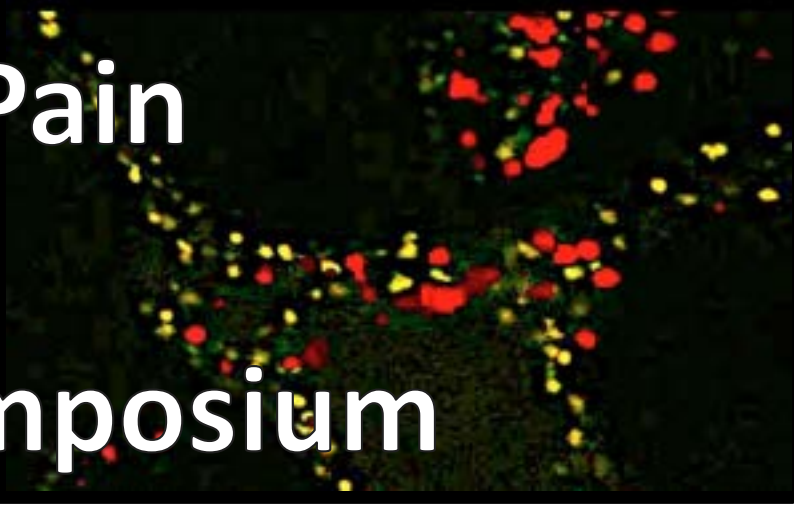
GCC Translational Pain Research 7th Annual Symposium



April 7, 2017

BioScience Research Collaborative

6500 Main St.

Houston, Texas

Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

RICE Unconventional Wisdom

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The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on **biomedical informatics, computational cancer biology, molecular biophysics, neuroengineering and pharmacological sciences.** GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include **bioinformatics, chemical genomics, magnetic resonance, protein crystallography, translational pain research, antimicrobial resistance, neuroengineering, addiction sciences, and regenerative medicine**. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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Agenda

8:45 am Breakfast and poster set up

9:15 am Welcoming remarks by Edgar T. Walters and Carmen Dessauer
Department of Integrative Biology and Pharmacology, UTHealth

9:20 am *Peripheral and Central Contributions to Cognitive and Sensorimotor Dysfunction in Patients with Type II Diabetes*
Stacey Gorniak, University of Houston

10:20 am *Innate Immune Signaling and the Chronic Pain Phenotype*
Tony Yaksh, University of California, San Diego

11:20 am Poster judging

12:00 pm Lunch and networking

1:00 pm Distinguished Trainee Talks

Cell type-specific Synaptic Plasticity and ROS in Neuropathic Mice
Alice Bittar, UTMB

Distinctions between Two Novel, Functionally Defined Classes of Primary Nociceptors Indicate Separate Roles in Driving Ongoing Pain and Evoked Pain After Spinal Cord Injury
Max Odem, UTHealth

The Systematic Analysis of Coding and Long Non-coding RNAs in the Sub-chronic and Chronic Stages of Spinal Cord Injury
Raquel Cuevas Diaz Duran, UTHealth

Inhibition of Opioid Responses in Sensory Neurons after Spinal Cord Injury
Anibal Garza Carbajal, UTHealth

A Novel Chemical Nociception Behavioral Assay in Drosophila Larvae
Roger López Bellido, MDACC

Inhibition of HDAC6 Effectively Treats Chemotherapy-Induced Peripheral Neuropathy
Jiacheng Ma, MDACC

2:30 pm Break

2:40 pm *Improving Evidence Based Pain Care*
Linda Porter, NINDS

3:40 pm Trainee award presentations and brief break

4:00 pm Keck Seminar: *Optogenetic Tools for the Study and Treatment of Pain*
Robert Gereau, Washington University

5:00 pm Reception

Gulf Coast Consortia
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In order of appearance

Stacey Gorniak

Assistant Professor, Department of Health and Human Performance
University of Houston

Peripheral and Central Contributions to Cognitive and Sensorimotor Dysfunction in Patients with Type II Diabetes

About Dr. Gorniak:
Dr. Gorniak is a tenure-track faculty member in the Department of Health and Human Performance at the University of Houston (UH). She is the current Director of the Center for Biomechanics Research (CNBR) at UH. She is also a member of the Texas Obesity Research Center (TORC) at UH. Dr. Gorniak completed her post-doctoral fellowship at the Cleveland Clinic in Biomedical Engineering. She earned her M.S. and Ph.D. in Kinesiology, focused in Biomechanics and Motor Control (with a minor in Statistics) from the Pennsylvania State University – University Park campus, where she was a teaching and research assistant. Dr. Gorniak received a B.S. in Physics from the Pennsylvania State University – Behrend campus. Dr. Gorniak's translational line of research focuses on evaluating both the central and peripheral changes in hand and finger function due to chronic health conditions and movement disorders. Her work in this area combines use of traditional clinical measurement tools with objective kinetic and kinematic measurements to assess hand and finger function. Dr. Gorniak's basic science line of research involves the creation and use of novel devices to evaluate hand and finger function. Her work is currently funded by the American Heart Association (AHA) and the National Institutes of Health (NIH).

Abstract:
Type II Diabetes has been linked to sensorimotor deficits of the hands in the absence of clinical diagnosis of peripheral neuropathy. Mild cognitive impairment (MCI) has also been found in diabetic patients, which also plays a role in one's ability to successfully perform basic self-care activities. In order to investigate the contribution of sensory and cognitive dysfunction on motor function, our lab seeks to elucidate the effects of each system on manual function. In the first project, our data indicate that tactile impairment does not account for all aspects of manual motor dysfunction, suggesting spinal and cortical involvement in motor impairment. In the second project, MCI was found in the diabetic group versus controls. When diabetic patients were asked to perform manual activities in conjunction with working memory tasks (dual-tasking), manual performance declined significantly versus controls while some aspects of cognitive performance were impaired. Similarly, when diabetic patients were asked to verbally recall different words while maintaining their balance (dual-tasking), their indices of balance were significantly impaired, as was cognitive performance. In both studies, health state markers such as blood pressure measures were found to co-vary with motor function. Overall, these data suggest the contribution of both central and peripheral mechanisms to motor disability in Type II Diabetes. In order to investigate these findings, our group has begun a series of imaging studies to better understand both the central and peripheral contributions to motor and cognitive dysfunction in patients with Type II Diabetes.

Tony Yaksh

Emeritus Professor, Department of Anesthesiology
University of California, San Diego

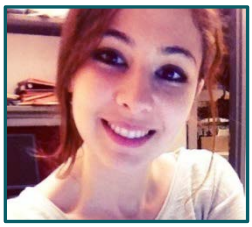
Innate Immune Signaling and the Chronic Pain Phenotype

About Dr. Yaksh:

Dr. Tony L. Yaksh obtained his Ph.D. degree from Purdue University (1971). He served in the U.S. Army in the Biomedical Laboratory at Edgewood Arsenal, MD (1971-73), was a research scientist in the School of Pharmacy, University of Wisconsin (1973-76) and an Associate Research Scientist in Anatomy at University College London with Pat Wall (1976-77). He worked at the Mayo Clinic with Dr. Frederick Kerr in Rochester, MN in Pharmacology and Neurosurgery (1977-1988), where he rose to the rank of Consultant and Professor. Dr. Yaksh joined UCSD in 1988 as Professor and Vice Chairman for Research in the Department of Anesthesiology. His research has been on the biology of pain processing. His studies have provided a basis for understanding the pharmacology of the spinal gating of pain information. He is an expert in spinal drug kinetics and evaluation of spinal drug safety. He has published more than 800 papers and edited 6 texts. His work has garnered over 46,000 citations in over 26,000 papers. He has been a mentor to more than 100 postdoctoral fellows and trainees. He has been funded consistently by NIH since 1977 and twice has been a Javitz Award recipient. Dr. Yaksh has received several honors, including the Kerr Award from the American Pain Society, the Seldon Memorial Lecturer award from the International Anesthesia Research Society, the American Society of Anesthesiologists award for Excellence in Research, the Torsten Gordh lecturer award from the Swedish Society of Medicine, the Bonica Award from the International Association for the Study of Pain and the lifetime achievement award from the North American Neuromodulation Society.

Abstract:

Pain persisting beyond the resolution or control of clinical signs after trauma or inflammation (as in rheumatoid disease) decreases quality of life for millions of people. Preclinical modeling has suggested that this reflects a complex interplay between peripheral and neuraxial mechanisms. The K/BxN serum transfer model of arthritis produces a long-lasting but reversible joint inflammation (15-20d) accompanied by an early onset allodynia that persists long after the resolution of inflammatory indices (>45d). This model is characterized by several properties. i) In the early phase of the model, pain behavior responds to nonsteroidal anti-inflammatory drugs and agents that block spinal sensitization, while in the post-inflammatory late phase, pain only responds to the latter agents. ii) This behavioral profile is accompanied by a persistent activation of dorsal horn microglia and expression of ATF3 (DRG marker of afferent injury). iii) Sprouting and neuroma-like structures in primary afferents and postganglionic sympathetic efferents occur at the peripheral terminals of the joint of the K/BxN in males and females, suggesting a transition in both sexes from an inflammatory to a neuropathic phenotype. iv) Unexpectedly, the female, despite evidence of nerve injury, displays a partial recovery in the postinflammatory state. v) Pharmacological studies and studies with mutant mice revealed that transition to a neuropathic phenotype is mediated by spinal Toll-like receptor 4 (TLR4) signaling as evidenced by a near complete resolution of late-phase pain in both males and females in relevant knock-out strains and after spinal TLR4 antagonism during the early, but not postinflammatory phase. vi) The TLR4 signaling is through MyD88. vii) TLR4 also signals through TRIF and interferon (IFN), which we found to attenuate the algesic effects of TLR4-MyD88 signaling (e.g. TRIF/IFN-r KO lead to an enhanced pain state in both males and females. Accordingly, we believe that both sexes use adaptive immune cells and TLR4 signaling, but to varying degrees. (NS 099338).



Alice Bittar

UTMB

Cell type-specific Synaptic Plasticity and ROS in Neuropathic Mice

About Ms. Bittar:

Ms. Bittar is a 5th year student in Dr. Jin Mo Chung's laboratory. They focus on investigating the mechanisms of peripheral nerve injury-induced neuropathic pain at both the neuronal physiology and behavioral levels. She is in specific interested in the role of reactive oxygen species in superficial dorsal horn neuronal excitability in a neuropathic pain model.

Abstract:

Peripheral nerve injury induces spinal synaptic plasticity which in turn underlies central sensitization and neuropathic pain. Using whole cell patch recording from identified dorsal horn neurons in a spinal cord slice preparation, we previously showed that, in response to the same frequency conditioning stimulation (CS), long-term potentiation develops in spinothalamic tract neurons (STTn-LTP), whereas long term-depression develops in GABAergic interneurons (GABAn-LTD). This phenomenon is thus termed “cell type-specific synaptic plasticity”, and is speculated to significantly contribute to central sensitization, GABA-disinhibition and neuropathic pain behavior induced by peripheral nerve injury. In addition, previous data showed that different ROS subtypes are differentially involved in the cell type-specific synaptic plasticity: superoxide radicals were shown to be important for both CS-induced STTn-LTP and GABAn-LTD, whereas hydroxyl radicals were involved only in CS-induced GABAn-LTD. In this study, we examined the establishment of cell type-specific synaptic plasticity in spinal nerve ligation (SNL) neuropathic pain model. We further examined the effect of specific superoxide and hydroxyl radicals’ scavengers on nerve injury-induced long-term changes in synaptic efficacy. We hypothesize that cell type-specific synaptic plasticity is established in STTn and GABAn of neuropathic mice, and that the different ROS subtypes are differentially involved in the established SNL-induced changes in synaptic efficacy. Long-term changes were assessed by comparing the following between naïve and neuropathic mice (7 days post-SNL): 1) The induction of synaptic plasticity following CS; 2) Amplitudes of raw excitatory postsynaptic currents (EPSCs) in STTn and GABAn; and 3) The effect of specific ROS scavengers on EPSCs in STTn and GABAn. Results showed that, following CS, LTP was induced in the STTn of the contralateral side of SNL, but was occluded in those of the ipsilateral side of SNL. LTD in GABAn of the ipsilateral side of SNL still developed after CS, but in a less robust fashion. Furthermore, baseline (before CS) EPSC amplitude in STTn of the SNL ipsilateral side was significantly greater than that of STTn of the contralateral side. Baseline EPSC of GABAn of the ipsilateral side of SNL was smaller than that of their contralateral counterparts. In addition, specific ROS scavengers alleviated SNL-induced changes in EPSC amplitudes in a cell type-specific manner. These results suggest that, in SNL mice, excitatory synaptic efficacy in STTn and GABAn are persistently potentiated and depressed, respectively, leading to central sensitization and neuropathic pain. The results also showed that ROS are important for the maintenance of such changes in synaptic strength observed in neuropathic pain model. The demonstrated importance of ROS subtypes in the maintenance of neuropathic pain is a novel finding and holds important translational implications that point towards targeting specific ROS species for better pain relief.

Max Odem

UTHSC

Distinctions between Two Novel, Functionally Defined Classes of Primary Nociceptors Indicate Separate Roles in Driving Ongoing Pain and Evoked Pain After Spinal Cord Injury

About Mr. Odem:

Mr. Odem is a 4th year graduate student in the UTHealth Graduate School of Biomedical Sciences. He obtained his B.S. in Biology from Tarleton State University in 2010 and his M.S. in Biology from Texas A&M – Corpus Christi in 2013. His scientific interests originally included marine biology, ecology, and invertebrate behavior; Max's early graduate research focused on defensive and non-defensive behavioral responses to noxious stimuli in the marine mollusk Aplysia californica and associated changes within the feeding neural circuit. He joined the lab of Edgar T. Walters, Ph.D. in spring 2014 because of their mutual interest in the evolutionary development of nociception and role that the peripheral nervous system may play in driving chronic pain. He immediately made a significant contribution to the ongoing pain behavioral studies using rodents with spinal cord injury (SCI; Yang et al., J Neurosci, 2014). In 2015, Max obtained a scholarship to attend the highly competitive Ohio State University SCI training course where he gained the behavioral expertise and surgical skills necessary to further investigate the neurobiological consequences of SCI in the peripheral nervous system. Nociceptor hyperexcitability and the spontaneous generation of action potentials are significant drivers of chronic pain after SCI. Max's current research is determining the contribution of irregular fluctuations in membrane potential to the generation of spontaneous activity in nociceptors and further charactering subpopulations of nociceptors based on these and other electrophysiological, pharmacological, and cellular characteristics.

Abstract:

The major complaint of many people with chronic pain is ongoing, apparently spontaneous pain, but very little is known about the underlying mechanisms. One plausible mechanism is persistent spontaneous activity (SA) generated in primary nociceptors. Previously we showed that ongoing pain produced by thoracic spinal cord injury (SCI) in rats requires chronic activity in nociceptors (Yang et al., J Neurosci, 34:10765, 2014). For many months after SCI, nociceptors generate SA in their somata, both in vivo and after dissociation (Bedi et al., J Neurosci, 30:14870, 2010; Wu et al., Pain, 154:2130, 2013). In principle, SA might be generated in normally silent neurons by 1) sufficient tonic depolarization of resting membrane potential (RMP), 2) decreased action potential (AP) threshold, and/or 3) increased amplitude of transient, depolarizing, spontaneous fluctuations of MP (DSFs). We have found that SCI causes all three effects in a newly recognized class of nociceptors that appear to be specialized for generating SA in persistent pain conditions. Small (15-30 µm soma diameter) DRG neurons tested 24 h after dissociation fell into 2 distinct classes. Ac nociceptors, defined by their strong AP accommodation during prolonged depolarization, never exhibited SA after SCI. In stark contrast, RF nociceptors, defined by non-accommodating repetitive firing during prolonged depolarization, usually showed SA after SCI (~60%). Both populations are considered nociceptors because most neurons within each class (~60% and 80%, respectively) were excited by 1 □M capsaicin similar RMP (-60 vs -63 mV), but after SCI only the RF neurons showed significant depolarization of the RMP (-53 vs -59 mV). AP voltage thresholds differed between RF and Ac neurons from control animals (-34 vs -28 mV), and showed opposite changes after SCI (decreasing to -40 mV and increasing to -24 mV, respectively). In A-fiber sensory neurons, regular, sinusoidal, subthreshold oscillations are known to drive SA after nerve injury (Amir et al., J Neurosci, 19:8589, 1999). In contrast, using a novel automated method for quantitative analysis of SFs, we

found that both RF and Ac neurons generated DSFs that were always irregular (non-sinusoidal) within the normal range of RMP in control and SCI conditions. SCI significantly increased the amplitude of DSFs in RF neurons (from 2.3 to 3.8 mV) but not in Ac neurons (from 2.5 to 2.7 mV). Under control conditions the incidence of somal SA was very low and large depolarizing SFs (DSFs, 5-25 mV) almost never occurred in either class. After SCI the incidence of large DSFs increased significantly in RF but not Ac neurons and their frequency (0.08-1.7 Hz) matched the SA firing rate (0.08-2.14 Hz). The combination of persistent depolarization, reduced AP threshold, and increased DSF amplitude accounts for the chronic generation of SA in RF nociceptors, and suggests that this class of nociceptors has specialized mechanisms that function to generate irregular, low-frequency SA in their somata, and possibly other cellular compartments, during ongoing pain conditions. The Ac nociceptors showed hyperexcitability after SCI in the form of reduced rheobase. However, Ac neurons never displayed SA and hence this hyperexcitability effect in Ac nociceptors, if also expressed in peripheral terminals, may contribute to evoked pain rather than ongoing pain. We are addressing the extent to which the novel functional distinction between RF and Ac nociceptors corresponds to existing phenotypic classes of nociceptors, and whether transitions can occur between the Ac and RF types. This discovery raises the possibility that molecular differences between RF and Ac nociceptors will allow specific targeting of each class of nociceptors for selective treatment of either ongoing or evoked pain.

Raquel Cuevas Diaz Duran

UTHSC

The Systematic Analysis of Coding and Long Non-coding RNAs in the Sub-chronic and Chronic Stages of Spinal Cord Injury

About Dr. Cuevas Diaz Duran:

Dr. Raquel Cuevas obtained her PhD degree in Biotechnology from Tecnologico de Monterrey University in Monterrey, Mexico in 2014 working in the Cell Therapy research group. Her thesis involved the genomic characterization of adipose-derived stem cells and the identification of regulatory modules driving differentiation using time-series gene expression profiles. In 2015, she joined Dr. Jiaqian Wu's lab in the Vivian L. Smith Department of Neurosurgery at McGovern Medical School as a postdoctoral research fellow. Her research is focused in studying gene expression and transcriptional regulation in stem cells and the central nervous system. She analyzes RNA-seq, ChIP-seq, ATAC-seq, and RIP-seq data of samples generated in her lab. She has special interest in the regulation exerted by long non-coding RNAs (lncRNA) on protein-coding genes in cells from the central nervous system during development and disease.

Abstract:

Previous studies of SCI have usually focused on a few genes and pathways at a time. In particular, the biological roles of long non-coding RNAs (lncRNAs) have never been characterized in SCI. Our study is the first to comprehensively investigate alterations in the expression of both coding and long non-coding genes in the sub-chronic and chronic stages of SCI using RNA-Sequencing.

Moderate contusive injury was performed using female Sprague-Dawley rats at T9 level of the spinal cord. Animals in the sham control group received a dorsal laminectomy without a contusive injury. At 1 month, 3 months, and 6 months post-SCI, a segment of the spinal cord (0.5 mm) at the epicenter was dissected and RNA was sequenced. The expression of protein-coding genes lncRNAs was calculated using our in-house pipeline and differentially expressed genes were found at each time point. Through pathway analysis and network construction, the functions of differentially expressed genes were analyzed systematically. We predicted the potential regulatory function of non-coding transcripts, revealed enriched motifs of transcription factors in the upstream regulatory regions of differentially expressed lncRNAs, and identified differentially expressed lncRNAs homologous to human genomic regions which contain single-nucleotide polymorphisms associated with diseases.

We created a combined annotation of 10,889 lncRNA transcripts derived from ENSEMBL and NCBI repositories. Additionally, we implemented a classification system for lncRNAs based on their location with respect to the most proximal protein-coding genes. We sequenced 36 RNA-seq libraries and obtained temporal expression profiles corresponding to 22,075 protein-coding and 8,368 lncRNA genes. Gene-set enrichment analysis highlighted the relation of gene expression profiles and functions. Using a "guilt-by-association" analysis, we inferred that differentially expressed (DE) lncRNAs are associated with biological functions including signaling cascades, epigenetic modification, immune responses, nervous system, and extracellular matrix. We found a high expression correlation between DE lncRNAs and DE protein-coding gene neighbors, suggesting cis-regulation of protein-coding genes by lncRNAs. Furthermore, we found transcription factor binding motifs enriched in the regulatory regions of many DE lncRNAs. Network and pathway analysis provided further insights into molecular signaling that regulates gliosis in the sub-chronic and chronic SCI.

Our systematic examination and analysis of post-SCI transcriptional alterations in rat has identified important pathways and networks for the pathological progression of SCI, and pinpointed novel target genes for further investigation, including a number of interesting lncRNA candidates with potentially important regulatory functions and human disease homologs.

Anibal Garza Carbajal

UTHSC

Inhibition of Opioid Responses in Sensory Neurons after Spinal Cord Injury

About Dr. Garza-Carbajal:
After graduating from Puebla Autonomous University (BUAP, Mexico) in biomedicine, he obtained his PhD in neuroimmunology from the University of Duisburg-Essen (Germany). During his first postdoctoral position, Anibal worked at the Max Planck institute of molecular Genetics (Berlin, Germany) and the University of Cologne (Cologne, Germany) studying neuronal and glial pain-related signaling via high content microscopy. Since April 2016 he joined Carmen Dessauer's group in the department of Integrative Biology and Pharmacology at UTHealth McGovern Medical School (Houston). His work is mostly focused on the crosstalk between signaling pathways in the context of pain sensitization on sensory neurons of the dorsal root ganglia.

Abstract:
Opioid insensitivity and tolerance are pathognomonic of different forms of pathological pain. Reduced opioid effects render these substances ineffective in pain management, which can contribute to substance abuse and opioid addiction. In the context of spinal cord injury (SCI), patients are refractory to the effects of opioids. We have previously shown that neurons from the dorsal root ganglia (DRG) of SCI animals show a marked increase in excitability and spontaneous activity and loss of sensitivity towards the inhibitory effects of G□on cAMP production, a key mechanism mediating opioid analgesic effects. Although the origin of these alterations is unknown, it has been suggested to contribute to the development and maintenance of different forms of chronic pain. In order to identify potential mechanisms that abolish inhibition of cAMP signaling by opioids, we have characterized the opioid responses of DRG neurons from rats cultured one month after either sham or SCI surgery via high content microscopy. This allowed us to evaluate the data on a single cell basis in the heterogeneous neuronal populations of the DRG. Phosphorylation of the PKA regulatory subunit RII (PKA-pRII) was used as a surrogate measurement of activity in the cAMP pathway. Detection of PKA-pRII was performed by immunocytochemistry. Further data analysis was performed using FACS analysis software.
We have shown that SCI and naïve/sham neurons do not differ in basal or forskolin- or serotonin- (5-HT) stimulated PKA-pRII, suggesting a lack of SCI effects on general cAMP production. This is consistent with measurement of total AC activity in DRGs, which is unaltered after SCI. Opioid inhibitory signaling was evaluated using the mu opioid agonist DAMGO and measured as a reduction of forskolin- or 5-HT-stimulated PKA-pRII. SCI neurons showed a small but significant decrease in DAMGO inhibition of 5-HT-stimulated cAMP generation, with an IC_{50} of nearly 3 times the value observed in naïve/sham animals. Similar effects were observed using forskolin as stimulus. These effects seem to be centered on the medium-sized and small neurons, suggesting subpopulation-specific effects.
Conclusions and significance: Together, our results show that after a spinal cord injury, sensory neurons from DRGs below the level of injury develop resistance to opioid effects on cAMP signaling. As endogenous opioids have been implicated in the inhibition of sustained pain in different forms of transient inflammatory hyperalgesia, such generalized resistance to opioid effects may sensitize SCI patients to stimuli unrelated to their SCI.

Roger López Bellido

MDACC

A Novel Chemical Nociception Behavioral Assay in Drosophila Larvae

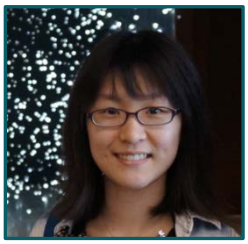
About Dr. López Bellido:

Roger Lopez Bellido is a postdoc at the University of Texas MD Anderson Cancer Center. He received his Degree in Dentistry in Peru and then he moved to Spain, where he got his doctorate in Neuroscience. His research interests include how different noxious stimuli (thermal, mechanical, and chemical) induce distinct nociceptive responses. His long-term career goal is to better understand pain mechanisms for developing novel devices to measure pain and discover new analgesic drugs.

Abstract:

Noxious stimuli of different sensory modalities (thermal, mechanical, electrical or chemical) provoke aversive pain behaviors in all animals. The molecular/genetic bases of thermal and mechanical nociception responses are beginning to be understood. However, chemically-provoked pain has not yet been studied intensively, in part due to a lack of simple genetically tractable models. **Objective**: Our goal is to use *Drosophila* genetics to establish a genetically tractable system to study the cellular and molecular genetic bases of chemically-induced nociception. **Methods**: We developed a new model of chemical nociception in *Drosophila* by exposing larvae to increasing concentrations of hydrochloric acid (HCl). Concentrations ranging from 1.0 % to 9.0 % produced an increasingly intense behavioral reaction which was manifest as an aversive rolling response, behaviorally similar to what is seen with heat and harsh touch. 0.5% HCl was subthreshold and provoked no rolling. Therefore, 0.5% HCl was used to study chemical allodynia (lowering of the response threshold in response to tissue injury). We also developed a physical wounding nociceptive sensitization model that elicited chemical allodynia in larvae. **Results**: To determine which sensory neurons are required for chemical nociception we genetically silenced the four different classes of multidendritic peripheral sensory neurons (classes I-IV). Class III, which mediates larval responses to noxious cold, was dispensable for chemical nociception. By contrast, classes I, II and IV were all required for the response to HCl, with class IV making the largest contribution. This response by multiple cell types has not been observed with other sensory modalities (heat, cold, mechanical). Physical puncture wounding induced a stronger chemical allodynia response, perhaps because this injury involves a breach in the external cuticle barrier. UV irradiation, which elicits strong thermal and mechanical sensitization was relatively ineffective with the chemical modality. At the genetic level, loss of function mutants of Piezo (an ion channel mediating mechanosensory transduction), or the transient receptor potential (TRP) channels Painless, Trpm, Trpml, Trpmγ, Trpa, Brv1, all displayed reduced sensitivity to noxious chemical stimuli. This was also true of Pvr (PDGFR/VEGFR like receptor tyrosine kinase) and its ligands.

Conclusion: We developed a novel assay to study chemically-induced nociception in *Drosophila* larvae. Nociceptive behavioral responses to HCl are mediated by class I, II and IV multidendritic sensory neurons. The spectrum of TRP channels required for chemical nociception appears partially overlapping with heat/mechanical. The high genetic resolving power of *Drosophila* will not only improve our basic understanding of fundamental mechanisms of chemical nociception, but also help us to identify novel gene targets for pain treatment that may ultimately prove useful in humans.



Jiacheng Ma

MDACC

Inhibition of HDAC6 Effectively Treats Chemotherapy-Induced Peripheral Neuropathy

About Dr. Ma:

Dr. Ma received her bachelor's degree from Beijing Institute of Technology in Pharmaceutical Engineering. In 2015, she received her PhD in Pharmacology and Toxicology from University of Kansas, where she studied diabetic peripheral neuropathy and targeting the heat shock proteins as a treatment for neurodegenerative diseases. She is currently a postdoctoral fellow in Dr. Annemieke Kavelaars and Dr. Cobi Heijnen's lab at MD Anderson Cancer Center, studying the neurotoxic side effects of cancer treatment, including chemotherapy-induced peripheral neuropathy and cognitive impairments. Her current project focuses on targeting histone deacetylase 6 (HDAC6) as a treatment for chemotherapy-induced neurodegenerative symptoms.

Abstract:

Chemotherapy-induced peripheral neuropathy is one of the most common dose-limiting side-effects of cancer treatment. Currently, there is no FDA-approved treatment available. Histone deacetylase 6 (HDAC6) is a microtubule-associated deacetylase whose function includes regulation of α-tubulin-dependent intracellular mitochondrial transport. Here we examined the effect of HDAC6 inhibition on cisplatin-induced peripheral neuropathy. We used a novel HDAC6 inhibitor ACY-1083, which shows 260-fold selectivity towards HDAC6 versus other HDACs. Our results show that HDAC6 inhibition completely reversed already existing cisplatin-induced mechanical allodynia, spontaneous pain, and numbness. These findings were confirmed using the established HDAC6 inhibitor ACY-1215 (Ricolinostat), which is currently in clinical trials for cancer treatment. Mechanistically, treatment with the HDAC6 inhibitor increased α-tubulin acetylation in the peripheral nerve. In addition, HDAC6 inhibition restored the cisplatin-induced reduction in mitochondrial bioenergetics and mitochondrial content in the tibial nerve, indicating increased mitochondrial transport. At a later time point, dorsal root ganglion mitochondrial bioenergetics also improved. HDAC6 inhibition restored the loss of intra-epidermal nerve fiber density in cisplatin-treated mice. Our results demonstrate that pharmacological inhibition of HDAC6 completely reverses all the hallmarks of established cisplatin-induced peripheral neuropathy. These results are especially promising because one of the HDAC6 inhibitors tested here is currently in clinical trials as an add-on cancer therapy, highlighting the potential for a fast clinical translation of our findings.

Linda Porter

Director, Office of Pain Policy, Program Director, Systems and Cognitive Neuroscience NINDS

Improving Evidence Based Pain Care

About Dr. Porter:

Dr. Porter joined the National Institute for Neurological Disorders and Stroke in 2003. She holds a bachelor's degree in Physical Therapy from McGill University and a Ph.D. in neuroscience from Boston University School of Medicine. Dr. Porter trained in neurophysiology at Rockefeller University with Hiroshi Asanuma. She was on the faculty of the Uniformed Services University of the Health Sciences for 15 years, where she directed an NIH funded research program aimed at elucidating mechanisms of sensory-motor integration. As a Program Director at NINDS, Dr. Porter was responsible for managing the institute's pain research portfolio. She played an essential role in promoting the objectives and activities of the NIH Pain Consortium, a trans-NIH entity whose mission is to advance the NIH pain research agenda. In 2012, Dr. Porter became Director of the Office of Pain Policy at NIH, which was established in response to recommendations from the 2011 Institute of Medicine report on pain. Her office supports and guides the activities of the NIH Pain Consortium and those of the Interagency Pain Research Coordinating Committee, a congressionally mandated advisory committee to the Secretary of Health and Human Services. She co-chaired the development of the National Pain Strategy report and now co-chairs the Strategy's implementation committee. She also is co-chair for the committee that oversees development of the Federal Pain Research Strategy on behalf of the IPRCC. Dr. Porter has been recognized for her work in advancing the federal pain research agenda and efforts on moving forward the National Pain Strategy.

Abstract:

The National Pain Strategy outlines the first broad-ranging federal effort to reduce the burden of pain for millions of Americans. The Strategy was developed in response to the 2011 IOM report, "Relieving Pain in America". It outlines steps in six key areas including population research, prevention and care, disparities, service delivery and payment, professional and public education and communication. The intent is to create a setting in which individualized, safe and effective treatment choices are available for people with pain. The current science indicates that this requires quality integrated care that is not solely reliant on prescription medications. Implementation of the Strategy is underway, and updates on the progress made by federal and external stakeholders will be presented. A strong evidence base is needed to develop, deliver, and support quality integrated health care. A companion effort to develop a long term Federal Pain Research Strategy is near completion. This strategic plan will guide the federal agencies and departments that support pain research to advance the science and ultimately improve patient outcomes. An overview of the strategy and its current status will be provided.

Robert Gereau

Professor, Anesthesiology and Neuroscience
Washington University

Optogenetic Tools for the Study and Treatment of Pain

About Dr. Gereau:

Dr. Gereau is the Dr. Seymour and Rose T. Brown Professor of Anesthesiology, and serves as Director of the Washington University Pain Center. He earned a Bachelor's degree from Missouri State University, and a PhD in Neuroscience from Emory University (1995). Following postdoctoral training at the Salk Institute (1998), he took a faculty position in Neuroscience at Baylor College of Medicine, serving as Assistant and Associate Professor until 2004, when he was recruited to Washington University School of Medicine.

Gereau's laboratory utilizes a combination of electrophysiology, optogenetics, and molecular approaches to understand mechanisms of maladaptive plasticity underlying the development of chronic pain. These studies include development of new enabling technologies for wireless measurement and manipulation of neural function. The lab also conducts translational research, including comparative studies of human and animal physiology, as well as healthy human volunteer studies aimed at establishing proof of concept for novel therapies. Dr. Gereau's work has been supported by the NIH for over 20 years, including an NIH SPARC Award and the NIH Director's Transformative Research Award.

Dr. Gereau has served as reviewing editor for Journal of Neuroscience, as Associate editor for Pain, Associate Editor for Pain Reports, and on the Editorial boards of Molecular Pain, Neurobiology of Pain, and Journal of Neurophysiology. He served as member or chair of multiple NIH review panels, and currently serves on the Board of Scientific Counselors for NIDCR. Dr. Gereau served on the Board of Directors for the American Pain Society for several years. In 2017, Gereau was awarded the Frederick H.L. Kerr Basic Science Research Award from the American Pain Society.

Abstract:

In the quest for new treatment options for patients suffering from chronic pain, a major hurdle is the perceived difficulty in predicting clinical efficacy of a new potential treatment based only on preclinical studies in animal models. In this lecture, Dr. Gereau discusses some of the issues in preclinical and early human studies that confront the field, and how his lab and others are working to overcome these obstacles to help bring new treatments to patients as quickly and efficiently as possible. He will describe work in his lab that goes from identifying mechanisms of central sensitization in preclinical models to initial testing of that mechanism in healthy human volunteers.

NOTES

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NOTES

Poster #1

Inhibition of EPAC Signaling Suppresses Persistent Spontaneous Activity in Primary Nociceptors after Spinal Cord Injury

Bavencoffe AG, Odem MA, Dessauer CW, Walters ET

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A majority of people with spinal cord injury (SCI) suffer from chronic neuropathic pain. In a rat T10 contusion model, nociceptors at and below the injury level exhibit chronic spontaneous activity (SA) generated in their somata in vivo and in vitro that persists for months after SCI (Bedi et al., J Neurosci 30:14870, 2010). The nociceptor hyperactivity is correlated with chronic pain-related behaviors and is necessary for the maintenance of both reflex hypersensitivity and an operant measure of spontaneous pain (Yang et al., J Neurosci 34:10765, 2014). Recently we found that SCI-induced SA in dissociated nociceptors requires ongoing cAMP signaling in a macromolecular complex composed of A-kinase anchoring protein (AKAP150), adenylyl cyclase (AC5/6) and PKA (Bavencoffe, Li et al., J Neurosci. 36:1660, 2016). The importance of cAMP signaling raises the possibility that multiple downstream cAMP effectors might contribute to SCI-induced SA in nociceptors.

We have begun to investigate the contribution of EPAC (Rap1 exchange protein activated by cAMP) to the maintenance of SA after SCI by examining the effects of EPAC inhibitors ESI-09, ESI-05 and CE3F4. Neurons were isolated from DRGs L3 to L5 1-2 months after SCI and recorded 18-24 h after dissociation. Presumptive nociceptors were identified by characteristic electrophysiological properties we found to be a reliable signature for capsaicin sensitivity in small DRG neurons. As reported previously, SA incidence recorded in current clamp (I=0) was significantly higher in nociceptors from SCI rats (30 of 39 neurons) compared to nociceptors from naïve, uninjured rats (4 of 34 neurons). In the SCI group, incubation of sensory neurons with ESI-09 (targeting both EPAC1and EPAC2, 5 μM), ESI-05 (targeting EPAC2, 5 μM) or CE3F4 (targeting EPAC1, 10 μM) significantly reduced SA incidence by hyperpolarizing resting membrane potential (RMP). In the naïve group, incubation of nociceptors with all three EPAC inhibitors did not significantly change the low incidence of SA. However, only ESI-09 produced a similarly large hyperpolarization of RMP in sensory neurons from the naïve group, comparable to the one observed in the SCI group. Incubation of sensory neurons isolated from uninjured animals with EPAC activator 8-pCPT-2-O-Me-cAMP-AM (007-AM, 10 μM) failed to generate repetitive firing or any depolarization but decreased slightly the rheobase, a sign of increased excitability. Finally, recent data tend also to imply a major role of PKC in modulation of nociceptor excitability. The importance of PKC in nociceptor hyperexcitability and its connection to the cAMP-EPAC signaling described above is currently under investigation.

These observations indicate that ongoing EPAC signaling contributes to normal nociceptor excitability under our culture conditions and might also contribute to resting excitability of nociceptors in uninjured animals in vivo. An important question is whether SCI further enhances cAMP-EPAC signaling to produce nociceptor SA. Taken together, our results encourage the study of cAMP-EPAC signaling as a potential therapeutic target for chronic pain.

Funding: This work is supported by NIH grant RO1 NS091759 (Dessauer and Walters).

Poster #2

EPAC2 and Its Role in Chronic Pain

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Chronic pain affects over 100 million people in the US alone, yet our understanding of the molecular mechanisms behind it remains incomplete. The canonical cAMP to PKA signaling pathway has long been known to play a role in chronic pain, but recently the novel cAMP sensors exchange protein activated by cAMP (EPAC) 1 and 2 have been to show to contribute. EPAC1 and 2 are implicated in the transition from acute to chronic pain as well as its maintenance. Much of the recent work has focused on EPAC1 in chronic pain; however, both EPAC1 and EPAC2 are highly expressed in the dorsal root ganglion, suggesting a role for EPAC2 in the context of pain.

Our study uses mechanical, thermal, and operant behavior tests to assess pain responses in EPAC2 knockout and wild type mice subjected to two murine models of chronic pain. We employ a hyperalgesic priming model to study the transition from acute to sustained pain; in this model, our early results suggest only the female mice enter into the hyperalgesic state. Our second chronic pain model utilizes spinal cord injured (SCI) mice. These mice receive a moderate spinal cord contusion at the T9 vertebrae. While this study is ongoing, our early results show a SCI pain effect in mice, as well as differences in the recovery times between males and females. Female mice recover to an average Basso Mouse score of 4 in 1-2 months (limited plantar stepping ability), while males remain at a score of 2 (extensive ankle movements). We are currently using these chronic pain models to explore our hypothesis that EPAC2 knockout can alleviate or prevent the transition to and maintenance of chronic pain.

Poster #3

The Systematic Analysis of Coding and Long Non-coding RNAs in the Sub-chronic and Chronic Stages of Spinal Cord Injury

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Abstract:
Previous studies of SCI have usually focused on a few genes and pathways at a time. In particular, the biological roles of long non-coding RNAs (lncRNAs) have never been characterized in SCI. Our study is the first to comprehensively investigate alterations in the expression of both coding and long non-coding genes in the sub-chronic and chronic stages of SCI using RNA-Sequencing.
Moderate contusive injury was performed using female Sprague-Dawley rats at T9 level of the spinal cord. Animals in the sham control group received a dorsal laminectomy without a contusive injury. At 1 month, 3 months, and 6 months post-SCI, a segment of the spinal cord (0.5 mm) at the epicenter was dissected and RNA was sequenced. The expression of protein-coding genes lncRNAs was calculated using our *in-house* pipeline and differentially expressed genes were found at each time point. Through pathway analysis and network construction, the functions of differentially expressed genes were analyzed systematically. We predicted the potential regulatory function of non-coding transcripts, revealed enriched motifs of transcription factors in the upstream regulatory regions of differentially expressed lncRNAs, and identified differentially expressed lncRNAs homologous to human genomic regions which contain single-nucleotide polymorphisms associated with diseases.
We created a combined annotation of 10,889 lncRNA transcripts derived from ENSEMBL and NCBI repositories. Additionally, we implemented a classification system for lncRNAs based on their location with respect to the most proximal protein-coding genes. We sequenced 36 RNA-seq libraries and obtained temporal expression profiles corresponding to 22,075 protein-coding and 8,368 lncRNA genes. Gene-set enrichment analysis highlighted the relation of gene expression profiles and functions. Using a "guilt-by-association" analysis, we inferred that differentially expressed (DE) lncRNAs are associated with biological functions including signaling cascades, epigenetic modification, immune responses, nervous system, and extracellular matrix. We found a high expression correlation between DE lncRNAs and DE protein-coding gene neighbors, suggesting *cis*-regulation of protein-coding genes by lncRNAs. Furthermore, we found transcription factor binding motifs enriched in the regulatory regions of many DE lncRNAs. Network and pathway analysis provided further insights into molecular signaling that regulates gliosis in the sub-chronic and chronic SCI.
Our systematic examination and analysis of post-SCI transcriptional alterations in rat has identified important pathways and networks for the pathological progression of SCI, and pinpointed novel target genes for further investigation, including a number of interesting lncRNA candidates with potentially important regulatory functions and human disease homologs.

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Prolactin and CGRP Produce Sex-specific Effects in Preclinical Migraine Models

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Migraine is the 3rd most common disease on earth, the 8th most disabling, and is 2-3 times more common in women. The pathophysiology that leads to migraine is poorly understood and treatment is only effective in about 50% of migraine sufferers. Although the intensity of headache attacks is similar between women and men, the duration of attacks is significantly longer in women and ≈50% of females migraineurs have attacks during specific times of their menstrual cycle. Thus, there is a clear hormonal contribution to migraine. One possible mediator contributing to the sex differences in migraine is the pituitary hormone prolactin (PRL). Serum PRL levels are tightly controlled and regulated by factors affecting and triggering migraine, such as menstrual cycles, menarche, pregnancy, lactation and psychological or physical stress. Moreover, elevation of serum PRL is *strongly correlated* to migraine attacks but mechanisms by which PRL may contribute to migraine pain are not known. Additionally, CGRP plays a critical role in migraine as it is elevated in the blood during attacks, systemic administration triggers attacks, and migraines can be treated with therapies blocking CGRP signaling. Levels and function of CGRP are also regulated by gonadal hormones but whether CGRP contributes to migraine equally between females and males is not known. The hypothesis of this project is that PRL and CGRP contribute to migraine in a female-specific manner via modulation of nociceptors innervating the dura/meninges.

Poster #5

A Genetically Tractable Platform for Identifying Regulators of the Acute to Chronic Pain Transition

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Tissue damage leads to an adaptive lowering of the threshold for pain behavior known as nociceptive (pain) sensitization. While injury-induced nociceptive sensitization is an adaptive pain modulation in response to tissue damage, when proper recovery fails to occur it can lead to maladaptive and debilitating chronic pain. Our unique platform takes advantage of the powerful genetics of *Drosophila* and combines UV radiation-induced tissue damage with quantitative behavioral assessment of thermal nociception. To identify regulators of the transition from acute to chronic pain, we performed an *in vivo* tissue-specific RNAi screen looking for genes that led to persistent thermal hypersensitivity when specifically knocked down in pain sensory neurons. Among 150 RNAi lines targeting conserved kinase-encoding genes, we found multiple potential regulators of the elusive acute-chronic pain transition. The most striking candidate to emerge from this pilot screen was the *insulin receptor* (*InR*). *InR* knockdown caused prolonged hypersensitivity toward thermal stimulation that was not accompanied by defects in baseline nociception (no injury) or in the acute adaptive sensitization to injury. Thus, the defect in *InR* knockdown larvae is specific to the transition from acute to chronic pain. The identification of *InR* as a regulator of the acute to chronic pain transition prompted us to model painful diabetic neuropathy (PDN), one of the most prevalent complications of diabetes that can manifest as disabling pain syndromes. Similar to knockdown of *InR*, *Drosophila* models of both type 1 and type 2 diabetes exhibited prolonged PDN-like thermal pain hypersensitivity. This data suggest that diabetic conditions in *Drosophila* lead to pathological pain hypersensitivity similar to that experienced by diabetic patients, advocating that our genetically tractable platform is useful for dissecting genetic/molecular mechanisms of PDN pain syndromes. This discovery suggests a novel hypothesis not yet investigated in the field, namely that Insulin signaling is required within nociceptive sensory neurons to prevent chronic pain development.

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Poster #6

Does Pain Make you Patient? Acute Laboratory Pain Predicts Decreased Impulsive Decision-Making.

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Clinical pain disorders are often treated with prescription analgesics that have high risk for substance abuse. Determining whether chronic pain patients are at risk for substance abuse is complicated by the co-morbidity of chronic pain with severe mood disorders. Therefore, using healthy young adults, the current study investigated the effect of acute, laboratory pain on delay discounting (DD), a behavioral process linked with impulsivity and substance use disorders, which quantifies the extent to which outcomes are devalued because of their delay. Using a within-subjects repeated measures design (DD before and during the pain manipulation), participants' delay discounting rates were assessed before and while experiencing a painful, inflammatory heat stimulus (n = 50). Hierarchical regressions indicated that pain intensity and unpleasantness after ratings after the delay discounting task, as well as pain intensity immediately prior to the task, inversely predicted delay discounting after controlling for baseline performance, meaning that the greater pain one perceived, the less impulsive they were. Consistent with our prior findings, these preliminary results suggest that acute pain may reduce impulsive decision-making. This finding may provide insight into the role of pain sensitivity on substance abuse. Future research should evaluate these effects in a chronic pain population.

Peripheral Nerve Activity Maintains Neuropathic Tactile Allodynia but Not Mechanical Hyperalgesia

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Peripheral neuropathic injury often causes chronic tactile allodynia (pain from an innocuous touch) and mechanical hyperalgesia (increased pain from a normally painful mechanical stimulus). Long-term central sensitization in the spinal cord has been suggested to be accountable for the chronic neuropathic pain symptoms. However, it remains unclear how the long-term central sensitization is persistently maintained. We hypothesized that it is maintained by ongoing peripheral nerve activities originating at the symptomatic area. To test this hypothesis, we injured L5 spinal nerve by tight ligation (L5 SNL) in the mouse and examined effects of local anesthesia on tactile allodynia and mechanical hyperalgesia at two symptomatic areas in the hind paw plantar skin. Tactile allodynia and mechanical hyperalgesia were measured as % occurrence of paw withdrawals in response to low- and high-intensity von Frey filament stimulation, respectively. L5 SNL-induced tactile allodynia was robust at the base of 3rd and 4th toes (Site 1) but less pronounced at the center of the paw (Site 2). Unlike the tactile allodynia at Site 1, that at Site 2 gradually declined over 3 weeks. Mechanical hyperalgesia at both Sites 1 and 2 persisted throughout the testing period. When a local anesthetic (bupivacaine 0.75%, 3 μl) was injected at Site 1, tactile allodynia, not mechanical hyperalgesia, at Site 2 was nearly abolished. Likewise, Site 2 anesthesia significantly attenuated only tactile allodynia at Site 1. These results suggest that long-term central sensitization for chronic neuropathic tactile allodynia is dynamically maintained by ongoing peripheral activities arising from the symptomatic areas, whereas that for chronic neuropathic mechanical hyperalgesia persists independently of such peripheral inputs.

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Poster #8

Resolution of Chemotherapy-induced Neuropathic Pain: a Key Role for CD8+ T Lymphocytes

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Background: Many chemotherapeutic agents, including paclitaxel and cisplatin, cause chemotherapy-induced peripheral neuropathy (CIPN) characterized by neuropathic pain and sensory loss in hands and feet and negatively affects quality of life of cancer survivors. Increased cancer survival rates result in enhanced numbers of survivors experiencing CIPN. Currently there is no FDA-approved treatment. In 30% of cancer survivors disabling CIPN persist long after treatment cessation. The reason for this are unknown. We propose that persistent CIPN is a consequence of a dysregulation of endogenous resolution pathways that depend on the activity of T cells.
Objective: Investigate the contribution of T cells to CIPN resolution.
Materials and methods: CIPN was induced by cisplatin (3 injections of 2 mg/kg) in wild-type (WT) and T cell-deficient mice (*Rag2*$^{-/-}$). CD8+ T cells were isolated from spleen of WT mice and intravenously transferred to *Rag2*$^{-/-}$ mice. Two symptoms of neuropathic pain were assessed; mechanical allodynia (von Frey test) and ongoing pain (conditioned place preference (CPP) test).
Results: WT mice treated with cisplatin have completely recovered from CIPN after 3 weeks, while T cell-deficient mice (*Rag2*$^{-/-}$) did not recover from CIPN and develop persistent neuropathic pain. Reconstitution of *Rag2*$^{-/-}$ mice with CD8+ T cells from WT mice normalized CIPN resolution in both males and females. CIPN resolution was similar in *Rag2*$^{-/-}$ mice reconstituted with CD8+ T cells with mutated T cell receptor (TCR) specific to an irrelevant antigen (chicken ovalbumin) or WT CD8+ T cells. These data indicate that T cell dependent resolution of CIPN does not require recognition of a specific antigen by the CD8+ T cells. We next investigated whether CD8+ T cells can be educated to improve endogenous resolution of CIPN. We compared the resolution of CIPN in *Rag2*$^{-/-}$ mice reconstituted with CD8+ T cells from cisplatin-treated mice that had recovered from CIPN or from naïve PBS-treated mice Interestingly, T cells isolated from cisplatin-treated WT mice prevented the development of CIPN in reconstituted *Rag2*$^{-/-}$ mice.
Conclusion: Our data demonstrate that CD8+ T cells are required for CIPN resolution and prevent the transition to persistent neuropathic pain via an antigen-independent pathway. Nevertheless, T cells can be educated to prevent CIPN. Taken together our data open the possibility to educate the CD8+ T cells *ex vivo* to prevent or reverse CIPN.

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Poster #9

A Novel Chemical Nociception Behavioral Assay in *Drosophila* Larvae

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Noxious stimuli of different sensory modalities (thermal, mechanical, electrical or chemical) provoke aversive pain behaviors in all animals. The molecular/genetic bases of thermal and mechanical nociception responses are beginning to be understood. However, chemically-provoked pain has not yet been studied intensively, in part due to a lack of simple genetically tractable models. **Objective**: Our goal is to use *Drosophila* genetics to establish a genetically tractable system to study the cellular and molecular genetic bases of chemically-induced nociception. **Methods**: We developed a new model of chemical nociception in *Drosophila* by exposing larvae to increasing concentrations of hydrochloric acid (HCl). Concentrations ranging from 1.0 % to 9.0 % produced an increasingly intense behavioral reaction which was manifest as an aversive rolling response, behaviorally similar to what is seen with heat and harsh touch. 0.5% HCl was subthreshold and provoked no rolling. Therefore, 0.5% HCl was used to study chemical allodynia (lowering of the response threshold in response to tissue injury). We also developed a physical wounding nociceptive sensitization model that elicited chemical allodynia in larvae. **Results**: To determine which sensory neurons are required for chemical nociception we genetically silenced the four different classes of multidendritic peripheral sensory neurons (classes I-IV). Class III, which mediates larval responses to noxious cold, was dispensable for chemical nociception. By contrast, classes I, II and IV were all required for the response to HCl, with class IV making the largest contribution. This response by multiple cell types has not been observed with other sensory modalities (heat, cold, mechanical). Physical puncture wounding induced a stronger chemical allodynia response, perhaps because this injury involves a breach in the external cuticle barrier. UV irradiation, which elicits strong thermal and mechanical sensitization was relatively ineffective with the chemical modality. At the genetic level, loss of function mutants of Piezo (an ion channel mediating mechanosensory transduction), or the transient receptor potential (TRP) channels Painless, Trpm, Trpml, Trpmγ, Trpa, Brv1, all displayed reduced sensitivity to noxious chemical stimuli. This was also true of Pvr (PDGFR/VEGFR like receptor tyrosine kinase) and its ligands.
Conclusion: We developed a novel assay to study chemically-induced nociception in *Drosophila* larvae. Nociceptive behavioral responses to HCl are mediated by class I, II and IV multidendritic sensory neurons. The spectrum of TRP channels required for chemical nociception appears partially overlapping with heat/mechanical. The high genetic resolving power of *Drosophila* will not only improve our basic understanding of fundamental mechanisms of chemical nociception, but also help us to identify novel gene targets for pain treatment that may ultimately prove useful in humans.

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Poster #10

Inhibition of HDAC6 Effectively Treats Chemotherapy-Induced Peripheral Neuropathy

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Chemotherapy-induced peripheral neuropathy is one of the most common dose-limiting side-effects of cancer treatment. Currently, there is no FDA-approved treatment available. Histone deacetylase 6 (HDAC6) is a microtubule-associated deacetylase whose function includes regulation of α-tubulin-dependent intracellular mitochondrial transport. Here we examined the effect of HDAC6 inhibition on cisplatin-induced peripheral neuropathy. We used a novel HDAC6 inhibitor ACY-1083, which shows 260-fold selectivity towards HDAC6 versus other HDACs. Our results show that HDAC6 inhibition completely reversed already existing cisplatin-induced mechanical allodynia, spontaneous pain, and numbness. These findings were confirmed using the established HDAC6 inhibitor ACY-1215 (Ricolinostat), which is currently in clinical trials for cancer treatment. Mechanistically, treatment with the HDAC6 inhibitor increased α-tubulin acetylation in the peripheral nerve. In addition, HDAC6 inhibition restored the cisplatin-induced reduction in mitochondrial bioenergetics and mitochondrial content in the tibial nerve, indicating increased mitochondrial transport. At a later time point, dorsal root ganglion mitochondrial bioenergetics also improved. HDAC6 inhibition restored the loss of intra-epidermal nerve fiber density in cisplatin-treated mice. Our results demonstrate that pharmacological inhibition of HDAC6 completely reverses all the hallmarks of established cisplatin-induced peripheral neuropathy. These results are especially promising because one of the HDAC6 inhibitors tested here is currently in clinical trials as an add-on cancer therapy, highlighting the potential for a fast clinical translation of our findings.

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Poster #11

The MNK-eIF4E Signaling Axis Contributes to Injury-induced Nociceptive Plasticity and the Transition to Chronic Pain

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Mechanisms governing the sensitization of nociceptors are widely believed to be responsible for the generation of chronic pain states, but are poorly understood. We investigated the role of phosphorylation of the 5' cap-binding protein, eIF4E, by MNK1/2 in nociceptive sensitization and the transition to chronic pain. Using mice harboring a point mutation at the MNK1/2 phosphorylation site on eIF4E, we find that pro-nociceptive and inflammatory factors fail to induce behavioral sensitization, hyperalgesic priming or electrophysiological measures of hyperexcitability in the absence of eIF4E phosphorylation. Our findings demonstrate that these effects are mediated by a peripheral mechanism of action pointing to a MNK1/2 – eIF4E signaling axis as a major contributing factor in controlling nociceptor plasticity. We conclude that eIF4E phosphorylation regulates nociceptor plasticity and is an integral mediator of the transition to a chronic pain state.

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Poster #12

Calcitonin Gene Related Peptide Promotes Pain Plasticity in Females in Hyperalgesic Priming and Spared Nerve Injury

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Objectives: Chronic pain disproportionately impacts women. Recently, numerous studies have demonstrated that there are male-specific mechanisms that promote pain plasticity, but no female-specific mechanisms have been identified. The objective of this study was to determine if Calcitonin Gene-Related Peptide (CGRP) is necessary for the development and maintenance of hyperalgesic priming in females. *We hypothesized the CGRP mediates the development and maintenance of hyperalgesic priming in females, but not in males. In addition, we also hypothesize that CGRP mediates maintenance of neuropathic pain in the Spared Nerve Injury (SNI) model.*

Methods: Adult Swiss Webster mice between the ages of 8 and 12 weeks were used in all experiments. Mechanical hypersensitivity was tested using the up-down method of Dixon with modification. In the first set of experiments animals were injected intrathecally (I.T.) with 10μg of olcegepant- a CGRP antagonist- or vehicle and intraplantarly (I.Pl.) with IL-6 (0.1ng). Mechanical withdrawal threshold was tested at 1h, 3h, 72h, and 5d post IL-6 injection. Animals were allowed to return to baseline levels of mechanical sensitivity and then injected I.Pl. with PGE_2 (100ng) and mechanical withdrawal threshold was tested at 3h and 24h. In the second set of experiments animals were injected I.Pl. with IL-6 (0.1ng), allowed to return to baseline, and then injected I.T. with 10μg of olcegepant or vehicle and I.Pl. with PGE_2 (100ng). Mechanical withdrawal threshold was tested at 3h and 24h post injection. In a third set of experiments, we injected olcegepant into mice that had been given a spared nerve injury 14d prior and measured their mechanical withdrawal threshold post injection. These experiments were then repeated in males and females using a second CGRP antagonist: $CGRP_{8-37}$.

Results: When given at the time of I.Pl. IL-6 injection, I.T. olcegepant blocked the initial response to IL-6 in female mice, but had no effect in male mice. I.T. olcegepant when given at the time of PGE_2 injection blocked response to PGE_2 in female mice, but not in male mice. In female mice, when olcegepant was given I.T. 14d post SNI surgery, there was a transient increase in mechanical withdrawal threshold at 30 minutes, but by 3h post injection there was no significant effect. $CGRP_{8-37}$ injected I.T. at the time of IL-6 blocked both the response to IL-6 and blocked hyperalgesic priming in females, but had no effect in males. $CGRP_{8-37}$ given at the time of PGE_2 injection in females reversed hyperalgesic priming. Given in I.T. in females, CGRP8-37 reversed mechanical hypersensitivity for 24h, but there were no significant effects after 48h.

Conclusions: CGRP antagonists given intrathecally in females block and reverse hyperalgesic priming, but have no effect in males. These same antagonists briefly increased mechanical withdrawal threshold, but the effect was transient. More work needs to be done in other models of chronic pain to determine if this is a phenomenon that occurs only in hyperalgesic priming. This finding serves as rationale for exploring sex-specific treatment of chronic pain in the clinic. 4

Poster #13

TRAPs and footprints reveal the landscape of protein synthesis in DRG and TG nociceptors

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While considerable progress has been made on profiling the transcriptome of mouse, rat and human DRG (and in some instances TG), including single cell resolution for mouse DRG neurons, the translateome remains uncharacterized. We capitalize on synergistic methodologies for high resolution, genome-wide snapshots of nascent protein synthesis in the DRG and TG. We have used translating ribosome affinity purification (TRAP) mice where the ribosomal L10a protein is tagged with GFP in a cell-type specific manner to label and purify translating ribosomes from Nav1.8-CRE expressing nociceptors ($TRAP^{Nav1.8}$). We have also used ribosome footprinting on cultured DRG neurons to examine ribosome-protected RNA fragments from these cells with or without stimulation by NGF and IL6. In both experimental paradigms we have used RNA sequencing and tophat/cufflinks to determine RNA abundances. From $TRAP^{Nav1.8}$ mice we have been able to ascertain translational efficiencies across the transcriptome and compare them in DRG and TG. We find striking differences between these two tissues that have not been described in previous reports. In ribosome footprinting experiments we find strong indications of upstream open reading frame (uORF) utilization in DRG neurons in culture and translational efficiencies for known coding genes that are similar to evidence from $TRAP^{Nav1.8}$ mice. In DRG neurons exposed to NGF and IL6 we find a signature of neuronal plasticity that is strikingly similar to changes reported in the long-term potentiation (LTP) literature. Our results reveal new insights into translational control in nociceptors and suggest that the evolutionary origins of translation regulation of synaptic plasticity may be grounded in the peripheral nociceptive system.

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Poster #14

A Genome-wide Snapshot of in Vivo and in Vitro Transcriptional and Translational Changes Underlying Pain Plasticity in Mouse Models

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Translational control in mammalian sensory neurons have been widely reported to be implicated in pain plasticity, but assays querying genome-wide profiles of such changes are challenging and expensive to design, difficult to quantify and inherently confounded with the transcriptional response. *Methods:* We use high-throughput assays used for sequencing ribosome-associated gene transcripts in sensory ganglia (Dorsal Root Ganglia / DRG) or sensory neurons in both *in vivo* (Translating Ribosome Affinity Purification/TRAP) and *in vitro* (Ribosomal FootPrinting/RPF) settings to quantify the active translatome in sensory neurons in mouse under baseline and pain states. We further quantify the steady-state transcriptome using QuantSeq / RNA-seq technology to tease apart changes that are due to transcriptional/post-transcriptional control versus translation control.

Under *in vivo* settings, we sequenced DRG mRNA from naïve and Paclitaxel-treated mice. The steady-state transcriptome was quantified using the QuantSeq assay. For our TRAP experiments, we used the reporter transgene that encodes an EGFP-ribosomal protein L10a driven by the nociceptor-specific promoter Nav1.8, allowing us to isolate transgenic ribosomes and their bound mRNAs in a nociceptor-specific manner. Under *in vitro* settings, we performed RNA-seq and Ribosomal Footprinting on cultured DRG under baseline conditions and upon exposure to NGF/IL6 plasticity-inducing cocktail.

A suite of computational algorithms were developed for identifying genes with differential abundance in the steady-state transcriptome versus active translatome, naive versus pain state, cultured ganglia versus tissue sourced *in vivo*; providing insight into the molecular changes and putative control mechanisms underlying nociception. Novel approaches to read trimming, *in silico* library selection, and normalization strategies for minimizing batch effects were developed to maximize signal to noise ratio. Identification of alternative exon usage in transcription and alternative reading frame usage in translation were also queried in our datasets.

Results Treatment of DRG cultures with NGF and IL6 result in transcriptional changes including positive regulation of kinases. It also changes the landscape of nascent protein synthesis, specifically appearing to target several ionotropic glutamate receptors and certain transcription factors for preferential translation. This suggests a complicated regulatory process with feedback, having potential for guiding pain therapeutics. In our TRAP experiments, while variance in gene abundances are higher due to the *in vivo* setting, exposure to Paclitaxel causes abundance increases in the active translatome for several genes in Wnt and Cadherin signaling pathways. *Future work* Our *in vitro* and *in vivo* experiments identify orthogonal information about the regulatory processes underlying nociceptor plasticity. Analysis driven by identifying solely nociceptor specific transcripts will allow us to resolve evidence from our two experimental setups, providing a cohesive, integrative picture of the translational landscape of pain plasticity in nociceptors.

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Poster #15

A Novel Electrophysiological Method to study Tumor-Nerve Interaction for Oral Cancer Pain

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Aim of Investigation: Pain in oral cancer patients is of multifactorial etiology and unlike other cancers is produced at the primary site of the tumor even when the tumor is still quite small in size. This suggests that tumor cells control the activity of surrounding nociceptors leading to the activation of pain pathways. It is therefore crucial to understand the interaction of oral tumor cells with surrounding nociceptors to delineate mechanisms by which oral cancer produces pain. Using an *in vivo* tongue cancer pain model in mice, we have established a novel electrophysiology method that allows us to study tumor-nerve interactions at the site of primary tumor growth.

Methods: Human oral squamous cell carcinoma cells (HSC2) were injected into the ventral anterior tongue of athymic mice. Upon tumor growth, animals were subjected to histological and immunological characterization as well as behavioral assays such as feeding behavior, conditioned place preference (CPP) test and Von Frey filament testing to reflect patient symptoms such as pain during chewing and swallowing, ongoing pain and facial pain. Additionally, we used this in vivo tumor model to dissect out the tumor growing tongue or normal tongue and its associated lingual nerve to determine and characterize lingual nerve fibers and their firing responses in response to the tumor. Data were analyzed by ANOVA and Bonferroni's test.

Results: Our data show that HSC2 injected mice produced observable tumors by day 9 post inoculation whereas NOK injected mice showed no visible growth. Tumor growth produced a significant reduction in feeding behavior as well as facial pain that was reversed by analgesics as well as produced a central nociceptive state as measured by CPP. Extracellular electrophysiological recordings showed that lingual nerve fibers produced spontaneous firing as well as increased mechanical hypersensitivity and decreased Von Frey threshold of C and Aβ fibers, in tumor-bearing preparations compared to normal preparations.

Conclusions: We have developed a novel electrophysiology method that permits evaluating tumor-nerve interactions in its naturally occurring environment. Further characterization of lingual nerve fiber discharges upon oral tumor growth will provide insight into the mechanisms by which oral cancer produces pain at the primary site of tumor development.

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Poster #16

Opioid Prescriptions through Texas Medicaid: Ramifications Of Hydrocodone Rescheduling

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Introduction: The misuse of prescriptions for chronic pain led to nearly 19,000 deaths from overdose in the U.S. in 2014. Hydrocodone combination products (HCPs) were the most commonly prescribed medication in the U.S. from 2008 to 2013. To combat this misuse, the Drug Enforcement Agency (DEA) rescheduled HCPs from schedule III to schedule II on October 6, 2014. Prescribers are now prohibited from issuing refills on HCPs and cannot orally transmit or fax prescriptions over the phone. This provides a negative incentive to the long-term use of HCPs, and we expect this to affect opioid prescribing habits.

Rationale: Prior studies demonstrated that increased regulation of HCPs has resulted in an increased prescribing of Schedule III opioid analgesics. A literature review revealed no studies examining HCP or opioid utilization using Texas Medicaid data since the rescheduling. Understanding current analgesic utilization can help target interventions and control usage. The primary purpose of this study is to describe the current trends in opioid and non-opioid analgesic prescription claims in the Texas Medicaid program. The primary hypotheses are: (1) after rescheduling, the total number of HCP prescriptions filled per month will decrease; and (2) after rescheduling, there will be a compensatory increase in the total number of non-opioid, other schedule II opioid, and non-schedule II opioid analgesics filled per month.

Method: We conducted a retrospective analysis of Texas Medicaid prescription claims data including all enrollees 18 to 64 years of age who filled analgesic prescriptions between April 1, 2013 and April 30, 2016 (the month of rescheduling, October 2014, was excluded from the analyses). Medicaid data files were provided by the Texas Health and Human Services Commission. Descriptive statistics were used to create trend graphs and calculate percent change in analgesic prescription claims by analgesic class and selected specific analgesic medications, and the mean number of prescriptions per patient. IBM SPSS v 24 was used for the analyses. The University of Texas IRB approved this study.

Results: During this time frame, 5,634,992 analgesic prescriptions were filled, and 67.4% (n=3,799,787) were for opioid narcotics. There were 76,739 HCP prescriptions per month for 10,135 patients in the 18 months prior to rescheduling, and 30,547 HCP prescriptions per month for 1,295 patients in the 18 months after rescheduling, resulting in a 60.2% decrease in HCP prescriptions. However, the mean number of HCP prescriptions per patient increased from 7.6 in the pre-reschedule period to 23.6 prescriptions in the post-reschedule period. The number of non-opioid analgesic prescriptions and other schedule II prescriptions increased slightly, but there was a sharp compensatory increase of 84.3% in the number of non-schedule II prescriptions dispensed.

Conclusion: The increase in non-schedule II opioid analgesic prescription claims after rescheduling did not balance the decrease in HCP claims. Given the reduced potency of non-schedule II opioids, this imbalance could indicate that patients are not receiving adequate pain management. However, it appears that the shift has primarily reduced prescriptions for short-term HCP therapy since the mean number of prescriptions per patient increased while the total number of patients dramatically decreased. It is unclear

if the rescheduling altered opioid-related morbidity and mortality. Future research should assess current pain management trends to determine if restrictions on opioid prescribing curb opioid overdose rates.

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Poster #17

Role of Wnt5a Signaling in NRTI-Induced Pain in Aging Mice

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Nucleoside reverse transcriptase inhibitors (NRTIs) are the central components in the regimens of anti-retroviral therapy for HIV-1 infection. However, long-term NRTI usage may contribute to the neurological complications developed in HIV patients. In this study, we investigate the effect of NRTIs on the development of chronic pain in aging mice. Mice at the age of 15.5 months (equivalent to human age of 50s) were administrated with representative NRTIs, including didanosine (ddI), stavudine (d4t) and zidovudine (AZT), all at the dose of 25mg/kg; i.p. every other day for two weeks. The NRTI treatment caused persistent hindpaw pain. In addition, dideoxycytidine (ddC) also induced visceral hyperalgesia. We observed up-regulation of Wnt5a and pro-inflammatory cytokines, the activation of astrocytes and microglia in the spinal cord dorsal horn (SCDH) of ddC treated aging mice. Administration of Wnt5a antagonist Box5 (20μg, one time i.t.,) attenuated ddC-induced up-regulation of TNF-α and GFAP expression, while Wnt5a agonist Foxy5 (20μg, one time i.t.,) potentiated the up-regulation. In addition, Box5 (20μg, one time i.t.) attenuated ddC-induced hyperalgesia. The results reveal a critical role of Wnt5a in the spinal cord pain circuit in NRTI-induced hyperalgesia in aging mice, probably by promoting the expression of neuroinflammation.

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Poster #18

Are External Contingencies of Self-Worth Related to Experimental Pain Ratings? The Moderating Role of Perceived Stress

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Pain is an integrative process that incorporates both somatosensory and affective-motivational components. Additionally, these components are malleable to both perceptions of threat to one's self in the environment and one's ability to cope with said threats. Threats of rejection and social disapproval are emotionally distressing, but people differ in how vulnerable they are to these experiences as a function of their bases of self-esteem, or contingencies of self-worth. Specifically, the threat of receiving negative interpersonal feedback on pain ratings and goal pursuit may depend on how contingent a person's self-worth is on others' approval and how great their perceptions of threat are. Therefore, the goal of this present study was to examine whether greater external self-worth contingencies (e.g. approval from others, competition) is associated with altered pain ratings and whether this relationship is moderated by trait levels of perceived environmental threat/coping. Participants prescreened for high (n = 19) and low trait perceived stress (n = 18) were invited to participate in a socio-evaluative cold pressor task where they were instructed to immerse their hand for two minutes or until they reached pain tolerance. After termination of the pain task, participants were asked to rate their pain intensity and unpleasantness to the experience. One-way ANOVAs indicated no differences in level of contingencies of self-worth, pain intensity, unpleasantness, and tolerance between the low and high perceived stress groups (p's > .05). After controlling for tolerance duration, partial correlations demonstrated that external contingencies of self-worth were negatively associated with pain intensity and unpleasantness for those in the high stress group, meaning that the more one's self-worth is contingent on others, the lower one's pain ratings in the context of a laboratory setting with an evaluator. However, these associations were not replicated in the low stress group. Additionally, no significant correlations were found for pain tolerance in either group. These results may provide insight into individual and social facts that affect somatosensory and affect-motivational components of experimental pain.