Sphingosine Kinase 1 (SphK1) but not SphK2 is necessary for the development of oxaliplatin-induced neuropathic pain

Oxaliplatin is a first line chemotherapeutic for the treatment of colorectal cancer; however it is associated with the dose-limiting side effect of neuropathic pain. Mechanisms of the development of oxaliplatin-induced neuropathic pain remain poorly understood and there is currently no FDA approved therapy. We have recently reported that various chemotherapies cause dysregulation of sphingolipid metabolism in the CNS with increased production of sphingosine 1-phosphate (S1P), a potent signaling sphingolipid. S1P is formed by the phosphorylation of sphingosine by sphingosine kinases (SphKs). Two isoforms have been identified: SphK1 and SphK2, however the contribution of these SphKs in S1P formation during oxaliplatin treatment is unknown. Here we show that when compared to their age and sex matched wild-type (WT) controls, global SphK1 knockout mice do not develop oxaliplatin-induced neuropathic pain. In contrast, the development of oxaliplatin-induced neuropathic pain was similar in SphK2 and WT mice. Our results further demonstrate that the development and maintenance of neuropathic pain is driven by S1P acting at the S1PR1 subtype. Our findings identify SphK1 as a novel target for therapeutic intervention and underscore the contribution of the SphK1/S1PR1 axis as a key driver of oxaliplatin-induced neuropathic pain. Noteworthy, SphK1 inhibitors are under preclinical development as anticancer agents.
Heat shock protein 90 promotes opioid anti-nociception in the brain and represses opioid anti-nociception in the spinal cord through the differential regulation of ERK MAPK signaling

Opioid drugs are efficacious in treating moderate to severe acute and chronic pain, but are limited by serious side effects. Recent efforts to create biased opioids without these side effects by selectively engaging downstream signal transduction (e.g. βarrestin2) has been limited by our lack of knowledge of the mu opioid receptor (MOR) signaling complex. In our earlier work, we identified Heat shock protein 90 (Hsp90) as a novel MOR signaling regulator. We found that intracerebroventricular (icv) 17-AAG, a well-established Hsp90 inhibitor, strongly blocked or abolished morphine anti-nociception in paw incision and HIV neuropathy pain in mice. In our current work, we’ve extended from these findings to examine the role of Hsp90 in the spinal cord. In contrast to our findings in the brain, we found that intrathecal (it) 0.5nmol 17-AAG for 24 hours in CD-1 mice caused strongly increased morphine anti-nociception in tail flick and paw incision pain (80.7-88.6% increase). This was confirmed using the alternate Hsp90 inhibitor KU-32 as well as Rotarod testing, ruling out off-target effects. We examined ERK MAPK signaling in the brain and spinal cord in response to opioids after 17-AAG inhibition, and found that ERK MAPK activation was lost in the brain, but potentiated in the spinal cord. By using icv and it administration of 5μg U0126, a selective ERK inhibitor, we demonstrated that the effects of Hsp90 inhibition in the brain and spinal cord could be completely explained by ERK signaling changes. By using selective inhibitors, we further found that the molecular isoform Hsp90α and the co-chaperones p23 and Cdc37 (but not Aha1) were responsible for the signaling and anti-nociception changes observed in the brain. Overall, these results demonstrate different regional effects of Hsp90 and ERK signaling that have a strong impact on opioid anti-nociception, which could be used to improve opioid drug discovery.
CGRPα sensory neurons drive chronic neuropathic pain whereas CGRPα peptide signaling mediates incisional pain

Cutaneous somatosensory neurons convey innocuous and noxious mechanical, and thermal stimuli from the skin to the CNS. Among those are neurons that express Calcitonin Gene-Related Peptide-alpha (CGRPα), which are known to be involved in pain. The role of peripheral CGRPα Neurons (CANs) has been studied using diphtheria toxin ablation. However, their role after injury remains controversial. Because ablation permanently deletes a population of neurons, compensatory changes may ensue that mask the physiological roles of CANs. Therefore, we sought to clarify their role under baseline and injury conditions by using non-invasive optogenetic silencing and assessing behavior and DRG soma-excitability. We generated mice that selectively express the outward-rectifying proton pump Archaerhodopsin-3 (Arch) in CANs, and silenced the cutaneous components of these neurons in models of neuropathic (spared nerve injury) and inflammatory (skin and muscle incision) pain using transdermal light activation of Arch. Brief activation of Arch reversed the chronic mechanical and thermal hypersensitivity and alleviated spontaneous pain following nerve injury, but did not affect inflammatory injury-related hypersensitivity. Instead, inflammation-induced mechanical and heat hypersensitivity was alleviated by blockade of peripheral CGRPα peptide signaling. These results reveal previously unknown functions for CANs in the establishment and maintenance of hyperalgesia following neuropathic and inflammatory injuries.
Genetic and physiological dissection of central amygdala neurons reveals Pain-ON and Pain-OFF cells in mouse models of persistent pain

It is well-established that the brain can bidirectionally modulate the perception of pain in both humans and rodents. The neural mechanisms underlying forebrain modulation of pain, however, remain largely unknown. The present study identifies a novel mechanism underlying bidirectional modulation of pain in the brain. Using molecular genetics in rodents, in combination with chemogenetic approaches in-vivo and histological and patch-clamp electrophysiology in-vitro, we targeted and manipulated specific populations of cells within the central nucleus of the amygdala (CeA), a forebrain structure that has been previously identified as an important site for pain modulation. The results from these experiments provided the first causal evidence for endogenous bidirectional, cell-type-specific, forebrain modulation of pain-related behaviors. The experiments specifically revealed the existence of “pain-ON” and “pain-OFF” cells within the CeA, that exert opposing control of tactile hypersensitivity in mouse models of persistent pain. Our experiments further defined the genetic identity, anatomical distribution and intrinsic membrane properties of these subpopulations of CeA cells, providing fundamental insight into the anatomical and cellular mechanisms that underlie bidirectional modulation of pain in the brain. Altogether, these innovative findings uncovered previously underappreciated complexities within the nociceptive amygdala and provide a novel mechanistic model for top-down forebrain modulation of pain.
Vulvodynia, an unexplained vulvar pain condition, is a major health concern that affects 8% of premenopausal women. Often considered as chronic, a significant proportion of women report remission of symptoms over time - even without treatment. However, the natural progression of vulvodynia and which factors may explain remission versus maintenance of symptoms remain unclear. This prospective seven-year study aimed to identify subgroups of pain trajectories in a sample of 173 women with vulvodynia, and to predict these different trajectories via treatments undertaken, pain characteristics and psychosocial factors. We identified two trajectories of pain, one that remains stable and one that decreases over time. The most noteworthy finding of this study was that having undergone a treatment or not was not predictive of the course of pain over time. Women who were older at first pain onset, had pain at another location than the entrance of the vagina, reported more anxiety and were married were more likely to have a stable pain trajectory relative to the decreased pain trajectory. Conversely, women with primary vulvodynia and who engaged in more positive activities with their partners were more likely to experience decreased pain over time.
Cerebellar White Matter Volume is Associated with Clinical and Experimental Pain in Older Individuals with Musculoskeletal Pain

Background/Aim of Study: Musculoskeletal pain in older adults is significantly associated with mobility-related disability, but the neurobiological mechanisms underlying mobility limitations in the presence of musculoskeletal pain are not well-understood. The cerebellum, in particular, plays a key role both in motor and pain processing and increased cerebellar activity has been associated with pain. Therefore, the aim of the present study was to determine the associations between cerebellar white matter (WM) volumes and musculoskeletal pain in older individuals. Methods: Participants (n=40) over 60 years of age filled out the Graded Chronic Pain Scale (GCPS) to assess characteristic pain intensity and pain disability during the past six months. A T1-weighted MPRAGE was obtained to determine cerebellar WM volume. Participants also underwent psychophysical assessments to determine thermal and mechanical pain thresholds as measures of acute pain processing, and a behavioral pain inhibition paradigm as a measure of pain modulation. Results: The majority (89%) of participants (mean age of 72±7.8 years) reported chronic musculoskeletal pain during the past six months. Given the large differences in cerebellar WM volume between males (n=10) and females (n=30) and the small sample size of male participants, associations were only examined within female participants. GCPS Characteristic Pain Intensity, but not Pain Disability, was negatively correlated with WM volume in the cerebellum in both hemispheres (Right: r=-0.49, df=25, p= 0.008 and Left: r=-0.52, df=25, p=0.005). Lower cerebellar WM volume was significantly associated with greater thermal and vibratory detection thresholds and heat pain thresholds even after adjusting for age, body mass index, and total intracranial volume (R2=0.50, df=25, p=0.001). There were no significant associations between cerebellar WM and behavioral pain inhibition (p>0.05).
Keratinocytes mediate mechanical sensation via a purinergic signaling mechanism

The first point of our body’s contact with tactile stimuli is the epidermis, the outermost layer of the skin that is largely composed of keratinocytes. Despite their ideal location for touch, the mechanisms through which keratinocytes communicate with sensory neurons is unknown. Therefore, we sought to determine the roles keratinocytes play in sensing acute tactile stimuli. Optogenetic inhibition of keratinocyte function via selective Archaerhodopsin expression elevates the mechanical thresholds of naive mice. Conversely, optogenetic activation of keratinocytes via Channelrhodopsin elicits attending behavior responses to stimulated skin in vivo. Further, we demonstrate that ATP is released from skin upon mechanical stimulation with the use of ATP biosensors (Sarissaprobes), and keratinocytes specifically (Cell Sniff assay). The degradation of ATP in the skin in vivo leads to decreased baseline behavioral sensitivity and decreases the responsiveness of afferent fibers ex vivo. We further investigated the signaling mechanisms via blockade of a P2X receptor, which also lead to a decreased normal behavioral sensitivity and afferent firing. This suggests that ATP released from keratinocytes acts via P2X receptors in non-injured skin. These data are the first to identify purinergic signaling as a critical component of mechanotransduction, specifically in the context of non-neuronal to neuronal cellular communication.
Activation of peripheral $\beta$ and $\beta_3$ARs leads to increased modality-specific nociceptor activity

Enhanced catecholamine tone resulting from decreased activity of catechol-O-methyltransferase (COMT; an enzyme that metabolizes catecholamines) contributes to functional pain syndromes, such as fibromyalgia and temporomandibular disorder. In line with findings from clinical studies, our lab has shown that acute delivery of a COMT inhibitor OR486 in rodents induces pronounced pain, which is mediated by $\beta$ - and $\beta_3$ARs-dependent increases in nitric oxide and cytokines. However, the effects of COMT inhibition on nociceptor activity remain unknown. The present study sought to directly investigate the effects of COMT inhibition on dorsal root ganglion (DRG) nociceptor activity using immunohistochemistry in wild-type mice and in vivo calcium imaging in pirt-GCaMP3 mice (that express a calcium indicator exclusively in peripheral nociceptors). Separate groups of mice received systemic delivery of the COMT inhibitor OR486 or vehicle alongside peripheral delivery of the $\beta_2$AR antagonist (ICI-118,551), $\beta_3$AR antagonist (SR59230A) or vehicle. We found that COMT inhibition increases nociceptor activity, characterized by increased ERK phosphorylation and heightened calcium response in DRG neurons responding to noxious stimuli. Notably, the majority of evoked DRG neurons are modality-specific, responding to either noxious mechanical or heat stimuli. Further, $\beta_3$ARs mediate neuronal responses evoked by both mechanical and thermal stimuli, while $\beta_2$ARs solely mediate responses evoked by mechanical stimuli. Collectively, these findings show that peripheral $\beta_2$- and $\beta_3$ARs drive the activity of nociceptors essential for the development of functional pain. Thus, treatments targeted towards peripheral $\beta_3$ARs and downstream effectors may prove useful in the management of functional pain syndromes.