generation in small DRG neurons. These data suggest that 1) the slow-repriming current in small DRG neurons was likely mediated by Nav1.7 in both rats and mice; 2) the slow-repriming and fast-repriming TTX-S currents were differentially expressed among small DRG neurons; 3) the slow-repriming and fast-repriming TTX-S currents of small DRG neurons were differentially expressed between rats and mice; and 4) differential expression of Nav1.7-like currents might contribute to the diversity of membrane excitability among DRG neurons. (This work was supported by Indiana State Department of Health [ISCBIRF grants ISDH-A70-4-079988 and ISDH-013504] and American Cancer Society [ACS-USCC, IRC-84-002-28]).

(104) VGLUT3-containing primary muscle afferents are a unique subpopulation that respond to innocuous metabolites

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Primary group III and IV muscle afferents respond to varying combinations of mechanical, thermal and chemical stimuli, and each subtype likely plays diverse roles in somatosensory processing from the muscles. While the basic characteristics of dorsal root ganglion (DRG) neurons that respond to specific peripheral stimuli in the muscles have been described, biomarkers for distinct subpopulations have yet to be identified. A previous report has shown that a subset of DRG neurons express the vesicular glutamate transporter 3 (VGLUT3), and these cells have been implicated in the development of mechanical allodynia after inflammation and nerve injury. We have also recently shown that alterations in mechanical and chemical responsiveness in muscle afferents may play an important role in the development of myalgia after ischemia/reperfusion injury. While VGLUT3 neurons have been widely studied in multiple tissues, their role in muscle afferents remains to be elucidated. Therefore, to determine the physiological function of VGLUT3-containing afferents innervating the muscles, we functionally and neurochemically characterized individual muscle sensory neurons using an ex vivo forepaw muscles/median and ulnar nerves/DRG/spinal cord recording preparation in transgenic mice that expresses a td-Tomato reporter protein in VGLUT3-containing cells. We found that 50% of group III/IV muscle afferents which respond to an innocuous metabolite mixture consisting of low ATP and lactic acid at pH 7.0 were VGLUT3 positive. Surprisingly, this was the only population of muscle sensory neurons found to express VGLUT3, and these cells responded to functional or heat stimuli, and only one of the innocuous metabolite responders also fired to cold. These results suggest that the VGLUT3-positive afferents innervating the muscles may play a distinct role from what has been reported in cutaneous afferents and could be the population of chemosensitive cells that are thought to be crucial in the development of ischemic muscle pain.

(105) mGluz/3 differentially modulate TRPV1 sensitization in mouse and human sensory neurons

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Chronic pain afflicts over 100 million Americans and costs the United States $635 billion annually. Despite the major societal and financial burdens of chronic pain, an effective treatment without adverse off-target side effects remains elusive. Given the central side effects such as addiction and abuse related to existing analgesics, identifying peripheral analgesic targets is of particular interest. Metabotropic glutamate receptors 2 and 3 (mGluz/3), a group of inhibitory G-protein coupled receptors, have been identified as putative analgesic targets in rodent models of inflammatory and neuropathic pain. We have previously demonstrated that peripheral activation of mGluz/3 attenuates rodent pain-like behavior, suggesting that mGluz/3 modulate pain at the peripheral sensory neuron level. We are currently testing whether these observations in rodent models translate to humans. As a first step, we are utilizing human sensory neurons obtained from organ donors without chronic pain to test whether mGluz/3 activation reduces the sensory neuron sensitization that contributes to chronic pain across species. We recently showed that mGluz/3 activation with the agonist APDC suppresses prostaglandin E2 (PGE2)-induced membrane hyperexcitability without affecting basal sensory neuron excitability in both species. Here, we demonstrate that APDC suppresses PGE2-induced sensitization of TRPV1 calcium responses in mouse, but not human sensory neurons. To determine whether the differential effects of APDC on PGE2-induced TRPV1 sensitization are due to differences in mGluz/3 and TRPV1 coexpression in mouse versus human sensory neurons, we are currently carrying out in situ hybridization for these transcripts in intact and dissociated dorsal root ganglia neurons. Taken together, our results suggest that mGluz/3 activation suppresses some aspects of human sensory neuron sensitization, and targeting peripheral mGluz/3 could have analgesic efficacy in humans. Further, our findings highlight the use human tissue as a powerful tool in the validation of putative analgesics targets identified in clinical animal models. Supported by NINDS R01NS042595.

(106) Peripheral GABA_A receptors regulate colonicafferent excitability

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The role of GABA_A receptors located at central terminals of primary afferents fibers in the regulation ofafferent input to the superficial dorsal horn has been well established. However, there is evidence that GABA_A receptors are trafficked to peripheral terminals as well. Because there are several sources of GABA in the colon, we hypothesized that the excitability of colonic afferents is established at least in part via GABA_A acting at GABA_A receptors on the peripheral terminals of these afferents. To test this hypothesis, we utilized an in vitro mouse colorectum-pelvic nerve preparation in which GABA_A receptor agonists and antagonists could be applied to the receptive field of functionally identified afferent fibers as a means of assessing changes in stimulus response properties. Using single-fiber recordings of the pelvic nerve we found that the application either of GABA or muscimol results in both a decrease in the amount of colon stretch required to evoke an action potential, a decrease in the number of stretch-evoked action potentials. Both agonists also increased the electrical-threshold and decreased the apparent conduction velocity of the evoked action potential. Conversely, the GABA_A-antagonist bicuculline or blocker picrotoxin increased the stretch threshold and increased the number of stretch-evoked action potentials. Picrotoxin and bicuculline also increased the apparent conduction velocity of the electrical stimulation evoked action potential evoked by electrical stimulation. These results suggest that peripheral GABA_A receptors are not only present and functional in the peripheral terminals of colonic afferents but that activation of these receptors via endogenous GABA release contributes to the establishment of colonic afferent stimulus-response properties. These results raise the intriguing possibility that approaches to selectively increase peripheral GABA_A receptor signaling could be used to treat visceral pain in the absence of central nervous system side effects. Work supported by NIH grant R01 DK107966.

(107) Vitamin B12 and Keturolac on pain and inflammation in Long Evans rats

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Effects of vitamin B12 on nociceptive pain, inflammatory pain were used in several experimental studies but comparison of these effect with ketorolac tromethamine (KT) and their combination has not been established. To assess the effects of vitamin B12 with KT against pain and inflammation after single administration in rat models and compare them with the combinations of vitamin B12 with KT. This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2015 to February 2016. For this, 60 (sixty) Long Evans rats (215±35 gm) of both sexes were divided into control (A, with 5 ml/kg normal saline) and experimental (B1, with 15 mg/kg; B2, with 10 mg/kg KT; B3, with B1+KT) groups with 15 rats in each group. All the drugs and vitamin were administered intraperitoneally in a single dose in rodents just one hour before the pain evaluation test (tail immersion, formalin and writhing test) followed by formalin induced paw edema test. Afterwards, all rats were deeply anesthetized with chloral hydrate followed by euthanasia. Statistical analysis was done by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results, p<0.05 was considered as significant. Single dose of vitamin B12 lowered (p=0.001) variabilities for nociceptive pain and inflammatory pain. Again, combined administration of vitamin B12 and KT lowered all variables for
nociceptive and inflammatory pain (p<0.001) than individual vitamin B12 and KT administration. In addition, statistically significant lowering of paw edema was observed after KT (p<0.01) and combination of B12 and KT (p<0.001) in formalin induced paw edema test. It may be concluded that, vitamin B12 possess analgesic as well as anti-inflammatory effects and combination of B12 with KT is more effective than those of their individual administration.

**Amygdala**

(108) Asymmetric nociceptive properties of the left and right central amygdala

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The left and right central nuclei of the amygdala (CeA) are homologous limbic regions capable of modulating visceral pain. Despite equivalent processing by peripheral and spinal nociceptive systems, recent data suggest that visceral pain, and specifically bladder pain, is is asymmetrically processed by the left and right CeA. Eaba from chronic bladder pain patients shows asymmetry in functional connectivity and activation of the left and right amygdala during experimental stimulation. We sought to determine if lateralization occurs in animal models of bladder pain and furthermore, identify the cellular mechanisms of such lateralization. In our studies, we first investigated the functional contributions of the left and right CeA to bladder nociception using excitatory and inhibitory optogenetic approaches. Both light-induced activation of the right CeA and light-induced inhibition of the left CeA increased bladder pain-like responses during noxious bladder distension. These data suggest divergent pro- and antinociceptive functions of the right and left CeA respectively. Similar functional properties were observed during a second nociceptive measure; light-induced activation of the right CeA increased mechanical and thermal sensitivity in naive mice while light-induced activation of the left CeA reversed referred pain-like measures in animals with cyclophosphamide-induced cystitis. Excitability of the left and right CeA was measured during noxious bladder distention in naive and cyclophosphamide-sensitized animals. Evidence was found for differences in both background and evoked excitability of the two hemispheres. Current studies are evaluating the cell-type specific projections that drive sensitized animals. Evidence was found for differences in both back-vitamin B12 and KT administration. In addition, statistically significant lowering of paw edema was observed after KT (p<0.01) and combination of B12 and KT (p<0.001) in formalin induced paw edema test. It may be concluded that, vitamin B12 possess analgesic as well as anti-inflammatory effects and combination of B12 with KT is more effective than those of their individual administration.

**Anterior Cingulate**

(109) Peripheral inflammation induces hyperexcitability in Group II mGluR-positive neurons in anterior cingulate cortex

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In this study, we focused exclusively on a group of neurons in mouse anterior cingulate cortex (ACC) expressing Group II metabotropic glutamate receptors subtype 2 (mGluR2). We hypothesized that mGluR2+ neurons in ACC could be potential targets to modulate pain perception. To identify mGluR2+ neurons, we crossed Grm2-cre mice with tdTomato reporter mice. Immunohistochemical studies showed that mGluR2+ neurons were localized to layer 2/3 in the ACC and did not co-express somatostatin or parvalbumin suggesting they are unlikely to be inhibitory interneurons. To study the intrinsic membrane properties of mGluR2+ neurons, we performed whole-cell patch clamp electrophysiology on brain slices containing ACC obtained from 6-8 week old male and female mice. To explore the characteristics of these cells under persistent peripheral inflammation, we injected the hind-paw with Complete Freund’s adjuvant (CFA) and examined mGluR2+ layer 2/3 neurons in contralateral ACC 24 hours later. We found that after inflammation, mGluR2+ neurons exhibited a significantly higher discharge level compared to the naive group. This hyperexcitability was reversed by bath applied (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC, 1uM), an agonist for mGluR2. With the goal of modulating mGluR2+ neural activity in the ACC in vivo, we generated Grm2xChR2-EYFP mice and examined light-induced activation of these neurons using whole-cell patch clamp in vitro. mGluR2+ neurons from ACC were activated with 470nm light and the optimal parameters for light intensity and pulse frequency for modulation of neural activity was determined. Animal behavior studies will be conducted in the future to further examine the role of mGluR2+ neurons in ACC. Funding provided by Rita Allen Foundation and American Pain Society.

**Cell Signaling**

(110) Tumor-derived therapeutic targets of oral cancer pain: a translational approach

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Oral cancer patients experience severe functional pain hypothesized to be due in part to secretion of pro-nociceptive mediators. We hypothesize that pro-nociceptive mediators secreted by human oral squamous cell carcinoma (oSCC) not only sensitize and activate primary afferent neurons but also attract immune cells to the cancer microenvironment. To simultaneously study the immune cell chemotactic and pain-producing effects of cancer-secreted mediators, we injected oSCC cell line supernatant into tongues of mice. Supernatant injection alone avoids the impact of tumor growth and peripheral involvement. Tongue inflammatory infiltrate was quantified with flow cytometry and pain behavior was measured with an objective operant nociceptive assay (Dolognawmeter). We found that oral cancer cell lines, HSC3 and SCC9, drive both significant pain behavior and increased inflammatory infiltrate, whereas, melanoma cell line, SKMel28, only drives inflammation but without pain (all p<0.05). Lastly, oral dysplasia and normal keratinocytes cell line supernatant result in neither pain nor inflammation. These data suggest that oSCC-secreted mediators are unique from malignant melanoma and precancerous keratinocytes. We evaluated levels of 37 unique cytokines/chemokines among cell line supernatants using a magnetic bead panel (MILLIPLEX® MAP multiplex) and found that both HSC3 and SCC9 released significantly higher amounts of about 20% of the cytokines tested (e.g., TNF-alpha, IL-1beta) compared to supernatants from oral dysplasia, normal keratinocytes, and melanoma (all p>0.05). While promising, it is unclear if additional mediators may be present that also contribute to the phenotypic differences, given the large number of proteins that may be secreted by cells lines. An evaluation of the subset of genes that are consistently expressed within each phenotypic group of cells lines (e.g., HSC3, SCC9) and not in the phenotypically contrasting cell lines (e.g., oral dysplasia, melanoma) is warranted to reduce the number of gene products worth pursuing to quantify their nociceptive and/or inflammatory effects.

(111) Epidermal growth factor receptor signaling in oral cancer pain

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Oral cancer patients have poor survival and experience excruciating pain. Epidermal growth factor receptor (EGFR) activation by its ligands (e.g., EGF, TGF-α, amphiregulin) is a known cause for oral cancer progression. EGFR inhibitors such as Cetuximab have been used to treat patients with EGFR positive cancer. Recently, EGFR signaling in sensory neurons has been demonstrated in animal models and patients with neuropathic pain. We therefore hypothesize that oral cancer pain results from EGFR activation and overexpression in peripheral sensory systems by EGFR ligands released by oral cancer cells. We report that oral cancer cells express high mRNA levels of ADAM17, a disintegrin and metalloprotease that regulates the bioavailability of EGFR ligands. Oral cancer cells release high protein levels of EGF, TGF-α, and amphiregulin. Oral cancer patients with pain have a high amphiregulin protein concentration in the saliva compared to healthy, pain-free subjects. Cancer supernatant