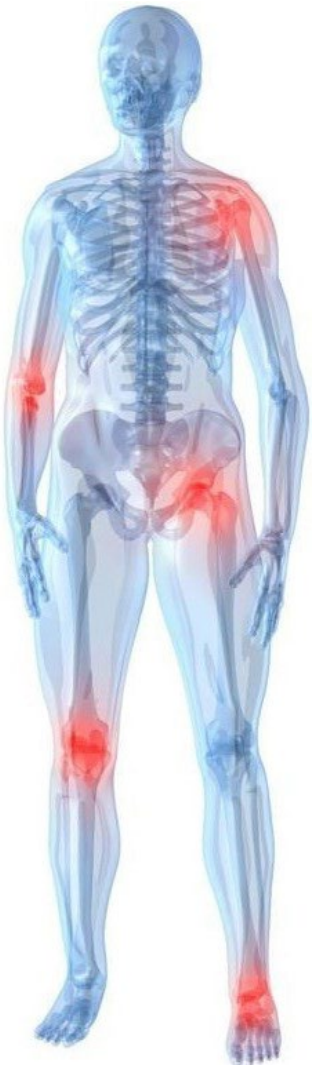
# GCC Translational Pain Research
# 9th Annual Symposium

## April 12, 2019

**BioScience Research**
**Collaborative**
**6500 Main St.**
**Houston, Texas**



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Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on **Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Neuroengineering and Pharmacological Sciences.** GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include **Antimicrobial Resistance, Nanox, Mental Health, Innovative Drug Discovery and Development, Translational Pain Research, Theoretical and Computational Neuroscience, Single Cell Omics, Regenerative Medicine, Translational Imaging and Cellular and Molecular Biophysics**. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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## Agenda

8:30 Breakfast and Poster Setup

9:00 Welcome
**Annemieke Kavelaars**, MD Anderson, Chair, GCC Translational Pain Research Consortium

9:10 Session 1
**Convener: Patrick Dougherty**, MD Anderson

*Exciting Touch: Synaptic Mechanisms of Epithelial-neural Signaling in Mechanosensation*
**Ellen Lumpkin**, Columbia University

9:55 *The Role of Opioids in the Development of Pain After SCI*
**Michelle Hook**, Texas A&M Health Science Center

10:15 *Antioxidant Carbon Nanoparticles for the Treatment of Pain After SCI*
**Juan Herrera**, University of Texas Health Science Center at Houston

10:35 Break

10:50 Session 2
**Convener: Dorina Papageorgiou**, Baylor College of Medicine

*Angiotensin Receptor Signaling in Chronic Pain States*
**Andrew Shepherd**, MD Anderson

11:10 *Nociplastic Pain*
**Jin Mo Chung**, University of Texas Medical Branch, Galveston

11:30 *Nociceptor Mechanisms that Drive Ongoing Pain*
**Terry Walters**, University of Texas Health Science Center, Houston

11:50 Poster data blitz
**Convener: Annemieke Kavelaars**, MD Anderson

12:20 Lunch

1:00 Poster session

1:40 Session 3
**Convener: Jin Mo Chung**, University of Texas Medical Branch, Galveston

*How does Physical Activity Modulate Pain? Basic Mechanisms and Clinical Implications*
**Kathleen Sluka**, University of Iowa

2:25 *Female-specific Meningeal Nociception: Potential Mechanisms for Sex Differences in Migraine*
**Greg Dussor**, University of Texas at Dallas

## Agenda

2:45 Selected Abstracts
**Convener: Carmen Dessauer**, University of Texas Health Science Center, Houston

*Targeting of the MNK-eIF4E Signaling Axis Reverses Prefrontal Cortex Cognitive Dysfunction in Neuropathic Pain*
**Stephanie Shiers** , University of Texas at Dallas

*Mechanisms Inducing Opioid Resistance after Spinal Cord Injury*
**Anibal Garza Carbajal**, University of Texas Health Science Center

*The Role of a Nutraceutical Intervention in an Animal Model of Surgical Recovery*
**Juan Cata**, MD Anderson Cancer Center

*The Unexpected Relationship between Pain, Cigarette Smoking, and Negative Affect*
**Andrew Rogers**, University of Houston

*Estrogen and Serotonin Interact to Modulate Rodent Trigeminal Sensory Neurons*
**Sukhbir Kaur Lulla,** Texas Women's University

3:35 Break

4:00 Keynote: "*Listening" and "Talking" to Neurons: Non-neuronal Cells Amplify Pain and Drug Reward ~ Pathways from Basic Science to Human and Veterinary Clinical Trials ~*
**Linda Watkins**, University of Colorado, Boulder

5:00 Reception

## Speaker Abstracts

*(in order of appearance)*

**Ellen Lumpkin**, PhD
Associate Professor, Physiology & Cellular Biophysics and of Dermatology
Co-Director, Thompson Family Foundation Initiative in CIPN & Sensory Neuroscience, Columbia University
*Exciting Touch: Synaptic Mechanisms of Epithelial-neural Signaling in Mechanosensation*

Ellen A. Lumpkin has been appointed as a professor of molecular & cell biology at University of California, Berkeley effective July 1, 2019. She is currently an associate professor of physiology & cellular biophysics and of somatosensory biology (in dermatology) at Columbia University. Dr. Lumpkin is also Co-director of the Thompson Family Foundation Initiative in Chemotherapy-Induced Peripheral Neuropathy & Sensory Neuroscience. She previously was a Sandler Fellow at UC San Francisco and an assistant professor of neuroscience, physiology & molecular biophysics, and molecular & human genetics at Baylor College of Medicine.

Abstract: The Lumpkin laboratory studies genes, cells and signals underlying skin sensations, such as touch, pain and itch. Our research has unveiled how mechanosensitive epithelial cells work in concert with the nervous system to generate different qualities of touch sensation. We have identified distinct sensory functions of epithelial Merkel cells using optogenetics, neurophysiology, mouse models and molecular approaches. Current studies are defining mechanisms of cell-cell signaling between epithelia and neurons, unravelling conserved functions of mechanoreceptors across tissues, and elucidating mechanisms that establish epithelial-neuronal connections during development. Lumpkin's translational research aims to develop non-invasive methods for modulating the peripheral nervous system.

**Michelle Hook**, PhD
Assistant Professor, Neuroscience & Experimental Therapeutics
Texas A&M Health Science Center
*The Role of Opioids in the Development of Pain After SCI*

Michelle Hook obtained her PhD degree in Physiology from the University of New England, Australia. She moved to the United States and completed post-doctoral training at the University of Memphis, MD Anderson Cancer Center and Texas A&M University. She joined the Texas A&M College of Medicine as an Assistant Professor in January, 2014. The focus of her research is on recovery of function following a spinal cord injury. She has received several national research grants (NIH, DoD), as well as funding from private foundations, to study the effects of morphine on recovery of function following spinal injury and the etiology of depression in a rodent spinal cord injury model. She received the Jerry Johnson Andrew Award for Spinal Cord Injury research in 2016.

Abstract: Opioids are among the most effective and widely prescribed medications for the treatment of pain following spinal cord injury (SCI). After SCI, patients receive opioids within hours of arrival at the emergency room, and prolonged opioid regimens are often employed for the management of post-SCI chronic pain. However, previous studies in our laboratory suggest that the effects of opioids, such as morphine, may be altered in the pathophysiological context of neurotrauma. Specifically, we have shown that morphine administration in a rodent model of SCI increases tissue loss at the injury site, decreases recovery of motor function, and increases pain, even weeks after treatment. Similarly, we have found a dose-dependent effect of opioids on pain in the clinical SCI population. High doses of opioids administered in the early phase of SCI are associated with increased pain levels in the chronic stages of injury. These data are alarming, but as opioids are among the most effective medications for the treatment of acute pain following SCI, simply discarding these analgesics is not an option. Instead it is critical to identify the molecular mechanisms engaged by morphine that lead to these adverse effects. The literature suggests that opioids may produce adverse effects by increasing glial activation and inflammation. Indeed, using flow cytometry we found that just three days of intravenous morphine administration increases the numbers of microglia and macrophages at the spinal cord injury site, compared with vehicle-treated SCI controls. Moreover, these glial cells appear to have increased expression of kappa-opioid receptors. Using immune-magnetic cell sorting, we have found that after one day of intravenous morphine administration there is not only increased expression of kappa-opioid receptors, but also increased $\beta$-arrestin and prodynorphin in the glial cells isolated from the injured tissue. Importantly, our pharmacological studies show that antagonizing the kappa-opioid receptor with nor-Binaltorphimine and reducing glial activation with minocycline, blocks the adverse effects of a single dose of intrathecal morphine on locomotor recovery and pain. These preliminary data suggest that agonists binding to kappa-opioid receptors on microglia may be biasing G protein coupled receptor signaling toward $\beta$-arrestin recruitment, increasing inflammation (IL-1$\beta$, TNF$\alpha$, and IL-6) and cell death, and undermining functional recovery.
Research support: This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Spinal Cord Injury Research Program under Award No. W81XWH 17-1-0629 to M. A. Hook. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

**Juan Herrera**, PhD
Assistant Professor, Diagnostic and Interventional Imaging
UT Health Science Center at Houston
*Antioxidant Carbon Nanoparticles for the Treatment of Pain after SCI*

Dr. Herrera is an Assistant Professor of Diagnostic and Interventional Imaging. He received his Ph.D. in Oncological Science from the University of Utah. He completed his postdoctoral training at UTHealth McGovern Medical School in the Department of Neurosurgery and was a recipient of Ruth L. Kirschstein National Research Service Award (NRSA) post-doctoral fellowship from National Institutes of Neurological Disorders and Stroke. Dr. Herrera recently received The Jerry Johnston Andrew Spinal Cord Research Project Award from Mission Connect a collaborative research project by the TIRR Foundation.

Abstract: Traumatic spinal cord injury (SCI) is devastating injury that results in motor neuron dysfunction and death at and below the lesion level. SCI patients not only live with the paralysis but as many as 80% of patients develop clinically significant neuropathic pain described as burning, stabbing, and/or electrical in sensation. One of the key underlying mechanisms contributing to both issues is systemic inflammation. Both motor and sensory dysfunction is a combination of early necrotic as well as delayed progressive cell death processes, collectively referred to as secondary injury. Oxidative stress which is the result of excess production of reactive oxygen species (ROS) and inflammation are widely considered hallmarks of the secondary injury cascade initiated after SCI. ROS are crucial contributors to a range of normal physiologic cell signaling such as energy production/regulation and contributors to the immune response to invading pathogens. As our understanding of oxidative stress involvement in various human diseases increases, studies involving antioxidants have shown some benefit in disease treatment. The need for efficient antioxidants has led to the development of nanoparticle antioxidants. Antioxidant carbon nanoparticles can scavenge ROS with far higher efficacy than dietary and endogenous antioxidants. Our study in collaboration with Dr. James Tour, Dept. of Chemistry at Rice University, demonstrates that an intravenous administration following a moderate to severe spinal cord contusion results in a significant reduction in neuropathic pain following SCI.

**Andrew Shepherd**, PhD
Assistant Professor, Symptom Research
MD Anderson Cancer Center
*Angiotensin Receptor Signaling in Chronic Pain States*

Andrew received his Bachelors' degree in Molecular Cell Biology in 2003 from the University of Manchester Institute of Science and Technology in the UK. He received his PhD in Neuroimmunomodulation in 2007 from the University of Manchester – where he investigated the impact of sensory innervation and neuropeptide signaling in a rodent model of allergic inflammation. He then relocated to the newly-established lab of Durga 'DP' Mohapatra in the Department of Pharmacology at the University of Iowa in 2008. During this time, he developed an interest in modulation of ion channel activity in the nervous system by GPCR signaling, particularly in relation to pain associated with bone-metastasized prostate cancer. Since becoming research-track faculty in the Department of Anesthesiology at Washington University in St. Louis in 2015, he has developed research related to macrophage involvement in chronic pain associated with neuropathy, inflammation and cancer. These projects will form the basis of his research in the department of Symptom Research at MD Anderson, where he was appointed as an assistant professor in December 2018.

Abstract: Peripheral nerve damage initiates a complex series of structural and cellular processes that culminate in chronic neuropathic pain. The recent success of a type 2 angiotensin II (Ang II) receptor (AT2R) antagonist in a phase II clinical trial for the treatment of postherpetic neuralgia suggests angiotensin signaling is involved in neuropathic pain. However, transcriptome analysis indicates a lack of AT2R gene (Agtr2) expression in human and rodent sensory ganglia, raising questions regarding the tissue/cell target underlying the analgesic effect of AT2R antagonism. We show that selective antagonism of AT2R attenuates neuropathic but not inflammatory mechanical and cold pain hypersensitivity behaviors in mice. Agtr2-expressing macrophages (MΦs) constitute the predominant immune cells that infiltrate the site of nerve injury. Interestingly, neuropathic mechanical and cold pain hypersensitivity can be attenuated by chemogenetic depletion of peripheral MΦs and AT2R-null hematopoietic cell transplantation. Our study identifies AT2R on peripheral MΦs as a critical trigger for pain sensitization at the site of nerve injury, and therefore proposes a translatable peripheral mechanism underlying chronic neuropathic pain.

**Jin Mo Chung**, PhD
Professor and Chair, Neuroscience and Cell Biology
University of Texas Medical Branch at Galveston
*Nociplastic Pain*

Dr. Chung received his PhD in Physiology from Loyola University of Chicago in 1977 and received his post-doctoral training in neurophysiology from Dr. William D. Willis, Jr. at the University of Texas Medical Branch, Galveston, TX. Dr. Chung's research is focused on the mechanisms of chronic pain, especially neuropathic pain. His most notable contribution to the field of pain is his rodent model of chronic pain referred to as the "spinal nerve ligation (SNL)" model. Currently his specific research focus is on the synaptic plasticity of pain transmission pathways, involvement of reactive oxygen species in chronic pain and development of various pain treatment strategies. Dr. Chung has published more than 155 papers in peer reviewed journals. These papers have been cited more than 22,000 times in the literature. Dr. Chung's research has been supported by NIH research awards for over 30 years, with multiple RO1s and Program Project Grants.

Abstract: "Nociplastic pain" is a new term recently defined by the IASP as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (IASP Taxonomy, 2017). We support the use of this term, "nociplastic pain", because there are many clinical pain conditions that can be categorized under this term, and the term gives mechanistic clues to many painful conditions that arise from altered nociceptive functions.

To study mechanisms underlying nociplastic pain, our lab recently developed an experimental procedure that can transition acute pain into a long-lasting nociplastic pain using a mouse capsaicin model. Specifically, intradermal injection of capsaicin in a mouse causes well-known pain behaviors for a short duration (less than 1 day). However, when capsaicin injection is followed, 2 hours later, by an intermittent brief series of innocuous stimuli (10 min of intermittent brush or warmth), the pain behaviors last for more than 2 weeks. We believe this is a good model to study the transition of acute pain into persistent or chronic nociplastic pain.

In addition, although this nociplastic pain model can be induced in both sexes of mice, there is a clear sexual dimorphism in underlying mechanisms. Specifically, the persistent nociplastic pain in female mice is found to be maintained by continuous peripheral input from the previously capsaicin-injected (original injury) site, whereas the nociplastic pain in male mice is independent of such peripheral input. The detailed mechanisms of nociplastic pain in both female and male mice are under study in our lab.

**Terry Walters**, PhD
Professor, Integrative Biology and Pharmacology
University of Texas Health Science Center
*Nociceptor Mechanisms that Drive Ongoing Pain*

Edgar (Terry) Walters is Professor of Integrative Biology and Pharmacology, and holder of the Fondren Chair in Cellular Signaling at McGovern Medical School. He received a Ph.D. in 1980 from Columbia University. Since 1982 he has been a faculty member in the Medical School at UT Houston, with active service to the Graduate School of Biomedical Sciences, and he has been Co-Director of the MD/PhD (now MSTP) Program for over a decade. He has used the sea slug Aplysia, squid, and rodents to investigate behavioral and neuronal phenomena important for adaptive responses to bodily injury, with a continuing emphasis on plasticity in primary nociceptors. Current research focuses on the functions and cellular signaling mechanisms associated with nociceptor hyperexcitability and ongoing electrical activity in rodent neuropathic and inflammatory pain models, including spinal cord injury.

Abstract: Ongoing or spontaneous pain is often the worst complaint of people with persistent pain conditions. This pain has been linked to ongoing activity (OA) in human C-fiber nociceptors, but little is known about how nociceptor OA is generated. Using novel algorithms to analyze irregular changes in membrane potential, we have shown in a common, non-accommodating type of probable nociceptor three alterations that promote OA: 1) prolonged depolarization of resting membrane potential, 2) a hyperpolarizing shift in the voltage threshold for action potential generation, and 3) an increase in the incidence of large depolarizing spontaneous fluctuations (DSFs). Large, randomly occurring DSFs in nociceptors had not been recognized previously as major contributors to OA, but they explain the irregularity, lack of accommodation, and low firing frequency in nociceptor OA that can promote ongoing pain. Increases in large DSFs paralleling OA have been found in nociceptors dissociated from rats with ongoing pain months after spinal cord injury or other neuropathic conditions, and acutely during in vitro exposure to inflammation-related ligands such as serotonin and capsaicin. Enhanced DSFs have also been found in nociceptors associated with ongoing pain in humans. Investigations into ion channels and cellular signals important for generating large DSFs, and potentially for selective therapeutic targeting of ongoing pain, will be described.

**Kathleen A. Sluka, PT, PhD, FAPTA**
Professor, Physical Therapy
University of Iowa
*Exercise-induced Pain and Analgesia: Basic Mechanisms and Clinical Implications*

Dr. Sluka is a professor in the Department of Physical Therapy and Rehabilitation Science at the University of Iowa. She received a physical therapy degree from Georgia State University and a PhD in Anatomy from the University of Texas Medical Branch in Galveston. After a postdoctoral fellowship with Dr. William D. Willis, she joined the faculty at the University of Iowa. Dr. Sluka's research focuses on the neurobiology of musculoskeletal pain as well as the mechanisms and effectiveness of non-pharmacological pain treatments. She has published over 200 peer-reviewed manuscripts, numerous book chapters, and a textbook on Pain Mechanisms and Management for the Physical Therapist. She has received numerous awards including the Marian Williams Award for Research in Physical Therapy and Catherine Worthingham Fellowship from the American Physical Therapy Association and the Frederick W.L. Kerr Basic Science Research Award from the American Pain Society. She is actively involved in the International Association for the Study of Pain, the American Pain Society, and the American Physical Therapy Association serving on committees, task forces and society boards.

Abstract: Exercise is an effective treatment for chronic pain, yet an acute bout of exercise can increase pain and interfere with activity participation. Using animal models of pain and exercise I will show how exercise modulates central excitability and inhibition, and how exercise can modulate peripheral and central neuroimmune mechanisms to produce both pain and analgesia. Clinical implications and translation of this basic science data will be discussed.

**Greg Dussor**, PhD
Associate Professor, Behavioral and Brain Sciences
University of Texas at Dallas
*Female-specific Meningeal Nociception: Potential Mechanisms for Sex Differences in Migraine*

Greg Dussor is currently an Associate Professor in the School of Behavioral and Brain Sciences at UT Dallas and is also a Eugene McDermott Endowed Professor. He received his PhD in Pharmacology at The University of Texas Health Science Center in San Antonio in 2002 where he worked on peripheral acetylcholine receptors and their contribution to pain signaling. Following his PhD studies, he did postdoctoral training from 2004-2007 at the Vollum Institute on the campus of The Oregon Health & Science University in Portland. There, he worked on biophysical properties of acid-sensing ion channels (ASICs) and also characterized a population of pain-sensing neurons that innervate the outer epidermis. In 2007, he joined the Faculty in the Department of Pharmacology at the University of Arizona in Tucson. While in Arizona, he shifted the focus of his laboratory to understanding the pathophysiology contributing to chronic headache disorders such as migraine. He remained on faculty there until joining UT Dallas in 2014. Greg has published over 60 peer-reviewed research articles, he was a recipient of a Future Leaders in Pain Research Award from the American Pain Society, he is on the editorial board of Pain, Pain Reports, Molecular Pain, and Headache, and he is a member of the Medical Advisory Board of the Migraine Research Foundation. He is also co-founder of CerSci Therapeutics and Ted's Brain Science Products.

Abstract: Migraine is the second-most disabling disease worldwide and is the most common neurological disorder. It is also three times more common in women than in men; reasons for this sex difference are not known. The hormone prolactin (PRL) is associated with migraine as levels rise during attacks and PRL-lowering agents have been shown to treat specific types of migraine in humans. Thus, PRL may also contribute to the sex differences observed in the disorder.

Using a mouse line where tdTomato is driven in cells that express the PRL receptor (Prlr), we found extensive expression of Prlr in nerve fibers in female dura mater but little to no expression in males. Consistent with female-specific Prlr expression on meningeal sensory neurons, application of PRL to cultured dural afferents caused increased excitability only in females and PRL potentiated evoked CGRP release only from female dura. Using a preclinical behavioral model of migraine in both rats and mice, application of PRL to the dura mater caused headache responses in females but not males. These findings show that PRL can enhance meningeal nociceptive signaling selectively in females.

The role of PRL and Prlr signaling was also evaluated in an additional preclinical behavioral model of migraine where mice are primed using 3 consecutive days of restraint stress (2-hours per day). Following recovery from stress-induced cutaneous hypersensitivity, mice are primed to subthreshold doses of sodium nitroprusside (SNP, 0.1 mg/kg), a nitric oxide (NO) donor. Since NO donors reliably trigger attacks in human migraine patients but not in healthy controls, this preclinical model mimics features of the human disorder. Using mice where the Prlr was conditionally deleted from Nav1.8-expressing sensory neurons, stress was found to prime male mice to SNP identical to wild-type. However, female conditional knockouts had significantly less response to SNP in the absence of Prlr on peripheral sensory neurons. Further, female mice showed attenuated acute stress behaviors and an absence of priming when treated with bromocriptine for 5-days during stress exposure. Since bromocriptine suppresses PRL release from

the pituitary, these data suggest that circulating PRL also contributes to stress responses and stress-induced priming in females.

Together, these data show that PRL release and Prlr signaling contribute to migraine-related mechanisms in females, but do not play a similar role in males. These dimorphic effects of PRL may contribute to the sex differences observed in migraine in humans and may offer critical insight for future therapeutic development.

**Research support:** NIH NS104200 and NS072204; The University of Texas System

**Disclosures:** Greg Dussor receives grant support from Alder Biopharmaceuticals and Teva Pharmaceuticals and receives consulting fees from Novartis/Amgen.

**Stephanie Shiers**
Immunohistochemist, PhD student
University of Texas at Dallas
*Targeting of the MNK-eIF4E Signaling Axis Reverses Prefrontal Cortex Cognitive Dysfunction in Neuropathic Pain*

Stephanie Shiers is a fourth-year PhD student in the Cognition and Neuroscience program at the University of Texas at Dallas, working with Ted Price on the central and peripheral mechanisms of chronic pain. She previously obtained a dual BA and BS degree from the University of New England in Maine in 2011. As an undergraduate researcher, she worked in the laboratory of Dr. Michael Burman, who studies the neurobiology of learning and memory during development. Post-graduation, she joined the laboratory of Dr. Tamara King and Dr. Frank Porreca where she conducted conditioned place preference experiments on osteoarthritic mice and rats. She then gained employment at the UCDavis/NIH NeuroMab facility in California where she worked as an Immunohistochemist for three years screening and characterizing antibodies against novel targets. In 2015, she joined the PhD program at UTD and has been conducting experiments to elucidate the mechanisms underlying medial-prefrontal cortex cognitive dysfunction during neuropathic pain.

Abstract:
Background:
Chronic pain presents with a variety of comorbidities including deficits in higher executive functions which are not treated by current analgesics. Targeting of the mitogen activated protein kinase-interacting kinase (MNK1/2) and mRNA cap binding protein, eIF4E, attenuates nociceptive behaviors in pain by regulating the translation of a subset of mRNAs involved in neuronal plasticity. However, whether manipulation of the MNK-eIF4E system reverses cognitive deficits has not been addressed.
Hypothesis/Goals: We hypothesized that genetic and pharmacological disruption of the eIF4E translational control system would reverse cognitive deficits in neuropathic pain.
Methods:We used genetics and pharmacology to inhibit MNK-eIF4E activity in animals with spared nerve injury and assessed their performance in a medial prefrontal cortex (mPFC)-dependent attentional set-shifting task. We assessed evoked and spontaneous measures of pain using Von Frey testing and a conditioned place preference paradigm. Additionally, we investigated maladaptive mPFC structural plasticity in the length of axon initial segments which we have previously shown are decreased after neuropathic pain and are correlated to poor performance in the set-shifting task.
Results: WT neuropathic animals were significantly impaired in the task while genetic and pharmacological inhibition of the MNK-eIF4E signaling axis protected against or reversed the cognitive impairment. Additionally, disruption of MNK-eIF4E signaling reversed maladaptive shrinkage in the length of axon initial segments in the mPFC, and attenuated ongoing pain, but not mechanical hypersensitivity.
Conclusions: Our findings reveal that neuropathic pain-related cognitive deficits, spontaneous pain, and mPFC morphology can be treated by analgesics that target the MNK-eIF4E signaling axis.
Acknowledgements This work was supported by NIH grants R01NS065926 (TJP).

**Anibal Garza Carbajal**, PhD
Postdoctoral Research Fellow, Integrative Biology and Pharmacology
University of Texas Health Science Center
*Mechanisms Inducing Opioid Resistance After Spinal Cord Injury*

After graduating in Biomedicine at Puebla Autonomous University (BUAP, Mexico), he obtained his PhD in neuroimmunology from the University of Duisburg-Essen (Germany). During his first postdoctoral position, Anibal worked at the Max Planck institute of molecular Genetics (Berlin, Germany) and the University of Cologne (Cologne, Germany) studying neuronal and glial signaling using high content microscopy. Since April 2016 he joined Carmen Dessauer's group in the department of Integrative Biology and Pharmacology at UTHealth McGovern Medical School (Houston). His work is mainly focused on signaling pathways related to pain sensitization in primary sensory neurons and glia.

Abstract: Background: Chronic, untreatable pain is a common complication after a traumatic spinal cord injury (SCI). Our group has shown that many of the functional alterations leading to sustained pain perception after SCI originate at the level of the sensory neurons in the dorsal root ganglia (DRG). Among other alterations, DRG neurons from SCI animals show a marked increase in electrical excitability and a loss of sensitivity towards the inhibitory effects of Gαi on cAMP production, a key component mediating opioid analgesic effects. While the cause of these alterations is unknown, they have been suggested to contribute to the development and maintenance of different forms of chronic pain.
Hypothesis: The alterations in opioid sensitivity observed after SCI in DRG neurons reflect covalent modifications at the level of adenylyl cyclase, reducing its responsiveness to the inhibitory effects of Gαi. These modifications are likely induced by specific factors released in response to SCI, acting on a defined neuronal subpopulation within the DRG to reduce sensitivity to effects of opioids.
Methods: Using an in vitro model based on cultured DRG neurons from naïve, sham operated and SCI injured rats, we explored the cellular and molecular alterations that induce nociceptor insensitivity to opioids. Opioid inhibitory effects on cAMP production were evaluated by a combination of immunocytochemistry, high content microscopy and cluster analysis, using the phosphorylation of PKA-RII as a surrogate measurement of the cAMP production. Morphological and molecular markers were used to correlate these responses to specific neuronal subpopulations.
Results and Conclusions: Our results show that while neurons from SCI and naive or sham-operated rats do not differ in their subpopulation-specific responses to cAMP-inducing agents (forskolin and serotonin), inhibition of these responses by DAMGO (a mu opioid receptor agonist) is reduced in SCI neurons. This effect occurs specifically in neurons stained with the marker IB4, with a change in the IC50 for DAMGO inhibition of forskolin responses of 0.12 +/- 0.03 µM versus 0.66 +/- 0.2 µM in naïve/sham versus SCI animals, respectively ($p=0.006$). We have found that a similar state of opioid insensitivity can be induced in vitro on naïve neurons by exposure to specific factors previously identified to increase after SCI. In both cases, neuronal opioid sensitivity can be restored by inhibition of components of the translation machinery, indicating that opioid insensitivity is actively maintained by translation-regulated components. In conclusion, we identify a novel form of opioid-independent opioid resistance induced by factors released after SCI, suggesting potential targets to restore opioid analgesia in chronic pain patients.
Acknowledgements:
National Institute of Neurological Diseases and Stroke (grant NS091759)
Craig H. Neilsen Foundation (project 545880)

**Juan Cata**, MD
Assistant Professor, Anesthesiology and Perioperative Medicine
MD Anderson Cancer Center

*The Impact of Nutraceutical Prehabilitation and Postsurgical Treatment With Ecoisapentanoic Acid and Acetylsalicylic Acid on a Novel Animal Model of Recovery After Surgery*

The goal of this research is to investigate the role specialized pro-resolving molecules (SPMs) in relation to cancer-related fatigue in patients with ovarian cancer. SPMs are endogenous mediators of the resolution of inflammation and potent anti-cancer molecules. I have recently demonstrated that there is a deficiency in the levels of specialized proresolvin molecules (SPMs) in patients undergoing cancer surgery. This exciting preliminary data opens an opportunity to investigate in depth the effect of pharmaco-nutrition to treat symptoms related to inflammation such as fatigue. Based on my training in clinical (Residency in Anesthesiology at the Cleveland Clinic) and basic science (Postdoctoral Fellowship at MD Anderson Cancer Center), I am well-suited for my role as the Co-P.I. of this application. After finishing my clinical training, I decided to focus my research on the effect of interventions that can impact patients' outcomes including surgical recovery. Specifically, I have been interested in understanding the mechanisms that modulate the inflammatory response after surgery, particularly the role of SPMs in resolution of surgery-induced inflammation. I consider of upmost importance to investigate the interaction between SPMs and fatigue because this if our hypothesis that women with ovarian cancer have significantly low level of SPM, then a simple dietary intervention such as the administration of eicosapentaenoic acid or docosahexaenoic acid could be rapidly tested in a human clinical trial. Furthermore, the understanding of the role of SPMs during surgery could change the manner that we treat patients symptoms: From inhibiting inflammation to promoting resolution.
My training in basic science and clinical anesthesiology, and the strong collaboration that I have developed with Dr. Imad Shureiqi (mentor) who is an expert in SPMs biology will allow me to rigorously test the central hypothesis of proposed research in this application. In summary, I am highly motivated and very committed to work on this project and I bring research skills, experience and departmental support to greatly facilitate its ultimate success.

Abstract:
Introduction: Every year thousands of patients undergoing major abdominal surgery worldwide fail to recover adequately after surgery.1 While an exaggerated inflammatory has been indicated as a main driver of failed postoperative recovery, the mechanisms that govern resolution of inflammation after surgery remains poorly understood.2 Recent data from our group suggest that specialized proresolvin molecules of inflammation (SPMs) might play a significant role in postsurgical recovery.3 The eicosapentaenoic acid (EPA) is a natural donor of SPMs that when it is co-administered in presence in of cyclooxygenase 2 acetylator such as acetylsalicylic acid (ASA) enhance the formation of SPMs.4
The goal of our research was to develop surgical animal model to understand recovery after surgery to then, test the efficacy of preoperative (pre-habilitation) and postoperative oral treatment with EPA in combination with ASA on recovery.5 First, we hypothesized that the integration of clinically relevant domains of recovery after surgery could be used to understand it in an animal model. Second, we tested the hypothesis that pre-habilitation and postoperative oral feeding with EPA+ASA could accelerate the functional recovery of animals that had abdominal surgery.

Methods: After obtaining Institutional Animal Care and Use Committee approval, we conducted under inhalational anesthesia with isoflurane (1%-3%) a right sided laparotomy or sham surgery in Sprague-Dawley rats that were subjected to different behavioral tests and physiological measurements before surgery and on postoperative days 1, 2, 3 and 7. Briefly, we measured their nocturnal food intake (aversion to food), gastrointestinal transient time (ileus), sucrose consumption preference (anhedonia), mechanically evoked abdominal hyperalgesia (pain) around the surgical wound, social interaction with juvenile (depression-like behavior) and nocturnal locomotive activity (overall animal activity) (Figure 1). Each behavior or parameter was graded based to their own baseline (2: within 75%, 1: within 50%-74%, 0: within 25%-49% or -1: within <25%). The sum of each behavior and parameter tested on a single day was scored with a maximum of 12 or minimum of -6). During the duration of the study, all animals had access to food and water at libitum. A cohort of 6 animals per group was used to compare the quality of postsurgical recovery among 6 different treatments (controls, isoflurane (ISO) sham surgery, laparotomy animals, EPA+ASA sham animals, EPA+ASA laparotomy animals, ASA laparotomy). EPA and ASA (10 mg/kg) were daily administered seven days before and after surgery (Figure 3). Animals were randomly assigned to each treatment group. Data is shown in means ± standard error of the mean (SEM). Two-way ANOVA was used to analyze and compare the effect of surgery and each intervention on quality of recovery. Tukey test was used for correcting for multiple comparisons. To further distinguish how recovery occurred between each treatment group we estimated their area under curve (AUC) and compared them with one-way ANOVA. Dunnett's test was used for correcting for multiple comparisons. An adjusted $p < 0.05$ was considered statistically significant.

Results: A total of 36 rats were included in the study. Two animals died during the experiment and were replaced accordingly. As expected, laparotomized rats showed a decrease in food consumption and sucrose preference (anhedonia), prolonged gastrointestinal time (ileus), mechanical wound hyperalgesia (pain), poor social interaction (depression-like) and a diminish locomotor activity. Collectively, we observed in these rats had a significant lower mean (SEM) quality of postoperative recovery score than their baseline (12±0) on days 1 (5.5±0.84. $p<0.001$), 2 (7.33±0.49, $p<0.001$) and 3 (8±0.68, $p<0.001$) after surgery (Figure 2). Compared to sham animals, laparotomy rats also showed a statistically significant decrease in the recovery score on postoperative days 1 (sham:10±0.77 vs. laparotomy:5.5±0.84. $p<0.001$), 2 (sham:10.66±0.42 vs. laparotomy:7.33±0.49), 3 (sham:10.83±0.54 vs. 8±0.68, $p<0.001$, Figure 2). Except for mechanical hyperalgesia, we observed improvements in each behavioral tests and physiological parameter in operated animals treated with EPA+ASA. As shown in Figure 4, it translated in a significantly higher postoperative quality of recovery score of EPA+ASA laparotomized animals in comparison to sham animals on days 1 (EPA+ASA+laparotomy:8.16±0.7 vs. laparotomy:5.5±0.84. $p=0.004$), 2 (sham:10±0.42 vs. laparotomy:7.33±0.57), 3 (sham:10.33±0.66 vs. 8±0.68, $p<0.001$, Figure 2) after surgery. Laparotomized rats treated only with ASA did not perform better than those treated with regular food and placebo. Nor isoflurane (sham animals) nor EPA+ASA in sham animals had any effect on behaviors or physiological parameters.

The area under curve analysis (figure 5) indicated that surgery caused a significant reduction in recovery (total area ± standard error: 31.67±2.31) compared to controls (44.92±1.13, $p<0.001$), sham-surgery rats (42.92±1.93, $p<0.001$) and shams that received ASA +EPA (44.42±1.12, $p<0.001$). Contrarily, operated rats that were treated with ASA only had a slightly but not statistically significant higher total area (37.4±2.22) than laparatomized animals that received no perioperative treatment (31.67±2.31, $p=0.14$).

Conclusion: Here for the first time we present an animals model to study recovery after surgery in a comprehensive manner. The model was sensitive enough to test the effect of a pre-habilitation and postoperative intervention that would stimulate the formation of molecules that promote resolution of inflammation. The role of pre-habilitation and postoperative feeding with EPA+ASA needs further investigation.

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**Andrew Rogers**, Doctoral Student
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University of Houston

*The Unexpected Relationship between Pain, Cigarette Smoking, and Negative Affect*

Mr. Andrew Rogers is a second-year doctoral student in the clinical psychology program at the University of Houston. He completed his B.A. in psychology at Tufts University in 2014, and completed his M.A. at the University of Houston in 2018. His research interests generally focus physical and mental health, with an emphasis on transdiagnostic predictors. More specifically, he focuses on the intersection of chronic pain and opioid use, and identifying underlying psychological mechanisms (e.g. anxiety sensitivity, emotion regulation, pain-related anxiety) of these relationships. Mr. Rogers is interested in developing brief interventions targeting these mechanisms to reduce the disease burden of chronic pain and opioid use.

Abstract: Chronic pain is a significant public health problem associated with numerous negative outcomes, including increased medical costs, functional impairment, and substance-related problems. While pain is most commonly managed with prescription opioid medications, there is more recent emerging evidence that other substances, such as tobacco, may have analgesic properties. Previous research suggests that pain is a potent motivator of tobacco use. However, much like other substances used to manage pain, tobacco-related hyperalgesia increases pain over time, indicating that individuals may need additional strategies to manage their pain. Among these individuals as well, there may be some that are at highest risk for these pain-smoking relations, and examining underlying psychological vulnerability factors may help identify these individuals. Distress tolerance, or the ability to withstand psychological distress, has been differentially associated with pain and smoking behavior, whereby individuals with *lower* distress tolerance report the highest pain and worst smoking outcomes. However, distress tolerance has never been explored in the relationship of smoking and pain.

Therefore, the current study employed a longitudinal design to examine the interactive effect of cigarettes per day with distress tolerance on pain outcomes, among 44 daily adult smokers. Adult smokers were enrolled in a self-quit study and followed, using EMA methodology, for 14 days. Multi-level models were employed to examine how within person distress tolerance levels moderates the association between cigarettes per day and pain. Results from the current study indicate a significant interaction of cigarettes per day and distress tolerance predicting pain ($b$ = 0.001, $SE$ = 0.0004, $p$ = 0.002). These results suggest that levels of distress tolerance may confer heightened vulnerability for some individuals that may exacerbate the relationship between cigarettes per day and smoking behavior. Existing clinical interventions targeting distress tolerance may be particularly useful for some individuals with chronic pain who may attempt to quit smoking.

**Sukhbir Kaur Lulla**, Doctoral Student
Molecular Biology Program
Texas Woman's University
*Estrogen and Serotonin Interact to Modulate Rodent Trigeminal Sensory Neurons*

I am a PhD candidate and Graduate Research Assistant in Dr. Dayna Averitt's Pain Neuroscience lab at Texas Woman's University (TWU). My research focuses on understanding why craniofacial pain conditions are more prevalent in women. Since gonadal hormones are implicated in pain, I am looking at the interaction between estrogen and serotonin, a pro-nociceptive and proinflammatory mediator in peripheral nervous system on sensitization of TRPV1 ion channels. Before coming to the States, I earned my Bachelor's degree in Biotechnology from Pune University (2011) and Master's Degree in Medical Biotechnology (2013) from MS University, India. Currently, I am a fourth year doctoral student at TWU and expect to graduate in December 2020 with a PhD in Molecular Biology.

Abstract:
Background: Serotonin (5HT), a major pronociceptive and proinflammatory mediator in the peripheral nervous system, elicits pain via the excitatory 5HT receptors co-expressed with transient receptor potential vanilloid 1 ion channels (TRPV1) on trigeminal sensory neurons. TRPV1, activated by noxious stimuli like capsaicin, heat, and protons, can be sensitized resulting in increased calcium influx and post-synaptic release of proinflammatory calcitonin gene-related peptide (CGRP) leading to peripheral sensitization. 5HT can thus trigger pain directly via its excitatory receptors or via sensitization of TRPV1. These previous studies were conducted in male rats, whereas the mechanism in females is unclear.
Hypothesis: As females have *2-3X greater prevalence* of craniofacial pain disorders, it is likely that gonadal hormones modulate trigeminal pain however the role of estrogen on trigeminal pain is controversial. Recent studies in our lab have reported that hindpaw injection of 5HT evokes significantly higher thermal hyperalgesia and mechanical allodynia in female rats in proestrus and estrus. Here we hypothesized that *17β-estradiol (E2) increases 5HT release during inflammation and enhances capsaicin-evoked pain signaling in the presence of 5HT in trigeminal sensory neurons.*
Methods: Adult male, cycling females, and ovariectomized (OVX) female Sprague-Dawley rats received one vibrissal pad (VP) injection of 1.5 µg or 3 µg 5HT, each dose combined with 1 µg capsaicin, or vehicle (50µl). Number of VP forelimb swipes was counted as nocifensive behavior. A separate group of OVX rats were given an acute subcutaneous injection of 2 µg E2, 20 µg E2, or vehicle one hour prior to 5HT-evoked nocifensive behavior assessment. We also extracted the trigeminal ganglia from OVX rats and cultured the sensory neurons for 5 days. Cells where then incubated in buffered saline for baseline measures (15-min) and then treated with various E2 receptor agonists or vehicle (15-min) followed by 5HT and capsaicin stimulation. After each treatment, fractions were collected and CGRP release was quantitated by ELISA. A separate set of rats received left VP injection of complete Freund's adjuvant (CFA) and right VP injection of saline (50 µl). Interstitial fluid was collected 24 hours post-injection and assayed for 5HT content by a rat-specific 5HT ELISA. All data was analyzed by ordinary or repeated-measures 2-way ANOVA ($p<0.05$) and individual groups were compared by Tukey's posthoc analysis.
Results: We report that 3 µg 5HT evoked significant orofacial nocifensive behaviors in *estrus and proestrus females.* With capsaicin, 3µg 5HT evoked nocifensive behaviors in estrus females and males, whereas, 1.5 µg 5HT only evoked significant nocifensive behaviors in proestrus females. In OVX rats, 2 µg E2 exacerbated and 20 µg E2 slowed the onset of nocifensive behaviors. Additionally, *E2 significantly*

*enhanced 5HT-potentiated CGRP release* from cultured trigeminal sensory neurons and this effect does not appear to occur via a membrane-bound estrogen receptor. Serotonin content was significantly higher in the orofacial interstitial fluid in saline-treated vibrissal pad from cycling females.

Conclusions: Overall, our data indicates that estrogen modulates the pronociceptive effects of 5HT on trigeminal sensory neurons and that trigeminal nociception is dependent on estradiol timing and concentration. We are currently standardizing live cell calcium imaging to study this neuromodulation.

Acknowledgements: This research is supported by NIH DE025970, TWU Research Enhancement Program Grants, and a TWU Chancellor's Research Fellowship awarded to DLA and a TWU Quality Enhancement Program grant and TWU Center for Student Research Small Grant awarded to SK.

**Linda Watkins**, PhD
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*"Listening" and "Talking" to Neurons: Non-neuronal cells amplify pain and drug reward ~ Pathways from Basic Science to Human and Veterinary Clinical Trials ~*

Dr. Linda Watkins received her PhD in Physiology from the Medical College of Virginia. After postdoctoral training there and at the University of California-Davis, she retrained in neuroimmunology. She joined the faculty at the University of Colorado-Boulder in 1990 and is currently a University of Colorado Distinguished Professor in Psychology and Neuroscience. She is the recipient of numerous research awards. She has authored over 400 original research papers, scholarly reviews and book chapters. Her research interests include immune and glial regulation of pain and opioids actions.

Abstract:
Work over the past 25 years has challenged classical views of pain & opioid actions as being mediated solely by neurons. Recently, this challenge to classical views has extended to drug abuse. Glia (microglia & astrocytes) in the CNS are key players in chronic pain, compromising the efficacy of opioids for suppressing pain, contributing to opioid tolerance and dependence/withdrawal, and potently contributing to the rewarding effects of opioids, cocaine, methamphetamine, and alcohol. Further, glial reactivity can be "primed" so to create enduring, amplified neuroinflammation, thereby contributing to the transition of acute-to-chronic pain.

Intriguingly, the glial activation receptor that creates neuroinflammation under conditions of chronic pain is one and the same receptor that is activated by opioids and other abused drugs. Importantly, this glial activation receptor is not the neuronal opioid receptor that suppresses pain. Indeed, clinically-relevant therapeutics targeting this glial activation receptor in particular or glially-drive neuroinflammation more generally have shown remarkable efficacy as stand alone treatments for neuropathic pain, improving the clinical utility of opioids and suppressing drug abuse. One such compound is now in human clinical trials and showing efficacy in pet dogs with chronic pain as well.

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**Poster #1**

# Expression and Functional Profiles of Sodium Channel Subtypes $Na_v1.7$ and $Na_v1.8$ are maintained in Adult Dorsal Root Ganglion Neurons in Vitro

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## Background

Pain which is chronic or present in the absence of a stimuli is maladaptive. Nociceptors, located in the dorsal root ganglion (DRG) respond to noxious external stimuli generating all-or-nothing action potentials which are dependent on voltage-gated sodium ion channels. Sodium ion channel subtypes, $Na_v1.7$ and $Na_v1.8$ are expressed preferentially by nociceptors and their dysregulation in specific pain modalities have been linked to neuropathic and inflammatory pain. The present study explores the expression and functional profiles of $Na_v1.7$ and $Na_v1.8$ in adult murine-derived DRG neurons using multi-well microelectrode arrays which allow long term and non-invasive extracellular recordings from a large population of excitable cells.

## Hypothesis

The expression and functional profiles of $Na_v1.7$ and $Na_v1.8$ channels are conserved *in vitro* and contribute to the aberrant spontaneous firing of adult DRG neurons after exposure to pro-inflammatory mediators – interleukin-6 (IL6) and nerve growth factor (NGF).

## Methods

DRGs derived from 4-6 week old adult mice (ICR-CD1) were cultured at a density of 90,000 cells/$cm^2$ on either PEI/laminin pre-treated multi-well MEA plates (Axion Biosystems).

## Results

Exposure to IL-6 and NGF caused an increase in the number of spontaneously firing DRG neurons (Fig 1A) as early as 3 hr after treatment and persisted for at least 72 hr in culture. The increased firing could be readily attenuated by 30 nM Huwentoxin (IV), specific $Na_v1.7$ channel blocker, and 300 nM A-803467, a specific $Na_v1.8$ blocker. Treatment with an antagonist to CNS specific ion channels Nav1.1/1.3 exhibited no effect.

## Conclusion

Our findings suggest that $Na_v1.7$ and $Na_v1.8$ modulation contributes in part to sensitization by IL6 and NGF *in vitro* which can be blunted by specific blockers to these subtypes.

## Acknowledgements

This work was supported by the University of Texas at Dallas

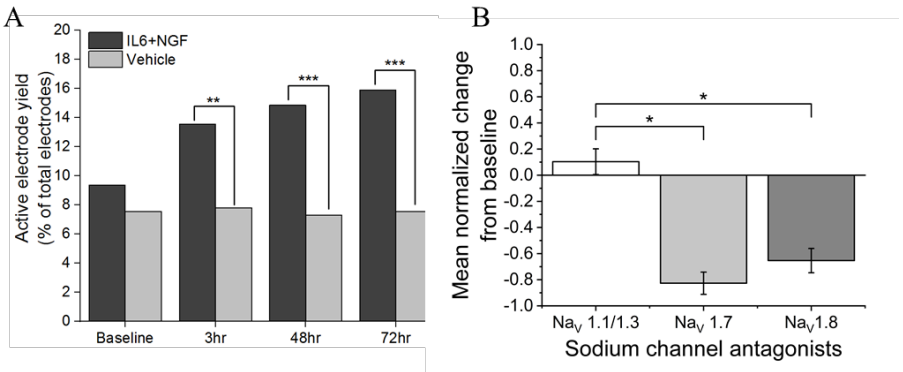


**Figure 1** (A) Active electrode yield post treatment with IL6+NGF or vehicle over 72 hr period. (B) Normalized change in firing rates after treatment with 100 nM ICA-121431 (Nav1.1/1.3 blocker), 30 nM Huwentoxin IV (Nav1.7 blocker), or 300 nM A-803467 (Nav1.8 blocker)

**Poster #2**

# *Euphorbia bicolor* Latex Extract Reduces Inflammatory Cytokines and Oxidative Stress in a Rat Model of Orofacial Pain

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**Background**: One target of pain management is the transient receptor potential V1 ion channel (TRPV1), a cation channel that is a pain generator located on nociceptors. Recent studies have reported that reactive oxygen species (ROS; oxidative stress) may cause pain through activation of TRPV1. ROS are key mediators in the development of peripheral and central sensitization in various pain etiologies, including neuropathic, inflammatory and opioid-induced pain. Furthermore, TRPV1 can be activated and potentiated by NADPH oxidase (NOX), which plays a key role in the production of ROS. ROS can activate TRPV1 by inducing its conformational change or by increasing the plasma membrane permeability to calcium ions. In support, inhibition of ROS is effective in reducing pain.

**Hypothesis/Goals**: Our previous studies reported that *Euphorbia bicolor* (*Euphorbiaceae*) latex extract evokes long-lasting peripheral analgesia, in part through TRPV1, in the inflamed hindpaw of male and female rats. The present study hypothesized that *E. bicolor* latex extract treament downregulates oxidative stress biomarkers and inflammatory cytokines in a rat model of orofacial inflammatory pain.

**Methods**: Orofacial pain behavior was tested using the von Frey method to assess mechanical allodynia in the rat vibrissal pad. Baseline sensitivity was recorded in male and female rats followed by injections of complete Freund's adjuvant (CFA) into the left vibrissal pad and mechanical allodynia was confirmed 24 hours later. Rats received one injection of either *E. bicolor* latex extract (300 μg/mg in 0.9% saline and <5% methanol) or vehicle (0.9% saline and <5% methanol) into the inflamed vibrissal pad and mechanical sensitivity was reassesed at 1, 6, 24, and/or 72 hours. All rats were then rapidly decapitated immediately following the last time point and trunk blood and trigeminal ganglia (TG) were collected. A separate group of rats received the same treatments and were used to collect blood and TGs at the 1- and 6-hour time points. ROS in the TGs were quantified by the fluorescent dye, 2',7'-dichlorodihydrofluorescein diacetate method. Oxidative stress markers were measured in the trunk blood by quantification of advanced oxidation protein product (AOPP) in plasma samples. ROS-induced NOX4 protein expression was quantified by western blot analysis. Furthermore, a Proteome Profiler Rat Cytokine Array Kit, Panel A (R&D Systems, Inc. Minneapolis, MN), coated with 29 cytokines were probed with the protein samples and the relative cytokine levels were compared.

**Results**: Here we report that *E. bicolor* latex extract significantly reduced orofacial mechanical sensitivity in both male and female rats at 24 hours and 72 hours, respectively and in concurrence with our previous reports in hindpaw-inflamed rats. Both ROS and NOX4 were significantly reduced in the trigeminal ganglia of extract-treated as compared to vehicle-treated rats at the same time points. In addition, plasma AOPP was also significantly reduced with the onset of analgesia. Overall, the latex extract reduced the oxidative stress biomarkers in both plasma and TGs of rats and downregulated NOX4 protein expression corresponding with the onset of analgesia. Also, extract treatment reduced proinflammatory cytokines in a sexually dimorphic manner in male and female rats.

**Conclusions**: These data indicate that the extract contains phytochemicals that may serve as novel therapeutics for treating pain with an oxidative stress component.

**Acknowledgements**: This research was supported by Texas Woman's University (TWU) Research Enhancement Grants awarded to CM and DA, TWU Quality Enhancement Program Learn by Doing and Center for Student Research Small Grants awarded to PB. The authors would also like to thank the technical assistance of Dr. Lionel Faure.

**Poster #3**

# EPAC1 and EPAC2 Promote SCI-Induced Nociceptor Hyperexcitability that Contributes to Chronic Pain

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**Background**: A majority of patients with spinal cord injury (SCI) suffer from chronic neuropathic pain. In a rat T10 contusion model, nociceptors at and below the injury level often exhibit chronic spontaneous activity (SA) generated in their somata in vivo and in vitro that persists for months after SCI (Bedi et al., J Neurosci 30:14870, 2010). The nociceptor hyperactivity is correlated with chronic pain-related behaviors and is necessary for the maintenance of spontaneous pain (Yang et al., J Neurosci 34:10765, 2014).
**Hypothesis/Goals:** Recently we found that SCI-induced SA in dissociated nociceptors requires ongoing cAMP signaling in a macromolecular complex composed of A-kinase anchoring protein (AKAP150), adenylyl cyclase (AC5/6) and PKA (Bavencoffe, Li et al., J Neurosci. 36:1660, 2016). The importance of cAMP signaling raises the possibility that multiple downstream cAMP effectors, such as EPAC1 and EPAC2, might contribute to SCI-induced SA in nociceptors.
**Methods:** To examine the roles of both EPAC1 and 2 in maintaining chronic pain we used electrophysiological and behavioral tests in both rat and mouse models of contusive SCI.
**Results:** Pharmacological inhibition of either EPAC1 or 2 in a rat SCI model was sufficient to reverse SCI-induced nociceptor hyperexcitability, suggesting that EPAC1 and 2 are complementary and are both required to maintain the hyperexcitable state in rat nociceptors after SCI. However, EPAC activation was not sufficient to induce a similar hyperexcitable state in naïve rats, and we observed no change in protein expression levels after SCI. In a mouse SCI model, inhibition of both EPAC isoforms through a combination of pharmacological inhibition and genetic deletion was required to reverse SCI-induced nociceptor hyperexcitability. This was consistent with our finding that EPAC2-/- mice were not protected against SCI-induced chronic pain as assessed with a novel operant mechanical conflict test. Thus, EPAC1 and 2 may play a redundant role in mouse nociceptors, although no corresponding change in EPAC protein expression levels was detected after SCI.
**Conclusion:** Despite some differences between these species, our data demonstrate a fundamental role for both EPAC1 and EPAC2 in mechanisms maintaining nociceptor hyperexcitability and chronic pain after SCI, encouraging further studies to consider the cAMP-EPAC signaling as a potential therapeutic target for chronic pain.
**Acknowledgements:** The authors thank Dr. Fang C. Mei for her expert technical assistance and for her management of the EPAC1 and 2 mouse colonies; she supplied all of the mice used in this project. We also thank Dr. Susan M. Carlton for the use of her custom MCS tunnel adaptor and Dr. Stephen Katzen for his assistance with animal surgeries.

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**Poster #2**

# Nasal Administration of Mesenchymal Stem Cells Reverses Chemotherapy-Induced Peripheral Neuropathy.

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most commonly reported adverse side effects of cancer treatment. CIPN affects 30 to 80% of patients, often persists after treatment completion and has detrimental effects on patient's quality of life. Currently, there are no effective FDA-approved drugs to prevent or reverse CIPN. The mechanisms associated with the pathogenesis of CIPN are not completely understood. However, growing evidence identifies mitochondrial dysfunction in the peripheral sensory system in the etiology of this condition. Mesenchymal stem cells (MSC) have been shown to stimulate tissue repair and ameliorate the outcome of various neurodegenerative diseases. We have shown previously that nasal administration of MSC resolves cisplatin-induced cognitive impairment.

**Hypothesis/Goals:** In this study we tested the hypothesis that nasal MSC treatment reverses peripheral neuropathy and restores mitochondrial bioenergetics in cisplatin-treated mice.

**Methods:** Male and female mice were treated with two cycles of cisplatin (2.3 mg/kg for 5 days), followed by nasal administration of MSC at 48 and 96 h after the last dose of cisplatin. Behavioral testing was performed prior to cisplatin and MSC treatment as well as 5-21 days after the last MSC dose. Mechanical allodynia was measured using von Frey hairs and spontaneous pain was tested using a conditioned place preference test. Mitochondrial function in the dorsal root ganglia (DRG) as well as peripheral nerve was determined by Seahorse Flux analysis.

**Results:** Nasal MSC administration resolved cisplatin-induced mechanical allodynia in both male and female mice, while mechanical allodynia persisted in mice treated with cisplatin only. MSC treatment also alleviated spontaneous pain. Furthermore, MSC administration normalized the cisplatin-induced decrease in mitochondrial dysfunction in DRG neurons as well as tibial nerve as compared to mice who received cisplatin alone.

**Conclusions:** Our results show that administration of two doses of nasal MSC is sufficient to reverse symptoms of cisplatin-induced peripheral neuropathy, including mechanical allodynia and spontaneous pain by restoring mitochondrial function in dorsal root ganglia and peripheral nerve.

**Poster #5**

## Effects Of Cluster Headache Medications On Circadian Reporter And Behavioral Rhythms

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**Background:** Cluster headache is an extremely painful headache disorder with a strong connection to circadian rhythms. This circadian link is supported by multiple lines of evidence: 1) most patients have headaches at precisely the same time each day, 2) radiographic changes are seen in the anterior hypothalamus, 3) alterations of CLOCK and NR1D1 have been reported in patients, and 4) effective treatments include dexamethasone, melatonin, and lithium which are known to modulate circadian rhythms. However, no systemic characterization of the effects of cluster headache medications on the circadian clock has been reported.
**Hypothesis/Goals:** We hypothesize that cluster headache medications modulate circadian rhythms.
**Methods:** We performed in vitro and in vivo studies. In cell studies, mouse embryonic *Period2::LucSV* fibroblasts were grown to confluency and treated with two cluster headache medications, verapamil (calcium channel blocker) and ergotamine (5HT 1D receptor activation as well as dopaminergic and alpha-adrenergic effects); these medications have primary mechanisms of action very different from each other and from dexamethasone, melatonin, and lithium. To examine in vivo efficacy, C57BL/6 mice were placed individually into cages with running wheels. Mice spent 1 week in normal light conditions (LD) and 1 week in 24 hour darkness (DD), followed by 2 weeks in DD with verapamil 1mg/mL added to the drinking water.
**Results:** Ergotamine showed a dose-dependent increase in *Period2::luciferase* expression, while verapamil showed some increased *Period2::luciferase* expression. Verapamil-treated mice displayed a distinct lengthening of the circadian period.
**Conclusions:** These data suggest that many cluster headache medications modulate core circadian gene expression and behavioral output, suggesting a fundamental circadian mechanism underlying a disorder with profound circadian features.
**Acknowledgments:** Grant support for this project includes funding from the American Headache Society and National Headache Foundation.

**Poster #30**

**Title.** Stabilizing dendrites protects against antiretroviral drug-induced neuropathy in a *Drosophila* Model

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## Abstract:

The success of anti-retroviral therapy (ART) has generally improved the survival of HIV-infected patients. However, patients on ART whose HIV disease is well controlled show peripheral sensory neuropathy (PSN), supporting the idea that the ART may cause PSN. Although the nucleoside reverse transcriptase inhibitors (NRTIs) used in the ART are thought to contribute to PSN, the mechanisms underlying the PSN induced by NRTIs are unclear. This is partly due to the lack of a good model for mechanistic investigations. In this study, we developed a *Drosophila* model of NRTI-induced PSN that recapitulates the salient features observed in patients undergoing ART: PSN and nociception. Furthermore, our data demonstrate that known pathways that have been shown to underlie both Wallerian degeneration and PSN induced by chemotherapy are not responsible for PSN in our model. Instead, compromised stability of peripheral neurons may underlie the nociception induced by NRTIs. Our model provides a robust platform to elucidate the pathogenic mechanisms of painful PSN induced by chronic exposure to NRTIs. This study included the equivalent numbers of both sexes.

**Funding:** Fellowship from the neuroscience and cell biology department of UTMB and Mitchell center for neurodegenerative diseases. We are also thankful for funding from the STARs award from the UT system and Bridging grant from the Mitchell Center for neurodegenerative disease. SJT was supported by NIH grants: R01NS095747, R01NS079166, R01DA036165.

**Poster #6**

# Sex Differences in the Amygdaloid Projections to the Periaqueductal Gray During Inflammatory Pain in the Rat

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**Background:** The central nervous system is involved in the modulation of nociceptive and antinociceptive activity and is sexually dimorphic in anatomical organization and functional activity. Further, sex hormones are implicated in pain modulation and studies suggest that estradiol increases pain and decreases opioid-based analgesia. Females typically require twice the amount of morphine to reach comparable levels of analgesia as males and previous studies have identified that sex differences in the PAG-RVM pathway contribute to sex differences in morphine analgesia. The midbrain periaqueductal gray (PAG) is a primary pain modulatory center for descending inhibition of pain. The PAG projects to the rostral ventromedial medulla (RVM), which in turn projects to the dorsal horn of the spinal cord to inhibit incoming pain signals from nociceptors and this system can be activated by endogenous (e.g. endorphins) during pain. Further, forebrain structures can modulate descending inhibition, however studies examining forebrain projections to the PAG have been conducted exclusively in males.

**Hypothesis/Goals:** The amygdala is one pain modulatory center that is known to send projections to the PAG in males. We hypothesized that the neural projections from the amygdala to the PAG are sexually dimorphic in anatomical organization and activation by inflammatory pain and are estradiol-sensitive.

**Methods:** The retrograde tracer fluorogold (FG) was injected into the ventrolateral PAG of adult male (n=4) and female (n=4) Sprague Dawley rats. After 10 days, complete Freund's adjuvant (CFA) was injected into the right hind paw to induce inflammatory pain. Twenty-four hours later, rats were perfusion fixed, brains were sectioned at 25 μm, then processed by immunohistochemistry. Fluorogold was visualized using diaminobenzidine (DAB) oxidation and Fos or estrogen receptor (ERα) was visualized using DAB oxidation in the presence of nickel.

**Results:** We found that projections were heavily observed from the central (CeA) and medial (MeA) nuclei of the amygdala to the ventrolateral PAG in females. CeA-PAG projections were comparable, however MeA-PAG projections were greater in females. Greater Fos expression was observed in both the CeA and MeA of males compared to females, however females had greater Fos expression in CeA-PAG projection neurons. On the other hand, MeA-PAG projection neurons in males had higher Fos expression. While more MeA neurons were ER-α positive in females, ER-α expression in the MeA-PAG projecting neurons was comparable. There were no sex differences in ER-α expression in the CeA or CeA-PAG projection neurons.

**Conclusions:** Together our data indicate that although the MeA-PAG projection is denser in females, inflammatory pain activates this pathway to a greater degree in males. This suggests that descending inhibition is more 'engaged' in males during inflammatory pain. On the other hand, inflammatory pain results in a greater activation of the CeA-PAG pathway in females, suggesting a pain faciliatory mechanism in females. We are currently using confocal microscopy to determine potential sex differences in the neurochemistry of this system that underlies dimorphic modulation of the PAG during pain.

**Acknowledgements:** This research was supported by NIH grants DA16272 and AR49555 to AZM and the STC Program of NSF IBN-9876754. This work was funded, in part, by a TWU Quality Enhancement Program grant and TWU Center for Student Research grant awarded to DJC.

**Poster #31**

# Peripheral Macrophage Recruitment to the Dorsal Root Ganglia Modulates Pain Development after Spinal Cord Injury

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**Background.** Spinal cord injury (SCI) damages sensory systems and causes chronic, intractable neuropathic pain, which is influenced by anatomical and functional plasticity of nociceptors in the dorsal root ganglia (DRG). Peripheral inflammation in the DRG in response to SCI may contribute to nociceptor dysfunction and the development of pain. Our published work has shown that increased macrophage presence in the DRG is associated with pain persistence at chronic time-points. There is a gap in our understanding of immune signaling events that transpire in the DRG after SCI that could induce pathological changes in the nociceptor and drive central, SCI-induced pain.

**Goals.** This study aimed to determine whether early macrophage recruitment to the DRG after SCI is essential to pain development and explore whether the phenotype and cytokine release profile of macrophages can dictate pain development.

**Methods.** Rats received a moderate, cervical (C5) contusive injury to the spinal cord causing neuropathic pain in 40% of the injured animals. We assessed the acute immune response to SCI in the C7-8 DRGs. At 12, 24, 48, 72- and 120-hours post-injury (hpi), macrophage chemoattractant CCL2 levels were assessed using ELISA and qPCR, along with expression of inflammatory cytokine and macrophage phenotype marker mRNA, and ED1+ labeling with immunohistochemistry. A cohort of rats was administered INCB3344, a CCL2 receptor antagonist intravenously acutely after SCI (0-72hpi) to prevent macrophage recruitment from the blood to the DRG. Rats were tested preoperatively, and 7,14,21 and 28 days post-injury (dpi) for allodynia in the forepaw (von Frey method) and cognitive perception of pain (mechanical conflict-avoidance paradigm).

**Results.** We observed a rapid, transient increase in CCL2 expression in the DRG at 12hpi, followed by recruitment of ED1+ cells to the DRG by 120hpi. mRNA analysis revealed that rats were distributed into two distinct clusters acutely after SCI based on mRNA expression- those with a large fold-change in pro- or anti-inflammatory markers in the DRG, or with near-normal expression. Rats that were administered a systemic CCR2 antagonist acutely after SCI had decreased number of macrophages in the DRG at 7 dpi compared to vehicle-treated SCI rats, however this was accompanied by transient pain that partially recovered by 28dpi.

**Conclusion.** Our data supports the hypothesis that there is a dichotomy in the peripheral immune response to SCI in the DRG in rats that may influence pain development. While macrophages at 28dpi are pro-inflammatory and associated with pain, early macrophage recruitment may be anti-inflammatory and necessary to prevent pain. Future studies will examine the influence of macrophages on nociceptor electrophysiological dysfunction as a mechanism of DRG neuroimmune interactions contributing to SCI-induced neuropathic pain.

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**Poster #7**

## Female Hormones Mediate Peripherally-Maintained Persistent Central Sensitization

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**Background**: Female patients are disproportionately represented in nociplastic pain conditions, in which pain may abnormally persist despite that the triggering injury has resolved. Central sensitization has been suggested as a critical driver of nociplastic pain, but the mechanisms how an acute injury-induced, normally resolving central sensitization becomes persistent to result in nociplastic pain are unclear. To address this, we are developing a novel animal model of nociplastic pain. Interestingly, we found that persistent central sensitization in females, but not in males, is dependent upon ongoing peripheral input of afferents innervating the previous injury site.

**Goals**: Our goals were to 1) identify the afferents at the injury site maintaining persistent central sensitization in females and 2) determine whether female sex hormones are critical for the development of peripherally-maintained persistent central sensitization.

**Methods**: Persistent central sensitization was induced by injecting capsaicin (as an acute experimental injury) intradermally into the hindpaw of female mice, then subsequently treating the injury site with innocuous warmth. For studies on sex hormones, females were bilaterally ovariectomized two weeks prior to capsaicin plus warmth treatments. Seven days after treatment with capsaicin and warmth, afferents at the previous injury site were silenced using bupivacaine or the membrane-impermeable lidocaine derivative QX-314. Central sensitization was assessed by measuring secondary mechanical hypersensitivity.

**Results**: We found that selective silencing of $TRPA1^+$ afferents, but not $TRPV1^+$ afferents or Aβ fibers, attenuated secondary mechanical hypersensitivity. Intradermal bupivacaine at the previous injury site failed to attenuate secondary mechanical hypersensitivity in ovariectomized females.

**Conclusions**: In females, ongoing activity of $TRPA1^+$ afferents innervating the previous injury site maintain persistent central sensitization. Additionally, female hormones are critical for the development of peripherally-maintained persistent central sensitization, as central sensitization in ovariectomized females was maintained independently of ongoing afferent activity at the injury site (i.e. like males).

**Acknowledgements**: NIH grant R01 NS031680 and JSMEF Data Acquisition Grant CON22735

**Poster #8**

# Allopregnanolone Attenuates Estrogen-Exacerbated Mechanical Allodynia in a Rat Model of Temporomandibular Joint Inflammation

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**Background:** It remains unclear why estrogen replacement therapy triggers reemergence of temporomandibular joint (TMJ) pain in post-menopausal women. Progesterone, however, is reported to have anti-inflammatory and antinociceptive properties that may be able to reduce pain. We recently reported ovariectomy (OVX) attenuates mechanical allodynia in a rat model of TMJ inflammation, which reemerges following estradiol benzoate (EB) treatment. Importantly, estrogen-exacerbated allodynia was attenuated one hour following progesterone treatment. Progesterone is an antagonist of the sigma-1 receptor, which is a chaperone protein and has nociceptive properties. It is probable that progesterone's attenuation of mechanical allodynia is due to its antagonism of the sigma-1 receptor. Alternatively, since the progesterone metabolite, allopregnanolone, can rapidly attenuate pain via activity at GABA, it is possible that progesterone's protective effects are due to allopregnanolone.

**Hypothesis:** We hypothesized that allopregnanolone would attenuate estrogen's nociceptive effects in a rat model of TMJ inflammation and that the sigma-1 receptor would be expressed in the trigeminal ganglia.

**Methods:** Adult, female Sprague Dawley rats (n=36) had vaginal lavages performed for 10 consecutive days to ensure normal estrous cyclicity. Baseline mechanical sensitivity was measured with von Frey filaments using the up-down method followed by a unilateral injection of complete Freund's adjuvant (30 µl; CFA) into the intra-articular area of the TMJ. Mechanical allodynia was confirmed 24 hours later, then rats were either OVX or received a sham surgery, and two weeks later mechanical allodynia was reassessed. Animals then received five days of the following injections: daily EB (50 µg/ml), daily allopregnanolone (0.16 mg/ml), daily EB and allopregnanolone, daily EB and allopregnanolone on days 1, 3, and 5, or daily vehicle (sesame oil). Allodynia was reassessed one hour after the last injection on days 1, 3, and 5. Data were analyzed by repeated measure two-way analysis of variance (ANOVA) and individual groups compared by Tukey's post hoc analysis.

A separate group of rats were rapidly decapitated, and TGs removed for immunohistochemistry (IHC) and western blot processing. For IHC, TG was incubated with mouse anti-AKR1C1/1C2 (AbCam; 3α-hydroxysteroid oxidoreductase; 3α-HSOR) primary antibody to determine progesterone metabolism and visualized by Confocal Microscopy. For western blots, TG tissue was processed with rabbit anti-Sigma-1 receptor primary antibody (Novus Biologicals) and imaged using a Licor Odyssey imaging system.

**Results:** We report that OVX reversed CFA-evoked allodynia at the TMJ and EB treatment significantly increased allodynia, confirming our previous reports. Administration of allopregnanolone (daily or every other day), attenuated estrogen-exacerbated allodynia within one hour of treatment on day 1 but not days 3 or 5. Our preliminary data indicate the expression of both sigma 1 receptor and 3α-HSOR in the TG.

**Conclusions:** Overall, our data show allopregnanolone attenuates orofacial mechanical allodynia in an acute manner, and the sigma-1 receptor and 3α-HSOR are present in the trigeminal ganglia making it a potential site for progesterone's protective effects. Allopregnanolone may provide short-term relief, whereas progesterone may provide continual relief, in the reemergence of TMD pain in post-menopausal women.

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**Poster #9**

**An individualized neuro-modulatory method targeted for patients with hypoglossal and glossopharyngeal injuries associated with neuropathic tongue and oral pain.**

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**Background:** The glossopharyngeal (CNIX) and hypoglossal (CNXII) cranial nerves control tongue movement along with pharyngeal and laryngeal function. Supranuclear or infranuclear injury to these nerves as a result of neurological insults, such as stroke, brain, or head and neck tumors, or following radio- and chemo-therapy is associated with neuropathic tongue and oral pain as well as partial paralysis of the tongue, swallowing, mastication, and speech articulation difficulties. The prevalence of cranial nerve neuropathy varies and can be as high as 48% following HNC radiotherapy treatment. In this study, we applied an innovative, and individualized approach with the goal to enhance the controlled, and voluntary movement of the tongue in healthy subjects. This approach is based on the induction of neuromodulation via individualized, real-time functional MRI neurofeedback (rt-fMRI nFb) training. The principle of this method as a treatment regimen is to bypass the lesioned pathway and capitalize on others that are intact and can become functionally associated to the lesioned one, as a function of neurofeedback training.

**Goal and Hypothesis:** Our study is first to develop, optimize, and apply individualized rt-fMRI nFb to neuro-rehabilitate lower cranial nerve injury. The immediate goal of our study is to enhance consistency of voluntary tongue movement in healthy subjects. The long-term goal is to apply this method as a therapeutic modality to patients following lower cranial nerve injury associated with oral neuropathic pain. Our hypothesis was that nFb in comparison to control-no nFb would increase: (i) the activity of spatial patterns that control voluntary tongue movement as evidenced by enhanced classification accuracies generated by machine learning approaches, and (ii) the magnitude of the blood-oxygen-level-dependent (BOLD) signal in somatosensory and somatomotor regions which control tongue movement.

**Methods:** Thirty healthy volunteers participated in a two-day study in the MRI environment. On study-day one, we decoded the cortical spatial patterns generated by voluntary tongue-movement activations in four directions (up; down; left; right), which were interleaved with periods of tongue-rest. The individualized networks associated with each participant's tongue movement were extracted and used for nFb delivery; i.e., on study-day two we delivered nFb to each subject's individualized network. We employed linear support vector machine (SVM) to classify brain patterns associated to each tongue movement generated during nFb and control scans.

**Results:** Neurofeedback-generated tongue movement is characterized by a somatosensory and somatomotor bilateral network, such as the thalamus, precentral gyrus, insula, as well as an attention and proprioceptive awareness network, such as the middle frontal, inferior (opercular) frontal and inferior parietal gyri. Our findings show that the SVM nFb-generated classification accuracy is higher than control-no nFb; i.e., when nFb is applied to both train-test machine learning permutations, it generates greater consistency of controlled tongue motor movement in healthy participants.

**Conclusions:** This study suggests that the purposeful induction of neuromodulation via individualized nFb can achieve enhanced control of voluntary tongue movement. This finding has significant implications as a neuro-rehabilitory method for lower cranial CNIX and CNXII injury associated with neuropathic pain.

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**Poster #10**

## ABSTRACT

**Title:**
**Autonomic Dysfunction Correlates With Chronic Neuropathic Pain Following Spinal Cord Injury**

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**Background:**
Spinal cord injury (SCI) persons with chronic neuropathic pain (NP) demonstrate maladaptive autonomic profiles compared to SCI counterparts without NP (SCI-NP) or able-bodied (AB) controls. These aberrations may be secondary to maladaptive neuroplasticity in the shared circuitry of the pain neuromatrix-central autonomic network interface (PNM-CAN).

**Hypothesis/Goals:**
In this study, we explored the proposed PNM-CAN mechanism in SCI+NP and AB cohorts following centrally-directed neuromodulation to assess if the PNM and CAN are capable of being differentially modulated.

**Methods:**
Central neuromodulation was administered via breathing-controlled electrical stimulation (BreEStim), previously evidenced to operate at the PNM. To quantify CAN activity, conventional heart rate variability (HRV) recordings were used to gather time and frequency domain parameters of autonomic modulation. SCI+NP (n=10) and AB (n=13) cohorts received null and active BreEStim randomly in crossover fashion. HRV data were gathered pre-test and 30 minutes post-test. Pain modulation was quantified at both time-points by visual analog scale (VAS) for SCI+NP persons and electrical detection and pain threshold levels (EDT, EPT) for AB persons.

**Results:**
Following active BreEStim only, SCI+NP persons demonstrated increased parasympathetic tone (increased NN50, $p=0.03$, and pNN50, $p=0.02$, HRV parameters). This parasympathetic restoration was associated with analgesia (VAS reduction, $p<0.01$). Similarly, AB persons demonstrated increased noxious tolerance (increased EPT, $p=0.03$, with preserved EDL, $p=0.78$) only following active BreEStim. However, this increased pain threshold wasn't associated with autonomic changes.

**Conclusions:**
Central modulation targeting the PNM produced autonomic changes in SCI+NP persons but not AB persons. These findings suggest that AB persons exhibit intact CAN mechanisms capable of compensating for PNM aberrations or simply that SCI+NP persons exhibit altered PNM-CAN machinery altogether. Our collective findings confirm the interconnectedness and maladaptive plasticity of PNM-CAN machinery in SCI+NP persons and suggest that the PNM and CAN circuitry can be differentially modulated.

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**Poster #11**

## The Cytokine IL-13 Plays a Key Role in Resolution of Chemotherapy-Induced Neuropathy

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**Background:** Chemotherapy has successfully improved the rate of cancer survival over the last decades. Unfortunately, chemotherapy also causes significant adverse effects affecting the health and wellbeing of cancer patients and survivors, leading to suffering and decreased quality of life. Chemotherapy-induced peripheral neuropathy (CIPN) is one of the main contributors to the suffering. Symptoms of CIPN include pain, numbness, and tingling, lasting for months or even years after completion of treatment. Previous reports indicate a critical role for inflammatory cascade activation, upregulation of pro-inflammatory cytokines and neuro-immune communication in initiation and progression of CIPN. In addition, we recently identified a key role of the immune system in the resolution of CIPN after completion of chemotherapy. Specifically, we showed that CD8 T cells and endogenous IL-10 signalling are required for resolution of CIPN.

**Goals**: We studied the contribution of interleukin 13 (IL-13) to resolution of CIPN. IL-13 is a cytokine that can be produced by CD8 T cells and is known to promote IL-10 production.

**Methods**: C57BL6 mice were treated with cisplatin (3 daily doses of 2 mg/kg) leading to mechanical allodynia. At 7 and 8 days after the first dose of cisplatin, anti-IL-13 antibody or control IgG was injected intrathecally (i.t.) to target spinal cord and dorsal root ganglia. Mechanical allodynia was measured over time using the von Frey test.

**Results**: Cisplatin treatment induced transient allodynia that started to recover 12 days after the first dose. Treatment with anti-IL-13 significantly prolonged cisplatin induced mechanical allodynia. Furthermore, FACS analysis revealed a reduction in anti-inflammatory M2 macrophage (CD206+ and CD200R+) polarization in response to anti-IL-13 administration.

**Conclusions**: Our data indicate a novel and thus far unappreciated role of endogenous IL-13 in resolution of CIPN that is likely mediated by promoting M2 macrophage polarization.

**Poster #12**

# A Preliminary Investigation of Itch Behaviors Using a PAR2 Agonist

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**Background**
Neurons in the dorsal root ganglion (DRG) that express Protease-activated receptor 2 (PAR2) also express the receptor for interleukin-31 (IL-31), a cytokine associated with inflammatory diseases like atopic dermatitis.
**Hypothesis/Goals**
We hypothesized that PAR2 neuronal activation by the PAR2 peptidomimetic agonist, 2at-LIGRL-NH2, would induce itching behavior similar to IL-31 administration, as previously reported.
**Methods**
We injected 30pmols and 100pmols of 2at-LIGRL-NH2 and 300ng of IL-31 intradermally into the cheek or paw of naïve male C57bl/6 mice. We video recorded for 30 minutes after drug administration and assessed wiping and itching behaviors. Additionally, we tested mechanical withdrawal threshold of the hind paw using Von Frey testing.
**Results**
IL-31 injected into the cheek induced robust itching behavior, while administration into the paw caused mechanical hypersensitivity. However, PAR2 activation through 2at-LIGRL-NH2 resulted in mechanical hypersensitivity of the paw, but no itching or wiping behaviors in the cheek.
**Conclusion**
Our preliminary data support that PAR2 activation via intradermal injection into the cheek does not cause itching nor nociceptive behaviors. Future experiments will address whether direct activation of PAR2 in DRG neurons will cause an itching phenotype. We will address this by using a Pirt-PAR2 knockout mouse in which PAR2 is not expressed in sensory neurons. These data will yield influential data for understanding the mechanism of PAR2 in the sensory system.
**Acknowledgements**
This work was supported by NIH grants R01NS065926 (TJP)

**Poster #13**

# Sham Surgeries for Central and Peripheral Injuries Enhance Pain-Avoidance Behavior as Revealed by an Operant Conflict Test

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**Background:** Pain experienced by laboratory animals is typically measured by withdrawal responses to noxious stimuli delivered by the experimenter. These reflex assays may not capture more complex emotional-affective dimensions of pain, and researchers have argued for the inclusion of non-reflex behavioral tasks. Operant assays of voluntary behavior that reveal negative motivational and cognitive aspects of pain may provide sensitive tools for defining pain-related alterations in neuropathic pain models and their surgical controls. In an operant mechanical conflict (MC) test, rodents may freely choose to escape from an aversive, brightly lit chamber by crossing over an array of sharp, noxious probes. However, most studies employing the MC test use lengthy familiarization and training procedures.

**Goals:** Here, we sought to develop a simple, rapid protocol for the operant MC test to measure pain-related behavior in rodents.

**Methods:** Sprague-Dawley male rats underwent differing models of neuropathic pain: thoracic spinal cord injury (SCI) or chronic constriction injury (CCI) of the sciatic nerve. Sham surgical treatment and naïve, uninjured rats were used as controls. Rats underwent a novel two-day MC training and testing procedure. The latency to escape the brightly lit chamber and cross over the noxious probes was recorded, as well as the total number of crosses between chambers.

**Results:** We found that both SCI and CCI injured rats exhibited heightened pain-like avoidance behavior compared to naïve, uninjured controls. Unexpectedly, we found that sham operated animals also demonstrated pain-like avoidance behavior that closely resembled their injured counterparts.

**Conclusions:** In central and peripheral neuropathic pain models, both injury and sham surgeries may enhance pain-avoidance behavior in an operant MC assay, which is not addressed by reflex testing. We speculate that the reduction in exploratory drive in the MC test is the result of tissue damage from the surgical intervention, resulting in postsurgical pain revealed by the MC assay. These findings have important implications for preclinical investigations into behavioral alterations and physiological mechanisms associated with postsurgical and neuropathic pain.

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**Poster #14**

# Cisplatin Educates CD8$^+$ T Cells to Prevent and Resolve Chemotherapy-Induced Peripheral Neuropathy in Mice

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**Background**. Many chemotherapeutic agents cause chemotherapy-induced peripheral neuropathy (CIPN). In 30% of cancer survivors CIPN persists long after treatment cessation and negatively affects their quality of life. The mechanisms responsible for the persistence of CIPN are still unknown. Our previous findings show that CD8+ T cells are necessary for the resolution of paclitaxel-induced mechanical allodynia in male mice (*Krukowski, et al., J Neurosci, 2016*).
**Hypothesis**. We first tested the hypothesis that CD81 T cells are essential for the resolution of all signs of CIPN in female and male mice treated with cisplatin. Second, we tested the hypothesis that CD81 T cells need to be educated by cisplatin to be capable of resolving CIPN.
**Methods**. CIPN was induced by cisplatin (3 daily injections of 2 mg/kg) in male and female wild-type (WT) and T cell-deficient (Rag2-/-) mice.
**Results**. We demonstrate that CD8+ T cells are not only essential for resolving cisplatin-induced mechanical allodynia, but also to normalize spontaneous pain, numbness, and the reduction in intra-epidermal nerve fiber density in male and female mice. Resolution of CIPN was not observed in Rag2-/- mice that lack T and B cells. Reconstitution of Rag2-/- mice with CD8+ T cells prior to cisplatin treatment normalized the resolution of CIPN. In vivo education of CD8+ T cells by cisplatin was necessary to induce resolution of CIPN in Rag2-/- mice because adoptive transfer of CD8+ T cells from naïve WT mice to Rag2-/- mice after completion of chemotherapy did not promote resolution of established CIPN. The CD8+ T cell-dependent resolution of CIPN does not require epitope recognition by the T cell receptor (TCR). Moreover, adoptive transfer of cisplatin-educated CD8+ T cells to Rag2-/- mice prevented CIPN development induced by either cisplatin or paclitaxel, indicating that the activity of the educated CD8+ T is not cisplatin-specific.
**Conclusion.** Resolution of CIPN requires in vivo education of CD8+ T cells by exposure to cisplatin. Future studies should examine whether ex vivo CD8+ T cell education could be applied as a therapeutic strategy for treating or preventing CIPN in patients.

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**Poster #15**

**Interleukin-10 signals to sensory neurons to resolve mechanical allodynia induced by cisplatin**

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**Background**. Many chemotherapeutic agents such as cisplatin may induce chemotherapy-induced peripheral neuropathy (CIPN). Symptoms of CIPN do not always resolve after treatment cessation and may persist for months. This suggest a dysregulation of the endogenous resolution pathways. Little is known about the resolution of CIPN. Whether resolution of CIPN is a spontaneous process or depends on engagement of endogenous resolution pathways remains elusive. Our previous work showed that resolution of paclitaxel-induced mechanical allodynia was impaired in Il10-/- mice (*Krukowski et al., J Neurosci, 2016*).

**Goal**. We investigated the contribution of endogenous IL-10 signaling to the resolution of neuropathic pain induced by the chemotherapeutic agent cisplatin in both sexes.
**Methods**. Mechanical allodynia was induced by injection of cisplatin 2 mg/kg daily for 3 consecutive days. We generated *avil*-cre:*Il10ra*$^{flox/flox}$ (*Il10*$^{DRG-KO}$) to study the contribution of IL-10R1 on sensory neurons. Neuronal activity was recorded from dissociated sensory neurons in whole-cell patch clamp experiments.
**Results**. Cisplatin induces mechanical allodynia that resolves in 15-21 days. Mechanical allodynia is markedly prolonged in mice intrathecally injected with neutralizing anti-IL-10 antibody and in Il10-/- mice compared to controls. IL-10R1 is expressed by DRG neurons in naive mice and its expression is upregulated during recovery from cisplatin-induced mechanical allodynia. The lack of IL-10R1 on the sensory neurons significantly delayed the resolution of mechanical allodynia following cisplatin treatment. The resolution of cisplatin-induced mechanical allodynia was similar in both sexes. Electrophysiologically, small to medium-sized sensory neurons isolated from cisplatin-treated mice exhibited depolarized resting membrane potential (RMP), reduced action potential threshold, and decreased rheobase associated with an increase in the incidence of spontaneous activity at RMP and ongoing activity during artificial depolarization. Bath application of recombinant IL-10 (1-10 ng/ml) reversed the elevated incidence of spontaneous activity in these neurons.

**Conclusions.** Our data show for the first time that the anti-inflammatory cytokine IL-10 signals directly to sensory neurons to induce resolution of mechanical allodynia and spontaneous neuronal activity. Taken together, our data indicate that deficiency in IL-10 signaling may impair the resolution of chemotherapy-induced neuropathic pain. Targeting endogenous resolution mechanisms such as IL-10 signaling, may offer a therapeutic opportunity to alleviate chemotherapy-induced mechanical allodynia.

**Poster #15**

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**Poster #16**

## Oral Treatment of Neuropathic Pain by Fumaric Acid Esters

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**Abstract**

**Background:** Currently available treatments for neuropathic pain have only have modest efficacy and have significant adverse effects, including abuse potential. As an alternative target for neuropathic pain, we focused on the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which is responsible for transcription of >200 antioxidant-related genes.

**Hypothesis:** We hypothesized that oral administration of pharmacological activation of Nrf2 with fumaric acid esters would alleviate established neuropathic pain.

**Methods:** Male Sprague Dawley rats and male and female Nrf2 knockout/wild type mice (Nfe2l2tm1Ywk/J, C57BL/6J) underwent spared nerve injury (SNI)/sham surgery. When neuropathic pain was established, rodents received daily oral administrations of dimethyl fumarate (100 and 300 mg/kg), diroximel fumarate (100 mg/kg), or tepilamide fumarate (100 mg/kg) for 5 days. Vehicle was used as a control. Mechanical allodynia, nociceptive hypersensitivity, and dynamic allodynia were measured in von Frey, operant conflict-avoidance, and paintbrush tests.

**Results:** Oral administration of fumaric acid esters progressively reversed allodynia and hypersensitivity established by SNI in rats and wild type mice. Meanwhile, fumaric acid esters treatment had no effect in Nrf2 knockout mice. Compared to vehicle group, oral treatment of dimethyl fumarate caused significant body weight loss in rats. Diroximel fumarate and tepilamide fumarate showed no influence on body weight of rats.

**Conclusions:** Our results highlight the potential for fumaric acid esters, non-addictive anti-nociceptive drugs, to be repurposed for disease-modifying treatment of neuropathic pain. Furthermore, second generation fumaric acid esters—diroximel fumarate and tepilamide fumarate—may have improved tolerability over dimethyl fumarate.

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**Poster #18**

# Mechanisms that Promote Ongoing Activity in Nociceptors During Exposure to Serotonin

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## Background
Hyperexcitability in nociceptors is an important driver of ongoing pain. Nociceptors in a hyperexcitable state often exhibit ongoing activity (OA), the continuing discharge of action potentials that may be driven by intrinsic or extrinsic sources of excitation. Two well-recognized electrophysiological alterations that promote OA are prolonged depolarization of resting membrane potential and reduction of action potential threshold. A recent study identified increased amplitude of depolarizing spontaneous fluctuations (DSFs) of membrane potential as a third important contributor to OA in nociceptors. OA can be induced acutely by the inflammatory mediator serotonin. Recent findings demonstrate serotonin increases nociceptor excitability by reducing action potential threshold and enhancing DSFs, leading to potentiation of OA under depolarized conditions. Thus, acute treatment with serotonin combined with artificial depolarization provides a model for detailed mechanistic studies of OA in DRG neurons from naïve rats.

## Hypothesis/Goals
The objective of this study is to identify the cell signaling mechanisms critical for enhancement of OA in the serotonin-induced model of nociceptor hyperexcitability. Based on previous studies and preliminary observations, I hypothesize that peripheral serotonin modulates specific conductances via cAMP signaling downstream of Gs-coupled receptor activation to generate large DSFs that promote OA in nociceptors.

## Methods
Neurons were dissociated from rat dorsal root ganglia (DRG, T10-L6) and cultured in serum-free medium. Incidence of OA was determined by whole-cell patch recording in current clamp at rest (spontaneous activity, SA) and during depolarization to ~-45 mV. Spontaneous fluctuations of membrane potential were quantified using novel automated algorithms.

## Results
Dose-response relationships indicate that relatively low concentrations of serotonin, 10-300 nM, are more effective at promoting nociceptor hyperexcitability than commonly used high concentrations (1-10μM). In whole-cell patch recordings, an inhibitor of protein kinase A (PKA) strongly reduced serotonin enhancement of DSFs and potentiation of OA. An inhibitor of exchange factor directly activated by cAMP1 (EPAC1) prevented serotonin reduction of action potential threshold but did not significantly attenuate serotonin-induced OA.

## Conclusions
These results suggest that ongoing activity potentiated acutely by relatively low concentrations of serotonin depends upon cooperative cAMP signaling through PKA and EPAC. Further investigation into the receptors, intracellular effectors, and ion channels that mediate ongoing activity in nociceptors may lead to novel therapeutic strategies for alleviating ongoing pain.

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**Poster #19**

## A Novel Chemical Nociception Assay in *Drosophila* Larvae

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**Background:** Noxious stimuli of different sensory modalities (thermal, mechanical, or chemical) provoke aversive pain behaviors in all animals. The molecular/genetic bases of thermal and mechanical nociception responses are beginning to be understood. However, chemically-provoked pain has not yet been studied intensively, in part due to a lack of simple genetically tractable models. **Hypothesis/Goals**: Our goal is to use *Drosophila* larvae to establish a genetically tractable system to study the cellular and molecular genetic bases of chemically-induced nociception. **Methods**: Our study employs a combination of behavioral assays, genetic analysis, and cell biological analysis in *Drosophila* larvae. **Results**: We exposed intact *Drosophila* larvae to increasing concentrations of hydrochloric acid (HCl). 0.5% HCl was subthreshold and provoked no response differing from water-exposed controls. Concentrations ranging from 1.0 % to 9.0 % produced an increasingly intense aversive rolling response, similar to what is seen with noxious heat and harsh touch. To determine which peripheral sensory neurons are required for chemical nociception we genetically silenced the four classes (I-IV) of multidendritic sensory neurons. Each class is required for the response to HCl, with class IV making the largest contribution. CaMPARI-based calcium imaging supported that class IV neurons are responsive to acid. We also examined the necessity of second-order interneurons, located in the ventral nerve cord, part of the larval central nervous system (CNS). We found that Basin-4 second-order neurons are the key regulators of chemically-induced nociception, with a slight contribution from other types. Moreover, subthreshold 0.5% HCl was used to study chemical allodynia. Physical puncture wounding provoked chemical allodynia in larvae 4 hours after tissue damage. Pinch wounding and UV irradiation, which do not compromise the outer cuticle layer, did not cause chemical allodynia. Finally, at the genetic level, loss of function mutants of tachykinin receptor and its ligand displayed reduced sensitivity to noxious chemical stimuli.

**Conclusions:** We developed a novel assay to study chemically-induced nociception and nociceptive sensitization in *Drosophila* larvae. Aversive behavioral responses to HCl are mediated by specific peripheral neurons (primarily class IV), and Basin-4 interneurons. A distributed response involving multiple peripheral nociceptors has not been observed with other sensory modalities (heat, cold, mechanical). Injury that breaks the cuticle barrier is capable of causing chemical allodynia. Genetically, Tachykinin and its receptor are required for chemical nociception. This new assay combined with the high genetic resolving power of *Drosophila* should improve our basic understanding of fundamental mechanisms of chemical nociception.

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**Poster #20**

# The Role of HDAC6 in Cisplatin-induced Mechanical Allodynia and Loss of Intra-Epidermal Nerve Fibers

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) is a serious healthcare issue that can impair cancer treatment and reduce quality of life for cancer survivors. Currently there is no FDA-approved medication to prevent or manage CIPN, emphasizing the urgent need to identify novel mechanism-based interventions. Histone deacetylase 6 (HDAC6) is an attractive target, given its pathogenic role in both cancer and neurodegenerative diseases.

**Hypothesis/Goals**: We hypothesize that HDAC6 is involved in the pathological development of CIPN, and that pharmacological inhibition of HDAC6 or genetic ablation of HDAC6 in sensory neurons will prevent the signs of CIPN, including mechanical allodynia and the loss of intra-epidermal nerve fibers.

**Methods:** CIPN was induced by 2-cycles of 5 daily intraperitoneal cisplatin injections. The HDAC6 inhibitor ACY-1215 was given via oral gavage 1-hour prior to each cisplatin dosing. Mechanical allodynia was measured using von Frey test in wild type (WT), global HDAC6 knockout (HDAC6 KO) mice, Advillin-HDAC6 KO mice or T cell-deficient Rag2 knockout (Rag2 KO) mice. Mitochondrial bioenergetics in DRG neuron and peripheral nerve were measured using the XF24 Flux Analyzer. For adoptive T cell transfer, T cells were isolated from spleens of WT or HDAC6$^{-/-}$ mice, and injected intravenously to Rag2$^{-/-}$ mice 1-week prior to cisplatin treatment.

**Results**: Cisplatin enhances HDAC6 expression and decreases acetylation of the HDAC6 substrate α-tubulin in the dorsal root ganglion (DRG). Pharmacological inhibition of HDAC6 with ACY-1215, an HDAC6 inhibitor that is currently in clinical trials as add-on to cancer therapy, prevented cisplatin-induced mechanical allodynia, loss of IENFs, and protected against mitochondrial dysfunction in the DRG neurons and peripheral nerves in male and female mice. Global genetic deletion of HDAC6 also protected against all CIPN signs. Interestingly, cell specific deletion of HDAC6 in Advillin-positive sensory neurons prevented loss of IENFs and mitochondrial deficits in the peripheral nerves, but did not protect against mechanical allodynia or DRG neuronal mitochondrial dysfunction. These findings indicate that inhibition of HDAC6 in other cells contributes to the protective effect of global genetic deletion of HDAC6. We previously showed that T cells are required for spontaneous resolution of mechanical allodynia in CIPN. Here we assessed the contribution of T cells to the beneficial effects of HDAC6 inhibition with ACY-1215. HDAC6 inhibition during chemotherapy in T cell deficient Rag2 KO mice prevented IENF loss and mitochondrial dysfunction in the peripheral nerves, but did not prevent mechanical allodynia or DRG mitochondrial deficits. Reconstitution with T cells normalized the protective effects of ACY-1215. Adoptive transfer of HDAC6 deficient T cells to Rag2 KO mice failed to protect against CIPN, indicating targeting HDAC6 in T cells alone was not sufficient.

**Conclusions:** Our findings identify cell specific mechanisms for the protective effects of HDAC6 inhibition on cisplatin-induced mechanical allodynia and IENF loss. We show that DRG mitochondrial function is essential for the prevention of allodynia, while mitochondrial function in the nerves is more closely associated with the IENFs. These outcomes also strengthen the rationale for using HDAC6 inhibitors like ACY-1215 for CIPN prevention.

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**Poster #21**

## Maintenance Mechanism of Nociplastic Pain in Males

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**Background:** Recently, the International Association for the Study of Pain defined a third form of pain: *nociplastic pain*. One important mechanism of nociplastic pain is central sensitization persistently maintained even in the absence of an underlying persistent injury. Using a novel mouse model of nociplastic pain, our lab has previously shown that the maintenance of central sensitization in this pain state depends on persistent, ongoing afferent activity at the previous injury site in female mice, but not in male mice, thus necessitating investigation of male-specific mechanisms maintaining the central sensitization underlying nociplastic pain.

**Hypothesis/Goals:** Based on the literature that spinal microglia and their inflammatory mediators play a key role in other models of chronic pain, specifically in males, we hypothesize that nociplastic pain in males is due to central sensitization maintained by activated microglia and subsequent release of inflammatory mediators such as prostaglandins generated through the cyclooxygenase (COX) pathway.

**Methods:** We utilized a male mouse model of nociplastic pain, which uses hindpaw capsaicin injection as a transient injury, followed two hours later by innocuous vibration stimulation. Our lab has found this to produce a robust phenotype of central sensitization, prolonging mechanical hypersensitivity in areas outside the injury site (i.e., secondary mechanical hypersensitivity). Spinal microglia and COX were inhibited by intrathecally injecting the microglia-targeting toxin Mac-1-saporin or the COX inhibitor indomethacin, respectively, following establishment of a nociplastic pain state (i.e., 7 days after capsaicin and vibration treatments). Behavioral data was collected using the von Frey behavioral assay and reported as percent paw withdrawal following ten trials.

**Results:** In the male nociplastic pain model, secondary mechanical hypersensitivity, a behavioral biomarker of central sensitization, is mitigated by intrathecal Mac-1-saporin. By contrast, intrathecal indomethacin had no effect on the secondary mechanical hypersensitivity.

**Conclusions:** Our results suggest that spinal microglia maintain central sensitization driving nociplastic pain in males. However, prostaglandins generated through the COX pathway do not seem to be the main inflammatory mediator involved in the maintenance of this central sensitization. It is possible that other inflammatory factors released from microglia act in concert to maintain this nociplastic pain-driving central sensitization in males.

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**Poster #22**

## Theta Burst Stimulation over Human Primary Somatosensory Cortex Elevates Electrical Pain Perception Threshold

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**Background:** Primary somatosensory cortex (S1) participates in the perception of pain and thus disruption of S1 using non-invasive neuromodulatory techniques has been suggested as a non-pharmacological treatment of neuropathic pain in clinical population. However, the effects of such an approach on pain perception in healthy adults have not been consistent across studies. **Hypothesis/Goals:** We investigated the time course of effect of MRI-guided continuous theta burst stimulation (cTBS) over S1 on electrical pain perception threshold in healthy young adults. We hypothesized that temporary disruption of S1 using cTBS would increase the pain and sensory perception thresholds in healthy individuals. **Methods:** In a sham-controlled, crossover design, we assessed pain and sensory perception threshold by delivering electrical pulse of 200 μs width over abductor pollicis brevis muscle. We also estimated tactile sensitivity using Semmes-Weinstein monofilaments over the thumb and index fingertips. The effects of cTBS over S1 on the sensory measures may result from the spread of current from S1 to M1 via reciprocal horizontal axonal connections. Therefore, we also investigated the effects of cTBS over S1 on corticospinal excitability (CSE) by delivering single pulse TMS over M1. Following initial sensory and CSE assessments, we delivered MRI-guided cTBS over S1. Subsequently, we performed sensory and CSE assessments immediately, 10 min, 20 min, 30 min, and 40 min post cTBS/sham. **Results:** In eight healthy young adults, we found that cTBS over S1 increased electrical pain threshold and reduced tactile sensitivity with effects lasting for ~30 min. Interestingly, cTBS over S1 showed no effect on electrical sensory threshold. We did not find any change in CSE following cTBS over S1. Sham stimulation did not change electrical sensory and pain thresholds, tactile sensitivity, or CSE. These findings demonstrate that a single application of cTBS over S1 can be used to alleviate pain for at least 30 min and these effects were not due to spread of stimulation current to M1. **Conclusions:** Neuromodulation of S1 using cTBS may prove to be an effective non-pharmacological intervention for alleviating pain in clinical populations. Future studies should investigate the effects of multiple sessions of cTBS over S1 on pain perception threshold.

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**Poster #23**

## New Mouse Model for Studying Comorbid Migraine and Temporomandibular Disorder

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**Background:** Previous epidemiological studies have suggested that migraine and temporomandibular disorder (TMD) are associated in the general population, and TMD is a risk factor for migraine attacks. However, the underlying mechanisms for the association remain elusive. Currently an animal model that mimics this comorbid condition is lacking.

**Goals:** In the present study, we developed a mouse model to study comorbid migraine-like pain and TMD as well as its sex difference. We also investigated whether the expression of calcitonin gene-related peptide (CGRP) in the spinal trigeminal nucleus caudalis (Sp5C) is involved in the comorbid migraine and TMD.

**Methods:** Male and female C57BL/6 mice were used in this study. Myogenic TMD was induced by unilateral of the masseter muscle tendon ligation (MMTL). Nitroglycerin (NTG) (1 and 10 mg/kg, i.p.) was injected to induce migraine-like pain. The von Frey test was used to measure head withdrawal threshold. Topiramate (30, 60 mg/kg, i.p.), an FDA-approved drug prescribed for migraine prophylaxis, was injected 30-min before NTG administration to verify migraine-like pain. The expression of CGRP in the Sp5C was examined using immunofluorescence staining.

**Results:** 1) MMTL significantly reduced mechanical threshold in the mandibular nerve (V3)-innervated area for up to 10 days, without affecting the ophthalmic nerve (V1)-innervated area. 2) NTG alone at a high dose (10 mg/kg), but not a low dose (1 mg/kg), induced acute migraine-like pain for 2 hours. 3) MMTL not only strongly prolonged the high-dose of NTG-induced migraine-like pain, but also enabled the low-dose of NTG to produce such pain for at least 5 days in male mice and 7 days in female mice. 4) Topiramate dramatically suppressed the MMTL-enhanced NTG-induced migraine-like pain in a dose-dependent manner. 5) CGRP was expressed in the superficial laminae of the Sp5C, and MMTL pretreatment plus NTG upregulated the expression of CGRP in V1-projected Sp5C area, compared with MMTL or NTG alone.

**Conclusions:** Our results indicate that myogenic TMD can promote the chronicity of migraine-like pain, which is showing different time courses in male and female mice and that trigeminal CGRP may contribute to the pathogenesis of the comorbid migraine and TMD.

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**Poster #24**

## Testing The Roles of Smoothened and Insulin Receptor Signaling in Mouse Pain Biology

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**Background:** Pain hypersensitivity is a protective response that accompanies tissue damage during repair. Sensitization is normally acute and resolves as tissue repair progresses. Under a variety of situations, however, chronic pain develops when injury-induced sensitization does not resolve normally. Despite decades of study, the molecular/genetic mechanisms underlying acute pain sensitization and the transition to chronic pain remain poorly understood. Prior studies using a *Drosophila* model of pain sensitization in our lab have identified multiple molecular signaling pathways that regulate injury-induced hypersensitivity. Hedgehog/Smoothened (Smo) signaling is required for acute pain sensitization. By contrast, fly larvae lacking the insulin receptor in pain-sensing sensory neurons have normal baseline pain responses (absence of injury) and normal acute sensitization, but they fail to shut off the acute sensitization response leading to a persistently hypersensitive state.

**Hypothesis/Goals:** We here extend our genetic analysis of Smo and Insulin receptor from fruit flies to mice to test the role(s) of these pain signaling pathways in a vertebrate model more similar to humans.

**Methods:** Nociceptor-specific Cre (NaV1.8- Cre) mice were bred to either Smo-flox/flox or InsR-flox/flox mice to produce mice with a nociceptor-specific deletion of either Smo or InsR. These mice, together with sibling controls, were injected intraplantarly with Complete Freund's Adjuvant (CFA) into their hind paws to induce inflammatory pain. Nociceptive responses were measured before CFA injection (baseline) and at different times after CFA injection until control mice nociceptive responses returned to baseline. Mechanical allodynia was tested using the von Frey test and thermal hyperalgesia using the Hargreaves test.

**Results:** Mice with *Smo* deleted in sensory neurons showed a normal mechanical nociceptive baseline and inflammation-induced mechanical allodynia. They also exhibited normal thermal nociception and inflammation-induced thermal hyperalgesia, however, female mice did not go back to normal baseline as same rate as their controls. Mice with *Insulin receptor* deleted in sensory neurons showed a normal mechanical nociceptive baseline. These mice overall showed normal inflammation-induced mechanical allodynia, however, inflammation-induced mechanical allodynia persisted in female mice.

**Conclusions:** Based on these preliminary data, Smo may not function or may function differently in sensory neurons than it does in fruit flies. Pharmacological experiments in rats suggest crosstalk between Smo and opiate signaling and this will be examined experimentally. The mouse insulin receptor seems to function similarly to what we found in fruit flies but only in female mice.

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**Poster #26**

# KCNQ channels contribute to prevent paclitaxel-induced mitochondrial dysfunction

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Abstract:

Paclitaxel-induced peripheral neuropathy (PIPN) and associated pain are the most common and serious adverse effects experienced by cancer patients receiving paclitaxel. It has been reported that paclitaxel-induced painful neuropathy is associated with mitochondria dysfunction in dorsal root ganglion neurons (DRG). We recently found activation of KCNQ channels prevents paclitaxel-induced painful neuropathy including the abnormal mitochondria. In this study, we will test if KCNQ channels contribute to prevent paclitaxel-induced mitochondrial dysfunction. Utilizing Seahorse XF Extracellular Flux Analyzer, we measured oxygen consumption rate (OCR) of isolated DRG neurons from vehicle/vehicle, paclitaxel/vehicle, and paclitaxel/retigabine-treated rats. In order to test if KCNQ channels contribute to prevent mitochondria dysfunction induced by paclitaxel, OCR was measured in CHO cell with and without cDNA of human KCNQ channel. We found that 1). Retigabine significantly preserve maximum respiration and spare reserve capacity of DRG neurons that induced by paclitaxel, 2). In KCNQ2&3 overexpressed CHO, ATP production, maximum respiration, and spare respiratory capacity are significant higher than control CHO; 3). Low concentration paclitaxel did not change mitochondria function of CHO cells with and without KCNQ2&3 channels. However, high concentration paclitaxel reduce mitochondria function in both groups; 4). Compared to control CHO cells, there are significantly high ATP production, maximal respiration and spare respiratory capacity in CHO cells with KCNQ2&3 channels in the presence of low concentration paclitaxel; 5). Maximal respiration of CHO cells with KCNQ2&3 channels is significantly higher than control CHO cells in the presence of 10 μM paclitaxel. Our result indicated that KCNQ2&3 channel may be potential target to prevent mitochondria function in the presence of paclitaxel.

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**Poster #25**

# Activation of KCNQ Channels Prevents Paclitaxel-induced Peripheral Neuropathy and Associated Neuropathic Pain

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**Abstract:**

Paclitaxel-induced peripheral neuropathy (PIPN) and associated neuropathic pain are the most common and serious adverse effects experienced by cancer patients receiving paclitaxel treatment. These effects adversely impact daily activities and consequently the quality of life, sometimes forcing the suspension of treatment and negatively influencing survival. Patients are usually at high risk of developing PIPN if paclitaxel induces acute pain, which strongly suggests that an acute increase in the excitability of nociceptors underlies the chronic alterations of PIPN. KCNQ/Kv7 channels are widely expressed in the primary sensory neurons to modulate their excitability. In the present study, we show that targeting KCNQ/Kv7 channels at an early stage is an effective strategy to attenuate the development of PIPN. We found that paclitaxel did not decrease the expression level of KCNQ/Kv7 channels in the primary sensory neurons as detected by qRT-PCR and Western blotting. However, retigabine, which is a specific KCNQ/Kv7 channel opener, significantly attenuated the development of PIPN, as shown by both morphologic and behavioral evidence. We also observed that retigabine had no obvious effect on the chemosensitivity of breast cancer cells to paclitaxel. While retigabine has been approved by the FDA as an anticonvulsant, our study suggests that this drug can be repurposed to attenuate the development of PIPN.

**Support:**

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**Poster #27**

# RE1-Silencing Transcription Factor Controls the Acute-to-Chronic Neuropathic Pain Transition and Chrm2 Receptor Gene Expression in Primary Sensory Neurons

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**Background:** neuropathic pain is associated with persistent changes in gene expression in primary sensory neurons, but the underlying epigenetic mechanisms that cause these changes remain unclear. The cholinergic receptor muscarinic 2 (Chrm2) is abundantly expressed at primary afferent terminals and critically involved in the regulation of spinal nociceptive transmission. However, little is known about how Chrm2 expression is transcriptionally regulated.

**Hypothesis/Goals:** to check the gene expression change of Chrm2 after nerve injury and investigate the underlying mechanism of how Chrm2 is controlled. Also, we determined the role of RE1-silencing transcription factor (REST, also known as neuron-restrictive silencing factor [NRSF]) in neuropathic pain development after nerve injury and Chrm2 expression in the injury DRG.

**Methods:** Spinal nerve ligation (SNL), spared nerve injury (SNI), RT-PCR, Western blotting, ChIP-PCR, promoter luciferase activity assay, whole-cell recording in spinal cord slices

**Results:** nerve injury persistently increased the expression of REST and decreased Chrm2 in DRG, respectively. The RE1 binding site on the Chrm2 promoter is required for REST-mediated Chrm2 repression, and nerve injury increased the enrichment of REST in the Chrm2 promoter in the DRG. Furthermore, Rest knockdown or genetic ablation in DRG neurons normalized Chrm2 expression and augmented muscarine's analgesic effect on neuropathic pain and fully reversed the nerve injury-induced reduction in the inhibitory effect of muscarine on glutamatergic input to spinal dorsal horn neurons. Remarkably, nerve injury-induced chronic, but not acute, pain hypersensitivity was attenuated in mice with Rest knockout in DRG neurons and rats with siRNA-mediated Rest knockdown.

**Conclusions:** nerve injury induces REST, which contributes to neuropathic pain development and the transcriptional repression of Chrm2. Rest-knockdown or knockout enhanced the analgesic effect of muscarine after nerve injury.

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**Poster #28**

# Microglia-like Cells Derived from Hematopoietic Stem and Progenitor Cells Are a Model System to Investigate Chronic Pain in Sickle Cell Disease

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**Background:**
Patients with sickle cell disease (SCD) often experience severe chronic pain (CP). In chronic pain, microglia are readily activated, stimulating neurons to send a pain signal. Human microglia are difficult to obtain. To study the microglial role in CP in SCD, a translational platform based on human microglia culture is needed.

**Hypothesis/Goals**
We proposed to culture induced microglia-like cells (MLC) from human peripheral blood (PB) to develop a model system to investigate chronic pain in sickle cell disease.

**Methods:** Peripheral blood mononuclear cells (PBMCs) were obtained from each of the following patient groups: SCD and chronic pain (SCD CP+, defined as pain most days for 3 months), SCD without chronic pain (SCD CP-), and normal donors (WT). PBMCs were cultured with human GM-CSF and IL-34 to induce peripheral blood derived microglia (PB-MLC). On day 7 of culture, cells were collected and morphology analyzed by microscopy, phenotyped by flow cytometry, and immunofluorescence with anti-CX3CR1, TMEM119, CD68, Iba1 antibodies. TNFα, IL-1β, IL-10 secreted by PB-MLCs were measured with ELISA. Microglia morphology was evaluated by quantitative analysis of cell body roundness and branch length.

**Results**: When cultured with GM-CSF and IL-34, PBMCs developed microglial morphology, were $CD11b^{high}$ and $CD45^{low}$ by flow cytometry, $CX3CR1^{+}$ and $TMEM119^{+}$ by fluorescence microscopy, consistent with microglia. LPS-treated PB-MLC cells had significantly higher CD68 and Iba1 positivity compared to resting microglia cells, indicating activation. PB-MLC differed significantly depending on donor group. SCD CP+ had shorter and fewer branches than WT; branching from SCD CP- were intermediate in number and length. Activated PB-MLC derived from patients with SCD secreted more inflammatory cytokines than PB-MLC derived from normal donors, suggesting that donor characteristics are retained by the PB-MLC. To evaluate the possibility of using this model system to screen compounds, we tested MLC cells with the following drugs: gabapentin, metformin, piceatannol, and resveratrol. All drugs suppressed the release of proinflammatory cytokine from LPS-induced PB-MLC in a dose-dependent manner and also reversed the deramification of activated PB-MLC upon LPS stimulation by quantitative analysis of cell body roundness and branch length, though gabapentin showed weaker effects than others.

**Conclusions:** We have established the microglia-like nature of the cultured peripheral blood cells derived from patients with SCD and normal blood donors. We propose to use this model system to derive mechanistic insights into the development of chronic pain in SCD, and to screen pharmacologic agents to treat chronic pain.

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**Poster #29**

# Single Cell RNA-seq Analysis Reveals Regional-Specific Heterogeneity and Plasticity of Microglia during Aging and Pathogenic Challenging

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## Abstract

**Background** Microglia are heterogeneous CNS resident immune cells. Emerging evidence indicates that microglia in different regions differentiate in their phenotypes. However, the molecular basis underlying the regional-specific microglial heterogeneity and functional plasticity is unclear.

**Goals** The purpose of this study is to determine the temporal and regional differentiation of microglia in response to aging and HIV-1 toxins.

**Methods** Single-cell RNA-seq was applied on total 120,000 cells dissociated from brain cortices and spinal cords from the 2-, 4- and 8-month wild type and an HIV-1 gp120 transgenic mice, followed by a comparative transcriptomic analysis.

**Results** We revealed 3 and 2 of resting microglial subtypes in the cortex and spinal cord from the 2-month mice respectively. The cortex was detected with the latent, homeostatic and proinflammatory subtypes. Spinal cord lacked the latent microglia but have a much larger population of proinflammatory microglia. Spinal microglia polarized toward a proinflammation subtype during aging, while cortical microglia showed non-inflammatory activation with the emerging of a subtype with an interferon I signature. In gp120 transgenic mice, functions of resting microglia were severely impaired in the cortex but not the spinal cord. We identified gp120-induced temporal and regional new microglia subtypes associated with interferon response, cell proliferation and myelination/demyelination in CNS.

**Conclusions** These results reveal differential microglial subtypes in the cortex and the spinal cord and their different plasticity in response to aging and HIV-1 gp120, and suggest functional differentiation of microglia in different CNS regions.