



2020 NIH Pain Consortium Symposium on Advances in Pain Research Technologies for Improved Understanding and Management of Pain

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All-Virtual Meeting

POSTER ABSTRACTS

(Listed in alphabetical order by Presenting Author)

Poster #1

NinaA: A Novel Mediator of Pain Memory in Adult *Drosophila Melanogaster*

Crocker, A., Sarikaya, K., Pil, B., Marriott, J., Taber, K., Vinton, E.

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Objective: To gain greater insight into the role NinaA plays in the formation of olfactory aversive memory we looked at whether the gene NinaA was involved in pain perception in the periphery, pain integration, or the behavioral output of pain avoidance.

Specific Aim: To determine where NinaA is involved along the pain processing pathway and how this impacts memory formation.

Background: The detection, learning, and recall of pain is crucial to all animals' survival. Dysregulation of these functions can contribute to a number of anxiety disorders. Here we characterize the role of the NinaA gene which appears to play a part in painful memory formation and stress behavior in the fly. NinaA is known to be important for trafficking Rhodopsin 1 (Rh1) out of the ER in the eye. Rh1 is also involved in determining temperature preference. However, our data suggests that the actions of NinaA are independent of Rh1.

Previously we found that the NinaA gene is down-regulated following memory formation. Flies carrying a mutation in the NinaA gene fail to form long-term, aversive odor memories.

Results and Significance: Our preliminary data suggest that these flies fail to centrally integrate the aversive nature of electric shock. NinaA mutant animals appear to detect peripheral pain stimuli normally. This deficit is seen when trained with electric shock or with noxious heat. Our data suggests that NinaA animals generate a stress response to noxious stimuli, as measured by an increase in wall-following behavior, but once that noxious stimuli is removed, the heightened stress response ends. This is in contrast to wild type flies who show enhanced stress responses for at least 10 minutes following painful stimuli exposure. We hypothesize that this failure to maintain a normal stress response to painful stimuli reduces their capacity to maintain long-term memories. This

work will allow us to explore non-cortisol mediated stress pathways in response to pain and memory formation in a genetically tractable organism.

Poster #2

A Randomized Double-Blind Placebo-Controlled Trial to Assess the Effectiveness of Low-Dose Naltrexone in Combination with Standard Treatment in Women with Chronic Pelvic Pain Secondary to Endometriosis

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Objectives

1. Study Objectives: This study aims to test the hypothesis that low dose naltrexone improves pain symptoms and quality of life in patients with endometriosis, by showing a decrease in VAS scores and increased endometriosis health profile scores.
2. Primary Endpoints: Primary outcome is change in averaged daily VAS (visual analog scale) pain score. Patients will be asked to report daily electronic VAS scores. Scores will be averaged at weekly intervals.
3. Secondary Endpoints: Secondary outcomes are Patient's Global Impression of Change (PGIC) surveys and patient reported Endometriosis Health Profile- 30 (EHP 30) surveys. Each survey will be completed at 4-week intervals. Additional outcomes include side effects, pain medication use, number of patients electing to continue LDN use.

Specific Aims: My central hypothesis is that LDN in combination with hormonal suppression of endometriosis (standard of care) will lead to significant improvement of endometriosis-related pain. The proposal seeks to: 1) determine if the addition of LDN to standard endometriosis treatments will improve patient- reported endometriosis associated pain using daily Visual Analogue Scale (VAS) scores and 2) measure the impact of treatment on quality of life as measured by validated questionnaires including the Endometriosis Health Profile-30 (EHP30) and the Patient's Global Impression of Change (PGIC). I propose a double-blind placebo-controlled randomized clinical trial to achieve the following aims:

Aim 1: Identify the impact of LDN on daily endometriosis associated pain. We hypothesize that LDN in combination with standard hormonal suppression of endometriosis will decrease endometriosis associated pain as reported by the VAS as compared with hormonal suppression alone.

Aim 2: Identify the impact of LDN on quality of life among women with endometriosis. The EHP30 and PGIC are validated surveys used to measure quality of life outcomes. We hypothesize that LDN in combination with standard hormonal suppression of endometriosis will improve quality of life as compared with hormonal suppression alone.

Background: Endometriosis is a common disorder, defined by the presence of endometrial glands and stroma outside the uterine cavity. Endometriosis affects up to 10% of reproductive age women, with affected patients experiencing chronic pelvic pain, including dyspareunia, and dysmenorrhea^{1,2}. Despite its prevalence,

endometriosis is still poorly understood with limited management options and no cure.

The etiology of endometriosis remains unknown, however is thought to be multifactorial. The most accepted theory involves retrograde menstruation, in the context of altered immunity, inflammatory changes, and genetic factors^{1,2}. Endometriosis is characterized by increased peritoneal inflammation. Inflammatory cytokines, including TNF-alpha, interleukins 1, 6, and 8 and activated macrophages are significantly increased in the peritoneal fluid of women with endometriosis³. These inflammatory changes may be associated with the implantation and proliferation of endometriosis, as well as a mechanism for the seemingly discordant pain symptoms associated with sometimes minimal appearing cytologic changes of endometriosis^{1,2,3}.

Traditional treatment relies on surgery and medical management, often focused on hormonal modulation. As such, existing treatment is often associated with significant side effects and is limited in both clinical outcomes and patient tolerance^{4,5}. Patient comorbidities, and fertility desires, may further restrict the use of hormonal agents in women suffering with endometriosis^{4,5}.

Naltrexone hydrochloride is a competitive opioid antagonist, traditionally used as a treatment for opioid addiction^{6,7,8}. More recently, naltrexone has been evaluated as a novel treatment for chronic pain and autoimmune disorders. When used for this indication, a significantly lower dosage of 3-4.5 mg is employed. This decreased, off-label daily dosing is typically referred to as “low-dose naltrexone” (LDN). Using lower doses exploits naltrexone’s ability to act (directly on microglia cells of the) on the central nervous system, in addition to its more widely known action at opioid receptors⁶. Prior studies suggest that LDN may be beneficial in patients with fibromyalgia, Crohn’s disease, multiple sclerosis, and complex regional pain syndrome.

Evidence shows that LDN can function as an anti-inflammatory agent, acting on non-opioid receptors of central nervous system microglia cells (Liu). The non-opioid pathway decreases activation of microglia, blocking TNF alpha synthesis, among multiple other inflammatory factors. Reduced plasma levels of pro-inflammatory cytokines have been seen with use of LDN^{7,8}. Given that endometriosis is characterized by increased peritoneal inflammation, patients may benefit from the nontraditional anti-inflammatory properties of LDN.

Additionally, LDN has been shown to intermittently block all 3 subtypes (μ, κ, δ) of opioid receptors. The transient blockade results in an upregulation of endogenous opioids and opioid receptors. This rebound elevation of endogenous opioid levels may improve endogenous analgesia and further enhance QOL in patients with chronic pain⁶.

This study aims to investigate the effects of low-dose naltrexone (LDN) in women with chronic pelvic pain secondary to endometriosis. We hypothesize that LDN will improve pain symptoms and quality of life in patients with endometriosis, reflected by decreased VAS scores and increased HR-QOL scores, when compared to placebo.

Results/Significance: To date we have recruited and enrolled our second study participant in the trial. We do not yet have any significant results for. We have discovered that our study design, which includes daily electronic diaries, has allowed us to continue to conduct study visits and gather study data remotely during COVID-19. We are utilizing a RedCap and email-based system to collect daily pain outcomes and monthly surveys. Study visits have been completed with coordinators using Zoom and our investigational pharmacy has been able to supply

study drugs via mail. Independent of the results of this clinical trial, we will be implementing many of these strategies in future trials to help facilitate recruitment and retention in clinical trials by easing the burden of participation on patients.

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Poster #3

Utilizing Liquid Chromatography-Tandem Mass Spectrometry to Identify Lipid Biomarkers of Chronic Pain

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Objective: To identify oxidized lipid mediators (oxylipins) as biomarkers of chronic pain.

Specific aim(s):

- 1) Utilize Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) to measure circulating concentrations of oxylipins acutely after a Traumatic Brain Injury (TBI).
- 2) Identify circulating oxylipins that are related to the development of chronic post-traumatic headache (PTH).
- 3) Determine if oxylipins that are related to PTH are modifiable by diet.

Background: Oxylipins are lipids that are synthesized from dietary polyunsaturated fatty acids (PUFAs). The roles of oxylipins in chronic pain have been studied since the discovery that aspirin blocks the synthesis of prostaglandins (a family of oxylipins synthesized from arachidonic acid, AA). Recently, advances in LC-MS/MS technology has facilitated the measurement of oxylipins in a variety of tissues at fMol concentrations. This

improved detection of oxylipins has led to the discovery of hundreds of new oxylipins and sparked a plethora of research investigating the bioactivity of newly identified oxylipins. This new research has identified oxylipins derived from linoleic acid [LA, the most common dietary omega-6 (n-6) PUFA] as pronociceptive and oxylipins derived from docosahexaenoic acid (DHA, an n-3 PUFA concentrated in fish oil) as anti-nociceptive. Additionally, a recent randomized trial from our group found that lowering LA and increasing n-3 PUFA (including DHA) in the diets of chronic daily headache patients evoked significant reductions in headache frequency, severity, and interference. These findings implicate dietary PUFA and oxylipins derived from dietary PUFA in chronic pain and support the role of diet as a potential non-pharmaceutical approach to treat chronic pain.

PTH is headache that develops acutely after a TBI. PTH is common in TBI patients but usually resolves. In a significant proportion of TBI patients PTH becomes chronic. Treatment approaches for chronic PTH are similar to approaches undertaken for primary headache disorders and, like with primary headache disorders, treatments for PTH have limited efficacy and negative side effects. In this project, we used LC-MS/MS to identify oxylipins as prognostic biomarkers of chronic PTH in TBI patients and investigated if these oxylipin biomarkers were modified by diet in a separate headache population. This will provide further understanding into the biochemical processes underlying headache disorders which can facilitate the identification of new treatment targets for this disorder.

Results and Significance: We identified 27 oxylipins in serum of patients acutely after TBI. Of these 27, 3 oxylipins were significantly associated with headache severity 90 days post TBI. Remarkably, the 2 oxylipins that were found to be inversely associated with headache severity, 4-hydroxy docosahexaenoic acid (4-HDHA) and 19,20-epoxy docosapentaenoic acid (19,20-EDP) are oxylipins derived from DHA, while the only oxylipin that was positively associated with headache, 11-hydroxy-9,10-epoxy linoleic acid, was derived from linoleic acid. We then analyzed serum samples that were archived from a dietary randomized trial in chronic daily headache sufferers. We found that increasing dietary n-3 PUFA and decreasing dietary LA in these patients increased serum concentrations of both 4-DHA and 19,20-EDP. Additionally, serum concentrations of 4-HDHA and 19,20-EDP in these patients were inversely associated with headache frequency and intensity. Therefore, utilizing LC-MS/MS technology we identified 2 oxylipins biomarkers of chronic PTH. We further validated these findings in a separate headache population and found that these biomarkers are modifiable by diet highlighting a role for dietary derived oxylipins in chronic pain.

Poster #4

Cortical Control Over Touch and Tactile Neuropathic Pain Sensitivity

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Objective: Current models of somatosensory perception emphasize transmission from primary sensory neurons to the spinal cord and on to the brain. Mental influence on perception is largely assumed to occur locally within the

brain. This project aims to investigate whether sensory inflow through the spinal cord undergoes direct top-down control by the cortex.

Specific aims:

- 1) To investigate whether the descending projections from the cortex to dorsal spinal cord play any roles in regulating tactile and mechanical allodynia sensitivity
- 2) To investigate whether and how S1 corticospinal neurons respond to peripheral sensory stimulation
- 3) To investigate spinal targets of CST projections that might coordinate the descending control and primary sensory afferents in sensory processing

Background: The corticospinal tract (CST) serves as the only direct pathway that connects the cortex with the spinal cord. While it is well known CST plays an important role in fine motor control, a long-standing observation is that their axons, besides projecting to the intermediate and ventral spinal cord, densely terminate in the deep dorsal horn in all mammals (Coulter & Jones, 1977, Kuyper 1982, Lemon, 2008). Our study thus starts with an intriguing question: Why does a traditional motor pathway terminate in the spinal dorsal horn that is known for sensory processing?

Results and Significance: We showed that somatosensory corticospinal neurons (CSNs) project to deep laminae of dorsal horn, where $A\beta$ afferents terminate. Ablation of somatosensory CSNs impairs physiological touch responses, and tactile allodynia after nerve injury.

On the ascending direction, we are interested to know how somatosensory CSNs respond to different sensory stimuli. By performing in vivo imaging in freely behaving mice, our results indicated that a subset of somatosensory CSNs show immediate response to tactile, but not noxious thermal stimuli. Detailed analysis suggested that distinctive population of CSNs respond to different forms (punctate vs dynamic) of tactile stimuli. However, after SNI, such neuronal ensemble becomes more convergent. In addition, we discovered that the activation of somatosensory CSNs is further enhanced upon SNI.

A remaining question is which neurons in the spinal dorsal horn mediate the CST-dependent facilitation of tactile processing. By using electrophysiology, we showed that CST facilitates $A\beta$ inputs to activate CCK^+ neurons located in the deep dorsal horn.

Taken together, our results represent a spino-cortico-spinal feed-forward sensitization loop that is crucial for controlling tactile sensation in normal conditions and allodynia in neuropathic pain states. Normalizing the excitability of somatosensory CSNs could, therefore, be a potential target for treating neuropathic pain by pharmacological or electromagnetic manipulation. Furthermore, by identifying direct cortical control of tactile processing within spinal circuits, these results provide a plausible explanation for how mental states could directly increase or decrease normal and pathological tactile sensations in different contexts or mood states, by controlling activity in transmission pathways from the spinal cord to the brain.

Poster #5

Brain Connectivity Patterns in Circuits Associated with Endogenous Pain Modulation and Pain Persistence

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Objective: To provide normative data for the functional and anatomical brain connectivity in brain circuits related to endogenous pain modulation and pain persistence, determined from high-quality neuroimaging data collected in a large sample of healthy participants.

Specific aim: To determine functional connectivity (FC) and anatomical connectivity (AC) for thalamocortical, antinociceptive and corticolimbic circuits in a large sample of healthy controls from the Human Connectome Project¹ (HCP), that may serve as normative data in comparison with clinical pain populations.

Background: Neuroimaging of brain circuitry has suggested that differences in connectivity patterns predict efficacy in endogenous pain modulation (EPM) and pain persistence. However, normative circuitry data from a large population of healthy subjects have not been reported.

Previously, a small study² of healthy controls (n=80, 40 females) reported that pain facilitation was positively correlated with FC in a thalamocortical circuit between primary somatosensory cortex Brodmann area 3a (SI-3a) and sensory nuclei in the thalamus, while another study³ including 51 controls (26 females) reported that pain inhibition was negatively associated with AC in an antinociceptive circuit between the medial prefrontal cortex (mPFC) and periaqueductal gray (PAG). A longitudinal observational study⁴ following subacute back pain patients (n = 69, 34 females) showed that those patients with greater FC and AC within a corticolimbic circuit composed of the mPFC, amygdala (Amyg) and nucleus accumbens (NAcc) at baseline presented pain persistence at 1-year follow-up. These findings suggest that measurement of FC and AC of these 3 brain circuits may improve our understanding of EPM function and risk for pain persistence. Our goal was to determine AC and FC of these brain circuits from a publicly available neuroimaging dataset from the HCP that included 500 healthy participants.

This dataset provided preprocessed resting-state functional magnetic resonance imaging (rsfMRI) and diffusion MRI (dMRI), from which connectivity measures were derived: for FC, full and partial correlations between the rsfMRI blood oxygenation-level dependent (BOLD) time series extracted from regions of interest (ROIs) relevant for each brain circuit; for AC, we used probabilistic tractography between ROIs included in those circuits to derive thresholded tractography spatial maps for volume, fractional anisotropy (FA) and mean diffusivity (MD).

Results and Significance: The HCP sample included 274 females (251 right-handed; mean age in years (SD) = 29.3 (3.6)) and 226 males (207 right-handed; mean age (SD) = 27.8 (3.7)). Summary statistics for FC and AC are reported in the Table. Most measures were similar between females and males as well as symmetrical between sides. However, asymmetries were seen between sides for full correlation for the thalamocortical circuit (mean Z value females (SD): left = -1.6 (2.4), right = 5.1 (3.6); males left = -1.7 (2.4), right = 5.1 (2.7)) and the mPFC-Amyg

component of the corticolimbic circuit (mean Z value females (SD): left = -5.4 (4.6), right = -0.3 (3.1); males left = -5.7 (5.4), right = -0.4 (3.0)). These asymmetries did not persist for partial correlations. Reliable FC and AC outcome measures for pain-related brain circuits were determined from high-quality neuroimaging data from a large sample of young adult healthy participants from the HCP. These results may serve as normative data for future studies assessing FC and AC in these brain circuits in clinical pain populations.

Conflict(s) of Interest: None.

Source(s) of Financial Support for the Project: Work partly supported by the National Institute of Dental & Craniofacial Research of the National Institutes of Health Award Number K99DE027414, NIH grants P41 EB027061, P30 NS076408 and Minnesota Supercomputing Institute (MSI) at the University of Minnesota. Data were provided by the Human Connectome Project, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research; and by the McDonnell Center for Systems Neuroscience at Washington University.

Ethical Permissions: The research results reported here involve analysis of data collected by the NIH Human Connectome Project (HCP), which are publicly available, de-identified clinical and neuroimaging data sets. Because these data were not collected specifically for this research project and no study member has access to the subject identifiers linked to the data, this is not considered human subjects research thus not requiring ethical permission from the University of Minnesota Institutional Review Board.

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Poster #6 – Mitchell Max Awardee

Repression of Sodium Channels via a Gene Therapy for Treatment of Chronic Neuropathic Pain

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Significance: Chronic pain affects between 19-50% of the world population, with over 100 million people in the US alone. Current treatments consist mainly of opioids, which present severe side effects and risk of addiction. This burgeoning health crisis demands urgent development of alternative non-addictive methods to manage pain. A therapeutic alternative to opioids that is effective and long lasting would therefore alter the current paradigm.

Background: The human genome encodes genes that can confer protection to unnecessary pain. There are nine voltage gated sodium channel subtypes, of which NaV1.7, NaV1.8, NaV1.9 have been implicated in nociceptive transmission and/or contribution to the hyperexcitability in primary afferent nociceptive and sympathetic neurons. Previous studies have demonstrated that reduction of NaV1.7, NaV1.8, and NaV1.9 activity leads to reduced inflammatory or neuropathic pain. In addition, characterization of mutations in these channels have confirmed a causative link of these channels to human pain disorders. Since the discovery of the relationship between humans with NaV1.7 (SCN9A) mutations and congenital insensitivity to pain, this sodium channel has been an attractive target for developing chronic pain therapies. However, efforts to develop selective small molecule inhibitors have been hampered due to the high sequence identity between NaV subtypes, and in fact, many small-molecule drugs targeting NaV1.7 have failed due to side-effects caused by lack of specificity. Antibodies have faced a similar situation since there is a tradeoff between selectivity and potency due to the binding to a specific (open or close) conformation of the channel. Thus, no drug targeting this gene has reached the final phase of clinical trials. In our proof of concept in mice, we have successfully repressed NaV1.7 in the Dorsal Root Ganglia (DRGs). We delivered a variant of CRISPR-Cas9 system without nuclease activity (dCas9) and a guide-RNA (gRNA) targeting NaV1.7 into mice via AAV9 to enable pain relief for weeks. This project utilizes a novel way of targeting NaV genes via epigenome repression instead of genome edition, for non-addictive and long-term pain relief without permanent changes in the genome, lowering potential side effects.

Objective: We have developed a non-permanent gene therapy via CRISPR-dCas9 to target pain that is non-addictive, highly specific, and long-lasting. During this Phase I SBIR, we will 1) test additional pain targets *in vitro*, and 2) evaluate the new targets *in vivo* in mice models of inflammatory and neuropathic pain. In addition, we will initiate our toxicology studies in mice. At the end of this Phase I work, we will know the efficacy and safety of our candidates to perform IND-enabling toxicology studies in Phase II.

Specific Aims:

Aim 1: *In vitro* optimization of gRNA designs. Apart from the NaV1.7 channel, other sodium channels have been involved in pain signaling, mainly NaV1.8 and NaV1.9. By taking advantage of single and dual gene targeting via multiple gRNAs, we will be testing single and dual inhibition of NaV channels to determine which one shows higher efficacy. We hypothesize that targeting two NaV channels will increase pain relief, and thus, will require lower dosage.

Aim 2: *In vivo* evaluation of new targets. The best constructs from Aim 1 will be tested in adult male and female mice and compared to a negative control (non-targeting gRNA) and a positive control (Gabapentin) in a neuropathic (mononeuropathy) nerve ligation pain model (Chung's model) and a Carrageenan induced inflammatory pain model.

Conclusions: These studies will help determine whether single NaV knockdown (1.7, 1.8, 1.9), or a combination of these is more effective to ameliorate pain in an inflammatory and/or neuropathic pain rodent models and assess sex differences. Unlike small-molecules or antibodies which would be very difficult to design for multiple-gene targeting, our precision medicine approach takes the advantage of being able to specifically target single or multiple genes.

Poster #7

Long-term Impact of Adolescent Chronic Pain on Young Adult Educational, Vocational, and Social Outcomes

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Background & Objective. The incidence of pediatric chronic pain peaks in adolescence, and can result in widespread disability including missed school, withdrawal from social activities, and over-dependency on parents. While research has advanced understanding of the initial impact of chronic pain during childhood, surprisingly little is known about its long-term consequences as youth transition to adulthood. Using data from the National Longitudinal Study of Adolescent to Adult Health, this is the first nationally representative study to comprehensively determine the impact of chronic pain in adolescence on key educational, vocational, and social outcomes in young adulthood (12 years later). This study took an integrative approach derived from life course perspectives of pediatric to adult health. Specifically, we examine (1) educational attainment, (2) vocational /economic outcomes (i.e., employment, income, and public assistance), and (3) social outcomes (i.e., independent living, interpersonal relationships, parenthood).

Methods. Data from the National Longitudinal Study of Adolescent to Adult Health was used, including 3,174 youth with chronic pain and 11,610 without chronic pain who completed Wave I at ages 11-17 (adolescence) and Wave IV at ages 24-32 (young adulthood). We conducted multivariate regression analyses to examine the main effect of adolescent chronic pain status (Wave I) in predicting young adult educational, vocational, and social outcomes (Wave IV) while controlling for sociodemographic variables (i.e., Wave I sex, race/ethnicity, and parent income and Wave IV age) and adolescent depressive symptoms.

Results. Adolescent chronic pain was associated with decreased odds of attaining a high school diploma (OR = 0.66, 95% CI: 0.57 – 0.82) and attaining a bachelor's degree (OR = 0.83, 95% CI: 0.71 – 0.96) in young adulthood. Adolescent chronic pain was also associated with decreased odds of receiving employer-provided insurance benefits (OR = 0.80, 95% CI: 0.68, 0.92) and increased odds of receiving public assistance (OR = 1.31, 95% CI: 1.16, 1.48). Finally, adolescent chronic pain was associated with earlier pregnancy/increased rate of having biological children (OR = 1.28, 95% CI: 1.13, 1.45) as well as lower romantic relationship quality ($b = -0.08$, SE = .02) in young adulthood.

Conclusion & Significance. Chronic pain in adolescence is associated with long-term risk for socioeconomic and social disparities. Our findings contribute to the limited knowledge base of the scope of adverse long-term outcomes in young adults with a history of adolescent chronic pain, informing the need for more focused screening and intervention efforts. While our results provide a window into the future of adolescents with chronic pain, several questions remain. Increased research attention is needed to understand the life course impact of pediatric chronic pain, including early risk factors and underlying mechanisms that drive adverse outcomes as they unfold across the lifespan.

Poster #8

Self-Management for Youth Living with Sickle Cell Disease

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Sickle cell disease (SCD) is a hereditary hemoglobinopathy that causes alteration in the shape of red blood cells and leads to blood vessel occlusion, inflammation, infarction, organ damage, and pain. In the US, approximately 100,000 individuals have SCD, 90% of whom are African American. Beginning in childhood, individuals with SCD experience pain and other symptoms, such as fever, fatigue, and sleep disturbance. As children with SCD age, quality of life declines. Over the past few decades, survival rates for individuals with SCD have improved. However, during late adolescence and early adulthood, rates of complications, risk of death, and symptom burden dramatically increase. In addition, young adults have the highest rates of hospitalization, emergency care use, and rehospitalization relative to other age groups of individuals with SCD. The transition to adulthood and adult care is challenging, particularly for individuals with SCD who face a shortage of available adult providers. The development of effective self-management behaviors is a key component of successful transition from pediatric to adult care, as day-to-day health management is critical to improved health and utilization outcomes.

Dr. Phillips and colleagues developed a mHealth self-management intervention (web-based application; “app”) called Voice Crisis Alert V2 following an end-user-based approach by which qualitative interviews were conducted with children and adolescents with SCD, their parents or primary caregivers, and healthcare providers of children with SCD. Subsequently, initial feasibility testing was conducted with 60 dyads of children or adolescents with SCD up to age 17 and their parent or primary caregiver. Findings of the study supported feasibility of use in the population; 94% of dyads used the app, and end-of-intervention satisfaction scores were high. In addition, preliminary findings suggest improvement from baseline to end-of-intervention in symptoms and quality of life scores in children and adolescents, though improvements were more marked in children with ages older than the mean (8 – 17 years). In this older group, improvements were noted in mean and median scores for fatigue, pain interference, total and all subscale scores for health-related quality of life, and total and all subscale scores for disease-specific health-related quality of life. During qualitative post-intervention interviews, dyads described the potential benefit of the application in facilitating the transition from parent-led management to adolescent self-management.

Thus, the purpose of this study is to further test the feasibility of the app with 30 dyads of pre-adolescents/adolescents ages 11 – 17 with SCD and the parent/primary caregiver, with a focus on transition readiness, responsibility allocation, and communication between the pre-adolescent/adolescent, parent/caregiver, and healthcare provider. The app will include shared access on pre-adolescent/adolescent and parent/caregiver respective devices (“mirror view” of the app), and secure messaging between the pre-adolescent/adolescent, parent/caregiver, and sickle cell provider. We hypothesize that the addition of these features to the app will facilitate transition from parent-led management to adolescent self-management of SCD, ultimately improving transition readiness, healthcare utilization, and quality of life and symptoms outcomes. In addition to assessing

feasibility of implementation guided by the RE-AIM (Reach, Efficacy, Adoption, Implementation, Maintenance) framework, we will explore the communication that occurs via the app between the adolescent/caregiver and healthcare providers during the transition period. We will assess the types of healthcare activities that occur (e.g. provider screening, supporting, informing, or referring) and the relationship to self-management behaviors (e.g. monitoring and tracking symptoms, clinic appointment attendance, adherence to home medications and treatments), and symptom outcomes. In addition, we will preliminarily investigate signals of efficacy for measures of transition readiness, symptoms outcomes in adolescents and caregivers, healthcare utilization, and quality of life to inform a future large-scale study. Currently, adaptations to the app have been completed and recruitment is underway.

Poster #9

Differentiation of Anesthetic from Antidepressant Transcriptomic Signatures Using High-Dose Ketamine Administration

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Objective To define unique systems level transcriptional alterations after ketamine administration, with the goal of understanding how these alterations both differ from those of classical anesthetics and can be leveraged to develop new analgesic and antidepressant therapeutics.

Specific Aim 1. To gain a better understanding of both isoflurane and ketamine anesthetic effects on central nervous system.

Specific Aim 2. To determine the unique and similar mechanisms of these drugs. Gene changes are examined that are highly anti-correlated between isoflurane, which is a classical anesthetic agent, and ketamine, an N-methyl-D-aspartate receptor antagonist with unique antidepressant and analgesic actions.

Background Chronic pain is highly comorbid with depression, and both can be intractable and debilitating. Growing evidence reinforces the centrality of the glutamatergic system in the pathogenesis and treatment of pain and major depression. Traditional antidepressants require weeks to attain therapeutic efficacy, whereas single doses of the N-methyl-D-aspartate receptor antagonist ketamine produce clinical improvement within hours. Despite the therapeutic utility of this effect, the underlying mechanisms of these actions are still being clarified. Recently, clinical progress on ketamine administration paradigms for pain and depression has driven new incisive investigations into how this drug acts distinctly from others in its class. Nonetheless, the systems-level downstream effectors remain unclear, creating substantial obstacles to improving the efficacy, durability, and specificity of ketamine.

In order to begin answering these central questions, we developed rat models of long-term isoflurane or ketamine anesthesia. Isoflurane was delivered to rats via inhalation at around 1.2 MAC, which produces a light anesthetic plane. Ketamine was given intravenously through a femoral vein catheter and titrated to loss of righting reflex. In both cases, transcriptomic sampling was performed at 1hr, 10hrs, and 24hrs after recovery from 10hrs of anesthesia. Brain regions examined included the frontal cortex, hippocampus and amygdala using RNA-Seq. Additionally, whole-brain coronal sections were imaged for gene alterations at the cellular level by multiplex in situ

hybridization and immunofluorescence.

Results Detailed transcriptomic analyses of isoflurane and ketamine administration paradigms, established downstream molecular signatures of each of these agents after 10hrs of administration. Most highly significant genes were correlated between isoflurane and ketamine, most likely representing a shared anesthetic response. Conversely, gene signatures corresponding to neuronal activity were divergent between isoflurane and ketamine. While isoflurane administration caused a profound decrease in all activity-correlated genes, ketamine administration showed a paradoxical *increase* in a number of transcripts related to neuronal activity and plasticity. These activation signatures include induction of the immediate early gene *Fos*, and transcript-specific induction of brain derived neurotrophic factor (*Bdnf*). Using multiplex in situ hybridization, this paradoxical increase was observed throughout superficial cerebral cortex, with the strongest induction noted in layer 2/3 pyramidal neurons in the retrosplenial cortex (a29). These results were also confirmed by their relative correlation with a dataset examining maximal electroconvulsive shock on gene transcription. This analysis demonstrated that the most divergent gene changes between isoflurane and ketamine were also correlated between ketamine and electroconvulsive shock.

In aggregate, these findings represent a pharmacological and transcriptomic dissection of the molecular mechanisms of these two agents. They are particularly divergent with respect to signaling through a variety of neuropeptidergic and receptor signaling systems including the cocaine and amphetamine related transcript (CART peptide), as well as several other potentially targetable receptor systems. The results of our analysis help to prioritize lead development for improving the clinical utility of ketamine. More broadly, this strategy of utilizing multiple large datasets in combination with anatomical and human genetic studies can be used for elucidating the mechanism and downstream signaling pathways of any number of complex drug administration paradigms. If applied systematically, this method may provide new routes to CNS drug discovery.

Poster #10 – Mitchell Max Award Finalist

Bidirectional Modulation of Pain-Related Behaviors in the Zona Incerta

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Objectives: To identify the neural circuits underlying bidirectional modulation of persistent pain in the central amygdala (CeA).

Specific Aim: To test the hypothesis that CeA neurons expressing protein kinase c-delta (CeA-PKC δ) project to and modulate zona incerta activity to regulate nociceptive behaviors in mice

Background: Pain is a distressing feeling that is conveyed to the brain via ascending pain pathways. Recent studies have shown that neurons expressing protein kinase c-delta in the central amygdala (CeA-PKC δ) are sensitized following nerve injury and increase pain-related responses in mice. However, the neural circuits underlying the modulation of pain-related behaviors by CeA-PKC δ neurons remains unknown.

Results and Significance: Using cre-dependent anterograde and retrograde anatomical tracers in mice in combination with optogenetic-assisted circuit mapping in acute brain slices, we identified a functional inhibitory neural circuit that originates from CeA-PKC δ neurons and terminates in the Zona Incerta (ZI). The ZI was a

particularly attractive CeA efferent target because previous studies have shown silencing of this region in the context of persistent pain. Consistent with this, our behavioral experiments show that activity of ZI-GABAergic cells robustly modulate pain-related behaviors in a mouse model of neuropathic pain. Specifically, chemogenetic inhibition of ZI-GABAergic cells is sufficient to induce pain-related tactile and pressure hypersensitivity in uninjured animals and in the uninjured paw, contralateral to nerve injury. Furthermore, chemogenetic activation of ZI-GABAergic cells in nerve injury mice reverses injury-induced pressure hypersensitivity. Together, our experiments describe a novel neural circuit where inhibition of ZI by CeA-PKC δ cells promotes pain-related behaviors. This study will enhance further the current knowledge in the field about the neural circuits that are altered in persistent pain conditions.

Poster #11

The Orphan Receptor GPR139 Opposes Mu Opioid Receptor Signaling Through Activation of Gq/11

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Signaling through G protein coupled receptors (GPCRs) is highly regulated and shaped not only by how and when a GPCR engages its ligand, but also through complex and varied protein interactions at the level of the receptor and at downstream effectors. Opioid receptors are a GPCR system whose signaling is finely tuned in the central nervous system. The mu opioid receptor (MOR) is the primary receptor that modulates acute pain responses and has been heavily targeted therapeutically for its anti-nociceptive properties. The duration and extent of endogenous MOR signaling is modulated by available endogenous opioid peptides, by β -arrestin recruitment and subsequent receptor desensitization, by regulator of G protein signaling (RGS) control of G α activation, and by interaction with other GPCRs that may positively or negatively alter signaling. Using an unbiased forward genetic screen in MOR-expressing *c. elegans*, we uncovered another possible regulator of MOR signaling: the orphan GPCR GPR139. Studies using *GPR139* knockout mice demonstrate hypersensitivity to opioid analgesics across multiple measures of antinociception. However, the exact molecular mechanism(s) of how GPR139 activation and signaling can modulate MOR downstream signaling is unknown. We ran a battery of *in vitro* signaling assays to determine how GPR139 signals in cells and how that could negatively modulate MOR signaling. GPR139 G protein coupling was tested using every G alpha protein and was found to couple to both Gq/11 and Gi/o family of G proteins. We examined the effect of GPR139 on multiple parameters of MOR signaling and trafficking and found that GPR139-Gq signals in an opposing manner to MOR-G α signaling at multiple downstream effectors *in vitro*. Further, MOR-mediated changes in habenular neuron firing are blunted by dynamic GPR139 activation and can be rescued by Gq inhibition. Overall, this research examines the molecular underpinnings of GPR139 negative regulation of MOR, explores mechanisms of GPCR cross-regulation, and evaluates the possibility of therapeutically targeting GPR139 to increase the safety and efficacy of opioids.

Poster #12 - Mitchell Max Award Finalist

During Acute Intermittent Pain, Midazolam and Ketamine Distinctly Alter Functional Connectivity Between Memory, Pain, and Fear Response Centers in the Human Brain

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Objective: Using functional MRI (fMRI) and experimental pain, we sought to determine how two distinct sedative anesthetic agents, midazolam and ketamine, modulated task-related activity within and functional connectivity (FC) between brain areas that process pain, form memory, and engage in fear learning.

Specific Aim & Hypotheses:

Aim: Determine the neural changes underlying the distinct mental states induced by midazolam and ketamine.

Hypotheses: We expected that midazolam would predominantly decrease hippocampal activity, while ketamine would cause widespread reductions in areas known to process pain. Both drugs were expected to decrease FC between distinct networks.

Background: Despite widespread clinical use of anesthetic drugs, there is much we do not know about the neural correlates of their actions, particularly when processing painful stimuli. Despite no previous corroborating human imaging, preclinical models have demonstrated that midazolam's amnestic effect occurs by inhibition of the hippocampus [1, 2]. Human imaging has shown that ketamine diffusely inhibits pain-related activity, most notably in the insula and thalamus [3]. In resting-state studies done without painful stimulation, both drugs have demonstrated widespread decreases in functional connectivity [4, 5].

Approach: Healthy volunteers (n=23, 11 female, age 25.6 ± 4.9) underwent fMRI (3T BOLD, TR=1 s) in a randomized single-blind within-subject crossover trial comparing midazolam and ketamine at equisedative (light sedation) levels. Starting under saline control, subjects performed a memory encoding task of auditory word items while receiving periodic painful (7/10) electric nerve stimulation. After a target-controlled drug infusion reached steady-state, the task was repeated. Pain scores were obtained after each experimental epoch. The next day, recognition was assessed outside the scanner using the Remember-Know paradigm. After at least 5 days, subjects returned and performed the experiment using the other drug. fMRI data timeseries were compared between saline and drug conditions for brain responses related to experimental events and for background functional connectivity, after task-related activity was removed by regression. Group average activation and FC were compared between saline and drug conditions. Clusters were thresholded at $p < 0.001$ and 27 contiguous voxels.

Results: Midazolam did not diminish the perception of pain but greatly reduced later recollection of items experienced under its effects. Ketamine reduced pain ratings and caused a smaller reduction of subsequent memory. Task-related fMRI activation under saline was seen in characteristic areas within different functional networks, including the: insula (pain processing network), hippocampus (memory encoding network), and amygdala (fear learning network). Both drugs broadly reduced task activation in these regions. Midazolam demonstrated increased FC between brain areas in these networks, while ketamine showed disorganized and predominantly decreased FC between them.

Significance: Anesthetics can produce dramatic changes in mental state, which makes them powerful tools in human neuroscience. We directly compared brain activity at equal levels of responsiveness during sedation with two common intravenous anesthetics, while subjects performed a memory task and experienced pain. Midazolam inhibited memory without changing pain perception, and this was characterized by increased FC between networks that process different aspects of this experience. In contrast, ketamine relieved pain but had a lesser impact on memory, and this was characterized by decreased FC. This demonstrates that high-level brain activity occurs during sedation, independent of behavioral measures of memory or pain perception.

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