

Texas Pain Research Highlights 2021

April 7-8, 2021

Hosted by

Translational Pain Research Consortium of the Gulf Coast Consortia
Annemieke Kavelaars, MD Anderson Cancer Center
Chair

Texas Pain Research Consortium
Ted Price, University of Texas Dallas
Chair

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, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include AI in Healthcare, Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. 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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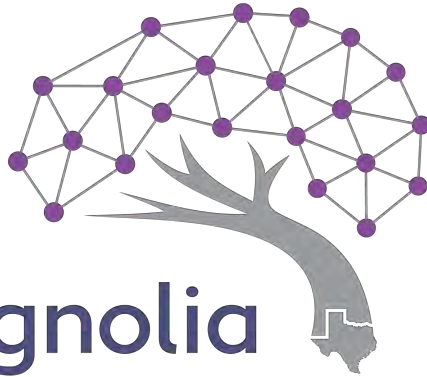
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April 7, Day 1

9:55-10:00 am Opening and Welcome

Annemieke Kavelaars, MD Anderson Cancer Center

Ted Price, University of Texas, Dallas

Session 1

Moderator: **Ted Price**, University of Texas, Dallas

10:00-10:15 *The Role of Microbiome in Pain Modulation*

Tor Savidge, Baylor College of Medicine

10:15-10:30 *The Impact of Inflammatory Mediators on the Landscape of Nascent Translation in the Dorsal Root Ganglion*

Zach Campbell, University of Texas, Dallas

June Bryan de la Pena, University of Texas, Dallas

10:30-10:45 *Targeting Neuroimmune Interactions for Relief of Neuropathic Pain*

Andrew Shepherd, MD Anderson Cancer Center

10:45-11:00 *The Scope of Translational and Stem Cell Biology in Studying Peripheral Pain Mechanisms*

Nikita Ruparel, University of Texas, San Antonio

11:00-11:30 Break Out Rooms:

- Peripheral and Spinal Mechanisms Driving Pain
- Brain Mechanisms of Pain

11:30-11:50 Poster Data Blitz Presentations

Parabrachial CGRP Signaling Contributes to Amygdala Lateralization in the Context of Bladder Pain

Poster 42

Heather Allen, Duquesne University

The Effects of Nitric Oxide on Migraine Headache are Mediated by Peroxynitrite Formation Poster 38

Jacob Lackovic, University of Texas, Dallas

Clinically Relevant Pain Assays for Fibromyalgia Applied to a Preclinical Model of Musculoskeletal Pain

Poster 36

Melissa Lenert, University of Texas, Dallas

The Role of Astrocyte Elevated Gene-1 (AEG-1), A Novel Multifunctional Protein, In Chronic Inflammatory and Neuropathic Pain

Poster 26

Bryan Mckiver, Virginia Commonwealth University School of Medicine

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Chronic THC Vapor Attenuates Hyperalgesia and Alters Neuronal Function in the Ventrolateral Periaqueductal Gray (vlPAG) Male Rats with Chronic Inflammatory Pain

Poster 24

Jason Middleton, Southeast Louisiana Veterans Health Care System

What can RNA-sequencing of human DRGs from pain patients teach us?

Poster 28

Pradipta Ray, University of Texas, Dallas

CCL2-Induced Macrophage Accumulation in the Dorsal Root Ganglia Correlates with Persistent Paw Hypersensitivity

Poster 40

Jonathan Richards, Drexel University

Single Cell Transcriptome of the Human DRG

Poster 44

Diana Tavares-Ferreira, University of Texas, Dallas

Effects of a High Omega-6 Diet on Orofacial Allodynia and Gene Expression Patterns in the Trigeminal Ganglia

Poster 32

Meilinn Tramm, University of Texas Health Science Center San Antonio

HEAL Initiative Preclinical Screening Platform for Pain: Evaluation of Novel Non-Opioid, Non-addictive Therapeutics

Poster 34

Sarah Woller, NIIH/NINDS

11:50-12:30 Poster session 1: Even number posters

12:30-1:00 Vendor Session and Lunch

Session 2

Moderator: **Ken Hargreaves**, University of Texas, San Antonio

1:00-1:15 *Underlying Mechanisms of Comorbid Depressive Symptoms in Chronic Pain*

Lingyong Li, Baylor College of Medicine

1:15-1:30 *Novel Selective Kappa-opioid Ligands as Modulators of Pain Responses*

Nadia German, Texas Tech University Health Sciences Center

Siavash Shahbazi Nia, Texas Tech University Health Sciences Center

1:30-1:45 *Unexpected Contributions of the Cytokine MIF to Persistent Nociceptor Hyperactivity and Chronic Pain*

Alexis Bavencoffe, University of Texas Health Science Center, Houston

1:45-2:00 *Neuromodulation of Somatomotor, Somatosensory and Pain Networks Using an MRI-compatible Brain Computer Interface*

Dorina Papageorgiou, Baylor College of Medicine

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April 8, Day 2

Moderator: **Annemieke Kavelaars**, MD Anderson Cancer Center
10:00-10:45 *NIH's HEAL Initiative in Pain Science*
Walter Koroshetz, NINDS

Session 3

Moderator: **Carmen Dessauer**, University of Texas Health Science Center, Houston

10:45-11:00 *Alterations in Histone Acetylation and Neuroinflammation in Diabetic Painful Neuropathy*
Munmun Chattopadhyay, Texas Tech University Health Sciences Center

11:00-11:15 *Pipeline for Evaluating Efficacy of Novel Non-opioid Compounds for Battlefield Injury-induced Pain*
John Clifford, United States Army Institute of Surgical Research
Natasha Sosanya, United States Army Institute of Surgical Research

11:15-11:30 *Understanding the Developmental and Molecular Function of Prdm12, a Gene Underlying Congenital Insensitivity to Pain*
Helen Lai, University of Texas Southwestern
Mark Landy, University of Texas Southwestern

11:30-11:45 *Antibody Receptor Signaling from Spinal Cord Glial Cells Promotes Neuropathic Pain*
Peter Grace, MD Anderson Cancer Center
Michael Lacagnina, MD Anderson Cancer Center

11:45-12:15 Break Out Rooms:
• Translational and Therapeutic Pain Research
• Novel Technologies and Methods for Studying Pain

12:15-12:35 Poster Data Blitz
Nasal Administration of Mesenchymal Stem Cells Reverses Chemotherapy-induced Peripheral Neuropathy
Poster 35
Nabila Boukelmoune, MD Anderson Cancer Center

Effect of Adolescent Alcohol Exposure and an Adult Pain Challenge on Nociception and CEA-PAG Circuitry
Poster 43
Lily Chen, Southeast Louisiana Veterans Health Care System

Characterizing Nociceptive Response Properties During the Acute Phase of Spinal Cord Injury
Poster 27
Olivia Eller, University of Kansas Medical Center

Modulation of Opioid Responses in Nociceptors by Electrical Activity After Spinal Cord Injury
Poster 41

All times listed are Central Time

Anibal Garza Carbajal, University of Texas Health Science, Houston

TRPA1+ Afferents Maintain Persistent Mechanical Hypersensitivity in Female Nociceptive Pain Model

Poster 23

Kali Hankerd, University of Texas Medical Branch at Galveston

Individualized fMRI-based Neuromodulation Increases Signal-to-noise-ratio in Somatomotor and Proprioceptive Awareness Networks: Implications in the Neuro-rehabilitation of Patients with Oral Neuropathic Pain

Poster 25

Rasoul Hekmati, Baylor College of Medicine

Tim-3 Positive CD8 T cells and IL-13 Promote Resolution of chemotherapy-induced Peripheral Neuropathy

Poster 33

Susmita Kumari, MD Anderson Cancer Center

Transition Mechanism of Nociceptive Pain in Males

Poster 37

Kathleen McDonough, University of Texas Medical Branch at Galveston

Inter- and Trans-generational Inheritance of Paternal Chronic Pain to Male and Female Offspring in Mice

Poster 31

Magali Millecamps, McGill University

A Pharmacological Interactome Platform for Discovery of Pain Mechanisms and Targets

Poster 39

Sanjay Neerukonda, University of Texas Dallas

The Cannabinoid Agonist CB-13 Produces Acute Peripherally-Mediated Analgesia in Mice and Reduces Measures of Neuronal Hyperexcitability in Mouse DRG Yet Repeated Dosing Elicits Tolerance and Signs of CNS Activity

Poster 29

Richard Slivicki, Washington University

TIMP-1 Attenuates Hypersensitivity Through CD63 Signaling

Poster 45

Rena Stair, University of Kansas Medical Center

Spinal Cord Injury-Induced Chronic Pain: A Role For Bacterial Translocation And DNA Damage In Bowel Pain After Injury

47

Adam Willits, University of Kansas Medical Center

12:35-1:15 Poster Session 2: Odd numbered posters

1:15-2:00 Vendor Session and lunch

All times listed are Central Time

Session 4

Moderator: **Patrick Dougherty**, MD Anderson Cancer Center

- 2:00-2:15 *Peripheral Interactions of Tongue Tumor and the Sensory Nervous System*
Shivani Ruparel, University of Texas, San Antonio
- 2:15-2:30 *Sexually Dimorphic Mechanisms of Nociceptive Pain*
Jun-Ho La, University of Texas Medical Branch at Galveston
- 2:30-2:45 *Sex Differences in the Endocannabinoid System in Acute, Inflammatory, Chronic and Cancer Pain*
Josee Guindon, Texas Tech University Health Sciences Center
Henry Blanton, Texas Tech University Health Sciences Center
- 2:45-3:00 *Neuroimmune Modulation of Prolactin Responses in Preclinical Migraine Models*
Bianca Mason, University of Texas, Dallas
- 3:00-3:10 Closing remarks and awards presentation
Annemieke Kavelaars, MD Anderson Cancer Center
Ted Price, University of Texas, Dallas



Alexis Bavencoffe, PhD
Research Assistant Professor
Integrative Biology and Pharmacology
*Unexpected Contributions of the Cytokine MIF to Persistent
Nociceptor Hyperactivity and Chronic Pain*

Dr. Alexis Bavencoffe obtained his License, Master's and PhD degrees from the University of Sciences and Technologies of Lille (France). Developing a strong interest in the roles of ion channels in the generation and maintenance of pain, Alexis came for his postdoctoral training to the Department of Integrative Biology and Pharmacology at McGovern Medical School in 2010. He worked in the Michael Zhu lab and thereafter joined Carmen Dessauer's and Terry Walters' labs where he became instructor and then Research Assistant Professor.

Alexis's research employs electrophysiological (patch-clamp) and behavioral approaches to define intracellular signaling pathways that induce and maintain the pain-related spontaneous activity in nociceptors that drives neuropathic pain after spinal cord injury or peripheral nerve injury. These efforts have led to 16 scientific papers, 3 book chapters and over 30 communications at national and international meetings.

Abstract: Background: Chronic neuropathic pain afflicts more than half of patients with spinal cord injury (SCI) and impairs quality of life. The mechanisms are poorly understood and treatments remain inadequate. Critical mechanisms promoting chronic pain are located within nociceptors, which exhibit hyperactivity, including spontaneous and ongoing activity (SA and OA), that contributes to ongoing pain after injury. Nociceptors after SCI also become hypersensitive to chemical signals linked to inflammation and injury, including TRPV1 agonists and serotonin. Due to the lack of an effective vascular permeability barrier in dorsal root ganglia, and their intrathecal location, nociceptor somata are exposed to chemical signals in both the blood and cerebrospinal fluid. In humans, SCI causes acute and chronic elevation of circulating levels of the cytokine, macrophage migration inhibitory factor (MIF), and MIF has been identified as a key factor in other models of inflammatory and neuropathic pain.

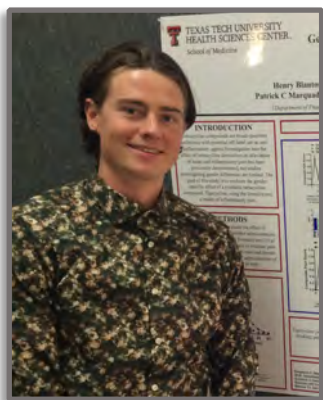
Hypothesis / goal: This work tested the hypothesis that MIF contributes to pain after SCI by promoting OA in primary nociceptors.

Methods: We used electrophysiological (patch-clamp) plus operant and reflex behavioral assays in a thoracic (vertebral T10 level) contusive spinal cord injury model in adult male rats.

Results: We previously identified two types or states of nociceptors in vitro based on differences in accommodation to prolonged depolarizing pulses: rapidly accommodating (RA) and nonaccommodating (NA), with only the latter exhibiting SA and OA. We find that MIF only excites NA neurons. Moreover, MIF, at levels reported in SCI patients' plasma (1 ng/ml), potently switches nociceptors into a hyperexcitable OA state comparable to the one observed after SCI. MIF dose-dependently enhances all three general electrophysiological properties that can promote OA: depolarization of resting membrane potential, hyperpolarization of action potential threshold, and enhancement of the amplitude and incidence of depolarizing spontaneous fluctuations. Nociceptors isolated from SCI rats displayed much greater responsiveness to MIF compared to sham controls. Conditioned place avoidance and conditioned place preference tests suggested that MIF evokes ongoing pain and contributes to chronic spontaneous pain after SCI.

Conclusions: MIF plays an important role in driving persistent SA, OA, and pain in a chronic spinal cord injury model, and our results indicate that this role involves both chronic increases in the sensitivity of nociceptors to MIF and increased availability of MIF to nociceptors. These findings suggest that therapeutic inhibition of MIF after SCI could reduce pain by reducing nociceptor hyperactivity.

Funding Sources: Supported by a grant from Mission Connect (017-107), a program of TIRR Foundation to Alexis Bavencoffe and NIH grants to Carmen W. Dessauer and Edgar T. Walters (NS091759) and to Edgar T. Walters and Michael X. Zhu (NS111521).



Henry Blanton Graduate Student

Translational Neuroscience and Pharmacology *Sex Differences in the Endocannabinoid System in Acute, Inflammatory, Chronic and Cancer Pain*

Henry Blanton is a 5th year Ph.D. candidate in the Texas Tech University Health Sciences Center Graduate School of Biomedical Sciences Ph.D. program in the concentration of Translational Neuroscience and Pharmacology. He received a B.S. in Psychology from Texas State University in 2014 and began his graduate studies at TTUHSC in the fall of 2016. His research is focused on the endocannabinoid system and its role in pain modulation. In his time as a graduate student, he has co-authored four research articles, two literature reviews, and one book chapter. Currently, he is investigating the influence of estrogen on endocannabinoid system function and the development of tolerance to cannabinoids in a neuropathic pain model in mice. He plans to defend his dissertation this summer and would like to continue to study the endocannabinoid system moving forward.

Abstract: Pain conditions and responses to analgesics have been demonstrated to be influenced by sex. Evidence is emerging that this is also true with cannabinoid-mediated analgesia. Cannabis use has been increasing in recent years, particularly among women, and one of the most common uses of cannabis for medical purposes is pain relief. Recent preclinical and clinical studies have demonstrated sex differences in response to the development of cannabinoid antinociceptive tolerance. In our studies, we find sex differences in the formalin test directly related to the stage of the estrous cycle with female showing significantly lower pain scores in the metestrus and diestrus stage whereas no sex differences was found in cisplatin-induced mechanical and cold allodynia between male and female wild type mice. Sex differences is found in the antinociceptive effects of ACEA (CB1 agonist, 0.5 mg/kg i.p.) and CP55,940 (0.3 mg/kg i.p.) following the formalin test. Moreover, antinociceptive tolerance to ACEA (0.5 mg/kg) developed after 5 days of chronic administration for females and after 9 days for males following mechanical and cold allodynia, respectively. CP55,940 (0.3 mg/kg i.p.) antinociceptive tolerance developed after 8 days for females and 11 days for males for mechanical allodynia and after 10 days for females and 15 days for males for cold allodynia. Chronic administration of ACEA and CP55,940 in females resulted in disturbance of the estrous cycle resulting in a sustained predominant

metestrus phase quantified for 27 consecutive days. We also found a tumor (OVCAR-5 human ovarian cancer cell lines)-dependent decrease and delay in chemotherapy-induced pain hypersensitivity in ovarian cancer models. These results illustrate the complexity of changes induced by sex-hormones. Further research into the role of sex hormones in endocannabinoid system function is critical as we gain deeper understanding of the impact of the endocannabinoid system in various diseases pain states.

Acknowledgements: Funded by NIH grants DA044999-01A1 (JG), CA155223 (KP), Cancer Prevention and Research Institute of Texas (CPRIT) RR140008 (KP) and Texas Tech University Health Sciences Center School of Medicine 121035 (JG).



Zachary T. Campbell, PhD

Assistant Professor

Biological Sciences

The Impact of Inflammatory Mediators on the Landscape of Nascent Translation in the Dorsal Root Ganglion

Zachary Campbell is currently an assistant professor at UT-Dallas. He earned his doctorate at the University of Arizona with Thomas Baldwin. He conducted post-doctoral work at the University of Wisconsin Madison with Marv Wickens on translational control and functional genomics. He joined UT-Dallas in 2015 and has established a research program that seeks to understand RNA control in pain. The lab focuses on nociceptors. We are broadly interested in unbiased identification of transcripts that drive plasticity. We have developed a new class of mechanism based inhibitors that disrupt post-transcriptional regulons in vivo. This has led to the identification of RNA-binding factors that are required for pain associated behaviors. Our work can be found online at www.RNAcentral.com.

Abstract: While acute pain enables injury avoidance and benefits survival, chronic pain is persistent and debilitating with very few effective treatment options. The transition from acute to chronic pain has been associated with sensitization of sensory neurons in the dorsal root ganglion. These neurons not only senses pain (i.e. nociceptors) but also innervates the response of non-neuronal cells to injury. Despite the pervasive role of translational regulation in nociception, the contribution of activity-dependent protein synthesis to inflammation is not well understood. To address this problem, we examined the landscape of nascent translation in DRG neurons treated with inflammatory mediators using ribosome profiling. We identify a remarkably small subset of transcripts that are preferentially translated in response to NGF and IL6, including the immediate early genes cFos and Arc. cFos translation is regulated by the ribosomal protein S6 kinase (S6K1). Antagonism of either S6K1 or cFos by DG2 and T5224, respectively, blocks mechanical and thermal hyperalgesia induced by inflammatory mediators, suggesting that S6K1 mediated translation of cFos is required for inflammation-related pain sensitization. Arc is locally translated in the skin. Arc deficient mice display exaggerated paw temperatures and vasodilation in response to an inflammatory challenge. Since Arc has recently been shown to be released from neurons in extracellular vesicles, we hypothesized that intercellular Arc signaling regulates the inflammatory response in skin. We found that the aberrant phenotype

observed in Arc defective mice are rescued by injection of Arc-containing extracellular vesicles into the skin, suggesting that Arc regulates neurogenic inflammation through intercellular signaling. Together, our findings expand our understanding of how dynamic translation of a specific subset of mRNAs contribute to inflammation and nociception and have clear implications for the development of novel pain therapeutics.



Munmun Chattopadhyay, PhD

Assistant Professor

Molecular and Translational Medicine

Alterations in Histone Acetylation and Neuroinflammation in Diabetic Painful Neuropathy

Dr. Munmun Chattopadhyay is an Assistant Professor at TTUHSC El Paso, TX, USA. Her research is focused on determining the impact of inflammatory mediators on the pathogenesis of diabetic complications: neuropathy, cardiomyopathy and gastroparesis. Her lab established that replication defective herpes simplex virus-mediated gene transfer of growth factors (NGF, NT-3, VEGF) can prevent diabetic and drug-induced peripheral sensory neuropathy in animals, and that gene transfer mediated release of inhibitory neurotransmitters (Enkephalin, GABA) as well as anti-inflammatory mediators (IL-10, sTNFR) in diabetic animals would reduce pain concomitantly with a reduction in sodium channel NaV1.7 levels in dorsal root ganglia. Her lab also demonstrated exercise-mediated alleviation of painful neuropathy with a decrease in neuro-inflammation. Currently, the lab is investigating the novel early biomarkers of inflammation and epigenetic modulators (histone modifications) involved in the progression of neuropathy, cardiac dysfunction and gastroparesis in diabetic animals and human subjects, and are exploring whether inhibiting inflammation or epigenetic changes will alter the progression of these complications. Dr. Chattopadhyay has been funded by NSF, ADA and other foundations, published more than 35 articles and serves as an editorial board member in peer reviewed journals and panel member in grant review committees.

Abstract: Objective: Peripheral sensory neuropathy is one of the most debilitating complications of diabetes. Our studies have shown that high mobility group box1 (HMGB1) and tumor necrosis factor alpha (TNF α), two important neuroinflammatory mediators, play significant roles in the development of diabetic painful neuropathy (DPN). Our study also revealed that increase in Class I histone deacetylases (HDAC) are crucial in DPN and inhibition of HDAC activity provides relief from DPN. In this study, we investigated whether Class I HDAC inhibition reduces neuroinflammation and neuropathic pain by evaluating the changes in inflammatory mediators and histone modifications in the dorsal root ganglia (DRG) and spinal cord dorsal horn neurons of diabetic animals. This was further compared with an anti-inflammatory agent, glycyrrhizin (GLC).

Methods: Type 2 diabetic (T2D) animals with pain were treated either with Class I HDAC inhibitor, FK228 1mg/kg; I.P. twice a week for 4 weeks or with GLC for 5 days/ week for 4 weeks at a dose of 50 mg/kg per day I.P. Animals were grouped into 4 categories: naïve control, diabetic alone, diabetic with GLC treatment and diabetic with FK228 treatment. All behavioral analyses were carried out before and after the treatment. The expression of inflammatory markers and changes in histone acetylation in the peripheral nervous system were measured by immunohistochemistry and Western blot analysis after the completion of the treatment.

Results: T2D animals demonstrated significant changes in thermal and mechanical hyperalgesia manifested by a decrease in withdrawal latency to heat and mechanical stimuli after 6 weeks of diabetes and also exhibited marked increases in HDAC1, HDAC2, HDAC3, IL1 β , TNF α , TLR4, CXCR4 and alteration H3 acetylation as determined by the Western blot analysis and immunohistochemistry. Our results show that animals treated with FK228 had significant alleviation in thermal hyperalgesia along with changes in histone acetylation and expression of inflammatory mediators; whereas GLC treatment improved the mechanical and thermal pain threshold in these animals. GLC also altered histone 3 acetylation in the microglial cells and prevented the release of HMGB1 from neuronal cells.

Conclusion: Our study suggests that Class 1 HDACs play an important role in the inflammatory aspect of DPN in T2D animals and anti-inflammatory mediators could offer another alternative towards a novel treatment approach for DPN.



John L. Clifford, PhD
Branch Chief
Battlefield Pain Management Branch
Pain and Sensory Trauma Management
Department

*Pipeline for Evaluating Efficacy of Novel Non-opioid Compounds for
Battlefield Injury-induced Pain*

Dr. Clifford is the Branch Chief and PI in the Battlefield Pain Branch of the Pain and Sensory Trauma Management Combat Casualty Care Research Team (CRT), at the US Army Institute of Surgical Research (USAISR) at Joint Base San Antonio, TX. He received his Ph.D. in Cancer Biology from the University of Texas Health Sciences Center Graduate School of Biomedical Science – MD Anderson Cancer Center - Houston, in 1992. He did his postdoctoral research at the INSERM (Institute of Genetics and Molecular and Cellular Biology) in Strasbourg, France. His independent research career followed in 1997 at MD Anderson in the Department of Clinical Cancer Prevention. In 2003 he took a position as Associate Professor at the Louisiana State University Health Sciences Center in Shreveport School of Medicine, in the Department of Biochemistry and Molecular Biology, where he was joint appointed at the Feist-Weiller Cancer Center. In 2011 he moved to the USAISR, where he is currently conducting translational research using animal models, in-vitro laboratory technologies, and systems biology (omics) approaches. Their projects are aimed at preclinical screening of non-opioid analgesics and analgesic devices (non-pharmacologic), as well as understanding pain signaling in chronic neuropathic pain and burn pain models.

Abstract: Recent advances in combat casualty care have resulted in an unprecedented survival rate for battlefield injuries of over 90%, and these injuries typically involve severe acute pain. Currently the standard of treatment for acute pain in the battlefield is opioid drugs, which can cause loss of consciousness, immobility, and inability to remain in the fight. Opioids also produce other negative effects such as dependence, tolerance, hyperalgesia, and cognitive and psychological impairment, that further reduce unit effectiveness. We are therefore testing a range of novel, non-opioid compounds with analgesic potential in battlefield-relevant models of pain and hemorrhage. These analyses combine three established pain models, two of which have been developed at our institution: 1) the full thickness thermal injury (FTTI) pain model, 2) a model for acute extremity trauma that includes hemorrhage (ET+HEM), and 3) the spinal nerve ligation (SNL) model. The FTTI and SNL models provide precise analgesic efficacy characteristics for the novel compounds, such as optimal

dosing, timing and routes of administration. The ET+HEM model determines effects of analgesics on the compensatory hemodynamic and respiratory responses to moderate and severe hemorrhage, and survival to severe hemorrhage.

We have previously used the FTTI model to study the effects of morphine and other standard of care opioids as follows: 1.) to assess tolerance and hyperalgesia (Cheppudira et al, BMC Anesthesiology, 16:73, 2016), 2.) to determine efficacy of topical application, in an effort to reduce the overall opioid requirements (Clifford et al., Burns, 43:1709-1716, 2017) and 3.) as a standard for comparison for testing the analgesic efficacy of the novel non-opioid candidate drugs. We have shown that morphine is highly effective in suppressing both thermal hyperalgesia (TH) and mechanical allodynia (MA) at several times post thermal injury (Days 3,4,5,6,7), at a range of doses when administered intraperitoneally (2,5, and 10 mg/kg IP), and that the analgesic effects lasted for up to 2 hr post administration. In addition, topical administration of morphine to the burn wound site (0.1ml, 5mg/ml) in the FTTI model, produced comparable suppression of TH, with a lesser effect on MA. We have used the SNL model at very early time points post ligation to test the plasma secretome's ability to reduce nerve-injury induced nociceptive behavior and found that bath application of the ligated nerve with a secretome derived product reduces MA at 1 and 2 hours post SNL. With the ET model (without hemorrhage), behavioral responses to trauma were characterized and effects of intravenous (iv) opioid analgesics (morphine, fentanyl, sufentanil) and ketamine were assessed (Xiang et al., J Trauma Acute Care Surg, 85:S49-S56, 2018). Compared with the saline vehicle (VEH) group, opioid analgesics reduced MA for at least 80 minutes post injury. Opioids and ketamine were tested further in the complete ET+HEM model. When the volume loss of 40% was analyzed, opioids caused an increase in blood pressure and decrease in respiration, while ketamine had no effect on compensatory responses. Interestingly, i.v. administration of opioids given immediately after severe hemorrhage (55% blood volume loss) did not affect survival ($P = .55$).

Combining the use of these three distinct pre-clinical models in a 'pipeline' provides optimal candidate analgesics for clinical testing. This is a unique platform for determining both the precise analgesic effectiveness and the suitability for use in a severe polytrauma setting, for novel, non-opioid analgesics.

Disclaimers: Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC.

The views expressed in this abstract are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.

Acknowledgements: Funding was provided through the Clinical and Rehabilitative Medicine Research Program (CRM RP) and the Combat Casualty Care Research Program (CCCRP).



June Bryan de la Pena, PhD Postdoctoral Research Scientist

The Impact of Inflammatory Mediators on the Landscape of Nascent Translation in the Dorsal Root Ganglion

June Bryan de la Pena is a postdoctoral research scientist in the laboratory of Dr. Zachary Campbell at UT-Dallas. He earned his MS and PhD degree in Neuropharmacology at Sahmyook University, South Korea. His current research is focused on understanding translational control mechanisms that govern chronic pain. With the use of next-generation sequencing technology, their team has identified RNA-binding factors, regulatory elements, and specific transcripts in nociceptors that are required for pain-associated behaviors.

Abstract: While acute pain enables injury avoidance and benefits survival, chronic pain is persistent and debilitating with very few effective treatment options. The transition from acute to chronic pain has been associated with sensitization of sensory neurons in the dorsal root ganglion. These neurons not only sense pain (i.e. nociceptors) but also innervates the response of non-neuronal cells to injury. Despite the pervasive role of translational regulation in nociception, the contribution of activity-dependent protein synthesis to inflammation is not well understood. To address this problem, we examined the landscape of nascent translation in DRG neurons treated with inflammatory mediators using ribosome profiling. We identify a remarkably small subset of transcripts that are preferentially translated in response to NGF and IL6, including the immediate early genes cFos and Arc. cFos translation is regulated by the ribosomal protein S6 kinase (S6K1). Antagonism of either S6K1 or cFos by DG2 and T5224, respectively, blocks mechanical and thermal hyperalgesia induced by inflammatory mediators, suggesting that S6K1 mediated translation of cFos is required for inflammation-related pain sensitization. Arc is locally translated in the skin. Arc deficient mice display exaggerated paw temperatures and vasodilation in response to an inflammatory challenge. Since Arc has recently been shown to be released from neurons in extracellular vesicles, we hypothesized that intercellular Arc signaling regulates the inflammatory response in skin. We found that the aberrant phenotype observed in Arc defective mice are rescued by injection of Arc-containing extracellular vesicles into the skin, suggesting that Arc regulates neurogenic inflammation through intercellular signaling. Together, our findings expand our understanding of how dynamic translation of a specific subset of mRNAs contribute to inflammation and nociception and have clear implications for the development of novel pain therapeutics.



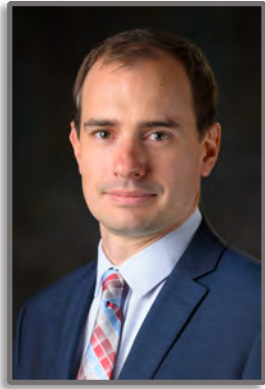
Nadia German
Assistant Professor

Pharmaceutical Sciences

Novel Selective Kappa-opioid Ligands as Modulators of Pain Responses

Nadezhda (Nadia) German is a tenured assistant professor at the Texas Tech University Health Sciences Center (TTUHSC). She received her Ph.D. with an emphasis in Medicinal Chemistry at the University of Iowa, working with Dr. Robert Kerns. Her training continued at Virginia Commonwealth University, where she worked in Dr. Richard Glennon's laboratory, followed by two years at the Research Triangle Institute in the group of Yana Zhang. Her current research focuses on developing molecules with two different biological activities: the ability to modulate GPCRs and kill cancer cells. Her work is funded by NIH and a variety of foundations' seed grants. She has published over 30 peer-reviewed papers and three book chapters.

Abstract: Using the structure of dehydrogliotoxin (fungal metabolite) as a starting point, we have prepared novel diketopiperazine-based ligands with a varying degree of selectivity between opioid subtypes. Selected compound with the preferential binding to KOR was tested in vivo, using the neuropathic pain model in rats. It showed the ability to modulate sensory and emotional pain-related behaviors in animals when administered 3 mg/kg intraperitoneally. These findings are in line with the existing data on the role of KOR-mediated signaling in the development of chronic pain conditions. Our compounds' chemical novelty, their favorable drug-likeness profile, and observed in vivo activity provide a platform for further developing these chemical agents as potential candidates for pain therapy.



Peter Grace, PhD
Assistant Professor
Symptom Research

Antibody Receptor Signaling from Spinal Cord Glial Cells Promotes Neuropathic Pain

Dr. Peter Grace is an Assistant Professor in the Department of Symptom Research at the University of Texas MD Anderson Cancer Center. The Grace lab investigates neuroinflammatory mechanisms that drive chronic pain, in order to identify new treatment strategies. Dr. Grace completed graduate training in pharmacology at the University of Adelaide, Australia, and a NHMRC CJ Martin postdoctoral fellowship in neuroimmunology at the University of Colorado Boulder. Dr. Grace is the recipient of awards from the American Pain Society, American Australian Association, Brain and Behavior Research Foundation, the Rita Allen Foundation, as well as grants from the NIH and Department of Defense.

Abstract: A hallmark of nerve injury is increased neuroimmune signaling from glial cells in the spinal cord, which can lead to enhanced activity of nociceptive neural circuits. However, it remains unclear what mechanisms control glial cell activation in the spinal cord following nerve injury. In this talk, we will present evidence that neuroimmune-mediator production by spinal cord glial cells is facilitated by activation of Fc gamma receptors (FcγRs), the receptors for immunoglobulin G (IgG) antibodies. Furthermore, inhibiting this signaling axis has the potential to arrest neuropathic pain behaviors. This raises the possibility that glial FcγRs may be targets for novel treatments to alleviate suffering from neuropathic pain.



Josée Guindon, PhD
Associate Professor
Pharmacology and Neuroscience

*Sex Differences in the Endocannabinoid System in Acute,
Inflammatory, Chronic and Cancer Pain*

Dr Josée Guindon is an Associate Professor in the Department of Pharmacology and Neuroscience at Texas Tech University Health Sciences Center (TTUHSC). She has obtained her Doctorate in Veterinary Medicine, Master and Ph.D. from Université de Montréal. She did her post-doctoral training at University of Georgia for which she received a substantial post-doctoral fellowship from the Fonds de la Recherche du Québec en Santé (FRQS). Dr. Guindon has a funded laboratory from NIH and got awarded an R01 from NIDA to study mechanisms of cannabinoid tolerance. Dr. Guindon also won several career awards. In 2019, she won the TTUHSC President Early Career Investigator Award and in 2016 the Unsung Hero Award honored by the Provost and Dean of TTUHSC. She has already published more than 42 manuscripts and 6 first-authored book chapters as well as giving more than 46 invited seminars for local and international conferences. Dr. Guindon is an expert in the behavioral, pharmacological, biochemical and transgenic analysis of pain mechanisms, using various pain models and investigating sex differences. She has greatly contributed and pioneered, neural and brain endocannabinoid mechanisms that regulate and modulate pain by studying sex hormones, thus opening up new and novel understandings of the ways in which pain is transmitted through the nervous system and opening up new and novel approaches to the pharmacological management of chronic and cancer pain.

Abstract: Pain conditions and responses to analgesics have been demonstrated to be influenced by sex. Evidence is emerging that this is also true with cannabinoid-mediated analgesia. Cannabis use has been increasing in recent years, particularly among women, and one of the most common uses of cannabis for medical purposes is pain relief. Recent preclinical and clinical studies have demonstrated sex differences in response to the development of cannabinoid antinociceptive tolerance. In our studies, we find sex differences in the formalin test directly related to the stage of the estrous cycle with female showing significantly lower pain scores in the metestrus and diestrus stage whereas no sex differences was found in cisplatin-induced mechanical and cold allodynia between male and female wild type mice. Sex differences is found in the

antinociceptive effects of ACEA (CB1 agonist, 0.5 mg/kg i.p.) and CP55,940 (0.3 mg/kg i.p.) following the formalin test. Moreover, antinociceptive tolerance to ACEA (0.5 mg/kg) developed after 5 days of chronic administration for females and after 9 days for males following mechanical and cold allodynia, respectively. CP55,940 (0.3 mg/kg i.p.) antinociceptive tolerance developed after 8 days for females and 11 days for males for mechanical allodynia and after 10 days for females and 15 days for males for cold allodynia. Chronic administration of ACEA and CP55,940 in females resulted in disturbance of the estrous cycle resulting in a sustained predominant metestrus phase quantified for 27 consecutive days. We also found a tumor (OVCAR-5 human ovarian cancer cell lines)-dependent decrease and delay in chemotherapy-induced pain hypersensitivity in ovarian cancer models. These results illustrate the complexity of changes induced by sex-hormones. Further research into the role of sex hormones in endocannabinoid system function is critical as we gain deeper understanding of the impact of the endocannabinoid system in various diseases pain states.

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Walter J. Koroshetz, MD

Director

NIH's HEAL Initiative in Pain Science

Dr. Koroshetz became Director of the National Institute of Neurological Disorders and Stroke (NINDS) in 2015. In that capacity, he is chair of the Interagency Pain Research Coordinating Committee that coordinates pain research across the federal government and is co-chair of the Acute to Chronic Pain Signatures Program, part of the NIH Common Fund. He leads a number of programs as part of the Helping to End Addiction Long Term (HEAL) Initiative. He has also held leadership roles in the NIH BRAIN Initiative, NIH Blueprint for Neuroscience, and the NIH Office of Emergency Care Research.

Dr. Koroshetz joined NINDS in 2007 as Deputy Director. Prior to that, he served as Vice Chair of the neurology service and Director of stroke and neurointensive care services at Massachusetts General Hospital (MGH). He was a professor of Neurology at Harvard Medical School (HMS) and led neurology resident training at MGH between 1990 and 2007. Over that same period, he co-directed the HMS Neurobiology of Disease Course with Drs. Edward Kravitz and Robert H Brown.

A native of Brooklyn, New York, Dr. Koroshetz graduated from Georgetown University and received his medical degree from the University of Chicago. He trained in internal medicine at the University of Chicago and Massachusetts General Hospital. Then trained in neurology and neuroscience at MGH and later at Harvard Neurobiology. A major focus of his clinical research career was to develop measures in patients that reflect the underlying biology of their disorders. This led to clinical research using brain imaging techniques including MR spectroscopy in Huntington's disease, diffusion/perfusion MR and CT imaging, CT angiography, and acute clot removal for large artery stroke.

Abstract: The Helping to End Addiction Long-term (HEAL) Initiative is an aggressive, trans-agency effort to speed scientific solutions to stem the national opioid public health crisis. Almost every NIH Institute and Center is accelerating research to address this public health emergency from all angles. Understanding, managing, and treating

pain is a core goal of the initiative, and this has taken the form of a number of diverse programs. The NIH HEAL Initiative aims to accelerate the discovery and preclinical development of new medications and devices to treat pain as well as advance clinical research on pain management. More effective medications for pain are needed, but poorly predictive animal models, changes in biopharmaceutical industry focus, and perceived regulatory and reimbursement concerns have made research challenging. NIH is supporting a suite of targeted research efforts to address this need. Additionally, the HEAL Initiative is supporting both new clinical trial programs and the expansion of existing programs to evaluate the safety and efficacy of innovative therapies for pain management. These clinical trials will help establish evidence-based guidelines for treating pain with non-opioid therapies to reduce use of prescription opioid medications. The HEAL Initiative is also taking steps to harness the power of patients and other stakeholders to advance research goals. In this talk, I will address the scope of the HEAL Initiative and current and future programs.



Jun-Ho La, DVM, PhD
Assistant Professor
Neuroscience, Cell Biology, & Anatomy
Sexually Dimorphic Mechanisms of Nociplastic Pain

Dr. La has a long-standing research interest in chronic pain and developing therapeutic tools to manage this debilitating condition. His current research focuses on mechanisms of pain chronification, namely transition from acute to chronic pain, without underlying persistent tissue injury. This type of chronic pain is recently termed 'nociplastic pain', implying long-term changes in the nociceptive neural circuit itself. There is growing appreciation that ongoing primary afferent inputs and glial activation play a pivotal role in triggering and maintaining these long-term changes at the level of the spinal cord where peripheral and central neurons, together with non-neuronal cells, form an integrated nociceptive neural circuit. Using multidisciplinary approaches such as behavioral, molecular biological, electrophysiological, and Ca²⁺-imaging tools, his lab investigates the mechanisms triggering and maintaining the long-term changes, which will be used for the development of mechanism-based therapies to convert chronic pain back to normally resolving pain.

Abstract: Background: Acute injury-induced pain can transition to chronic nociplastic pain, which predominantly affects women. However, it is unknown how the transition occurs, whether females are more susceptible to the transition, and how the chronic nociplastic pain state is maintained despite resolution of the inciting acute injury. Goals: To address the above questions, we developed a mouse model in which post-injury stimulation of injured area triggers the transition from normally resolving pain to chronic nociplastic pain. Methods: Intraplantar capsaicin injection was used as an initial injury and intermittent immersion of the capsaicin-injected paw into the warm (30°C or 40°C) water as post-injury stimulation. The degree of capsaicin-induced pain hypersensitivity and local inflammation was measured by assessing nocifensive behaviors in response to thermal/mechanical stimulation and quantifying local inflammatory reactions (increased vascular leakage and proinflammatory cytokine gene expression), respectively. Results: Capsaicin injection alone induced mechanical and thermal hypersensitivity that resolved more slowly in females. The post-injury 40°C stimulation at 2 hr post-capsaicin prolonged capsaicin-induced mechanical, but

not thermal, hypersensitivity up to 3 weeks in both sexes. When post-injury stimulation was given at a lower intensity (30°C) or at later time points (40°C at 1-3 days post-capsaicin), chronification of mechanical hypersensitivity occurred only in females. Notably, the post-injury 40°C stimulation did not prolong capsaicin-induced inflammation in the hindpaw, indicating that the prolonged mechanical hypersensitivity in these mice arises without clear evidence of ongoing injury, thus reflecting nociplastic pain. Although morphine and gabapentin commonly alleviated this persistent mechanical hypersensitivity in both sexes, sexually dimorphic mechanisms mediated the hypersensitivity. Specifically, local anesthesia of previously capsaicin-injected site inhibited the hypersensitivity outside the injection site only in females, and inhibiting spinal microglia alleviated the hypersensitivity only in males. Conclusions: These results demonstrate that post-injury stimulation of injured area can trigger the transition from acute pain to chronic nociplastic pain more readily in females. The nociplastic pain state is maintained peripherally by ongoing nerve activity at the initial injury site in females but centrally by activated spinal microglia in males.



Michael J. Lacagnina, PhD
Postdoctoral Fellow
Symptom Research

Antibody Receptor Signaling from Spinal Cord Glial Cells Promotes Neuropathic Pain

Dr. Michael Lacagnina is a postdoctoral fellow with Dr. Peter Grace in the Department of Symptom Research at the University of Texas MD Anderson Cancer Center. He received his PhD at Duke University under the mentorship of Dr. Staci Bilbo, where he examined the role of neuroinflammatory signaling involved in opioid drug reinforcement. Dr. Lacagnina's current research has centered on delineating neuroimmune mechanisms in the spinal cord and brain that contribute to the manifestation of chronic pain. He is particularly interested in uncovering the role that astrocytes and microglia play in shaping the emergence of pain after injury, and how targeting these glial cells may offer new therapeutic strategies for treating the symptoms of pain.

Abstract: A hallmark of nerve injury is increased neuroimmune signaling from glial cells in the spinal cord, which can lead to enhanced activity of nociceptive neural circuits. However, it remains unclear what mechanisms control glial cell activation in the spinal cord following nerve injury. In this talk, we will present evidence that neuroimmune-mediator production by spinal cord glial cells is facilitated by activation of Fc gamma receptors (FcγRs), the receptors for immunoglobulin G (IgG) antibodies. Furthermore, inhibiting this signaling axis has the potential to arrest neuropathic pain behaviors. This raises the possibility that glial FcγRs may be targets for novel treatments to alleviate suffering from neuropathic pain.



Helen Lai, PhD
Assistant Professor
Neuroscience | Anesthesiology & Pain
Management

*Understanding the Developmental and Molecular Function of
Prdm12, a Gene Underlying Congenital Insensitivity to Pain*

Helen Lai attended graduate school at UCSF in the laboratory of Dr. Lily Jan assessing the biophysical constraints of voltage-gated potassium channels. Having graduated with a Ph.D. in Biophysics, Helen switched fields to study transcription factor regulation in neuronal development with Dr. Jane Johnson at UT Southwestern Medical Center. As the Frank and Sara McKnight Fellow at UTSW, Helen combined her biophysics and development backgrounds to study the development of somatosensory circuits in the spinal cord. Her lab continues to focus on the development and function of nociceptive and proprioceptive circuits.

Abstract: *Prdm12* is a key transcription factor in nociceptor neurogenesis. Mutations of *Prdm12* cause Congenital Insensitivity to Pain (CIP) due to failure of nociceptor development. However, precisely how deletion of *Prdm12* during development or adulthood affects nociception is unknown. Here, we employ tissue- and temporal-specific knockout mouse models to test the function of *Prdm12* during development and in adulthood. We first show that constitutive loss of *Prdm12* causes deficiencies in the proliferation of nociceptor precursor cells. We then generate a new strain of mice in which *Prdm12* has been conditionally knocked out of neurons in dorsal root and cranial ganglia. We show that these *Prdm12*^{AviCKO} mice have reduced sensitivity to mechanical and cold pain, as well as pruriception, and correlate this with transcriptional and molecular evidence of loss of specific sensory populations. In contrast, using a tamoxifen-inducible CRE model, we find that *Prdm12* is dispensable for most pain sensation and injury-induced hypersensitivity in adult dorsal root ganglia (DRGs). Using transcriptomic analysis, we find mostly unique changes in adult *Prdm12* knockout DRGs compared to embryonic knockout, and that PRDM12 is likely a transcriptional activator in the adult. Overall, our findings suggest that the function of PRDM12 changes over developmental time.



Mark Landy
Graduate Student
MD/PhD program

Understanding the Developmental and Molecular Function of Prdm12, a Gene Underlying Congenital Insensitivity to Pain

Mark Landy is a graduate student in the MD/PhD program at UT Southwestern Medical Center, where he studies the role of *Prdm12* in nociceptor development and function under the mentorship of Helen Lai, PhD. Mark completed his undergraduate studies at Cornell University, where he earned a BS in Applied & Engineering Physics in 2013. While there, he was introduced to basic research in the lab of Lois Pollack, PhD, where he studied techniques of end-to-end distance measurement of macromolecules using gold nanoparticles and small-angle x-ray scattering. After graduating, Mark spent two years working at the Tisch Multiple Sclerosis Research Center of New York, under the direction of Violaine Harris, PhD, and Saud Sadiq, MD, before enrolling at UT Southwestern Medical Center. Mark joined the Lai Lab in 2017, where he has received funding through a Ruth L. Kirschstein Predoctoral Individual NRSA (F31) from NINDS, and the William F. and Grace H. Kirkpatrick Award. Mark's work was recently accepted for publication in *Cell Reports*.

Abstract: *Prdm12* is a key transcription factor in nociceptor neurogenesis. Mutations of *Prdm12* cause Congenital Insensitivity to Pain (CIP) due to failure of nociceptor development. However, precisely how deletion of *Prdm12* during development or adulthood affects nociception is unknown. Here, we employ tissue- and temporal-specific knockout mouse models to test the function of *Prdm12* during development and in adulthood. We first show that constitutive loss of *Prdm12* causes deficiencies in the proliferation of nociceptor precursor cells. We then generate a new strain of mice in which *Prdm12* has been conditionally knocked out of neurons in dorsal root and cranial ganglia. We show that these *Prdm12*^{AviCKO} mice have reduced sensitivity to mechanical and cold pain, as well as pruriception, and correlate this with transcriptional and molecular evidence of loss of specific sensory populations. In contrast, using a tamoxifen-inducible CRE model, we find that *Prdm12* is dispensable for most pain sensation and injury-induced hypersensitivity in adult dorsal root ganglia (DRGs). Using transcriptomic analysis, we find mostly unique changes in adult *Prdm12* knockout DRGs compared to embryonic knockout, and that PRDM12 is likely a transcriptional activator in the adult. Overall, our findings suggest that the function of PRDM12 changes over developmental time.



Lingyong Li, PhD
Assistant Professor/Principal Investigator
Neuroscience

Underlying Mechanisms of Comorbid Depressive Symptoms in Chronic Pain

Lingyong Li, PhD, is an Assistant Professor/Principal Investigator at the Department of Neuroscience, Baylor College of Medicine. Lingyong Li got his PhD from Nanjing Agricultural University in 2006. After finished his postdoctoral training at Vanderbilt University and MD Anderson Cancer Center, Lingyong Li moved to the Department of Neuroscience, Baylor College of Medicine as an assistant professor in 2017. The research of his group mainly focuses on uncovering novel mechanisms underlying chronic pain, chronic pain-induced mood disorders, and opioid analgesic tolerance at molecular, cellular, and synaptic network levels; and their translational research in chronic pain management. To address these issues, his group performs a multi-disciplinary approach, including mouse genetic tools, Patch-seq, electrophysiology, chemogenetic techniques, behavioral assays, viral-mediated gene transfer, confocal imaging, molecular biology, and biochemistry.

Mood disorders, such as depression and anxiety, are often observed in chronic pain patients, which intensify patients' suffering and are clinically difficult to treat. Hyperactivity of pyramidal neurons in the anterior cingulate cortex (ACC) is essential in driving depression-like behaviors in chronic pain. However, the cause of ACC hyperactivity remain elusive. Rho GTPases, activated by guanine nucleotide exchange factors (GEFs) and inhibited by GTPase-activating proteins (GAPs), play important roles in dendritic spine morphogenesis and synaptic plasticity by controlling the organization of the actin cytoskeleton in response to extracellular cues. We previously identified the Rac1-GEF Tiam1 as a critical regulator of dendritic spine morphogenesis, which couples synaptic the N-methyl-D-aspartate receptors (NMDARs) to Rac1 signaling and actin cytoskeletal remodeling during brain development. In this study, we found that Tiam1 is activated in the ACC of chronic pain mice displaying depressive-like behaviors and that ACC Tiam1 determines chronic pain-induced depressive symptoms. The mechanism study found that ACC Tiam1 mediates the effect of chronic pain-activated NMDAR on synaptic structural plasticity via actin cytoskeleton remodeling and synaptic functional plasticity via synaptic NMDAR stabilization, which together determines the ACC hyperactivity and comorbid depressive symptoms in chronic pain. Our study identify ACC Tiam1 as a key factor in the development of chronic pain-induced depression.



Bianca Mason, PhD
Post-doctoral Fellow

Neuroimmune Modulation of Prolactin Responses in Preclinical Migraine Models

Bianca Mason is a post-doctoral fellow in the laboratory of Gregory Dussor in the School of Behavioral and Brain Sciences at The University of Texas at Dallas. Throughout her research career, she has had a continued interest in understanding mechanisms that contribute to migraine and uncovering potential targets for therapeutic intervention. She received her PhD from the University of Iowa under the mentorship of Andrew Russo where her graduate work focused on identifying mechanisms and peripheral sites of CGRP action in a model of migraine-like photophobia. Currently, in the Dussor lab, she is focused on elucidating neurological and immunomodulatory signatures that contribute to prolactin-mediated migraine-like pain responses in preclinical models of migraine.

Abstract: Migraine is a complex, neurological disorder that is not completely understood. Although migraine is well known to have a much higher incidence in women, a key unsolved question in the migraine field is what mechanisms contribute to the increased susceptibility of migraine in females. Female-selective hormone regulation has long been implicated as key players in this disparity, however, there has only been indirect evidence linking migraine occurrence to gonadal hormones. Reports suggest that prolactin, a female-selective, cytokine-like, luteotropic hormone that is mostly released from the pituitary, may be a worsening factor in migraine. We have generated data that suggests prolactin administration on the dura can cause migraine-like behavior in rodents and this behavior can be blocked by prolactin receptor antagonists. The objective of this study was to determine how prolactin modulates and can be modulated in the dural microenvironment to induce pain responses. To determine whether prolactin receptor activation on sensory neurons contributed to migraine-like facial hypersensitivity, we administered prolactin on the dura of female mice that have the prolactin receptor excised from sensory neurons (PrLr CKO). Facial hypersensitivity was partially attenuated in the PrLr CKO mice suggesting that prolactin receptor on sensory neurons contributes to the prolactin-induced pain. Given that loss of prolactin receptor on sensory neurons did not completely inhibit facial hypersensitivity, we then asked what other prolactin receptor-expressing cells could

contribute to the pain phenotype observed. In the dura, nociceptive afferents reside near an abundance of immune cells with females having a higher basal content in the dura than males. In fact, prolactin receptor is widely expressed on immune cells and exerts cytokine-like actions to modulate immune cell activity. It is reported that the most macrophages are the most abundant immune cell in the dura and they express the prolactin receptor. So, we posited that prolactin may have a bidirectional relationship with immune cells to contribute to these pain responses. We found that loss of macrophages using clodronate attenuates prolactin-induced mediated hypersensitivity and that inhibition of basal prolactin release by bromocriptine administration modulates dural immune cell infiltration and possibly composition. These data give insight into a role of how prolactin may activate both the immune and local nociceptive system in the dura to cause migraine-like pain in mice.



T. Dorina Papageorgiou, PhD

Assistant Professor

Psychiatry, Neuroscience, Physical Medicine and Rehabilitation

Neuromodulation of Somatomotor, Somatosensory and pain Networks Using an MRI-compatible Brain Computer Interface

Dr. T. Dorina Papageorgiou obtained a BA in Psychology and Sociology (University of Georgia), a M.H.Sc. in Psychiatric Epidemiology (Johns Hopkins Bloomberg School of Public Health), and a Ph.D. in the Biomedical Sciences The (University of Texas - M.D. Anderson Cancer Center; MDACC) with a focus on human brain neuroimaging, specifically the effects of morphine in the pain matrix networks. She continued with three postdoctoral fellowships: (i) neuroimaging of cancer symptoms and its treatment (MDACC); (ii) cortical neuromodulation of speech using real-time functional MRI neurofeedback (Baylor College of Medicine; BCM); and (ii) cortical neuromodulation of visual perception in cortical blindness (BCM). As an Assistant Professor of Psychiatry, Neuroscience, Physical Medicine and Rehabilitation at BCM and Electrical and Computer Engineering, Neuroengineering, and Applied Physics at Rice University her lab's research focuses on the development and application of targeted, and individualized real-time fMRI neurofeedback methods to elucidate the mechanisms of cortical plasticity in health, and neuro-rehabilitate cortical blindness, speech impairment and, chronic pain syndromes following neurological disorders, traumatic brain injury or, cancer-related symptoms. The Papageorgiou - Investigational Targeted Brain Neurotherapeutics Lab has developed a novel, targeted and individualized MRI-compatible brain computer interface (BCI), individualized real-time functional MRI neurofeedback (iRTfMRI nFb), which is based on promoting the reorganization of networks by bypassing lesioned pathways and capitalizing on redundant, intact but functionally associated pathways to the injured ones. The Papageorgiou - Investigational Targeted Brain Neurotherapeutics Lab's research is funded by the McNair Medical Institute, the McNair Foundation, the TIRR Foundation, various other foundations, and NIH mechanisms. She is the Chief Editor of the internationally successful book "Advanced Brain Neuroimaging Topics in Health and Disease – Methods and Applications" (ISBN: 978-953-51-1203-7; DOI: 10.5772/58256; eBook(PDF) ISBN: 978-953-51-7209-3), which has been downloaded 40K times to date.

The Papageorgiou - Investigational Targeted Brain Neurotherapeutics Laboratory examines the reorganization and neuromodulation of human cortical networks as a function of induced learning in the MRI environment. Our goal is to understand the mechanisms involved in the neurorehabilitation of somatomotor and somatosensory networks, as a result of medulla-oblongata-supranuclear or, -infranuclear glossopharyngeal (CN IX) and hypoglossal (CN XII) cranial nerves injuries following stroke, brain tumors and head and neck tumors following radio- and chemotherapy. The medulla oblongata is comprised of the sensory tract responsible for relaying touch, pressure, and pain. Lesions in the neuronal branches with inputs and outputs to the medulla oblongata can be associated with neuropathic tongue and oral pain. These lesions are accompanied by partial paralysis of the tongue and swallowing, chewing and speech articulation difficulties. The prevalence of cranial nerve neuropathy can be as high as 48% following head and neck cancer radiotherapy treatment.

The Papageorgiou lab has developed an innovative, targeted and individualized Brain Computer Interface in the MRI environment referred to as individualized real-time functional magnetic resonance neurofeedback (iRTfMRI nFb), the goal of which is to induce neuromodulation and neuro-rehabilitation of somatosensory and somatomotor networks that directly control CN IX and CN XII. We show that iRTfMRI nFb upregulates the magnitude of the Blood-Oxygen-Level-Dependent (BOLD; the ratio of oxygenated to deoxygenated hemoglobin) signal and the spatial extent of these networks, while it decreases its variance spatiotemporally compared to the control-no nFb condition. Additionally, classification accuracies in sensory and motor control show greater consistency in the activation of the somatosensory networks (primary motor and somatosensory cortices, insula and claustrum, anterior cingulate cortex, thalamus, cerebellum, basal ganglia) under iRTfMRI nFb compared with the control condition. We quantify the iRTfMRI nFb-induced spatial and temporal neuromodulatory changes that occur in these sensory networks with dynamic causal modeling and machine learning approaches. Upregulation in the BOLD of these networks accompanied by a decrease in their variance will result in better motor and sensory control in patients with CN IX and CN XII injuries.



Nikita Ruparel, DDS, MS, PhD

Associate Professor

Endodontics

*The Scope of Translational and Stem Cell Biology in Studying
Peripheral Pain Mechanisms*

Niki Ruparel is an Associate Professor and Director of the Advanced Education Program in Endodontics at UT Health San Antonio. She is a Diplomate of the American Board of Endodontists and maintains both, a clinical and research program at UT Health. Her research program has been funded by foundation and federal grants and is currently the Principal Investigator for her study on endogenous peripheral pain regulatory systems in orofacial pain patients and a co-investigator for a study on sexually dimorphic pain mechanisms mediated by serotonin in the dental pulp. Her other areas of research include 1) development of novel non-opioid drugs using stem cells for treatment of infection-induced pain using a clinically translational orofacial model of apical periodontitis-induced pain; 2) study the role and function of stem cells in tooth regeneration, specially the role of bacteria/biofilms on stem cell fate and the immuno- regulatory role of stem cells in wound healing and regeneration; and 3) clinical trials in patients to evaluate the role of endodontic procedures on healing and bacterial reduction using cone beam computed tomography and RNA sequencing, respectively.

Abstract: The effectiveness and mechanisms of stem cell-induced analgesia in treating dental pain is unknown. We demonstrate that i.v. injections of human Stem Cells of Apical Papilla (hSCAP) reverse apical periodontitis (infection of a tooth; AP)-induced mechanical allodynia. The objective of this study is to define mechanisms mediating mesenchymal stem cell (MSC)-induced anti-allodynia in a mouse model of dental pain namely, apical periodontitis. To this end, our novel preliminary data using a mouse model of AP-induced mechanical allodynia demonstrate that:

- Injection (i.v.) of human MSCs (i.e. human Stem Cells of Apical Papilla; hSCAP) reverse AP-induced mechanical allodynia in mice without altering the disease progression
- Injected hSCAP homes to periapical granulomas (peripheral sites of tooth infection, but not to the CNS, suggesting a peripheral site of action.
- After homing to periapical granulomas, hSCAP express a **133-fold** upregulation of macrophage migratory inhibitory factor (MIF).

- Conditioned media (CM) generated from co-cultures of hSCAP with mouse periapical granulomas (hereafter described as “primed” hSCAP) release MIF **>5-fold** compared to control media.
- CM from primed hSCAP significantly attenuates capsaicin (CAP)-evoked $[Ca^{2+}]_i$ from mouse trigeminal (TG) neurons and this effect is reversed by anti-human MIF-Ab.

Collectively, these data support a **novel** peripheral mechanism for human MSC-induced inhibition of nociception due, at least in part, by MIF. However, mechanisms mediating MIF inhibition of TG sensory neurons remain largely unknown. This study will therefore test the central hypothesis that apical periodontitis-induced mechanical allodynia is reversed by hSCAP-derived release of MIF that **directly** inhibits TG neuronal activity



Shivani Ruparel, PhD
Associate Professor and Director of Research
Endodontics
Peripheral Interactions of Tongue Tumor and the Sensory Nervous System

Shivani Ruparel, PhD, is associate professor and director of Research in the Department of Endodontics at University of Texas Health San Antonio (UTHSCSA). She obtained her doctoral degree in cancer biology in the Department of Cellular and Structural Biology at UTHSCSA under the guidance of Dr. Robert Marciniak and Dr. Linda deGraffenried in 2009. This was complemented by her postdoctoral training, under the guidance of Dr. Ken Hargreaves, which focused on pain neuropharmacology and biochemistry. She started her independent research program in 2012 on cancer and pain. Alongside, she also obtained a master of science in clinical investigation at UTHSCSA. Her research program focuses on peripheral mechanisms of oral tumorigenesis, oral cancer-induced pain, and pain associated with cancer treatment. She was inducted to the Omikron Kappa Upsilon National Dental Society in 2018. She has been well funded throughout her career by several private and federal agencies.

Abstract: My lab focuses on peripheral interactions of oral tumors and the peripheral sensory system. Specifically, we are interested in investigating the cross-talk of tongue tumors and the peripheral sensory afferents in mediating tumor-induced pain as well as tumorigenesis. To study mechanisms of oral cancer pain, we are focusing on the role of neurotrophins like BDNF that is released from cancer cells and control activities of the lingual sensory fibers via the TrkB receptor. Additionally, we are also studying how the oral tumor microenvironment plays an important role in the interaction of the tumors with the innervating afferents. Most of my talk will focus on the role of newly identified lymphotoxin beta and its ligands in mediating oral cancer pain. While lymphotoxin-beta receptor and its ligands; LIGHT and Lymphotoxin-beta are known to play an in autoimmune and inflammatory conditions, its role in pain is entirely known. The signaling pathway is traditionally known to mediate pro-inflammatory effects; however, our data revealed that activation of LTBR reverses oral tumor-induced pain. Interestingly, we also found that the effect of LTBR pathway on nociception could be tissue specific.



Tor Savidge, PhD
Associate Professor
Pathology & Immunology
The Role of Microbiome in Pain Modulation

Dr. Savidge is Associate Professor in the Department of Pathology & Immunology at Baylor College of Medicine and is the Associate Director of the Texas Children's Microbiome Center. His research interests include studying microbial-neuroimmune interactions in the gastrointestinal tract and nervous systems. This work has established new disease susceptibility biomarkers of abdominal pain in functional gastrointestinal disorders, as well as identifying new host-microbiome signaling mechanisms that modulate chronic pain which are being pioneered as "precision microbial therapeutics".

Abstract: The gut microbiome is receiving increasing attention as a modulator of neurological disease, including chronic pain. Well accepted as a regulator of visceral pain by altering dorsal root ganglia neuronal excitability, the gut microbiota also plays an important role in modulating inflammatory and neuropathic pain, migraine, and opioid tolerance. These findings offer unique therapeutic scope because the gut is more externally modifiable compared to central or peripheral nervous systems, and treatments addressing pain through modulation of the gut microbiota (e.g., targeted microbial therapy) may have long-term benefits for chronic pain management. Although gut microbiota composition and function is known to be heavily influenced by diet and other malleable factors, a major deficiency in the field is understanding which microbes are involved and how they are beneficial to patients with chronic pain. Targeted metagenomic sequencing is an emerging strategy to survey pain-specific microbes for clinical diagnosis and prognosis. However, this approach often yields inconsistent or conflicting results due to inadequate study power and experimental bias. A comprehensive re-analysis of individual patient microbiome datasets using a bioinformatics pipeline that compensates for technical and demographic bias offers tremendous potential in identifying microbiome features that can reliably alleviate or induce chronic pain at a population scale-level. We designed Taxa4Meta for accurate taxonomic binning and metagenome function prediction of microbiome amplicon data acquired from different sequencing strategies. We used this workflow to facilitate combined meta-analysis of individual microbiome surveys to define the healthy human

microbiome, in order to reliably identify pain related signatures. Taxa4Meta was then used to profile 5,691 matched controls and patients with functional gastrointestinal disorders (irritable bowel syndrome), neuropathic and inflammatory pain across North America, Europe, Asia and Australasia. Combined “pan-microbiome” profiles generated from individual microbiome surveys identified distinct enterotype clusters that were significantly associated with different types of gastrointestinal pain, anxiety and depression. By linking functional omics to disease-specific enterotypes, we identified new regulatory neurotransmitter circuits associated with chronic gastrointestinal pain, which were confirmed in humanized animal models.



Siavash Shahbazi Nia, PhD

Research Assistant

Pharmaceutical Sciences

Novel Selective Kappa-opioid Ligands as Modulators of Pain Responses

Having completed his Pharm-D in 2018 from RGUHS, India, Siavash Shahbazi Nia joined the Graduate Program at Texas Tech University Health Sciences Center in 2019. Siavash's research focuses on medicinal chemistry, and he is interested in developing novel ligands targeting opioid receptors. His project includes the design and synthesis of selective kappa-opioid ligands as a potential therapy for neuropathic pain. Siavash is a member of Dr. German research group.

Abstract: Using the structure of dehidroglutinin (fungal metabolite) as a starting point, we have prepared novel diketopiperazine-based ligands with a varying degree of selectivity between opioid subtypes. Selected compound with the preferential binding to KOR was tested in vivo, using the neuropathic pain model in rats. It showed the ability to modulate sensory and emotional pain-related behaviors in animals when administered 3 mg/kg intraperitoneally. These findings are in line with the existing data on the role of KOR-mediated signaling in the development of chronic pain conditions. Our compounds' chemical novelty, their favorable drug-likeness profile, and observed in vivo activity provide a platform for further developing these chemical agents as potential candidates for pain therapy.



Andrew J. Shepherd, PhD

Assistant Professor

Symptom Research

Targeting Neuroimmune Interactions for Relief of Neuropathic Pain

Dr. Shepherd received his Bachelors' in cell biology and PhD in neurobiology from the University of Manchester, UK. He developed an interest in the neuropharmacology of pain as a Postdoc and Instructor at the University of Iowa and Washington University in St. Louis. His research in the Department of Symptom Research at MD Anderson seeks to understand the changes in crosstalk between macrophages and sensory nerves in chronic pain states, with the goal of identifying new therapeutic opportunities to treat debilitating forms of pain.

Abstract: Background:

Hypothesis/Goals: The recent reports of analgesic efficacy of a type 2 angiotensin II (Ang II) receptor (AT2R) antagonist in phase II clinical trials suggests angiotensin signaling is involved in neuropathic pain. However, the mechanism of action was unclear.

Methods: We used the spared nerve injury model of neuropathic pain in mice, macrophage cell culture RNA sequencing and live cell imaging of sensory neurons to investigate the role of AT2R signaling in neuropathic pain and identify the mechanism of action underlying the analgesic efficacy of AT2R antagonists.

Results: AT2R expression was not detected in DRG neurons. Macrophages infiltrate sites of nerve injury and express AT2R. Activation of macrophage AT2R by elevated Ang II induces production of reactive oxygen species, which induce hyperexcitability via the redox-sensitive ion channel TRPA1. Experiments are underway to assess the generalizability of this mechanism to other forms of neuropathic pain (chemotherapy-induced neuropathic pain, painful diabetic neuropathy).

Conclusions: Macrophage AT2R signaling, and Ang II production at sites of tissue damage are crucial drivers of chronic neuropathic pain, which AT2R antagonists target to achieve pain relief.



Natasha Sosanya, PhD
Staff Scientist
Battlefield Pain and Sensory Trauma
Management

*Pipeline for Evaluating Efficacy of Novel Non-opioid Compounds for
Battlefield Injury-induced Pain*

Dr. Sosanya received her PhD in molecular neuroscience from the University of Texas at Austin in May 2014. There, she studied the local dendritic translation of a key potassium channel and its role in learning, memory, and temporal lobe epilepsy. During my postdoctoral fellowship, she developed the idea to study the role of plasma-derived extracellular vesicles (EVs) in the induction and maintenance of neuropathic pain. As a staff scientist, she has expanded on this research and identified biomarkers of nerve-injury and pain. This EV miRNA biomarker panel is currently under patent review. Furthermore, she has studied the use of EVs as analgesics in various animal models of pain. She has published seminal work from both her graduate and post-graduate research. She has also presented her research at various conference meetings, where it has been well-received. She is also the co-coordinator of the USAISR Summer Undergraduate Research Program. This program supported research performed by 16 undergrad interns with 10 USAISR PIs serving as mentors in 2020. They also support summer research performed by interns from the MRDC program, Historically Black Colleges and Universities (HBCU), and Alamo Colleges.

Key Publications

- Sosanya, N. M., Kumar, R., Clifford, J., Chavez, R., Dmitrov, G., Srinivasan, S., Gautam, A., Trevino, A. V., Williams, M., Hammameih, R., Cheppudira, B. P., Christy, R. J., Crimmins, S.L. (2019). Identifying Plasma Derived Extracellular Vesicle (EV) Contained Biomarkers in the Development of Chronic Neuropathic Pain. *J Pain*. 2020 Jan-Feb;21(1-2):82-96. doi: 10.1016/j.jpain.2019.05.015.
- Sosanya, N. M., Stacey, W., Garza, T., Christy, R. J., & Cheppudira, B. P. (2019). Brain- Derived Neurotrophic Factor Contributes to Chronic Stress-induced Enhanced Pain in a Thermal Injured Rat Model. *BMC Neurosci*. 2019 Apr 24;20(1):17. doi: 10.1186/s12868-019-0500-1.
- Sosanya, N. M., Brager, D. H., Wolfe, S., & Raab-Graham, K. F. (2015). Rapamycin reveals an mTOR-independent repression of Kv1.1 expression during epileptogenesis. *Neurobiology of Disease*, 1(73), 96-105. doi:10.1016/j.nbd.2014.09.011

• Sosanya, N. M., Huang, P. P. C., Cacheaux, L. P., Chen, C. J., Nguyen, K., Perrone-Bizzozero, N. I., & Raab-Graham, K. F. (2013). Degradation of High Affinity HuD Targets Releases Kv1.1 mRNA from miR-129 Repression by mTORC1. *Journal of Cell Biology*, 202(1), 53-69. doi:10.1083/jcb.201212089

Abstract: Recent advances in combat casualty care have resulted in an unprecedented survival rate for battlefield injuries of over 90%, and these injuries typically involve severe acute pain. Currently the standard of treatment for acute pain in the battlefield is opioid drugs, which can cause loss of consciousness, immobility, and inability to remain in the fight. Opioids also produce other negative effects such as dependence, tolerance, hyperalgesia, and cognitive and psychological impairment, that further reduce unit effectiveness. We are therefore testing a range of novel, non-opioid compounds with analgesic potential in battlefield-relevant models of pain and hemorrhage. These analyses combine three established pain models, two of which have been developed at our institution: 1) the full thickness thermal injury (FTTI) pain model, 2) a model for acute extremity trauma that includes hemorrhage (ET+HEM), and 3) the spinal nerve ligation (SNL) model. The FTTI and SNL models provide precise analgesic efficacy characteristics for the novel compounds, such as optimal dosing, timing and routes of administration. The ET+HEM model determines effects of analgesics on the compensatory hemodynamic and respiratory responses to moderate and severe hemorrhage, and survival to severe hemorrhage.

We have previously used the FTTI model to study the effects of morphine and other standard of care opioids as follows: 1.) to assess tolerance and hyperalgesia (Cheppudira et al, *BMC Anesthesiology*, 16:73, 2016), 2.) to determine efficacy of topical application, in an effort to reduce the overall opioid requirements (Clifford et al., *Burns*, 43:1709-1716, 2017) and 3.) as a standard for comparison for testing the analgesic efficacy of the novel non-opioid candidate drugs. We have shown that morphine is highly effective in suppressing both thermal hyperalgesia (TH) and mechanical allodynia (MA) at several times post thermal injury (Days 3,4,5,6,7), at a range of doses when administered intraperitoneally (2,5, and 10 mg/kg IP), and that the analgesic effects lasted for up to 2 hr post administration. In addition, topical administration of morphine to the burn wound site (0.1ml, 5mg/ml) in the FTTI model, produced comparable suppression of TH, with a lesser effect on MA. We have used the SNL model at very early time points post ligation to test the plasma secretome's ability to reduce nerve-injury induced nociceptive behavior and found that bath application of the ligated nerve with a secretome derived product reduces MA at 1 and 2 hours post SNL. With the ET model (without hemorrhage), behavioral responses to trauma were characterized and effects of intravenous (iv) opioid analgesics (morphine, fentanyl, sufentanil) and ketamine were assessed (Xiang et al., *J Trauma Acute Care Surg*, 85:S49-S56, 2018). Compared with the saline vehicle (VEH) group, opioid analgesics reduced MA for at least 80 minutes post injury. Opioids and ketamine were tested further in the complete ET+HEM model. When the volume loss of 40% was analyzed, opioids caused an increase in blood pressure and decrease in respiration, while ketamine had no effect on compensatory responses. Interestingly, i.v. administration of opioids given immediately after severe hemorrhage (55% blood volume loss) did not affect survival ($P = .55$).

Combining the use of these three distinct pre-clinical models in a 'pipeline' provides optimal candidate analgesics for clinical testing. This is a unique platform for determining both the precise analgesic effectiveness and the suitability for use in a severe polytrauma setting, for novel, non-opioid analgesics.

Disclaimers: Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC.

The views expressed in this abstract are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.

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Poster presenters in alphabetical order

First Name	Last Name	Institution	Abstract Title	Poster #
Hamsini	Ajjan	UT Dallas	<i>Headache and Psychiatric Outcomes in Adults after Mild Traumatic Brain Injury</i>	22
Heather	Allen	Duquesne Univ.	<i>Parabrachial CGRP Signaling Contributes to Amygdala Lateralization in the Context of Bladder Pain</i>	42
Bryan	Anthonio	Rice Univ.	<i>Decoding Sensorimotor Brain Networks Associated with Tongue Motor Control Generated in the fMRI and real-time fMRI neurofeedback environment using Linear Support Vector Machines</i>	16
Neha	Arora	MD Anderson Cancer Center	<i>Meningeal Fibroblasts Control Pain through PI16</i>	17
Dominic	Arris	UT San Antonio	<i>Sensory Nerve-Immune Interactions Regulate Oral Cancer Progression</i>	8
Amelia	Balmain	UT Dallas	<i>Physical Activity Counteracts Up-Regulation of Potential Pain Drivers in Chronic Bedrest Patients</i>	9
Henry	Blanton	Texas Tech Univ. Health Science Center	<i>The G-protein Coupled Estrogen Receptor Agonist G1 Antagonizes The Anti-Allodynic Effects Of The Cannabinoid Receptor Agonist CP 55,940 In A Cisplatin Model Of Chemotherapy-Induced Neuropathic Pain Using Ovariectomized Female Mice</i>	18
Nabila	Boukelmoune	MD Anderson Cancer Center	<i>Nasal administration of mesenchymal stem cells reverses chemotherapy-induced peripheral neuropathy</i>	35
Victoria	Brings	Washington Univ.	<i>Paw Incision Injury and Analgesic Doses of Opioids Induce Gait Alterations in Mice.</i>	20
Lily	Chen	Southeast Louisiana Veterans Health Care System	<i>Effect of Adolescent Alcohol Exposure and an Adult Pain Challenge on Nociception and CEA-PAG Circuitry</i>	43
Adam	Dourson	Cincinnati Children's	<i>Early Life Neuroimmune Interactions Modulate Neonatal Nociceptive Priming</i>	15
Olivia	Eller	Univ. of Kansas Medical Center	<i>Characterizing Nociceptive Response Properties During the Acute Phase of Spinal Cord Injury</i>	27
Anibal	Garza Carbajal	UT Health Science Center Houston	<i>Modulation of opioid responses in nociceptors by electrical activity after spinal cord injury.</i>	41
Stacey	Gorniak	Univ. of Houston	<i>Sex differences in tactile and proprioceptive function in persons with Type II Diabetes</i>	10
Kali	Hankerd	UT Medical Branch at Galveston	<i>TRPA1⁺ Afferents Maintain Persistent Mechanical Hypersensitivity in Female Nociceptive Pain Model</i>	23
Rasoul	Hekmati	Baylor College of Medicine	<i>Individualized fMRI-based Neuromodulation Increases Signal-to-noise-ratio in Somatomotor and Proprioceptive Awareness Networks : Implications in the Neuro-rehabilitation of Patients with Oral Neuropathic Pain</i>	25
Georgene	Hergenroeder	UT Health Science Center Houston	<i>Identifying Measurable Phenotypes for Pain after Spinal Cord Injury (SCI)</i>	1
Rebecca	Hornung	Texas A&M	<i>In-vivo Calcium imaging of the Parabrachial of Adult male Rats</i>	2
Duong	Huynh	Baylor College of Medicine	<i>Individualized Real-Time fMRI Neurofeedback Enhances Consistency of Voluntary Tongue Motor Control: Implications for Patients with Oral Neuropathic Pain</i>	3
Susmita	Kumari	MD Anderson Cancer Center	<i>Tim-3 Positive CD8 T cells and IL-13 Promote Resolution of chemotherapy-induced Peripheral Neuropathy</i>	33

First Name	Last Name	Institution	Abstract Title	Poster #
Jacob	Lackovic	UT Dallas	<i>The Effects of Nitric Oxide on Migraine Headache are Mediated by Peroxynitrite Formation</i>	38
Melissa	Lenert	UT Dallas	<i>Clinically Relevant Pain Assays for Fibromyalgia Applied to a Preclinical Model of Musculoskeletal Pain</i>	36
Jiahe	Li	MD Anderson Cancer Center	<i>Endogenous Activation of Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Alleviates Peripheral Neuropathic Pain</i>	4
Katherine	Lillis	UT San Antonio	<i>Trigeminal Ganglia Nociceptors Modulate Periapical Bone Loss in Apical Periodontitis</i>	5
Kathleen	McDonough	UT Medical Branch at Galveston	<i>Transition Mechanism of Nociceptive Pain in Males</i>	37
Bryan	Mckiver	Virginia Commonwealth University School of Medicine	<i>The Role Of Astrocyte Elevated Gene-1 (AEG-1), A Novel Multifunctional Protein, In Chronic Inflammatory And Neuropathic Pain</i>	26
Jason	Middleton	Southeast Louisiana Veterans Health Care System	<i>Chronic THC Vapor Attenuates Hyperalgesia and Alters Neuronal Function in the Ventrolateral Periaqueductal Gray (vlPAG) Male Rats with Chronic Inflammatory Pain</i>	24
Magali	Millecamps	McGill Univ.	<i>Inter- and Trans-generational Inheritance of Paternal Chronic Pain to Male and Female Offspring in Mice</i>	31
Sanjay	Neerukonda	UT Dallas	<i>A Pharmacological Interactome Platform for Discovery of Pain Mechanisms and Targets</i>	39
Amanda	Phang	Southeast Louisiana Veterans Health Care System	<i>Pramipexole Treatment Attenuates Mechanical Hypersensitivity in Rats with Chronic Inflammatory Pain</i>	6
Zhenpeng	Qin	UT Dallas	<i>PAR2 inactivation by molecular hyperthermia</i>	7
Pradipta	Ray	UT Dallas	<i>What can RNA-sequencing of human DRGs from pain patients teach us ?</i>	28
Jonathan	Richards	Drexel Univ.	<i>CCL2-Induced Macrophage Accumulation in the Dorsal Root Ganglia Correlates With Persistent Paw Hypersensitivity</i>	40
Daneil	Sauter	Sophion Bioscience Inc.,	<i>Biophysical and Pharmacological Profiling of Multiple Voltage-Gated Sodium Channel Subtypes on QPatch II</i>	13
Meredith	Singer	Drexel Univ.	<i>Unbiased Analysis of Primary Afferent Fibers in Spinal Cord Injured Rats with and without Strength Training</i>	21
Anand	Singh	MD Anderson Cancer Center	<i>A Highly Selective A3 Adenosine Receptor (A3AR) Agonist, MRS5980 Prevents and Reverses Cisplatin-Induced Cognitive Impairments, Motor Incoordination and CIPN in Mice</i>	14
Richard	Slivicki	Washington Univ.	<i>The Cannabinoid Agonist CB-13 Produces Acute Peripherally-Mediated Analgesia in Mice and Reduces Measures of Neuronal Hyperexcitability in Mouse DRG Yet Repeated Dosing Elicits Tolerance and Signs of CNS Activity</i>	29
Rena	Stair	Univ. of Kansas Medical Center	<i>TIMP-1 Attenuates Hypersensitivity Through CD63 Signaling</i>	30
Diana	Tavares-Ferreira	UT Dallas	<i>Single cell transcriptome of the human DRG</i>	44
Meilinn	Tramm	UT San Antonio	<i>Effects of a High Omega-6 Diet on Orofacial Allodynia and Gene Expression Patterns in the Trigeminal Ganglia</i>	32
Adam	Willits	Univ. of Kansas Medical Center	<i>Spinal Cord Injury-Induced Chronic Pain: A Role For Bacterial Translocation And DNA Damage In Bowel Pain After Injury</i>	47

First Name	Last Name	Institution	Abstract Title	Poster #
Sarah	Woller	NIN/NINDS	<i>HEAL Initiative Preclinical Screening Platform for Pain: Evaluation of Novel Non-Opioid, Non-addictive Therapeutics</i>	34
Muhammad Saad	Yousuf	UT Dallas	<i>Taking the Road Less Traveled: Unconventional Translation Control in Pain Pathogenesis</i>	11
Jixiang	Zhang	MD Anderson Cancer Center	<i>HDAC6 Inhibition Reverses Chemotherapy-Induced Neuropathic Pain in an IL-10 Dependent Manner</i>	12

Headache and Psychiatric Outcomes in Adults after Mild Traumatic Brain Injury

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Background: More than 2.8 million Americans experience a traumatic brain injury (TBI) every year. The vast majority of these TBI incidences are of mild severity. Headache is one of the most common persistent post-injury problems in individuals with mild traumatic brain injury (mTBI). Inhibition of pain modulatory functions post mTBI can lead to post-traumatic headache (PTH). Persistent headache is associated with anxiety and depression. There may worse outcomes for people with comorbid mTBI and PTH, particularly for anxiety and depression. There is recent evidence to suggest worse depression with comorbid mTBI and PTH. However, differences in anxiety have not been explored.

Hypothesis: In this study, we examined differences in psychiatric outcomes among adults with and without PTH. It was expected that individuals with PTH would have worse psychiatric symptoms.

Methods: Participants consisted of 81 adults who received care at a university-based concussion clinic following mTBI (confirmed by clinician). Self-reported headache was used to determine PTH status at the time of evaluation. Individuals were grouped into those with PTH ($n=64$) and those without PTH ($n=17$). Each participant completed the Psychiatric Diagnostic Screening Questionnaire (PDSQ) as part of the clinical intake process. The PDSQ is a brief measure to screen for common psychiatric diagnoses. The anxiety and depression scales were examined for this study. Raw scores were used as well as categories of people with scores above and below the clinical cut-off scores for indication of anxiety and/or depression. A t-test was conducted on PDSQ raw scores to investigate differences in symptom reporting between PTH groups. In addition, a chi-square test of independence was conducted to evaluate the relationship between PTH status and individuals with positive screens for anxiety and/or depression.

Results: There were no significant differences ($p > .05$) in age, gender, race, education, and days since injury by PTH status. Participants, on average, were 37 years old with 14 years of education. The sample was near even for men (46%) and women (54%). Majority of the participant were Caucasian (89%). There were no significant differences in depression ($t(72) = -0.75, p = .457$) or anxiety ($t(72) = -0.28, p = .779$) symptoms between PTH groups. There were no association between psychiatric screen status for depression and PTH status, $X^2(1) = 0.59, p = .442$. No association for anxiety screen status ($X^2(1) = 0.12, p = .732$).

Conclusions: Comorbid PTH and mTBI did not reveal worse psychiatric symptoms in this study, surprisingly.

Parabrachial CGRP Signaling Contributes to Amygdala Lateralization in the Context of Bladder Pain

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Background

Urologic chronic pelvic pain syndromes are among the most common visceral pain conditions in the United States. The central amygdala (CeA) is a bilateral, mid-brain limbic region that processes both pain and emotion. Sensory neurons that relay information from the bladder to the brain express high levels of calcitonin gene-related peptide (CGRP). Ample evidence suggests CGRP is pro-nociceptive in the right CeA across pain models, but few studies target CGRP in the left CeA.

Hypothesis/Goals

The aim of this study is to explore the potential differential effects of CGRP in the left and right CeA. We hypothesize that CGRP contributes to amygdala lateralization in a mouse model of bladder pain.

Methods

Bladder pain was induced using repeated cyclophosphamide injections. Adult female C57 mice used for pharmacology received implantation of a cannula in either left or right CeA for drug delivery. Adult female *Calca-Cre-GFP* (CGRP-Cre^{+/-}) mice used for optogenetics had a Channelrhodopsin or Halorhodopsin virus injected into the right or left parabrachial nucleus and a fiberoptic implanted in the corresponding CeA. Bladder pain-like behavior was assessed using von Frey, and bladder pain-like physiology was assessed using urinary bladder distention.

Results

CGRP decreased bladder-pain like physiology when infused into the left CeA but increased bladder pain-like physiology when infused in the right CeA. Infusion of a CGRP antagonist in the right CeA decreased bladder pain but infusion in the left CeA increased bladder pain. Optogenetic activation of CGRP terminals in the right CeA increased bladder pain-like physiology while optogenetic inhibition decreased bladder pain-like physiology. Optogenetic activation of CGRP terminals in the left CeA decreased bladder pain-like behavior and physiology, and optogenetic inhibition increased visceromotor responses. Use of CGRP knockout animals (CGRP-Cre^{+/-}) confirmed the role of CGRP in modulating bladder pain-like responses.

Conclusions

Our data suggests a novel anti-nociceptive role for CGRP in the left CeA as well as an opposing pro-nociceptive role of CGRP in the right CeA in the context of bladder pain.

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Decoding Sensorimotor Brain Networks Associated with Tongue Motor Control Generated in the fMRI and Real-time fMRI Neurofeedback Environment using Linear Support Vector Machines

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Background and Significance: The development of brain-computer interfaces (BCI) has attracted significant attention in neuroscience research. BCIs, outside the MRI environment offer neuro-rehabilitative function to patients with motor and sensory impairments by decoding brain activity with the goal to operate external devices, such as prosthetics. The Papageorgiou lab has developed an MRI-compatible BCI, referred to as individualized real-time fMRI neurofeedback (iRTfMRI-nFb) for the neurorehabilitation of motor disorders, which affect speech, swallowing, and eating. Current BCI research focuses on increasing the sensitivity of decoding cortical networks using machine learning algorithms.

Goal: Our aim was to assess the ability of linear support vector machines (SVMs) to decode tongue movement, using blood-oxygen-level-dependent (BOLD) signal of the entire brain as input, in the iRTfMRI nFb and fMRI-control conditions.

Hypothesis: Linear SVM will achieve higher classification accuracies for brain networks generated in the iRTfMRI nFb condition as opposed to those generated in the fMRI-control condition.

Methods: We decoded participants' brain networks generated by tongue-motor-control in four directions (up; down; left; right) using Linear SVMs. The models were trained to distinguish between brain activity occurring during either up versus down, or left versus right tongue movement for the iRTfMRI nFb and fMRI-control conditions, respectively. We evaluated the performance of the linear SVM classifiers using a five-fold cross-validation procedure: in each iteration, the models were trained on brain data from 15 participants and predictive accuracy was evaluated on data from 5 participants not included in the training dataset.

Results: For decoding left versus right tongue movement in the iRTfMRI nFb and fMRI-control conditions, the linear SVM achieved classification accuracies of 74.70% and 74.00%. For decoding up versus down tongue movement in the iRTfMRI nFb and fMRI-control conditions, the classifier achieved accuracies of 63.25% and 60.94%.

Conclusion: These findings show that we can decode sensorimotor networks associated with tongue motor control. Thus, the optimization of machine learning algorithms for decoding brain activity is of utmost importance in iRTfMRI-nFb for the effective neuro-rehabilitation of cortical sensorimotor lesions.

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Meningeal Fibroblasts Control Pain through PI16

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Background: Chronic pain is a debilitating condition with limited treatment options. Dorsal root ganglia (DRG), which contain cell bodies of nociceptive neurons have been studied extensively for their role in chronic pain. DRG also contain non-neuronal cells including glia, macrophages and T cells, that participate in maintenance and resolution of pain. So far, fibroblasts of DRG meninges have been misconstrued as an inert cell type with no role in pain etiology.

Hypothesis: Fibroblasts play a key role in pain via release of Peptidase Inhibitor 16 (PI16), which promotes chronic pain by modulating vascular permeability.

Methods: Male and female Pi16^{-/-} and WT mice were exposed to spared nerve injury (SNI) or treated with intraplantar Complete Freund's adjuvant (CFA) to induce pain. PI16 protein expression was visualized by immunofluorescence and the underlying mechanisms were identified by RNA sequencing and immunofluorescence analysis on cellular infiltrates. Vascular permeability was examined *in vivo* using sodium fluorescein; trans-endothelial migration was assessed in a trans-well migration assay.

Results: Under baseline conditions, we detected PI16 in meningeal and perineurial fibroblasts, with no detectable protein in other cells in DRG, nerve, spinal cord or brain. SNI increased PI16 in fibroblasts in meninges of DRG and peripheral nerve, whereas CFA increased PI16 in the perineurium of sciatic nerve only. *In vitro*, overexpression of PI16 promoted the trans-endothelial migration of monocytes in response to chemoattractant CXCL2. *In vivo*, male and female Pi16^{-/-} mice are protected against neuropathic pain. This was associated with reduced endothelial permeability, lower leukocyte infiltration and reduced activation of myosin light chain kinase (MLCK). Moreover, female mice recover more quickly from inflammatory pain, whereas male mice are partially protected. Intrathecal administration of anti-Pi16 antibody promoted recovery from CFA-induced mechanical allodynia in males and females. We are investigating the contribution of leukocyte infiltration and inflammatory activity to the protective effect of PI16 deletion on inflammatory pain.

Conclusion: We identified a novel and critical role of fibroblasts in neuropathic and inflammatory pain through production and release of PI16, a poorly defined protein that may act as a peptidase inhibitor. We propose that PI16 secretion enhances endothelial permeability via a pathway involving endothelial MLCK, leading to leukocyte migration into the DRG and nerve, and persistent pain. In view of limited cellular and organ distribution of PI16, it could be an attractive novel and safe target for pain management.

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Peripheral Contribution of BDNF/TrkB Signaling in Mediating Oral Cancer Pain

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Background: More than 61,000 people are estimated to develop head and neck cancer (HNC) each year. Persistent and inadequately treated pain due to HNC causes many patients to seek health care. Indeed, 70-85% of HNC patients report pain as their top symptom. The greater prevalence and severity of pain experienced by HNC patients underlines the critical need for research on the mechanisms of HNC pain in order to develop novel analgesics with lesser side effects. Recently, the role of brain derived neurotrophic factor (BDNF) has been observed to play a role in inflammatory and neuropathic pain conditions. Importantly, BDNF has been showed to mediate pain maintenance in bone cancer models. These studies have primarily focused on central mechanisms of BDNF which canonically mediates and is isolated to the central nervous system. Interestingly, prior studies have seen that BDNF signaling is significantly increased in head and neck cancer (HNC) tumors and contributes to tumor progression. Considering the severity and large gap in knowledge regarding mechanisms of OSCC HNC pain, we thought it appropriate to clarify this contribution of peripheral BDNF signaling to debilitating pain state.

Goals: The current study investigates the role of OSCC derived BDNF and how it activates adjacent sensory fibers, contributing to OSCC-induced pain.

Methods/Results: We found that OSCC derived BDNF is increased in multiple oral cancer cell models and that blocking local BDNF reverses pain behaviors in tongue tumor bearing animals. Moreover, animals with the BDNF receptor - TrkB - knocked down in sensory afferents had decreased pain under tumor burden. Ex vivo evidence suggests that tumor-induced TrkB activities contributed to the hypersensitivity of A-slow HTMR fibers but not of C-fibers.

Conclusion: Taken together, our data suggest that TrkB+ sensory afferents are sensitized and exert a nociceptive effect on OSCC bearing animals via local release of OSCC derived BDNF.

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Physical Activity Counteracts Up-Regulation of Potential Pain Drivers in Chronic Bedrest Patients

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Background: Exercise is a well-established means of reducing the prevalence and intensity of chronic pain, thus it is typically inferred that physical inactivity is a risk factor for chronic pain onset. Studies have demonstrated skeletal muscle undergoes transcriptional changes following acute physical inactivity, although how these changes may contribute to nociceptive hypersensitivity remains unclear.

Hypothesis: We hypothesized that chronic physical inactivity would induce transcriptional changes in skeletal muscle tissue associated with the promotion of nociceptive hypersensitivity.

Methods: Skeletal muscle biopsies (n=21) from a NASA bedrest study were donated by Dr. Benjamin D. Levine. Briefly, healthy subjects (20-54 years) were randomly assigned to control group (35 days bedrest only; 'CON', n=7, 1 female) or intervention (35 days bedrest with exercise; 'EX', n=14, 2 female). RNA sequencing of muscle biopsies taken pre- and post-bedrest was performed and differential expression analysis conducted using DESeq2. Functional enrichment analysis was used to identify biological functions and molecular pathways associated with differentially expressed genes (DEGs). DEGs with potential for effects on sensory nervous system were then curated using a genome-wide ligand-receptor pair database curated for pharmacological interactions relevant to sensory neurons.

Results: Of 58,763 total genes (includes all possible splice variants) in the reference genome, we detected 36,072 gene variants in our samples. Differential expression analysis found 1352 DEGs in CON subjects. Gene ontology enrichment analysis of CON DEGs found transcription regulation, interferon- γ mediated signaling, and major histocompatibility complex class I to be key up-regulated biological processes. In EX subjects, 133 DEGs were identified, 22 of which were found to be differentially expressed in both EX and CON groups. Pharmacological interactome analysis of CON DEGs identified 48 genes which coded for secretory ligands with known human DRG receptors, including known pain modulators adrenomedullin and CCL2.

Conclusions: Chronic physical inactivity results in significant transcriptomic changes in skeletal muscle, including up-regulation of multiple ligands with potential for effects on the sensory nervous system. These may represent potential drivers of chronic pain associated with physical inactivity, and warrant further investigation. The introduction of an exercise countermeasure during bedrest prevented most of the transcriptional changes observed, including those with potential for effects on the sensory nervous system, further supporting a role for the protective effects of exercise.

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The G-protein Coupled Estrogen Receptor Agonist G1 Antagonizes the Anti-Allodynic Effects of the Cannabinoid Receptor Agonist CP 55,940 in a Cisplatin Model of Chemotherapy-Induced Neuropathic Pain Using Ovariectomized Female Mice

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Background: Women are at a greater risk for chronic pain, and relief from chronic pain is the most commonly reported reason for use of cannabis/cannabinoids for medical purposes. Preclinical studies have demonstrated sex differences in endocannabinoid system expression and sensitivity, and in females differences have been reported as a function of estrous cycle stage. Studies have shown that in ovariectomized females, response to exogenous cannabinoids in the context of pain can be influenced by estrogen replacement. To date, however, no studies have evaluated the G-protein coupled estrogen receptor (GPER) in the context of pain modulation by the endocannabinoid system.

Hypothesis/Goals: GPER and the type 1 cannabinoid receptor (CB1R) are expressed in many of the same regions of the brain, and activation has been shown to have opposing effects on pain, GPER activation promoting pain, and CB1R activation decreasing pain. Our goal was to determine if co-administration of a GPER agonist (G1) would block the pain relieving effects of a synthetic cannabinoid (CP 55,940), and to determine if the development of tolerance to CP 55,940 was altered by co-administration with this compound.

Methods: Female C57BL/6J mice were ovariectomized and allowed to recover. Mice were treated with cisplatin once per week, for four weeks, to model a chronic neuropathic pain state. We administered vehicle, CP 55,940 (0.3 mg/kg), G1 (0.2 mg/kg) and a combination of CP 55,940 and G1, daily, starting at experiment day 8. We evaluated mechanical and cold allodynia (von Frey and acetone tests), hypothermia, and motor impairment (rotarod and open field test).

Results: Cisplatin produced mechanical and cold allodynia which were reversed by the cannabinoid CP 55,940. Conversely, the GPER agonist G1 potentiated mechanical and cold allodynia. When the two compounds were combined the anti-allodynic effects of CP 55,940 were antagonized by G1, however the development of tolerance to CP 55,40 alone and in combination with G1 did not significantly differ. Notably, tolerance to the hypothermic and motor-impairing effects of Cp 55,940 developed much faster than development of tolerance to the anti-allodynic effects.

Conclusions: This work suggests that through GPER the pain-relieving effects of cannabinoids may be diminished. However, if estrogen contributed to the development of tolerance to cannabinoid compounds, it likely acts through mechanisms independent of GPER such as through estrogen's nuclear steroid receptors ER α and ER β .

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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 frequently reported adverse side effects of cancer treatment. CIPN has a prevalence of 30-80%, often persists after treatment completion, and affects patient's quality of life. Currently, there are no effective FDA-approved drugs to prevent or reverse CIPN. Growing evidence implicates mitochondrial dysfunction in the peripheral nervous system in the etiology of CIPN. Mesenchymal stem cells (MSC) have been demonstrated to stimulate tissue repair and ameliorate outcome in preclinical models of cerebral insults, neurodegenerative disorders and peripheral nerve damage. We showed previously that nasal administration of MSC reverses cisplatin-induced cognitive impairment in mice. In this study, we examined the therapeutic effects of nasal administration of MSC on CIPN.

Hypothesis/Goals: Nasal MSC treatment reverses peripheral neuropathy and restores mitochondrial bioenergetics in cisplatin-treated mice.

Methods- Mice received cisplatin (2.3 mg/kg/day) or saline for 2 cycles of 5 daily injections with 5 days of rest in between. MSC were administered nasally at 48 and 96 h after the last dose of cisplatin. CIPN was assessed prior to cisplatin and nasal MSC treatment as well as 5-21 days after the last dose of MSC. Mechanical allodynia was measured using von Frey hairs; spontaneous pain was tested using a conditioned place preference test. Mitochondrial function of dorsal root ganglia (DRG) was determined by Seahorse Flux analysis. Intraepidermal nerve fiber density and the fate of MSC were analyzed using confocal microscopy. Intracellular IL-10 production by macrophages was determined by flow cytometry.

Results- Nasal MSC administration after cisplatin completely resolved established mechanical allodynia, spontaneous pain, and loss of intraepidermal nerve fibers in the paw. Resolution of CIPN after MSC administration was associated with normalization of cisplatin-induced decrease in mitochondrial bioenergetics of DRG. Nasally administered MSC entered the meningeal compartment of the brain, spinal cord and peripheral lymph nodes. Macrophages in the meninges of the brain and in lymph nodes increased their IL10 production after MSC. MSC from *Il10*^{-/-} mice failed to promote resolution of CIPN. Finally, MSC did not resolve CIPN symptoms in mice lacking IL-10 receptors on peripheral sensory neurons.

Conclusions- Nasal administration of MSC reverses the various symptoms of CIPN. Resolution of CIPN is associated with normalization of mitochondrial bioenergetics in the peripheral nervous system. Mechanistically, we propose that MSC act through an IL10-dependent pathway via IL-10 derived from MSC and/or via inducing IL10 in macrophages.

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Paw Incision Injury and Analgesic Doses of Opioids Induce Gait Alterations in Mice

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Background: Mice are widely used to study the behavioral effects of opioids, including analgesia and dependence. Anti-nociceptive properties of opioids are often tested in uninjured mice using reflexive hot plate and tail flick assays. To study analgesia in injured mice, scientists evoke pain responses by directing noxious stimuli to the site affected by the injury, which is often the hind paw. However, applying stimuli to the hind paw in an opioid-treated mouse is challenging due to opioid-induced increases in locomotor activity.

Hypothesis/Goals: We aimed to overcome this challenge by using gait analysis to observe hind paw usage during walking in mice. We hypothesized that hind paw injury would alter usage of the injured limb and analgesic opioids would ameliorate sensitization phenotypes.

Methods: Using C57BL/6 mice, we measured changes in paw print area following induction of post-surgical pain (using the paw incision model) and reversal with oxycodone.

Results: Paw incision surgery reduced weight bearing on the incised section of the paw, resulting in a decrease in the paw print area of the injured hind paw. At a dose that reversed incision-induced mechanical allodynia in the von Frey assay (10 mg/kg), oxycodone caused a tiptoe-like gait in mice, resulting in a reduced paw print area in both hind paws. Further investigation of this opioid-induced phenotype revealed that analgesic doses of oxycodone or morphine dose-dependently reduced front and hind paw print area in uninjured mice. Interestingly, the gait changes caused by opioids were not caused by opioid-induced increases in locomotor activity; speed and paw print area had no correlation in opioid-treated mice, and other analgesic compounds that alter locomotor activity did not affect paw print area.

Conclusions: Whereas post-surgical injury produced a change in hind paw usage measurable through gait analysis, the opioid-induced “tiptoe” phenotype prevented gait analysis from being a viable metric for demonstrating opioid reversal of pain sensitization in injured mice. The effect of opioids on paw usage suggests that scientists should exercise caution when using hind paw-directed nociceptive assays to test opioid analgesia in mice. Additionally, this study adds to the literature on opioid-induced muscle rigidity, providing evidence of how that stiffness affects function by impacting walking activity in mice. Therefore, our characterization of how opioids affect gait has important implications for the use of mice to study opioid pharmacology.

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Effect of Adolescent Alcohol Exposure and an Adult Pain Challenge on Nociception and CEA-PAG Circuitry

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Background

Adolescence is commonly a time when alcohol use is initiated, and the manner of consumption is often binge-like. This adolescent alcohol exposure has been linked to increased likelihood of developing an alcohol use disorder (AUD) in adulthood. Chronic alcohol use has been shown to cause hyperalgesia and exacerbate existing pain. This effect is particularly concerning because adults often report utilizing alcohol to alleviate pain, when in fact it may result in opposite effects. One important pathway shown to mediate pain and hyperalgesia is the central amygdala (CeA) to the ventrolateral periaqueductal gray (vlPAG) circuit. The CeA sends GABAergic projections to the PAG and modulates descending pain pathways.

Hypothesis/Goals

The current study sought to elucidate the long-term effects of adolescent alcohol exposure and a subsequent adult pain challenge on behavior and regulation of the CeA-PAG circuit.

Methods

To model adolescent alcohol use, male adolescent Wistar rats (PND30-56) were subjected to four weeks of intermittent ethanol exposure (AIE) and then aged into adults with no alcohol (four weeks). Throughout this eight week period, we assessed nociception using behavioral assays, the Von-Frey and Hargreaves test. The rats were then injected with green retrobeads in the vlPAG and then slices were taken from the CeA to identify the CeA-PAG circuit. An adult pain challenge (paw injection of formalin) was then given to assess behavior and CeA-PAG activation through electrophysiology, immunohistochemistry, and RNAscope.

Results

Our results show that AIE produces stable blood alcohol levels in adolescent rats, and that AIE rapidly induces thermal hyperalgesia and mechanical allodynia. Consistent with previous data in adult alcohol-exposed rats, we find a significant reduction in synaptic drive and excitatory/inhibitory ratio in rats with a history of AIE. Moreover, these AIE-exposed rats have a significant reduction of cFos staining in the CeA→PAG projectors compared to controls. Behaviorally, these AIE rats also have higher pain sensitivity to application of a noxious stimulus (formalin).

Conclusions

Long-lasting AIE hyperalgesia may drive future alcohol use and/or increased sensitivity to pain challenges. Further studies will be conducted to further characterize this circuit.

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Early Life Neuroimmune Interactions Modulate Neonatal Nociceptive Priming

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Background: Young children perceive nociceptive stimuli and experience pain differently than adults, but the underlying mechanisms that cause these differential effects are unknown. The result of this uncertainty can lead to incomplete pain management. Further, a vulnerable period occurs during early life whereby aversive stimuli can be biologically encoded, but the mechanisms that underlie this are not well understood. As macrophages are unique in neonates, control the microenvironment, effect pain-like behaviors, and can retain memory in their epigenome (trained immunity), we are investigating how altered early life neuroimmune and endocrine interactions influence sensory responsiveness later in life. We evaluated the role of a specific neonatal mechanism of nociception through macrophage control of growth hormone (GH) signaling to the primary afferent, and the role that macrophages play in neonatal priming.

Hypothesis: We hypothesize that early life endocrine and neuroimmune interactions and macrophage epigenetic memory contribute to neonatal acute phenotypes and neonatal priming.

Methods: We assessed pain-like behaviors, sensory neuron response properties with *ex vivo* electrophysiology, and tissue specific gene regulation in neonatal and adolescent animals. Animals experienced either early life acute incision with some animals receiving repeat adolescent incision. Genetic manipulations included sensory and macrophage specific knockout of the GH receptor (GHR) and conditional knockout of macrophages in macrophage fas induced apoptosis animals (MaFIA) animals. ATAC-seq was completed on isolated macrophages.

Results: We first found that sensory neuron deletion of the receptor for the endocrine signaling molecule GH induced pain-like behaviors in neonates. Acute peripheral sensitization after incision injury was prevented by local GH treatment, and macrophage ablation of GH receptor also prevented incision-related effects. Macrophages appear to sequester injury-site GH, which releases a tonic inhibition on the sensory neurons and drives primary afferent sensitization. In MaFIA animals, neonatal treatment with AP20187, which induces cell-specific apoptosis, was found to blunt incision related pain-like behaviors, and change the injury microenvironment of pro- and anti-inflammatory factors. Manipulations in GH signaling or ablating neonatal macrophages was found to be critical for the development of neonatal priming. New ATAC-seq data indicates differences in chromatin accessibility in immature macrophages which may underlie how early life neuroimmune interactions modulate neonatal priming effects.

Conclusions: Results demonstrate that the periphery has unique modulators of neonatal nociception that often require distinct immune cell and endocrine signaling activities that influence sensory neuron function. We are currently investigating how altering these cells may affect neonatal priming through epigenetic modifications in macrophages.

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Characterizing Nociceptive Response Properties During the Acute Phase of Spinal Cord Injury

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Background: Spinal cord injury (SCI) affects 276,000 people in the United States alone and costs up to \$4.5 million over a patient's lifetime. The majority of individuals with SCI report persistent pain and half of these patients report severe pain. This pain is often located below the level of injury, usually does not present for weeks to months, and is refractory to medical treatment. The exact mechanisms that lead to SCI pain are not fully understood, but SCI-induced alterations in nociceptor function are hypothesized to contribute to its development and persistence.

Goals: It is not known how SCI contributes to functional changes in individual nociceptors acutely following injury. The goal of this project was to quantify electrophysiological, anatomical, and molecular changes in peripheral below-level tissues during the acute phase of SCI.

Methods: Female C57BL/6J mice were randomly assigned to SCI and naïve conditions, and SCI mice were subjected to a T9 spinal contusion injury (65 kD, 1 s dwell). Mice were sacrificed 24 hr or 7 days following SCI. In a subset of mice, electrophysiological recordings were performed 24 hr following SCI, using our *ex vivo* skin/nerve/DRG/ spinal cord preparation, and primary afferent response properties were characterized in the presence and absence of mechanical and thermal stimulation of the skin. In a separate cohort of mice, hindpaw thickness was assessed to track the development of edema, and skin was collected to measure levels of calcitonin gene-related peptide (CGRP), substance P, and nerve growth factor β . Intraepidermal nerve fiber density (IENFD) counts were also performed on hindpaw skin following staining with PGP9.5.

Results: Our studies reveal that SCI increased the incidence of spontaneous activity and after discharge, as well as reductions in mechanical and heat firing thresholds in specific subsets of nociceptors 24 hr following SCI. We also found that injured animals display significant hindpaw edema that is associated with increased levels of CGRP and decreases in IENFD.

Conclusions: These results indicate that electrophysiological and anatomical changes occur shortly after SCI in afferents that innervate the skin. Acute alterations in nociceptor response properties, increased peripheral CGRP release, and distal nerve degeneration may play a significant role in the development of pathologic pain during times when pain is unreported and often untreated.

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TRPA1⁺ Afferents Maintain Persistent Mechanical Hypersensitivity in Female Nociplastic Pain Model

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Background: Normally resolving pain can transition into nociplastic pain, which is persistent pain despite the resolution of a non-neuropathic injury. While nociplastic pain conditions predominately affect women, it is unknown whether sex-specific mechanisms are present. To this end, we developed murine model in which normally resolving mechanical hypersensitivity after an injury transitions to persistent hypersensitivity without ongoing inflammation, mimicking a nociplastic pain state. This model showed sexually-dimorphic mechanisms of the nociplastic pain state maintenance; specifically, in females but not in males, persistent mechanical hypersensitivity was maintained by ongoing afferent activity at the previous injury area. **Goals:** Our goals were to 1) identify the afferent population(s) sustaining persistent mechanical hypersensitivity in females, 2) determine how these afferents are sensitized, and 3) determine whether estrogen is a critical mediator of the sexually-dimorphic mechanisms. **Methods:** Normally resolving pain was induced by injecting capsaicin intradermally into the hindpaw of C57BL/6N mice. Two hours later, the injected hindpaw was stimulated with 40°C water immersion (i.e. “capsaicin plus warmth”) to induce the transition from acute to persistent mechanical hypersensitivity. Seven to ten days later, behavioral studies or *ex vivo* skin-nerve electrophysiology recordings were conducted. For behavior studies, bupivacaine or the lidocaine derivative QX-314 together with TRPA1 agonist were injected into the capsaicin-injected area. Percentage withdrawal to a normally innocuous (0.98 mN) von Frey filament was recorded. For skin-nerve recordings, spontaneous firing frequency and response profiles to ramp mechanical stimulation were obtained from mechano-nociceptors. For estrogen studies, females were bilaterally ovariectomized (OVX) at 5 weeks of age; a subset of OVX females were implanted with a 17 β -estradiol containing mini pump at 6 weeks. **Results:** Selective silencing of TRPA1⁺ afferents at the previous injury area in females attenuated persistent mechanical hypersensitivity. Skin-nerve electrophysiology revealed that TRPA1⁺ afferents innervating the injury area of capsaicin plus warmth females 1) had a greater cumulative number of action potentials during ramp stimulation and 2) were more likely to be spontaneously active than TRPA1⁻ afferents or TRPA1⁺ afferents innervating the capsaicin injection area in females treated with capsaicin alone. Silencing afferents at the previously capsaicin-injected area with local bupivacaine attenuated persistent mechanical hypersensitivity in sham-OVX and 17 β -estradiol-reconstituted OVX females, but not OVX females. **Conclusions:** These results indicate that sensitized TRPA1⁺ afferents at the previous injury area maintain the nociplastic pain in females. The presence of female hormones, specifically 17 β -estradiol, is a critical determinant as to whether females develop ‘peripherally maintained’ nociplastic pain state.

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Individualized fMRI-based Neuromodulation Increases Signal-to-noise-ratio in Somatomotor and Proprioceptive Awareness Networks: Implications in the Neuro-rehabilitation of Patients with Oral Neuropathic Pain

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Background: Tongue movement control is accomplished by the glossopharyngeal (CNIX) and hypoglossal (CNXII) cranial nerves. Injury to these nerves can be supra- or infra-nuclear to the brainstem's medulla oblongata, following stroke, brain, or head and neck tumors. The sequelae of CN IX and/or XII injuries can result in neuropathic tongue and oral pain, partial paralysis of the tongue, swallowing, mastication, and speech impairment. The prevalence of cranial nerve neuropathy varies and can be as high as 48% following head and neck cancer radiotherapy treatment. In this study, we applied an fMRI-based brain computer interface with the goal to enhance voluntary tongue movement selectivity in each direction in a consistent fashion. This method aims to induce upregulation of the Blood-Oxygen-Level-Dependent (BOLD) signal via individualized real-time fMRI neurofeedback (iRTfMRI nFb). Our method is based on bypassing the lesioned pathway and providing nFb to individualized networks that are intact and can become functionally associated to the lesioned one.

Hypothesis/Goals: The overall goal is to quantify the BOLD neuromodulation generated by iRTfMRI nFb for its use in the neuro-rehabilitation of lower cranial nerve injury. The immediate goal of this study is to enhance consistency of voluntary tongue movement in four directions in healthy subjects. Our long-term goal is to apply this method as a therapeutic modality to patients following sustenance of lower cranial nerve injury. We hypothesize that nFb in comparison to control-no nFb will: (i) increase the area under the curve (AUC), generated by the BOLD's percent signal change in somatomotor and attention areas for each of the tongue movements, and (ii) decrease the BOLD variance in these same networks.

Methods: Healthy subjects (n=30) participated in a two-day iRTfMRI nFb study. On day one, we decoded the individualized cortical patterns generated by tongue movement in four directions (up; down; left; right). On study-day two, each subject's individualized network for tongue movement direction selectivity was upregulated using iRTfMRI nFb. The AUC generated by the BOLD's PSC between nFb and control-no nFb, was computed using a sensitivity index D prime across networks, as a function of time. We measured the difference in the BOLD's variance for the nFb versus the control across subjects and visualized it using t-SNE, a dimensionality reduction technique.

Results: iRTfMRI nFb is characterized by somatosensory and somatomotor (intraparietal lobule, basal ganglia, thalamus), attention (middle and inferior frontal gyri) and proprioceptive awareness (insula) bilateral networks. These areas are significantly activated 16 secs after onset of tongue movement for a total of 20 secs, as quantified by signal detection (d' prime) and area under the curve (AUC; Simpson's rule) analyses. To quantify t-SNE variances in each of these networks as a function of time, we calculated the variance across blocks for each nFb-generated network, which showed a significant decrease in variance when compared to the control's networks for each of Motor, Sensory, and Attention networks (10% decrease for Motor; 14% for Sensory; 18% for Attention; $p < 0.009$).

Conclusions: This study shows that the purposeful induction of upregulation via iRTfMRI nFb can achieve enhanced control of voluntary tongue movement in each of the four directions by increasing the AUC and decreasing the variance of the BOLD signal. Our quantified findings can lead to clinical applications for the neuro-rehabilitation of patients who have sustained lower cranial CNIX and CNXII injury.

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Identifying Measurable Phenotypes for Pain after Spinal Cord Injury (SCI)

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Background: The authors previously identified autoantibodies to GFAP and/or CPM2 as prognostic in the development of SCI neuropathic pain (NP). As there is no universally accepted objective test for diagnosing NP, the clinical diagnosis remains the standard on which most tools are based. Interpatient variability in perception of pain and response to analgesics is recognized and, therefore, identifying measurable phenotypes for pain and treatment outcomes is essential to provide personalized treatment for pain. A personalized treatment approach may improve pain control and reduce the misuse and morbidity associated with opioids. Recommended NP phenotyping domains include quantitative sensory testing (QST).

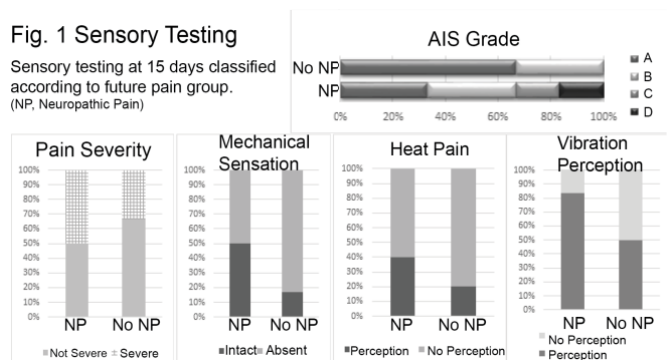
Hypothesis/Goals: The goal of this project is to develop an early pain phenotyping measure that predicts NP after SCI and as such could be another clinical tool that will contribute to the development of objective biomarkers of SCI-related NP.

Methods: Pilot data assessed includes American Spinal Injury Association Impairment Scale (AIS) grade at time of injury, pain qualifiers, and QST (mechanical, thermal, vibratory) at 15 days post-injury of 12 acute SCI subjects. The clinical diagnosis of NP at 6 months based on the International SCI Pain Classification serves as the primary endpoint. For this pilot, pain group is defined as those with NP vs those without NP (No NP).

Results: SCI subjects' below level thermal pain (spinothalamic anteriolateral function) as well as mechanical allodynia and vibration perception (both dorsal column functions) were classified according to subsequent pain group and showed variability in different perceptions and by pain group (Fig. 1). The location of NP was at or below level of the lesion in those identified as having NP.

Fig. 1 Sensory Testing

Sensory testing at 15 days classified according to future pain group. (NP, Neuropathic Pain)



Conclusions: These data from below the level of injury indicate variability of sensory perception among patients. Previous QST assessments on chronic SCI patients found NP only in regions with impaired thermal sensation. QST is expected to strengthen our endpoint by supporting clinical diagnosis data and identifying patients with thermal and/or mechanical pathway pathology.

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In-vivo Calcium imaging of the Parabrachial of Adult male Rats

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Objectives: The parabrachial nucleus (PBN) plays a role in the affective-motivational component of the orofacial pain pathway as the trigeminal region projects to the PBN and amygdala. The central amygdala, which has projections to the lateral PBN, has been shown to inhibit neurons of the PBN. GABA mediates estradiol's inhibitory effect in the PBN. In our lab, we utilize a chronic post herpetic neuralgia model by infecting rats with the human varicella zoster virus (VZV). This results in herpes zoster associated pain (HZ; shingles) affecting one or adjacent dermatomes that can last for at least 3 months. The prevalence of HZ increases with age and immunosuppression. Our long-term goal is to test that inhibiting GABA release in the amygdala would increase evoked calcium events in the PBN and increase orofacial affective-motivational pain.

Hypothesis: The excitatory neuronal response of neurons within the PBN of adult rats are visible through calcium imaging.

Methods: GAD67 transgenic adult male Long Evans rats were infused with an adeno-associated virus (AAV) containing a calmodulin dependent protein kinase II (CAMKII) GCAMP6f virus construct to the PBN. Following infusion, a glass lens was surgically implanted to the depth of the PBN to visualize activity of the PBN neurons in-vivo using Inscopix nVoke Imaging system. Calcium imaging was measured.

Results: Results indicated calcium events of individual excitatory neurons can be measured in the PBN of in-vivo animals.

Conclusions: The results suggest that modulation of PBN neuronal calcium events of GAD67 transgenic rats can be measured in-vivo.

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Individualized Real-Time fMRI Neurofeedback Enhances Consistency of Voluntary Tongue Motor Control: Implications for Patients with Oral Neuropathic 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 can be as high as 48% following head and neck cancer radiotherapy treatment. In this study, we applied an innovative brain computer interface approach with the goal to enhance voluntary movement of the tongue in a consistent fashion in healthy subjects. This approach is based on the induction of neuromodulation via individualized real-time functional MRI neurofeedback (iRTfMRI nFb) training. The principle of our innovative 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 result of neurofeedback.

Goal and Hypothesis: The overall goal is to develop, optimize, and apply individualized iRTfMRI nFb therapeutics 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 iRTfMRI nFb study. On day one, we decoded the cortical spatial patterns generated by voluntary tongue 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. On study-day two, we delivered nFb to each subject's individualized network. Linear support vector machine (SVM) was used 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 proprioceptive awareness (inferior parietal lobule, insula) and somatomotor (thalamus, basal ganglia, precentral gyrus), attention (middle and inferior (opercular) frontal), and a reward network (amygdala, and substantia nigra). The iRTfMRI nFb condition shows a significant decrease ($p < 0.0001$) in the variability of BOLD signal in each network during tongue movement intervals compared with the control-no nFb condition. SVM nFb-generated classification accuracy is higher than control-no nFb (94.3% vs. 89.6%, $p < 0.001$). Our findings show that iRTfMRI nFb generates greater consistency of controlled tongue 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-rehabilitation method for patients who have sustained lower cranial CNIX and CNXII injury.

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Tim-3 Positive CD8 T cells and IL-13 Promote Resolution of chemotherapy-induced Peripheral Neuropathy

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a common adverse effect of cancer therapy that persists after completion of treatment in 25-30% of affected patients. We showed that CD8 T cells and endogenous IL-10 are essential for CIPN resolution but CD8 T cells are not the source of the IL-10.

Hypothesis/Goals: We investigated the contribution of IL-13 and Tim-3 in the CD8 T cell and IL-10-mediated resolution of CIPN. IL-13 is a regulatory cytokine produced by CD8 T cell and Tim-3 is a receptor on CD8 T cells that allows cells to recognize damage and reduces inflammation.

Methods: Male and female C57BL6 (WT) or Rag2^{-/-} mice (lacking T cells) reconstituted with WT, IL-13^{-/-}, or Tim3^{-/-} CD8 T cells were treated with cisplatin (3 days, 2 mg/kg, i.p.), and mechanical allodynia was using von Frey hairs. Anti-Tim-3 or anti-IL-13 antibodies were injected intrathecally. Flow cytometry was used to quantify cytokines and phenotype T cells and macrophages.

Results: Male and female Rag2^{-/-} mice have delayed resolution of cisplatin-induced mechanical allodynia. Resolution is restored by adoptive transfer of WT CD8 T cells but not by IL-13^{-/-} CD8 T cells. In WT mice, intrathecal administration of anti-IL-13 delayed resolution. Blocking IL-13 intrathecally also suppressed M2 macrophage polarization and IL-10 production by F4/80+ DRG macrophages. In co-cultures of macrophages and CD8 T cells, cisplatin treatment stimulated macrophage IL-10 production, and this was prevented by addition of anti-IL-13. Cisplatin increased IL-13 production by WT CD8 T cells but not by Tim3^{-/-} CD8 T cells. Co-culture of CD8 T cells with cisplatin-induced apoptotic cells or with phosphatidyl serine liposomes upregulated IL-13 production by CD8 T cells via Tim-3. There was no effect of control phosphatidylcholine liposomes. Intrathecal administration of anti-Tim-3 prolonged cisplatin-induced mechanical allodynia and adoptive transfer of Tim-3^{-/-} CD8 T cells in Rag2^{-/-} mice failed to restore resolution of cisplatin-induced mechanical. FACS analysis revealed that DRG from Rag2^{-/-} mice reconstituted with Tim3^{-/-} CD8 T cells, contain fewer IL-13 producing CD8 T cells.

Conclusions: We conclude that Tim-3 is the receptor on CD8 T cells that senses cells damaged by cisplatin leading to induction of IL-13 production by these CD8 T cells. This IL-13 promotes M2 macrophage polarization and IL-10 production and thereby resolution of CIPN.

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The Effects of Nitric Oxide on Migraine Headache are Mediated by Peroxynitrite Formation

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Background. Despite its prevalence as the third most common disorder worldwide, the underlying pathophysiology associated with migraine is still poorly understood. Nitric oxide (NO) donors have been well-documented as being one of the most consistent experimental triggers, in which 75% of migraineurs develop an attack within six hours of NO donor administration. NO reacts with superoxide radicals to form peroxynitrite (PN), a molecule that activates and sensitizes sensory neurons in preclinical models of neuropathic, inflammatory, and cancer pain. While endogenous PN likely contributes to these other types of pain, migraine is the only pain state that is triggered by administration of a PN-producing stimulus. PN scavengers (molecules that react stoichiometrically with PN) and PN decomposition catalysts (molecules that destroy PN) have demonstrated efficacy in preclinical pain models; however, no studies to date have examined the effects of these molecules in a preclinical migraine model.

Hypothesis. We hypothesize that the effects of NO on migraine are due to PN formation.

Methods. Female mice were either subjected to three days of repeated restraint stress for two hours per day or administered a dural injection of IL-6 to induce acute periorbital hypersensitivity as measured by von Frey and grimace. Following resolution of pain, mice received i.p. injections of either vehicle, MnTBap (PN scavenger), or FeTMPyP (PN decomposition catalyst) 30 mins prior to an i.p. injection of the NO donor sodium nitroprusside (SNP) or dural injection of pH 7.0 to test for priming. To determine whether modulating PN affects acute hypersensitivity, female mice were administered an i.p. injection of FeTMPyP approximately 24 hrs following repeated restraint stress and tested for periorbital hypersensitivity and grimace.

Results. Both MnTBap and FeTMPyP partially blocked the effects of SNP in stress-primed mice, but did not prevent the hypersensitivity caused by dural pH 7.0. Additionally, FeTMPyP was not effective in preventing stress-induced acute hyperalgesia.

Conclusions. Our results indicate that neutralizers of PN are capable of partially blocking NO donor-induced periorbital hypersensitivity following repeated stress, but are not effective in blocking the acute phase of stress or hypersensitivity caused by dural pH 7.0. These findings highlight the potential for targeting PN in the treatment of migraine headache.

Clinically Relevant Pain Assays for Fibromyalgia Applied to a Preclinical Model of Musculoskeletal Pain

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Background

To date, there are few therapeutic strategies for fibromyalgia (FM), a chronic musculoskeletal pain disorder primarily diagnosed in women. There is a dearth in successful (basic-to-clinic) translated therapies as preclinical literature primarily uses evoked pain measures. However, therapeutics that successfully relieve evoked pain may not relieve the primary forms of pain reported by FM patients, spontaneous and movement-evoked pain (MEP).

Goals

The goal of this study is to apply pain and functional assays used in FM clinical trials to a preclinical model of chronic musculoskeletal pain.

Methods

Female C57BL/6J mice (2-5 months old) were used for all experiments. Two injections of acidic saline (pH4, 0.9%) into the left gastrocnemius muscle were used to induce bilateral sensitivity. MEP was tested by assessing mechanical sensitivity and facial grimacing before and after trials on the rotarod. Two spontaneous pain assays were employed, facial grimacing and conditioned place preference (CPP) using clonidine as the conditioning stimulus. Measures of functionality, grip strength and weight bearing were performed for three weeks following saline injections.

Results

Surprisingly, spontaneous facial grimacing persists for three weeks following acidic saline injections and peaks at 9 days after the first (4 days after the second injection). Facial grimacing significantly increases after rotarod trials, with no change in mechanical hypersensitivity. Mice that received pH4 saline injections, but not pH7, showed place preference for clonidine in the CPP assay. Acidic saline induces sustained decrease in grip strength across both hindlimbs and shifts in weight bearing behaviors.

Conclusions

Preclinical models of chronic musculoskeletal pain induce robust ongoing pain, MEP, and decreased muscle function. Using a range of assays to test multiple aspects of pain behaviors should be considered when testing effectiveness of potential therapeutics to maximize success potential in clinical trials.

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Endogenous Activation of Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Alleviates Peripheral Neuropathic Pain

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Background: We and others have shown that pharmacological activation of Nrf2—a transcription factor regulating expression of >200 antioxidant-related genes—can reverse neuropathic pain and underlying mechanisms.

Hypothesis/Goals: In this study, we tested whether deletion of Nrf2 would exacerbate oxidative stress, hence impairing mitochondrial function, and increasing neuroinflammation and transient receptor potential (TRP)-channel dependent neuronal sensitization. These processes are driven by oxidative stress and promote neuronal hyperexcitability that underlies neuropathic pain.

Methods: Chronic constriction injury was used as a pain model. Male and female *Nrf2*^{-/-} mice as well as wild type controls were used. Dorsal root ganglia neurons were isolated and cultured for TRPA1 sensitization recording.

Results: We show that evoked and ongoing pain behaviors induced by chronic constriction injury were exaggerated in male and female *Nrf2*^{-/-} mice. Dorsal root ganglia neurons from *Nrf2*^{-/-} mice also displayed enhanced TRPA1 sensitization, compared to wild-type controls.

Conclusion: Together, these data indicate that endogenous activation of Nrf2 after peripheral nerve injury reduces the severity of neuropathic pain and increases TRPA1 sensitization.

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Trigeminal Ganglia Nociceptors Modulate Periapical Bone Loss in Apical Periodontitis

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Background Apical periodontitis (AP) is a painful inflammatory disease following tooth infection characterized by increased mechanical allodynia and distinct bone loss surrounding the apex of the tooth. Dental structures are densely innervated by nociceptors, and there is growing evidence that sensory neurons also impact AP development. However, the mechanisms by which nociceptors modulate the progression of AP and osteolytic activity is largely unknown. **Hypothesis** We hypothesized that nociceptors are the primary subtype of trigeminal ganglia (TG) afferents in the periapical tissue modulating the bone metabolism activities in a murine model of AP. **Methods** Transgenic mice with selective denervation of nociceptive neurons (Nav1.8^{Cre}/DTA^{Lox}) and appropriate controls were used to assess the development and progression of AP using an infection-induced murine model. AP was induced by creating standardized pulp exposures in the lower left first molar, allowing for a periapical lesion to form. Micro-Computed Tomography (μ CT) was used to quantify the size of osteolytic lesions at 7-, 14- and 21-days following induction of AP. Additionally, RNA sequencing was used to analyze changes in the transcriptomic profile of the periapical lesion. Immunohistochemistry was used to quantify immune cells and markers of bone metabolism surrounding the apex of the tooth. Lastly, primary TG neuronal cultures from wild-type or nociceptor-denervated mice were co-cultured with precursor cells for osteoblasts (bone-building cells) or osteoclasts (bone-resorbing cells) to evaluate effects on mineralization and bone resorption, respectively. **Results** Nociceptor-denervated animals had earlier and faster progression of AP with larger osteolytic lesions compared to controls, and greater expression of crucial inflammatory mediators, significant increase in CD68⁺ and CD3⁺ cells, and an increased RANKL:OPG ratio at earlier timepoints. Moreover, *in vitro* assays demonstrated that co-culture with primary TG neurons had an overall inhibitory effect on osteoblastic and osteoclastic function. This inhibitory effect on osteoblasts is further increased with ablation of nociceptors, whereas the effect on osteoclasts is partially reversed. **Conclusions** The findings suggest that TG nociceptors modulate the development of AP, at least in part, by delaying the influx of immune cells, promoting osteoblastic function, and decreasing osteoclastic activities. This newly uncovered mechanism can potentially be an important therapeutic target for the treatment of AP. **Acknowledgements** Funded by R01 DE027929 (NIDCR) and T32 DE014318-19 (NIDCR).

Transition Mechanism of Nociplastic Pain in Males

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Background: *Nociplastic pain* is a form of chronic pain which is maintained despite no detectable tissue damage. Using a novel mouse model showing the transition from acute injury-induced pain to chronic nociplastic pain, our lab has previously shown that the maintenance of central sensitization in the nociplastic pain state is sex-dependent, mediated by activated spinal microglia only in male mice. The transition by which normally resolving acute pain becomes chronic nociplastic pain in males is yet unknown.

Goals: In this study, we investigate the transition mechanisms underlying the transition from acute injury-induced pain to a chronic nociplastic pain state in male mice.

Methods: We utilized a male mouse model of nociplastic pain, which uses hindpaw capsaicin injection as an acute injury, followed two hours later by vibration (post-injury stimulation) of the capsaicin-injected paw. We have found that this vibration stimulation produces a robust phenotype of central sensitization chronification, prolonging the capsaicin-induced secondary mechanical hypersensitivity. Spinal microglia were inhibited by intrathecally injecting minocycline following capsaicin injection but prior to vibration stimulation. In a second experiment, GABAergic inhibition was increased following capsaicin injection but prior to vibration stimulation by intrathecally injecting GABA. Percent paw withdrawal from ten trials of von Frey filament stimulation or paw withdrawal threshold was measured.

Results: In the male nociplastic pain model, inhibition of spinal cord microglia by minocycline prior to the application of vibration stimulation prevents the transition to chronic nociplastic pain. Boosting GABAergic inhibition prior to vibration stimulation decreases the degree of prolonged secondary mechanical hypersensitivity, compared to controls.

Conclusions: Our results suggest that microglia activation is necessary for the transition from an acute pain state to a chronic nociplastic pain state in males. Furthermore, GABAergic signaling may play a key role in this transition phase where microglia are activated by normally innocuous vibratory sensory inputs.

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The Role of Astrocyte Elevated Gene-1 (AEG-1), a Novel Transcriptional Regulator, in Chronic Inflammatory and Neuropathic Pain

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Background: Astrocyte Elevated Gene 1 (AEG-1) is a multifunctional protein shown to be a regulator of transcription and multiple intracellular signaling pathways. The role of AEG-1 in cellular inflammation appears to be primarily facilitated by its direct interaction with the transcription factor NFκB, a key transcriptional regulator of inflammatory cytokine expression. Inflammatory cytokines have been shown to play an important role in models of both chronic inflammatory and neuropathic pain. **Goal:** To investigate if AEG-1 contribute to the development of chronic inflammatory and neuropathic pain, making it a potential therapeutic target for these conditions. **Methods:** 12-24 weeks old AEG-1 wild type (WT) and global knockout (KO) male and female mice on C57BL/6J background were investigated in 3 mouse models of chronic pain: Chronic inflammatory pain (i.pl. injection of 50% CFA), The nerve injury chronic neuropathic pain (Chronic constrictive injury model or CCI) and the chemotherapy-induced peripheral neuropathy (CIPN) induced via four 8 mg/kg, i.p. injections of paclitaxel. Mechanical hypersensitivity was assessed via von frey filament assay. Thermal withdrawal latency was assessed via Hargreaves test. Cold sensitivity was assessed via acetone test. **Results:** AEG-1 KO mice displayed protection from CFA-induced mechanical hypersensitivity, thermal sensitivity, and reduced paw edema compared to WT counterparts. In addition, AEG-1 KO mice displayed protection from paclitaxel induced mechanical hypersensitivity and cold sensitivity compared to WT counterparts. In contrast, the mechanical hypersensitivity and thermal sensitivity of the AEG-1 KO mice showed no significant difference from that of the WT counterparts in the CCI model. Interestingly, the AEG-1 KO mice that underwent CCI surgery displayed a significant increase in morphine anti-allodynia compared to WT CCI mice. **Conclusions:** Our data suggest that AEG-1 is involved in the development of inflammatory pain and CIPN associated pain-like behaviors in mice. Additionally, AEG-1 may function as part of an anti-mu-opioid receptor (MOR) signaling system that reduces the antinociceptive effects of MOR agonists.

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Chronic THC Vapor Attenuates Hyperalgesia and Alters Neuronal Function in the Ventrolateral Periaqueductal Gray (vlPAG) Male Rats with Chronic Inflammatory Pain

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Background: Chronic pain conditions are a huge societal problem, and inflammatory pain conditions are the leading cause of disability among U.S. adults. The first-line treatment approach for chronic pain has been opioid drugs, but this approach has created a global health crisis in the form of opioid drug abuse and mortality. In an effort to reduce reliance on opioids, ongoing work is testing the utility of cannabinoid drugs as an alternative or adjunct to opioids.

Hypothesis: THC vapor inhalation will improve inflammatory pain-related behaviors and modify neural and synaptic properties in the midbrain (i.e., ventrolateral periaqueductal gray [vlPAG]).

Methods: Male Wistar rats received an injection of Complete Freund's Adjuvant (CFA) or saline in the hindpaw and were exposed to THC (100 mg) or vehicle (propylene glycol) vapor daily over 10 consecutive days. Male Wistar rats were tested for baseline thermal nociception using the Hargreaves test prior to THC vapor exposure then again on days 2, 5 and 8 of vapor exposure (immediately after removal from vapor), and also 24 hours after the 10th and final THC exposure. For slice electrophysiology experiments, rats were treated with CFA, exposed to THC (100 mg) or vehicle (propylene glycol) vapor for 10 days, then sacrificed 24 hours after the final vapor exposure and their brains were prepared for *in vitro* brain slice electrophysiology.

Results: A two-way ANOVA in CFA-treated rats revealed that THC vapor reduced thermal nociception over days, $F(2,72)=4.242$, $p<0.05$ (**Fig. 1A**). Tukey's post-hoc analyses showed THC vapor reduced thermal nociception in CFA-treated rats on multiple days during the 10-day THC vapor exposure ($p<0.05$). For slice electrophysiology experiments, chronic THC vapor exposure in CFA rats did not change intrinsic sub-threshold firing properties of vlPAG neurons. Chronic THC vapor inhalation reduced firing rates of vlPAG neurons (**Fig. 1B-D**; $F(1,174)=4.19$, $p=0.042$), and also reduced spontaneous inhibitory postsynaptic current (sIPSC) amplitude (**Fig. 1E**; $F(1,150)=13.91$, $p=0.0003$) and rate (**Fig. 1F**; $F(1,150)=8.23$, $p=0.0047$). All of these effects were enhanced specifically in neurons that displayed a delayed onset firing phenotype. Finally, we found that chronic THC vapor inhalation enhanced the suppression of presynaptic inhibition by bath application of the mu-opioid agonist DAMGO ($F(1,35)=8.35$, $p=0.0066$).

Conclusions: The results of this work may have implications for opioid drug prescriptive strategies in humans that have a history of vaping THC.

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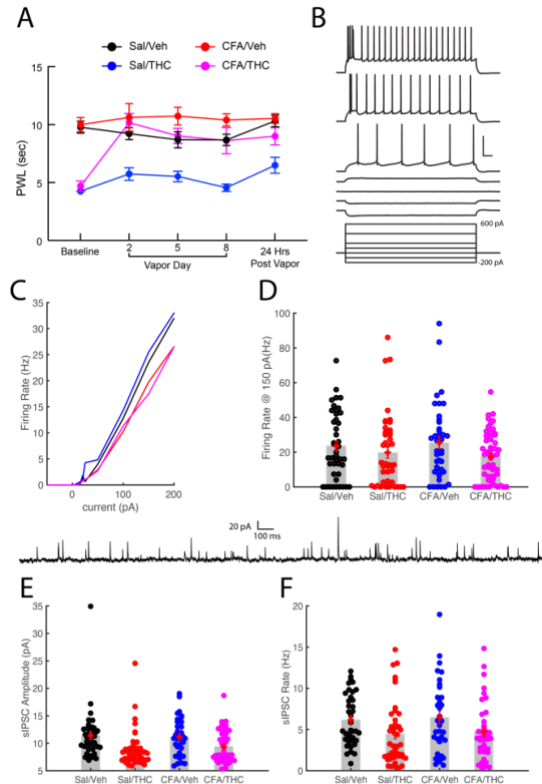


Figure 1

Inter- and Trans-generational Inheritance of Paternal Chronic Pain to Male and Female Offspring in Mice

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Background: Major life events impact behaviour and alter an individual's molecular profile. In many circumstances, these alterations will be transmitted to the offspring through biological (germline) or environmental inheritance. For example, both inter- and trans-generational transmission of stress and trauma have been documented in both humans and rodents. More recently, a growing body of literature suggests chronic pain and related outcomes can be intergenerationally transmitted in humans.

Hypothesis/Goals:

The goal of the present study was to assess inter- and trans-generational inheritance of paternal chronic pain. We hypothesized that males and females in lineages sired by male mice with chronic neuropathic pain would have altered baseline pain thresholds and increased risk of chronic pain development and severity over 2 generations.

Methods:

Animals: F0: 9-month-old CD1 male mice without or with chronic neuropathic pain for 6 months (Sham and Spared Nerve Injury (SNI), respectively) were mated with naïve young CD1 females. Breeders were kept together, and both parents remained with the pups until 3 weeks of age. Intergenerational inheritance was assessed in their direct progeny (F1) and transgenerational inheritance in further descendants (F2). F2 was generated by mating unrelated females and males from F1 SNI or Sham lineages.

Behavioural assays: Sensitivity to mechanical and cold stimuli were assessed using von Frey filament and acetone assays. Males and females from F1 and F2 were tested at 2.5 months of age (n=15-70/group). A subset of animals of each sex and generation received intra-plantar CFA (Complete Freund Adjuvant) and were monitored for 3 days (n=11-15/group).

Results:

No differences between mice from the two lineages were observed in mechanical sensitivity at baseline or in response to CFA. In contrast, F1 females and F2 males from SNI lineages were hypersensitive to cold at baseline compared to sham lineages. Interestingly, F1 females and F2 males from SNI lineages demonstrated weaker behavioural responses to acetone after CFA-injection compared to sham lineages.

Conclusions: Paternal experience can influence offspring development via germline and environmental inheritance. Our results provide evidence that a family history of chronic pain can lead to sex-specific inter- and trans-generational inheritance of cutaneous hypersensitivity but did not increase risk or severity of chronic pain after CFA.

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A Pharmacological Interactome Platform for Discovery of Pain Mechanisms and Targets

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Background: Cells communicate with each other through ligand and receptor interactions. In the case of the peripheral nervous system, these ligand-receptor interactions shape sensory experience. Nociceptive sensory neurons are responsible for detecting changes in the environment through specific receptors and then transmitting this signal to the central nervous system (CNS) via the generation of action potentials. These nociceptors innervate almost every tissue in the body, playing a critical role in detecting injury and/or pathology to skin, joints, bones and visceral organs. RNA sequencing (RNA-seq) experiments have defined tissue-wide and cell-specific transcriptomes for much of the body in both mice and humans. Cell profiling experiments on normal and diseased tissues have identified key molecular players in an increasing number of disease processes, including disorders with a strong pain component.

Hypothesis/Goals & Methods: Using RNA sequencing datasets from mouse and human, we created an interactome map for how mammalian sensory neurons potentially interact with peripheral cell types. We used this knowledge base to gain insight into how specific cell types and sensory neurons might interact in disease states. We created interactomes of knee joint macrophages from rheumatoid arthritis patients and pancreatic cancer samples with human dorsal root ganglion (DRG).

Results: A common theme was heparin binding EGF-like growth factor (HBEGF) interaction with sensory neurons through epidermal growth factor receptor (EGFR), a member of the ErbB family of receptors (encoded by EGFR and ERBB2-4). We validated that HBEGF causes pain in mice, likely acting on DRG neurons through ErbB family receptors.

Conclusions: Collectively, these interactomes highlight ligand-receptor interactions in mouse models and human disease states that reflect the complexity of cell to neuron signaling in chronic pain states.

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Pramipexole Treatment Attenuates Mechanical Hypersensitivity in Rats with Chronic Inflammatory Pain

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Background: Prescription opioids are a critical first-line treatment for chronic pain. However, chronic treatment with opioids can lead to paradoxical increases in pain sensitivity, termed hyperalgesia, which may promote use of opioids and/or alcohol to manage worsening pain symptoms. Functional abnormalities in the nucleus accumbens (NAc), including dopamine signaling deficits, have been reported in both chronic pain and drug dependent states. This is consistent with our finding that increased pain avoidance-like behavior is associated with changes in dopamine biosynthesis marker (pTH40) in the NAc and our evidence of increased intrinsic excitability of NAc neurons in rats with chronic inflammatory pain.

Hypothesis/Goals: In the present study, we tested the hypothesis that acute and repeated dopamine agonist treatment would attenuate mechanical hypersensitivity in rats with chronic inflammatory pain. We also examined the neurobiological effects of dopamine agonist treatment on glutamatergic and presynaptic signaling in pain- and reward-related brain regions. **Methods:** We tested the functional role of dopamine regulation in an animal model of chronic inflammatory pain (CFA) at both a dopamine biosynthesis and dopamine receptor level using clinically available therapeutics, L-DOPA (dopamine precursor) and pramipexole (dopamine receptor 2/3 agonist). 1W after paw injections, male Long-Evans rats were split into four groups (saline-vehicle, saline-drug, CFA-vehicle, CFA-drug). In the drug conditions, L-DOPA and Pramipexole were administered on separate test days. The order of drug presentation was counterbalanced. L-DOPA was always co-administered with a DOPA decarboxylase inhibitor, benserazide. Von Frey testing or brain collection for Western blotting occurred 1 hour after drug administration.

Results/Conclusions: We found that acute and repeated pramipexole treatment increased paw withdrawal thresholds in CFA animals, but had no effect on thresholds in saline animals. There was no effect of acute or repeated L-DOPA treatment on paw withdrawal thresholds in CFA and saline animals. Together these results indicate that pramipexole treatment produces anti-hyperalgesic effects on mechanical hypersensitivity, attenuates pain-like behavior in CFA animals, and does not produce analgesic effects in saline animals. In the NAc, we found that pramipexole treatment decreased pGluR1845, indicating that pramipexole reduces excitatory currents mediated by AMPA receptors in the NAc. Marker of presynaptic vesicle release, pSynapsin, was increased in saline animals, but not in CFA animals following pramipexole treatment. This indicates that presynaptic neurotransmitter release is blunted in the NAc of animals with chronic inflammatory pain. **Acknowledgements:** The work was funded by research grants from the Department of Veteran Affairs (IK2BX004334, AP) and the NIAAA (R01AA025996, SE).

CCL2-Induced Macrophage Accumulation in the Dorsal Root Ganglia Correlates with Persistent Paw Hypersensitivity

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Background: Inflammation in the nervous system can mediate the development and persistence of pathological pain states.

Hypothesis: Intraganglionic microinjection of CCL2 is sufficient to induce macrophage accumulation and concomitant hypersensitivity of the corresponding dermatomes.

Methods: Unilateral injections of recombinant rat CCL2 or vehicle were administered to the C7-8 DRG of uninjured Sprague Dawley rats. Neuropathic pain was assessed using von Frey and mechanical conflict avoidance paradigms (MCAP). Immunohistochemistry was used to quantify macrophages (ED1+) and microglia (IBA1+) in the DRG and ipsilateral dorsal horn. DRG macrophage phenotype and cytokine profile was analyzed using qPCR.

Results: Rats that received CCL2 injections exhibited persistent ipsilateral forepaw hypersensitivity starting at 3-days post-injection compared to naïve and vehicle-treated rats as measured by von Frey ($p < .05$). In contrast, there was no significant difference between groups when comparing escape latency in the MCAP. Immunocytochemistry and qPCR revealed that rats treated with CCL2 displayed increased macrophage presence in the DRG compared to naïve and vehicle-treated animals ($p < .05$ CD68 mRNA). Interestingly, analysis of macrophage phenotype suggests a pro-reparative rather than pro-inflammatory phenotype. In addition to recruitment of macrophages to the DRG, intraganglionic CCL2 caused significant microglial activation in the dorsal horn of the spinal cord compared to naïve and vehicle-treated animals ($p < .05$).

Conclusion: Intraganglionic delivery of CCL2 in the otherwise intact animal increases paw hypersensitivity but it does not impact behaviors requiring supraspinal processing of sensory information, suggesting that DRG CCL2 affects the local DRG and spinal cord circuits but has little impact on supraspinal centers. While we show that CCL2 recruits pro-reparative macrophages to the site of injection, CCL2 may also be acting directly on nociceptors to initiate pain development. Further research is necessary to examine the dual nature of this cytokine in the DRG.

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Biophysical and Pharmacological Profiling of Multiple Voltage-Gated Sodium Channel Subtypes on QPatch II

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Background

Voltage - gated sodium channels (VGSC) are responsible for the initiation and propagation of action potentials in excitable cells. VGSC have been identified as excellent drug targets for treatment of pain, epilepsy and to other neurological disorders. Early compounds, however, were developed using empirical approaches. The identification of the molecular identity of VGSC in combination with technological advances, such as the automated patch clamp technique, provide the basis for a rational design of subtype – selective compounds.

To date, 9 functional mammalian isoforms (NaV1.1–1.9) have been described in the literature. The various subtypes differ in their expression pattern and exhibit distinct biophysical and pharmacological profiles. All have in common that they produce a transient inward current in response to membrane depolarization. During this process the VGSC transitions from a closed to and open into an inactivated state. Interestingly, inhibitor compounds often exhibit different pharmacological profiles dependent upon the ion channel conformational state.

Methods and Results

In the present study, the second generation QPatch (QPatch II; Sophion Bioscience) was used in combination with adaptive voltage protocols to investigate state-dependent inhibition of tetrodotoxin (TTX) and tetracaine on 8 different VGSC subtypes (NaV1.1-8). A first step was to determine the half inactivation potential $V_{1/2}(\text{inactivation})$ for each individual cell. This value was then used during the next steps as preconditioning pulse. Such an adaptive protocol allowed to determine IC_{50} values for both the closed and the inactivated state and reduce heterogeneity of the cells. Both IC_{50} values and biophysical parameters of the different subtypes align well with literature values.

Unbiased Analysis of Primary Afferent Fibers in Spinal Cord Injured Rats with and without Strength Training

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Background: More than two-thirds of individuals with spinal cord injury (SCI) develop chronic neuropathic pain. SCI-induced neuropathic pain is associated with nociceptor plasticity. We have previously shown that early, aerobic exercise prevents development of neuropathic pain but does not ameliorate it once established. While locomotor training is used clinically, the standard of care in post-SCI rehabilitation focuses on improving muscle strength.

Hypothesis: Strength Training (ST) after SCI affects nociceptor plasticity and pain development after SCI

Methods: Female Sprague-Dawley rats received a C5 unilateral spinal cord contusion corresponding to handedness. A subset of SCI rats underwent isometric forelimb ST 5 days/week starting at 5 days post-injury (dpi) for 5 weeks. Briefly, rats complete 50 successful repetitions of ≥ 50 g force in an isometric forelimb pull task to receive a food reward. Animals each group received microinjection of cholera toxin-B into the ulnar nerve to identify large diameter afferents 3 days before sacrifice. Immunocytochemistry identified CTB+, CGRP+ and IB4+ nociceptive afferent fibers in the dorsal horn of C7-8. Fibers were analyzed with a custom MATLAB program that automates image-level adjustments and analyzes colocalization characteristics, axon density, projection distance, and regional axons projections within the dorsal horn (DH) laminae. This involves image restoration, individual clustering of CGRP+ and IB4+, line detection, line modeling, and cluster analysis.

Results: Mean pulling force returned to near normal after 10 ST sessions regardless of early or delayed initiation of exercise ($p > .05$ vs baseline). The recovery of forelimb strength corresponded to improvements in a single pellet-retrieval task, and ST reduced paw hypersensitivity and pain behavior as measured by von Frey and mechanical conflict avoidance operant tests compared to unexercised and acute ST SCI groups. Analysis of primary afferent fibers revealed that both SCI and ST altered the laminar distribution and in some cases the density of nociceptive afferent fibers that corresponded to pain phenotype.

Conclusions: Early ST reduces pain development after SCI. Our unbiased automated analysis of evaluating axon fibers sharing identical spatial parameters within immunofluorescent images revealed that this reduction in pain incidence correlates with a more “normal” distribution of nociceptive afferents in the dorsal horn. Together, these data suggest that afferent driven rehabilitative or exercise therapies may be important for reducing pain incidence after spinal cord injury.

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A Highly Selective A3 Adenosine Receptor (A3AR) Agonist, MRS5980 Prevents and Reverses Cisplatin-Induced Cognitive Impairments, Motor Incoordination and CIPN in Mice

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Background: Cisplatin is a widely used treatment to combat solid tumors. However, patients treated with cisplatin often develop cognitive impairments, deficits in fine motor activity, and peripheral neuropathic pain. There is no FDA-approved treatment for these neurotoxicities. In the central nervous system, the adenosine pathway is an important neuromodulator that regulates neuronal and glial function through adenosine receptors including the A3AR. Previous findings indicate that stimulation of the A3AR is an effective strategy to reverse mechanical allodynia in chronic constriction injury (CCI) and prevent chemotherapy (paclitaxel and oxaliplatin)-induced neuropathic pain (CIPN).

Hypothesis/ Goal: To investigate the role of A3AR activation by a highly specific A3AR agonist MRS5980 in the prevention and reversal of cisplatin-induced cognitive impairments, fine motor deficits, and CIPN.

Methods: C57BL6 mice were treated with 2 rounds of cisplatin (2.3 mg/kg/day, i.p. for 5 days) with 5 days of rest in between. Mice were treated with the brain penetrant A3AR agonist, MRS5980: for prevention: daily from one day before until one day after the last cisplatin injection; for reversal: daily injection for 24 days starting 2 days after the last dose of cisplatin. Two weeks after completion of treatment, mice were tested for cognitive behaviors, fine motor activity, mechanical allodynia, and spontaneous pain. Synaptic proteins were visualized via immunohistochemistry. Mitochondrial respiration was determined via Seahorse Flux analysis of brain synaptosomes. Cellular and regional expression of A3AR in the brain was determined using RNA *in situ* hybridization.

Results: MRS5980 not only prevents but also reverses cisplatin-induced mechanical allodynia, spontaneous pain, fine motor deficits and impaired executive functioning, working memory, and spatial memory in both sexes. In the brain, MRS5980 prevents cisplatin-induced loss of synaptic proteins, synaptophysin and PSD95, mitochondrial dysfunction and reduces the expression of nitrosylated-MnSOD. We detected *Adora3* mRNA in neurons, microglia, astrocytes, and oligodendrocytes in the mouse brain and expression was increased in response to cisplatin.

Conclusions: Our results highlight the critical role of A3AR in preventing and reversing cisplatin-induced cognitive impairments, motor incoordination, and CIPN. Since A3AR agonists are already in clinical trials to improve the effects of cancer treatment, our results are underlining the importance of the use of MRS5980 in preventing the neurotoxic effects of cancer treatment.

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The Cannabinoid Agonist CB-13 Produces Acute Peripherally-Mediated Analgesia in Mice and Reduces Measures of Neuronal Hyperexcitability in Mouse DRG Yet Repeated Dosing Elicits Tolerance and Signs of CNS Activity

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Introduction: Peripherally restricted cannabinoid type 1 receptor (CB₁R) agonists have been developed with the hope of providing analgesia without unwanted effects, including psychoactivity and tolerance, associated with CB₁R activation in the central nervous system. The present study sought to evaluate the impact of long-term administration of a peripherally restricted cannabinoid agonist, CB-13, on reducing inflammatory nociception and producing CB₁R dependence in mice in vivo and evaluate cellular mechanisms underlying CB-13-induced antinociception in vitro using cultured mouse dorsal root ganglion (DRG) neurons.

Methods: A within-subjects dose-response of CB-13 in reducing mechanical allodynia induced by CFA was generated and the maximally efficacious dose (3 mg/kg i.p.) was evaluated for efficacy in reducing CFA-induced thermal allodynia. The peripherally restricted CB₁ antagonist AM6545 was used to evaluate the specificity of CB-13's anti-allodynic effects. Tolerance and CB₁R dependence was evaluated following repeated dosing. To evaluate for other cardinal signs of central CB₁R activation, catalepsy, tail-flick antinociception and changes in body temperature were measured in naïve mice using the same dosing paradigm as stated above. The effect of CB-13 on prostaglandin E₂ (PGE₂)-induced TRPV1 sensitization and neuronal hyperexcitability was measured in lumbar DRG cultures isolated from naïve mice.

Results: CB-13 reduced inflammation-induced mechanical allodynia in a peripheral CB₁R-dependent manner and relieved inflammatory thermal hyperalgesia. Phenotypes associated with central CB₁R activation occurred only at a dose of CB-13 approximately 10-fold its ED₅₀ for reducing allodynia. In cultured mouse DRG neurons, CB-13 reduced TRPV1 sensitization and neuronal hyperexcitability induced by the inflammatory mediator (PGE₂)-providing potential mechanistic explanations for the analgesic actions of peripheral CB₁R activation. Strikingly, repeated dosing with CB-13 resulted in both analgesic tolerance and CB₁R dependence, even at a dose that did not produce central CB₁R-mediated phenotypes on acute dosing.

Conclusions: These results suggest increased CNS exposure with repeated CB-13 dosing, leading to unwanted engagement of central CB₁Rs. Thus, caution is warranted regarding the use of CB-13 as a therapy. Nonetheless, the clear analgesic effect of peripheral CB₁R activation suggests that the approach of developing peripherally restricted cannabinoids as novel analgesics should be pursued.

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TIMP-1 Attenuates Hypersensitivity Through CD63 Signaling

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Background: Unresolved inflammation is a major predictor for the development of chronic pain. Often treated with opioids, chronic pain affects millions of Americans per year and is recognized as one of the most common reasons people seek healthcare. Understanding the processes behind resolving inflammation is becoming increasingly important for developing non-opioid therapeutics to manage pain. Towards this goal, we have identified tissue inhibitor of metalloproteinases (TIMP-1) as a regulator of inflammatory pain. Mainly known for its ability to regulate tissue remodeling after injury, TIMP-1 inhibits the expression of matrix metalloproteinases (MMPs). MMPs escalate immune responses during injury which increases inflammation and nociceptor sensitization. Although the N-terminus of TIMP-1 binds MMPs to inhibit behavioral sensitivity, the cell surface receptor binding C-terminus also attenuates sensitivity through unknown receptor-mediated signaling interactions.

Hypothesis/Goals: The goal of the current work is to characterize a cellular signaling pathway TIMP-1 initiates through activation of CD63, and we hypothesize that CD63-dependent intracellular signaling is required for the antinociceptive effects of TIMP-1

Methods: Male C57BL/6J mice underwent a right hindpaw injection of CFA, rmTIMP-1, or CFA and rmTIMP-1. Mice were sacrificed 1 day, 3 days, 5 days, and 7 days after undergoing injection, and qPCR was performed to determine expression levels of CD63 pathway components including: *Pten*, *Akt*, and *Pik3ca*.

Results: Analysis revealed injection of rmTIMP-1 alone increased expression of *Pten*. *Akt* and *Pik3ca* levels of expression were attenuated following injections of CFA.

Conclusions: These results suggest that PI3K/AKT/PTEN signaling downstream of TIMP-1 and CD63 activation contributes to TIMP-1-mediated resolution of inflammatory hypersensitivity. Characterizing the non-canonical receptor-mediated effects of TIMP-1 signaling will identify novel molecular targets that may increase medical efficacy in resolving inflammation and subsequent prevention of chronic pain development.

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Effects of a High Omega-6 Diet on Orofacial Allodynia and Gene Expression Patterns in the Trigeminal Ganglia

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Background: Over 39 million adults in the United States experience chronic orofacial pain, with the common complaint from mechanical stimuli, although spontaneous pain also occurs in several orofacial pain conditions. Treatments for orofacial pain conditions are limited, and available treatments suffer from incomplete efficacy or adverse side effects. Health care providers recommend dietary interventions for management of cardiovascular disease and diabetes.

Hypothesis/Goals: It is possible that diet may also contribute to chronic orofacial pain conditions, as omega-6 fatty acids such as linoleic acid (LA) and arachidonic acid (AA), which are essential polyunsaturated fatty acids (PUFA), are regulated by dietary intake. However, there is a large gap in knowledge on the role of diet as a risk factor or potential therapy for chronic orofacial pain.

Methods: To measure the effect of a high omega-6 diet (H6D) on orofacial nociception, male and female C57 mice were fed standardized diets containing either a high 10% omega-6 diet (Dyets, Inc) or a low 0.5% omega-6 diet (Dyets, Inc) for 8 weeks with weekly monitoring of mechanical nociceptive withdrawal thresholds (n=10/group) using von Frey filaments by a blinded observer. After, we obtained the TG homogenates from mice fed a H6D and L6D and performed RNA-sequencing to identify the transcriptional changes.

Results: Data were analyzed by 2-way ANOVA. The results demonstrate that a H6D, significantly decreased mechanical nociceptive thresholds in the orofacial region. From the RNA-sequencing, we found 35 significant differentially expressed genes which were further evaluated with pathway analysis (raw p-val<0.05 and |FoldChange|>1.5).

Conclusions: These findings indicate that a H6D may be a risk factor for orofacial allodynia and may trigger functional changes in sensory neurons via altered gene expression.

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Spinal Cord Injury-Induced Chronic Pain: A Role for Bacterial Translocation and DNA Damage in Bowel Pain After Injury

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Background: Patients with spinal cord injury (SCI) identify pain as one of their highest priority concerns, even over recovery of locomotor function. Visceral pain, pain that originates in the abdominal and pelvic organs, is more likely to be described as unrelenting and more severe than musculoskeletal pain and often co-occurs with alterations in bowel function. The few available treatments primarily target gastric motility, but do not treat visceral pain. Nociceptors (pain-sensing neurons) can become hypersensitive after SCI, but the mechanisms involved in this process remain incompletely understood, representing a significant barrier to effective prevention and treatment.

Hypothesis/Goal: We hypothesize that colon-specific DNA damage results from microbiome-induced genetic reprogramming in host intestine and primary nociceptors, providing a potential novel target for the treatment of chronic abdominal pain in patients with SCI.

Methods: We use a mouse model of contusion spinal cord injury at the T6 level. Mice are sacrificed 24 hours or seven days after injury for collection of colon tissues. Snap frozen colons are used for protein and DNA assays. Proximal and distal colon segments are fixed in either formalin or carnoy's fixative, cut in cross-sections, and mounted on slides for H&E, IHC, and FISH stains.

Results: We have identified rapid (24 hour) and persistent (7 days) suppression of transcripts involved in the response to DNA damage in the colon and an accumulation of double strand DNA damage breaks. Subsequent experiments indicate the presence of inflammatory cells and disruption of the intestinal mucus bilayer. Without the protective mucus barrier, microbiota translocate into the intestinal wall, posing problems since bacteria are known to initiate host gene expression changes. This translocation corresponds to an upregulation in transcripts related to cellular response to biotic stimulus and defense responses in primary nociceptors.

Conclusions: Our preliminary data suggest an association between colonic genomic instability and translocating microbiome, offering a potential mechanism for induction and maintenance of hypersensitivity. Ongoing studies will 1) explore the impact of these changes within the colon on vagal and spinal sensory afferent excitability and 2) determine the impact of SCI on the composition of the intestinal microbiome.

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HEAL Initiative Preclinical Screening Platform for Pain: Evaluation of Novel Non-Opioid, Non-addictive Therapeutics

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Background: The NIH Helping to End Addiction Long-termSM Initiative, or NIH HEAL InitiativeSM Preclinical Screening Platform for Pain (PSPP) aims to accelerate the preclinical development of non-opioid, non-addictive therapeutics for pain.

Methods: PSPP accepts small molecules, biologics, natural products, and devices from academic and industry asset owners worldwide. Under NINDS direction, preclinical testing of submitted assets is performed by an NINDS contract facility, PsychoGenics Inc., in a blinded and confidential basis at no cost to the PSPP participants. PSPP works with PsychoGenics to develop and validate preclinical models and endpoints to enable the screening and profiling of assets.

Results: Assets are evaluated in a tiered approach within the program. In Tier 1, assets are screened in cell-based functional assays to assess activity at opioid receptors and other receptors associated with abuse liability. Also, in Tier 1, the pharmacokinetic (PK) profile of the asset in both plasma and brain is determined. In Tier 2, a side effect profile is assessed using an accelerating rotarod and modified Irwin test. Subsequently, assets are evaluated using evoked and non-evoked pain endpoints in two pain models representative of acute to sub-chronic pain and persistent pain mechanisms. Finally, in Tier 3, assets are evaluated *in vivo* for abuse liability and in disease specific pain models. Representative examples demonstrating the merits of evaluating promising assets rigorously in a tiered approach as well as efforts to enhance novelty and reproducibility within the NINDS PSPP program will be highlighted. A key feature of the PSPP is the flexibility to continuously acquire and validate innovative new models and endpoints that more closely represent human pain conditions and will be discussed. The novel approach within this program to enhance the search for non-opioid, non-addictive pain therapeutics will be elaborated.

Conclusion: This presentation will describe the progress made within this program and its efforts to engage the academic and industry drug discovery and development community towards evaluating novel non-opioid, non-addictive therapeutics.

Taking the Road Less Traveled: Unconventional Translation Control in Pain Pathogenesis

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Background: Changes in nociceptor phenotype, particularly increased excitability and ectopic activity, are caused by *de novo* protein synthesis. The processes governing the translation of certain genes that underlie chronic pain remain poorly understood. Translation initiation is the rate-limiting step in protein synthesis and is tightly regulated by the cell in response to inflammation, injury, and disease. In a process known as integrated stress response (ISR), specific kinases (PERK, PKR, HRI, and GCN2) transduce cellular stress by inhibiting the eukaryotic initiation factor 2 (eIF2) complex via phosphorylation of its α subunit (eIF2 α) at Ser51. The ISR results in the suppression of global protein synthesis and a paradoxical translation of *stress-resistant genes*. How these stress-resistant genes continue to be translated even when eIF2 is prevented from participating in translation remains to be investigated. eIF2A, not to be confused with eIF2 α , also recruits methionine-bound tRNA to the translation initiation complex.

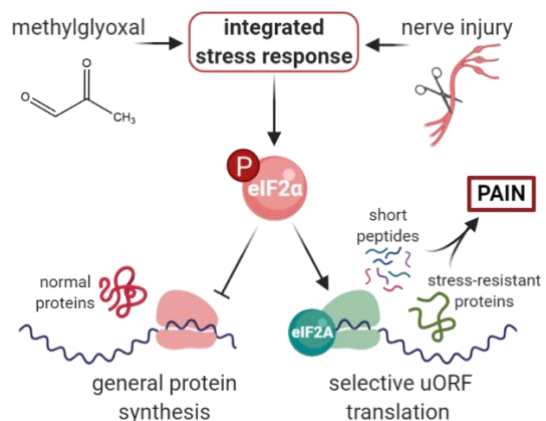
Hypothesis/Goals: Our *hypothesis* is that the ISR is a key signaling pathway that promotes increased excitability in nociceptors leading to pain sensitization. Recent reports have implicated the ISR in various chronic pain conditions, such as nerve injury, chronic inflammation, multiple sclerosis, and diabetic neuropathy. We postulate that the translation regulation capability of eIF2A becomes most apparent refractory to eIF2 α phosphorylation.

Methods: By employing newly established eIF2A-null mice, we explore how eIF2A regulates *de novo* protein synthesis and thereby regulates pain pathophysiology in models of ISR. Methylglyoxal (MGO), a toxic derivative of glycolysis associated with diabetic neuropathy, and tunicamycin are known inducers of the ISR.

Results: Male and female wild-type mice injected with MGO and tunicamycin developed mechanical hypersensitivity while eIF2A^{-/-} mice were protected against mechanical hypersensitivity. Systemic delivery of MGO also induced mechanical hypersensitivity in wild-type animals but not in eIF2A^{-/-}, suggesting that eIF2A contributes to MGO-induced pain hypersensitivity. Interestingly, wild-type and eIF2A-null animals both developed mechanical pain following a spared nerve injury (SNI), a commonly used neuropathic pain model. The ISR is not known to contribute to SNI-induced pain positing that the lack of pain in eIF2A^{-/-} is restricted to ISR-mediated neuropathy. Using human DRG explants, we further show that MGO treatment (1 μ M) for 24 hours produces a robust increase in p-eIF2 α and eIF2A.

Conclusions: These data point toward a previously unknown role of eIF2A in mediating ISR-induced pain hypersensitivity.

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HDAC6 Inhibition Reverses Chemotherapy-Induced Neuropathic Pain in an IL-10 Dependent Manner

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Background: Novel interventions to treat chemotherapy-induced peripheral neuropathy (CIPN), a common adverse effect of cancer therapy, can benefit a growing group of cancer survivors. HDAC6 inhibitors are attractive candidates because they fully reverse established CIPN and may also enhance the anti-tumor effects of chemotherapy. We showed recently that spontaneous resolution of CIPN after completion of chemotherapy depends on endogenous IL-10 signaling.

Hypothesis/Goals: We tested the hypothesis that IL-10 signaling to sensory neurons is required for the reversal of CIPN in response to HDAC6 inhibition.

Methods: CIPN was induced by treating mice with two rounds of cisplatin (2.3 mg/kg/day i.p. for 5 days followed by 5 days rest, and then another 5 days of injections). Mice were treated with the HDAC6 inhibitor ACY-1083 starting 3 days after completion of cisplatin treatment. IL-10 knock out (*Il10-ko*) mice and mice with conditional deletion of the IL-10 receptor (IL-10R) in advillin-positive peripheral neurons *Adv-il10r-ko* mice were used. Mechanical allodynia was followed over time using the von Frey test. RT-PCR was used to check mRNA levels. Mitochondrial bioenergetics in tibial nerve were measured with the XF24 Flux Analyzer.

Results: Treatment with the HDAC6 inhibitor reversed cisplatin-induced mechanical allodynia in WT male and female mice. HDAC6 inhibitor ACY-1083 treatment significantly increased IL-10 mRNA levels in the spinal cord. Intrathecal but not intraplantar administration of a neutralizing anti-IL-10 antibody to WT mice prevented reduction of mechanical allodynia in response to the HDAC inhibitor. Moreover, the HDAC6 inhibitor was not effective in *Il10-ko* and *Adv-il10r-ko* mice, indicating a key role of endogenous IL10 signaling to primary sensory neurons in reversal of CIPN. Cisplatin-treated mice showed mitochondrial dysfunction in the tibial nerve, with a significant decrease in both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). ACY-1083 normalized mitochondrial bioenergetics in the tibial nerve even when IL-10 signaling was blocked by intrathecal injection of anti-IL-10 antibody.

Conclusions: HDAC6 inhibition reverses cisplatin induced allodynia in an IL-10 dependent manner. However, the reversal of cisplatin-induced mitochondrial deficits in the tibial nerves in response to HDAC6 inhibition is not IL-10 dependent. These findings indicate that normalization of mitochondrial function in the peripheral nerve is not sufficient to resolve cisplatin-induced mechanical allodynia. Moreover, IL-10 signaling via IL10 receptors on peripheral sensory neurons is required for resolution of CIPN in response to HDAC6 inhibition.

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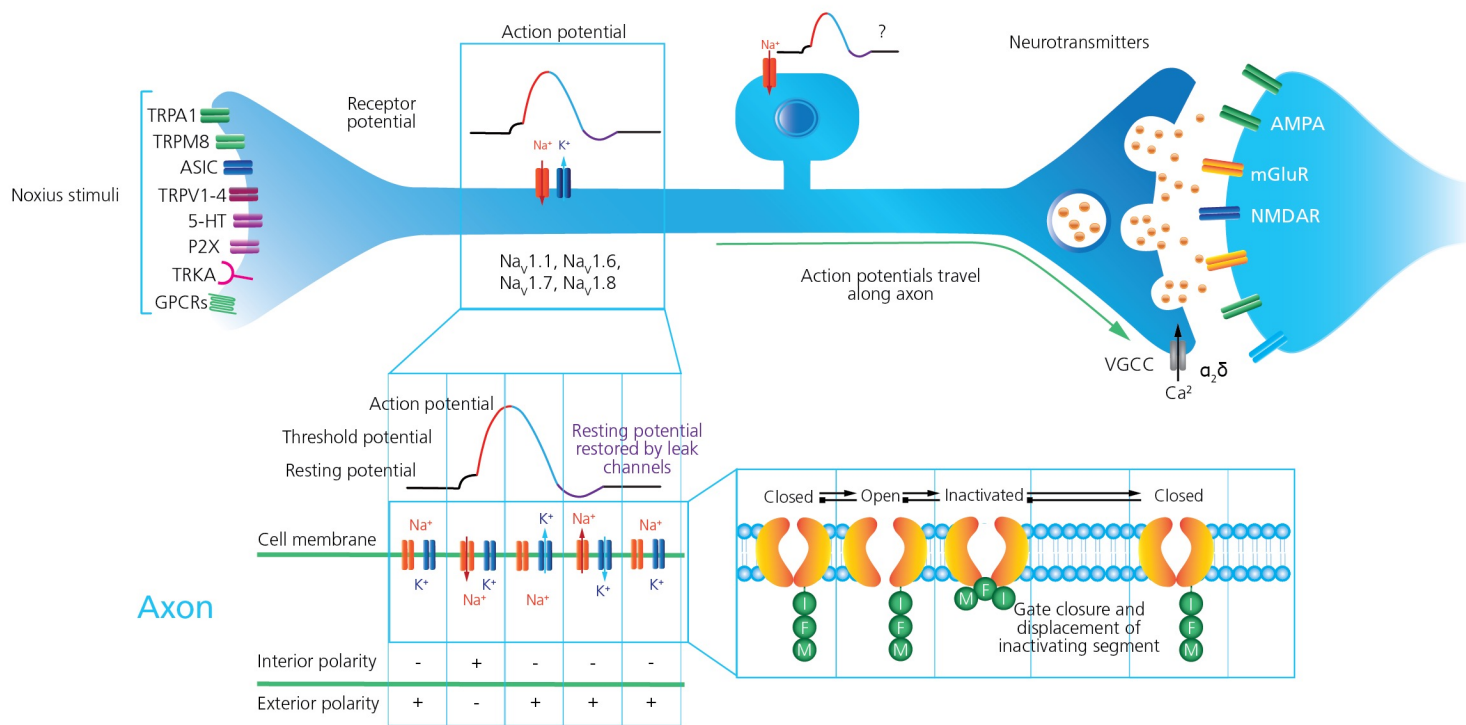


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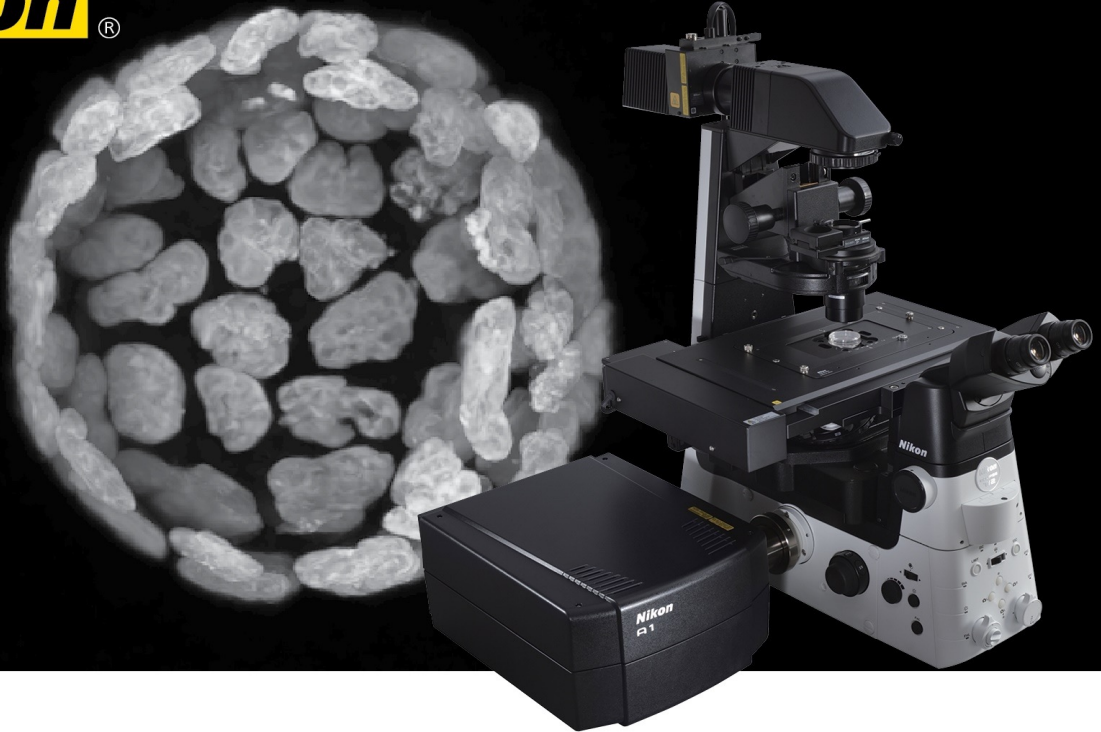
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