreased CD25+ T-cells ($p=0.02$) compared to no intervention group. Thus, stress with a mild insult produces a long-term reduction in activity that is not associated with alterations in immune cell phenotype. Funded by the Foundation for Physical Therapy Research: PODS I and II.

6 Post-exertional Malaise in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-Acute Sequelae of SARS-CoV-2 Infection

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and post-acute sequelae of SARS-CoV-2 infection (PASC) are complex clinical conditions sharing several symptoms hallmarked by post-exertional malaise (PEM). PEM is a severe exacerbation of symptoms after minor physical or mental exertion starting 6-24 hours after activity and can last for days or even weeks. Little is known about the relationship between PEM severity and fatigue, pain, function, and psychological measures. Our purpose was to compare two methods of collecting PEM data: (1) DePaul Symptom Questionnaire – PEM (DSQ-PEM) taken at baseline and (2) a sum score of different 0-10 scales measuring physical and mental fatigue, pain, function, and psychological constructs taken pre-exercise and 24hr post-exercise. A change score was calculated between the sum of these 0-10 scales which we termed task-specific PEM (TS-PEM). Survey data was collected from 60 individuals with ME/CFS (n=30) and PASC (n=30). Univariate analyses were used to separately correlate the DSQ-PEM and TS-PEM with pain, fatigue, function, and psychological factors respectively. Stepwise model selection was performed to determine the best fit model for each PEM method. Higher DSQ-PEM severity was associated with higher depression (HADS:Depression), lower levels of function (PROMIS Physical Function-6b), and individuals with ME/CFS who reported greater symptom severity (total model R2=0.63). Higher levels of CS-PEM severity were associated with lower levels of baseline fatigue (Brief Fatigue Inventory) and lower levels of early life stress (ACES) (total model R2=0.12). The DSQ-PEM and CS-PEM demonstrate different associations with baseline fatigue, pain, function, and psychological measures suggesting they capture different constructs. Funded by the National Institutes of Health (R01AR077418) and the Foundation for Physical Therapy Research: PODS I and II.

7 Exploring the Role of Fitness and Physical Activity in Pain Sensitivity or Inhibition

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Introduction: Evidence suggest physical activity (PA) may reduce or prevent chronic pain. However, there are mixed findings on the associations between select assessments of PA and quantitative sensory testing (QST), indirect measures of peripheral and central pain sensitivity. None have evaluated self-report and objective PA as well as fitness level in the same cohort. Thus, this study aimed to investigate the relationships between multiple QST measures with PA and fitness levels in a healthy cohort. Methods:68 healthy adults (33F) completed a QST battery, including pressure pain threshold (PPT), mechanical pressure temporal summation (TS), and conditioned pain modulation (CPM) using both PPTs and mechanical TS as test stimuli and a cold pressor conditioning stimulus. 7-day PA was assessed objectively using wrist-worn accelerometers (ActiGraph™ wActisleep+) and self-reported using the International Physical Activity Questionnaire (IPAQ) to estimate energy expenditure (MET*min/wk), minutes of moderate and vigorous PA (MVPA) and sedentary time. Cardiorespiratory fitness was assessed using the YMCA step test. Correlation and regression analyses were employed for the data analyses (SPSS, IBM). Results: Higher PPTs (lower pain sensitivity) were related to several PA and fitness metrics: greater self-reported PA (vigorous PA, MVPA and leisure PA) ($p \leq 0.01$), accelerometry vigorous activity ($p \leq 0.01$), and high fitness levels ($p \leq 0.01$). TS was only correlated with self-reported leisure activity ($p = 0.01$), but no other PA or fitness metrics. CPM was not significantly correlated with any PA or fitness metrics. Discussion: Similar to prior studies, we observed mixed results, with some measures of reduced pain sensitivity observed in those with greater PA (self-reported and accelerometry-based) but most without a significant relationship. Fitness level provided an additional metric not often assessed, again reinforcing that only some pain sensitivity measures appear to be related. However, all relationships supported either reduced pain sensitivity with greater PA or fitness, as hypothesized or no relationship. Never was higher pain sensitivity correlated with greater PA or fitness.

8 **Ultrasound Guided Tenotomy and Debridement for Managing Achilles Tendinopathy: A Longitudinal Cohort Study**

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Achilles tendinopathy (AT) is a prevalent musculoskeletal pain condition characterized by activity-related heel pain, elevated pain-related psychological factors, and decreased function. Although exercise is recommended as a front-line treatment for AT, up to 60% of individuals experience persistent pain and could benefit from the availability of second-line treatment options. Ultrasound-guided tenotomy and debridement (USGTD) is a minimally invasive treatment for individuals who do not respond to first-line treatments, yet the impact on pain, psychological factors, and function remains insufficiently studied. This prospective cohort study aimed to investigate the effect of USGTD on clinical outcomes in individuals with chronic AT who had tried first-line treatments for at least 3 months and identify imaging biomarkers of treatment success. 56 individuals with chronic Achilles tendinopathy (Mean(SD): Age=55.9(11), BMI= 34.8(8.2), female (n = 38)), insertional (n = 48), and midportion tendinopathy (n =8) participated. Linear mixed models were used to assess the effect of time on pain (NRS: 0-10), kinesiophobia (TSK-17), pain catastrophizing (PCS), and function (FAAM-ADL). Logistic regression models were built using purposeful model selection to assess the association between imaging biomarkers and response in pain and function outcomes. Participants were dichotomized into improved and not-improved groups based on the criteria of 20% reduction in their worst pain rating (Pain Responder) and 20% improvement in the FAAM (Function Responder). Baseline pain was 6.1(2.2), TSK-17 was 40.8(7.1), PCS was 13.7(10.2) and baseline FAAM-ADL was 55.9(17.3). By 6 weeks there were significant improvements in pain (Mean change (95% CI): NRS: -1.85 (-2.6 to -1.1), function (FAAM-ADL: 14.4 (9.3 to 19.5), and pain-related psychological variables (TSK -17: -5 (-6.9 to -3.2), PCS: -7 (-9.1 to -4.9)). These improvements were sustained at 12 weeks (NRS: -2.84(-3.6 to -2.1), TSK:-7.5 (-9.4 to -5.6), PCS:-7.6 (-9.8 to -5.4), FAAM-ADL: 23.5 (18.1 to 28.9)) and 52 weeks (NRS: -2.99 (-3.8 to -2.2), TSK:-7.5 (-11.4 to -6.1), PCS: -8.5 (-10.8 to -6.1), FAAM-ADL: 25.1 (19.6 to 30.7)) following the procedure. Retrocalcaneal bursitis OR: 0.07 (95% CI: 0.01 to 0.57). No imaging biomarkers were associated with function outcomes. No procedure-related complications were reported by participants. Our findings suggest that USGTD yields clinically meaningful, enduring improvements in clinical outcomes. Presence of retrocalcaneal bursitis is associated with decreased odds of improved pain. Future studies are needed to evaluate the efficacy of USGT and validate imaging biomarkers.

9 **Targeting noradrenergic tone to compensate the glymphatic dysfunction in post-traumatic headaches after mild traumatic brain injury**

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Mild traumatic brain injury (mTBI) is a common and complex head injury. It results in many symptoms, including migraine-like post-traumatic headaches (PTH). The mechanisms underlying these headaches are still unknown. A clue to PTH may be the shared clinical associations between mTBI, migraine, and sleep-wake disruption (SWD). Indeed, sleep disruption, common after mTBI, is also a migraine trigger. Instead, sleep is known as migraine abortive. In this setting, sleep disruption may impair the glymphatic system, a brain-wide network of perivascular spaces that supports the rapid exchange of cerebrospinal and interstitial fluid. Glymphatic function is most rapid during sleep, while it is inhibited during wakefulness by central noradrenergic tone and is reduced by mTBI. Our hypothesis is that the disruption of glymphatic function after mTBI may contribute to trigger and endure PTH symptoms.

Repetitive impact mTBI mouse models have been used for developing PTH phenotypes. The mice were then treated with the α1-adrenergic antagonist prazosin. Their PTH symptoms, induced by the injection of a sub-threshold calcitonin gene-related peptide (CGRP) dose, the main migraine mediator, were assessed through light aversion and mechanical facial allodynia tests. Glymphatic system impairment was measured via glymphatic CSF tracer influx compared to sham.

Our preliminary data demonstrate that treatment with prazosin prevents CGRP sensitivity in mice, mostly in males. Furthermore, prazosin increases glymphatic CSF tracer influx compared to vehicle-treated mice.

Our results suggest noradrenergic tone as novel target to improve PTH symptoms and glymphatic function, potentially improving sleep disruption as therapeutic strategies for mTBI patients.
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10 Exploration of Rural-Urban Differences in Pain, Depression Symptoms, and Quality of Life during Third Trimester of Pregnancy

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Background: Guided by the Integrated Perinatal Health Framework, pain and depression were two factors shaping quality of life for pregnant women. Rural residency has previously been associated with increased prevalence of perinatal depression in U.S. women, increased lower back pain in the general non-pregnant population, and decreased access to healthcare services for both mental health and pain in the general non-pregnant population. However, rural versus urban residency variances with pain, pain intensity, depression, and quality of life have not been explored in U.S. pregnant women.

Purpose: The purpose of this secondary data analysis was to assess rural-urban differences in pain, pain interference, depressive symptoms, and quality of life among US women in their third trimester of pregnancy.

Methods: Data were drawn from a descriptive, cross-sectional study in which a convenience sample of 114 U.S. women completed a demographic questionnaire that included zip code of residency, the Brief Pain Inventory, the Edinburgh Postnatal Depression Scale, and the SFQoL-36 scale using a pregnancy tracker app. Group differences were assessed using a Mann-Whitney U.

Results: Rural (n = 28, 25%) and urban (n = 86, 75%) groups experienced similar rates of depression and pain for all variable mean ranks, with no significant differences between groupings.

Conclusions: Our study suggests similarly high rates of pain and depression for rural and urban U.S. women. Although the small rural sample size may have impacted these results, we have identified that rural women have significant pain and depression that require perinatal healthcare. However, clinically relevant findings related to pain, anxiety, and social determinants were noted that need further exploration with a larger sample size.

11 **Spinal Cord Expression and Function of Complement C5aR1 Receptor in Neuropathic Pain**

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Chronic pain affects approximately 100 million Americans and only a minority of patients experience satisfactory relief of their pain with currently available pharmaceutics. One type of chronic pain is caused by direct injury to the nerve called neuropathic pain, which affects ~10% of the overall population. Despite the prevalence, the underlying mechanisms of neuropathic pain are not well-defined, and better understanding of the mechanisms that promote central sensitization after injury could lead to better treatment of this condition. Notably, a recent meta-analysis of microarray studies of pain-related genes demonstrated a remarkable enrichment of genes related to the complement system activation. Among the complement products, C5a and its receptor C5aR1 seem to be especially important in the pathogenesis of neuropathic pain. However, it is not well understood how C5a/C5aR1 signaling contributes to pain amplification in the spinal cord and which cells are involved. Here, we used immunohistochemistry (IHC) to examine which cells within the spinal cord express C5aR1. Our data show that C5aR1 is expressed primarily in microglia and astrocytes, but not in neurons. To further investigate the function of C5aR1, we have generated two mouse lines with C5aR1 deletion specifically in microglia and astrocytes, respectively to test the roles of C5aR1 in these cell types during neuropathic pain. Our data suggest that C5aR1 expressed in microglia is essential for the initiation of neuropathic pain. Collectively, these results provide a new mechanistic insight into the role of C5a/C5aR1 signaling in neuropathic pain.

Acknowledgments: This research was supported by NIH grant R01 NS127428

12 The neuropeptide amylin induces diarrhea in mice and the amylin receptor contributes to CGRP-induced diarrhea

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Migraine is a neurovascular disorder defined by many sensory symptoms and can include gastrointestinal issues. Calcitonin gene-related peptide (CGRP) is a neuropeptide that is necessary and sufficient for migraine in humans and can induce migraine-like symptoms and diarrhea. Prolonged use of a gepant or monoclonal antibody that target the CGRP receptor has been shown to cause constipation in patients. Amylin is a hormonal peptide that shares similarities with CGRP. In addition to sharing strong homology with CGRP, amylin and CGRP also bind to the calcitonin receptor/RAMP1 complex (AMY_1). We hypothesized that amylin may cause diarrhea in mice and that CGRP-induced diarrhea may also be mediated by the AMY_1 receptor.

Male and female C57BL/6J and CD1 mice were intraperitoneally (IP) injected with PBS, CGRP, or amylin. These agonists were also co-injected with antagonists of amylin receptors (AC187) and human CGRP receptors (olcegepant). Transgenic mice were bred to globally over-express human RAMP1 (hRAMP1). These global mice and litter mates were IP injected with the same set of agonists and antagonists. To measure diarrhea, mice were IP injected then placed in a holder cage for 30-minutes. Filter papers from the holder cages were weighed after removal of loose (non-diarrheal) excretion.

CGRP and amylin induced diarrhea post-IP treatment in CD1 and C57 mice. CD1 mice experienced higher amounts of diarrhea when injected with either agonist compared to C57 mice. In both strains of mice, amylin induced half the amount of diarrheal excretion than CGRP. In our transgenic model, we saw the similar effect of amylin inducing half as much diarrhea compared to CGRP. There was no difference between the transgenic mice and its littermates. AC187 inhibited CGRP and amylin with a 10mg/kg dose. In C57 mice, olcegepant blocked CGRP-induced diarrhea at 1mg/kg and amylin-induced diarrhea with a dose of 0.001mg/kg. In the CD1 mice, 10mg/kg olcegepant inhibited CGRP; amylin was blocked at 100-fold lower dose of 0.1mg/kg agonist.

CGRP and amylin can induce diarrhea, but amylin is less potent. AC187 and olcegepant more effectively block amylin-induced diarrhea compared to CGRP. Olcegepant inhibits amylin at a lower dose compared to CGRP in both strains of mice. This suggests both CLR-based CGRP receptor and CTR-based AMY_1 receptor mechanisms may be important. Our amylin-induced diarrhea data suggest a more complex receptor biology with AC187 and olcegepant blocking amylin's effects. Together, these data highlight the complex nature of the CGRP family of peptides and receptors in gastrointestinal pathophysiology.

13 C5a / C5aR1 signaling in spinal mechanisms of neuropathic pain

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Recent research indicates a strong correlation between neuropathic pain and the significant increase of complement effectors in the spinal cord, leading to the creation of a highly potent complement product, C5a. This study sheds light on the spinal mechanisms dependent on C5a, that contribute to the development of neuropathic pain in mice subjected to spared nerve injury (SNI). Initially, the reduction of mechanical allodynia in SNI mice was observed through the pharmacological or genetic suppression of C5a receptors (C5aR1). This pain-relieving effect strongly implies the crucial role of C5a/C5aR1 signaling in the genesis and persistence of neuropathic pain post-SNI. Subsequently, two mouse strains were developed that expressed fluorescent Ca^{2+} indicators in microglia and neurons within the spinal cord, respectively. Multiphoton Ca2+ imaging in an ex vivo intact spinal cord preparation from these mice revealed that the application of C5a triggered rapid [Ca2+] increases in superficial dorsal horn microglia, but not in neurons, , which were blocked by a C5aR1 competitive antagonist, PMX205. Using patch-clamp recordings, we found that the application of C5a significantly amplified the number of action potentials triggered by the saturating dorsal root stimulation in both unidentified and spinoparabrachial projection dorsal horn neurons. The application of C5a also initiated burst firing and increased the frequency of spontaneous firing in certain neurons. Interestingly, the afferent synaptic input remained largely unaffected by the application. Concurrently, C5a induced a significant reduction in the threshold for action potential generation in a subset of dorsal horn neurons, thereby amplifying the spinal cord output to supraspinal structures. In conclusion, these observations suggest that C5a/C5aR1 signaling enhances the intrinsic excitability of dorsal horn neurons, likely through microglial activation.

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14 Examining the role of cerebellar CGRP-expressing neurons in migraine-like sensory hypersensitivity in mice

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To better understand and treat migraine, identification of neuroanatomic contributors has been an integral component of ongoing studies. The cerebellum has become an auspicious region of interest regarding migraine. Notably, the cerebellum is a sensory integration center that communicates with migraine-related brain regions, is altered during migraine in human imaging studies and contains a high density of binding sites for calcitonin gene-related peptide (CGRP). CGRP injection centered on the deep cerebellar medial nucleus (MN) of mice induced migraine-like behaviors including light aversion, tactile hypersensitivity, and spontaneous squint. The objective of this study was to discern which cell types in the MN may contribute to these migraine-like phenotypes through selective optogenetic stimulation and DREADD inhibition of different cell types within that nucleus.

To stimulate all neuronal cell bodies, we used synapsin-driven channelrhodopsin (ChR2) expression in the medial nucleus. To stimulate CGRP-expressing neuronal cell bodies in the medial cerebellar nucleus (MNCGRP) and fibers that project to the posterior thalamus (PoT) and zona incerta (ZI), we expressed Cre-dependent ChR2 in the medial nucleus of CalcaCre/+ mice, which have Cre recombinase inserted into the α-CGRP encoding gene Calca. To inhibit CGRP-expressing neuronal cell bodies in the MNCGRP and fibers that project to the posterior thalamus (PoT) and zona incerta (ZI), we expressed synapsin-driven, Cre-dependent DREADDs in the medial nucleus of CalcaCre/+ mice.

Optical stimulation of synapsin-driven ChR2, which targets all MN neurons, did not induce migraine-like behaviors. However, optical stimulation of MNCGRP neurons in the medial nucleus of CalcaCre/+ mice induced light aversive behavior and tactile hypersensitivity. No significant increase in spontaneous squint, anxiety-like behavior, or changes in gait were observed. Inhibition of MNCGRP neurons by synapsin-driven DREADD did not prevent CGRP-induced light aversive behavior or tactile hypersensitivity.

15 Similarities and Differences of Reported Baseline Symptomology among Fibromyalgia, Osteoarthritis, and Carpal Tunnel Syndrome

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Fibromyalgia (FM) is characterized by multiple symptoms including widespread pain, fatigue, sleep, and psychological dysfunction. However, other forms of chronic pain can also impact several of these domains. Using data from a broader study investigating metabolic biomarkers of FM we aimed to characterize pain, fatigue, and dysfunction in individuals with FM compared to those with hip and knee osteoarthritis (OA), carpal tunnel syndrome (CTS), and pain-free controls (HC). 53 individuals with FM (Sex: 18 m, 35 f, age=47.0±15.3, BMI=30.3±7.0), 46 with OA (Sex: 10 m, 35 f, 1 unknown, age=54.3±11.2, BMI=31.9±8.1), 47 with CTS (Sex: 8 m, 38 f, 1 unknown, age=46.9±12.8, BMI=33.4±9.5), and 65 HC (22 m, 43 f, age=45.9±13.1, BMI=29.2±5.7) were recruited via mass mailing, flyers, clinic visits, and mailed postcards. Using validated self-report outcome measures completed remotely via REDCap or paper, several similarities and differences were noted between patient groups. Individuals with FM reported higher levels of widespread pain (FM: 9.1±4.1; OA: 1.9±2.3; CTS: 2.1±2.9; HC: 0.1±0.5), symptom severity (FM: 7.1±2.5; OA: 3.1±2.2; CTS: 4.9±3.1; HC: 1.6±2.0), pain severity (FM: 4.3±2.1; OA: 2.3±1.6; CTS: 2.9±2.2; HC: 0.3±0.8), pain interference (FM: 4.3±2.5; OA: 1.8±1.8; CTS: 2.4±2.4; HC: 0.2±0.5) compared to OA, CTS, HC. Individuals with FM reported different degrees of cognitive impairment (FM: 59.8±9.8; OA: 51.4±8.3; CTS: 55.2±11.1; HC: 43.7±9.8), cognitive functioning (FM: 46.4±5.1; OA: 50.5±4.1; CTS: 48.2±4.7; HC: 53.6±4.9), sleep disturbance (FM: 60.0±8.7; OA: 50.1±8.8; CTS: 55.5±9.8; HC: 44.7±10.5), and neuropathic pain (FM: 15.9±8.5; OA: 6.8±5.1; CTS: 13.3±6.7; HC: 1.3±2.7) compared to OA and HC but not CTS. Individuals with FM reported elevated pain catastrophizing (FM: 9.5±6.3; OA: 3.7±4.5; CTS: 5.1±6.3; HC: 1.5±3.5), and depression (FM: 55.2±7.3; OA: 49.0±7.7; CTS: 49.1±13.3; HC: 43.2±6.4) compared to all groups but similar anxiety (FM: 58.1±8.9; OA: 49.6±11.1; CTS: 53.2±12.1; HC: 47.7±8.1) compared to CTS. Individuals with FM present with elevated symptoms of pain, fatigue, pain interference, cognitive impairment, and cognitive dysfunction compared to HC and OA but share some characteristics to CTS. Future research is necessary to elucidate biomarkers that differentiate conditions with similar clinical profiles such as FM and CTS. An additional 15 CTS will be recruited for further comparison.

16 Providers' Role in Building Patient Buy-In for Treating Chronic Pain and PTSD: Qualitative Findings from Rural Veterans' Perspectives on their Interactions with Providers

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PTSD and chronic pain are two overlapping conditions prevalent among Veterans. Half of all Veterans with PTSD experience chronic pain, and nearly one-quarter of Veterans with chronic pain also have PTSD. Individuals with chronic pain and PTSD experience greater levels of functional interference and disability, more intense pain, higher affective distress, less sustainable coping strategies, and utilize more healthcare services and medications than those with either of the conditions alone. Compared to their urban counterparts, rural Veterans with comorbid chronic pain and PTSD disproportionately face a range of barriers to accessing specialty care and potentially risky prescribing practices. Importantly, the quality of patient-provider communication has the potential to shape patients' perceptions of the quality of their care as well as their willingness to try certain non-pharmacological treatments. As part of a needs assessment, semi-structured interviews were conducted with 22 rural-dwelling Veterans diagnosed with chronic pain and PTSD about experiences related to chronic pain and PTSD diagnoses and treatments, as well as perceptions of the interplay between symptoms and treatments of both conditions. Thematic analysis identified patterns in how Veterans described their interactions with their healthcare providers regarding their chronic pain and PTSD. Veterans' impressions of interactions with pain and PTSD providers greatly depended on provider validation and empowerment. First, when Veterans did not understand the rationale for their treatment, they described feeling distrusted and dismissed by their providers. Second, when Veterans were satisfied, they expressed appreciation for transparent clarification of treatment rationale and/or clear education on the overlap of chronic pain and PTSD. Lastly, Veterans identified medical records as a source of stress on the patient-provider relationship.

The present data highlight potential breakdowns that occur when patients are unsure of their providers' treatment rationale and underscore the importance of direct (conversational) and indirect communication (medical charts) in establishing trust and optimizing care for this population.

17 Increased nociception in a *Drosophila* larvae model of Neurofibromatosis type 1

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Neurofibromatosis type 1 (NF-1) is an inherited monogenetic disorder caused by loss of function mutations in a single gene, neurofibromin 1. This gene encodes a large protein called neurofibromin (Nf1), a known tumor suppressor. In humans, the disease is diagnosed early in development and is characterized by the formation of tumors in the nervous system and neuronal dysfunction. One of the most common symptoms of NF-1 is chronic pain, suggesting that nociceptive circuit function is altered. The fruit fly, *Drosophila melanogaster*, is a powerful model for studying this disease; in *Drosophila*, the Nf1 protein is ~60% homologous to the human Nf1 protein at the amino acid level. In this well-established animal model, *nf1* mutants display neuronal and behavioral phenotypes reminiscent of human symptoms. In *Drosophila* larvae, loss of Nf1 induces neuronal hyperexcitability and tactile hypersensitivity; however, the link to pain and underlying mechanisms are currently unknown. Larvae exhibit sophisticated nocifensive behavioral responses to noxious stimuli, which are produced by a well-characterized neuronal circuit. Using this paradigm, we found that loss of Nf1 increases the intensity of nocifensive behaviors in response to noxious heat, without altering the threshold for induction. We are further dissecting the neuronal population responsible using the sophisticated genetic toolkit available in flies.

18 A comparison of self-reported symptom profiles among individuals with mylagic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and post-acute sequelae of SARS-CoV-2 infection (PASC

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Individuals with mylagic encephalomyelitis/chronic fatigue syndrome (ME/CFS),post-acute sequelae of SARS-CoV-2 infection (PASC) experience activity-induced fatigue and pain which limits participation in exercise interventions. The goal of this study is to characterize pain and fatigue in individuals with ME/CFS and PASC prior to and during exercise compared to healthy controls (HC). These data are part of a larger study investigating the relation between immune system changes and changes in pain and fatigue following an exercise task. Thirty individuals from each cohort completed baseline measures and performed a 25-minute session on a whole-body upright cycle ergometer, adapted to age predicted maximal heart rate and symptom intensity for subjects with PASC and ME/CFS. Subjects were asked to rate their pain, fatigue, and exertion before and during the exercise task. ANOVA with Bonferroni adjustment for multiple comparisons were used to evaluate differences among the cohorts. Individuals from all cohorts were of similar age, BMI, and self-reported physical activity. Individuals with ME/CFS and PASC reported elevated symptoms across all domains compared to HC. Individuals with ME/CFS reported greater pain severity (ME/CFS: 3.3 ± 1.6, PASC: 2.2 ± 1.4), pain interference (ME/CFS: 4.2 ± 2.7, PASC: 2.3 ± 2.3), widespread pain index (ME/CFS: 6.6 ± 4.7, PASC: 4.1 ± 3.8), and post-exertional malaise (ME/CFS: 21.9 ± 6.5, PASC 14.5 ±8.2) compared to PASC. Individuals from ME/CFS and PASC reported similar fatigue severity (ME/CFS: 6.7 ± 1.6, PASC 6.2 ± 2.0), fatigue impact (ME/CFS: 59.2 ± 11.5, PASC 52.3 ± 14.9), cognitive impact (ME/CFS: 25.1 ± 8.0, PASC 26.2 ± 8.0), pain catastrophizing (ME/CFS: 11.3 ± 6.5, PASC 8.1± 6.0), kinesiophobia (ME/CFS: 39.2 ± 8.0, PASC: 34.9 ± 9.3), depression (ME/CFS: 7.7 ± 3.8, PASC 7.2 ± 4.1), anxiety (ME/CFS: 8.5 ± 4.9, PASC 6.9 ± 4.3), sleep disturbance (ME/CFS: 57.4 ± 6.6, PASC 54.8 ± 8.2), and quality of life (ME/CFS: 51.7 ± 18.4 , PASC 60.4 ± 20.6). This study highlights similarities and differences in baseline symptom presentation among individuals with ME/CFS and PASC, notably individuals with ME/CFS reporting greater pain and pain interference compared to PASC. Future research will investigate changes in symptoms and the immune system profile following exercise exposure.