19th Annual

SAFAR SYMPOSIUM

Acute Brain Care: Bringing the Future to the Bedside

ABSTRACTS

Multi-Departmental Trainees' Research Day

Thursday May 26, 2022 | 2 pm to 3:50 pm | University Club

Table of Contents

DEPARTMENT OF ANESTHESIOLOGY AND PERIOPERATIVE MEDICINE

Abstract 1

PARABRACHIAL NEUROPEPTIDE Y Y1 RECEPTORS MODULATE NEUROPATHIC PAIN IN MICE

Allen HN,[1,2] Nelson TS,[1,2] Taylor BK[1,2]

[1]Department of Anesthesiology and [2]Pittsburgh Center for Pain Research
University of Pittsburgh School of Medicine

Introduction: Chronic pain is often associated with psychiatric co-morbidities such as anxiety and depression, indicating pathology in the brain. The parabrachial nucleus (PBN) is a brainstem hub that relays ascending nociceptive information from the dorsal horn of the spinal cord to higher order brain regions. Activation of glutamatergic PBN neurons produces pain-like behaviors in naïve mice, and inhibition of glutamatergic PBN neurons reverses nerve injury induced neuropathic pain-like behaviors. Neuropeptide Y Y1 receptor mRNA is robustly expressed in the PBN, and pharmacological evidence indicates that NPY signaling in the PBN decreases acute inflammatory nociception. Based on these findings, we used mouse behavioral pharmacology, functional immunohistochemistry, and cell type-specific chemogenetics to test the **hypothesis** that *Npy1r* -expressing neurons of the PBN contribute to neuropathic pain.

Methods: Immunohistochemistry: $Npy1r^{Cre}$ mice received PBN mCherry injections followed by sham or SNI surgery. Animals received non-noxious light brush stimulation of the plantar left paw. Brains collected 60 min post stimulation were processed for Fos and mCherry immunohistochemistry.

Intracranial surgery: *Pharmacology*. C57Bl6/J mice were implanted with cannulae in the parabrachial nucleus for *in vivo* drug delivery. *Chemogenetics.* $Npy1r^{Cre}$ mice received 200nL injection of AAV8-hsyn-DIO-hM4DI-mCherry in the PBN for chemogenetic inhibition. Control animals received AAV8-hsyn-DIO-mCherry. Injections were verified via immunohistochemistry.

Spared nerve injury (SNI) model: One week after intracranial surgery, the left sciatic nerve was exposed and the tibial and common peroneal branches were ligated, leaving the sural nerve intact. Sham control surgery included exposure of the sciatic nerve, leaving all three branches intact. Testing for mechanical (up/down von Frey assay) and cold (acetone droplet test) sensitivity of the hindpaw was conducted before (baseline, prior to induction of NP) and 3 weeks after surgery.

Experimental Timeline. *Pharmacology*. Bilateral 200nL infusion of $Leu^{31}Pro^{34}$ (0.1ug, 0.01ug, 0.001ug), BIBO3304 (0.1ug), $Leu^{31}Pro^{34}$+BIBO3304, or vehicle was followed by testing for mechanical and cold sensitivity at 30, 60, 90, and 120 min timepoints. *Chemogenetics*. Intraperitoneal injection of saline or clozapine-N'-oxide (CNO, 3mg/kg) was followed by behavioral testing after 30 minutes.

Results*: Fos*. Compared to sham surgical controls, SNI increased stimulus-evoked Fos expression as well as Fos co-expression in *Npy1r*-expressing neurons in the PBN. *Pharmacology.* Bilateral intra-PBN $Leu^{31}Pro^{34}$ decreased mechanical and cold hypersensitivity in animals with SNI but not sham surgery, and this was blocked by co-administration of BIBO3304. BIBO3304 had no effect when injected alone. *Chemogenetics.* Inhibition of PBN *Npy1r* neurons with CNO alleviated mechanical and cold hypersensitivity in SNI mice but not sham surgery controls. Saline vehicle injections had no effect on pain-like behaviors.

Conclusions: Our Fos results demonstrate that SNI sensitizes *Npy1r* cells in the PBN to non-noxious mechanical stimulation. Chemogenetic inhibition of these cells can reverse pain-like behaviors in a mouse model of chronic neuropathic pain.

Significance: The *Npy1r*-expressing cells in the PBN contribute to the modulation of neuropathic pain and can be therapeutically targeted with Y1-selective agonists.

Grant Support: NINDS NS073548 (PI: Michael Gold, PhD) NS45954 (PI: Bradley Taylor, PhD)

Abstract 2

CREATION OF A WEB-BASED SIMULATED PATIENT SCENARIO FOR EVALUATING UNDERSTANDING OF HEMODYNAMIC MONITORING IN MEDICAL EDUCATION

Anglin E,[1] McIvor W[2]

[1]University of Pittsburgh School of Medicine
[2]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine

Introduction: The use of new hemodynamic monitoring techniques mandates educational tools to assess trainees' use of the information they provide. In medical education, simulation has consistently been associated with better learning outcomes than traditional lecture-based approaches. Creation of a web-based simulated patient scenario to evaluate learners' understanding of hemodynamic monitoring requires fewer resources than traditional in-person simulation, provides immediate objective feedback to the learner and instructor, and is an excellent option for remote learning.

Hypothesis: Learner understanding of hemodynamic monitoring can be evaluated by a web-based patient scenario, and individuals with higher levels of training will have superior performance during the scenario compared to less experienced individuals.

Methods: Using the University of Pittsburgh Lab for Educational Technology's vpSim program, a scenario was created of a simulated patient with hypertension undergoing noninvasive hemodynamic monitoring in the preoperative period. Vital signs and hemodynamic parameters are provided via images of monitor screens. The scenario contains decision points where learners choose between treatment options based on the hemodynamic parameters provided and are then given feedback and rationale as to whether their chosen treatment is indicated. An initial trial of the scenario was performed using mobile polling software to aggregate responses of a group of learners.

Results: During a test run of the scenario, 49% of SRNAs chose the correct treatment option at the first decision point, with the remaining respondents choosing a variety of incorrect options. After feedback was provided regarding rationale for correct and incorrect answers at the first decision point, 85% responded correctly at the second decision point.

Conclusions: Web-based simulated patient scenarios are a viable educational tool for teaching hemodynamic monitoring. Preliminary data indicates that the scenario created in this project has an appropriate level of difficulty and is likely to help improve learner understanding of hemodynamics.

Significance: The scenario developed during the project may be used to train medical students, residents, and nurse anesthesia students in hemodynamic management in the perioperative setting. The format may also be used to create similar scenarios to address a variety of clinical conditions that may be encountered in practice.

Abstract 3

THE ROLE OF lncRNA *MALAT1* IN CONTROLLING ETHANOL-INDUCED NEUROINFLAMMATION AND DRINKING BEHAVIOR

Baratta AM,[1] Plasil SL,[2] Farris SP,[1,2] Homanics GE[1,2]

[1]University of Pittsburgh, Center for Neuroscience, Pittsburgh, PA, 15213 USA
[2]University of Pittsburgh, Department of Anesthesiology & Perioperative Medicine, Pittsburgh, PA, 15261 USA

Introduction: Long noncoding RNA (lncRNA) are increasingly being recognized as important regulators of normal biological functions, such as transcriptional regulation and alternative splicing, as well as mediating disease processes. Originally examined in the context of cancer, further study of the lncRNA metastasis associated lung adenocarcinoma transcript 1 (*Malat1*) has found it to be involved in inflammatory processes throughout the body, as well as a number of diseases. *Malat1* expression is elevated in the brain of human AUD subjects and chronically drinking mice, and single cell RNA-Seq revealed that this increased expression is observed only in astrocytes.

Hypothesis: As neuroinflammation is a common consequence of excessive drinking and is thought to drive consummatory behavior, we hypothesize that changes in *Malat1* expression impact neuroinflammatory responses to ethanol and drinking behavior.

Methods: Using CRISPR/Cas9 in primary mouse astrocyte cultures, we knocked down *Malat1* expression and performed a preliminary RNA-Seq screen to quantify changes in gene expression. Concurrently, we used a novel CRISPR approach to rapidly create *Malat1* global knockout animals and subsequently tested ethanol drinking behavior.

Results: Results from our RNA-Seq pilot indicated knockdown of *Malat1* expression in primary astrocytes led to a significant upregulation in a number of interferon-signaling related genes, including *Isg15*, *Ifit1*, *Ifit3*, *Iigp1*, and *Irf7*. Every other day two-bottle choice (EOD-2BC) drinking revealed a female specific decrease in ethanol consumption in *Malat1* knockout animals. Taken together, our results indicate that *Malat1* plays a role in the perpetuation of drinking behavior, possibly through regulation of neuroimmune responses.

Conclusions: As our results indicate that astrocytes may play an outsized role in controlling *Malat1* dependent effects when compared to other central nervous system cell types, future studies will observe the effect of astrocyte specific *Malat1* knockout on drinking behavior and neuroinflammatory responses.

Research/Grant Support: We gratefully acknowledge the support of NIH/NIAAA grants AA020889, AA10422, AA024836, and T32 NS007433-22.

Abstract 4

NEUROPEPTIDE Y Y2 RECEPTORS IN SENSORY NEURONS TONICALLY SUPPRESS NOCICEPTION AND ITCH, BUT FACILITATE BEHAVIORAL SIGNS OF NEUROPATHIC AND POSTSURGICAL PAIN

Basu P,[1] Maddula A,[1] Nelson TS,[1] Prasoon P,[1] Taylor BK[1]

[1]Department of Anesthesiology and Perioperative Medicine, Pittsburgh Center for Pain Research, and Pittsburgh Project to end Opioid Misuse, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Exogenous or endogenous neuropeptide Y (NPY) acts at its cognate Y1 receptor in dorsal horn to tonically inhibit signs of inflammatory and neuropathic pain. The actions of NPY at the Y2 receptor (Y2R) on thinly myelinated primary afferent neurons are not as clear. Y2 receptor antagonists appear to suppress acute nociception, but few studies have addressed their impact in models of persistent pain.

Hypothesis: We predicted that intrathecal administration of Y2 receptor agonists or antagonist will act at the central terminals of primary afferent neurons to modulate acute nociception and/or chronic pain.

Methods: Experiment #1: to determine whether Y2Rs tonically inhibit nocifensive/itch behaviors in naïve mice, we intrathecally administered the Y2R antagonist BIIE0246 at doses ranging from 0.1 ng to 3.0 µg in C57BL/6 male and female mice, and measured mechanical (von Frey), heat, and cold sensitivity, as well as ataxia (rotarod), affective component of pain (conditioned place aversion), as well as ongoing nocifensive and itch like behaviors. To determine whether the effects of BIIE0246 were mediated by Y2Rs in DRG neurons, we crossed $Pirt^{cre}$ mice with $Npy2r^{lox/lox}$ mice to create $Npy2R^{DRG-/-}$ conditional knockout mice. Experiment #2: we intrathecally adminstered the Y2R selective agonist, PYY_{3-36} at doses ranging from 0.01 to 3µg in the plantar incision model of postsurgical pain or the spared nerve injury (SNI) model of neuropathic pain, and then evaluated mechanical (von Frey), heat, and cold sensitivity at the plantar hindpaw.

Results: Experiment #1. PYY_{3-36} had minimal effect on either mechanical or thermal hypersensitivity when tested 2 days after incision or 14 days after SNI. (This unexpected result could be attributed to endogenous NPY release and saturation of Y2Rs leading to unavailability of Y2Rs to bind to exogenous Y2 agonists). Experiment #2. Intrathecal administration of Y2R antagonist BIIE0246 in naïve mice dose-dependently (0.01-3µg) elicited robust pain-like behaviors including mechanical and cold hypersensitivity, but did not change heat hypersensitivity or motor coordination, and did not induce conditioned placed aversion. BIIE024 also elicited itch-like behaviors including scratching and back biting. As expected, $Npy2R^{DRG-/-}$ mice expressed Y2 protein in hippocampus (positive control) but not DRG. BIIE0246 (3µg, i.t.) induced pain- and itch-like behaviors in $Npy2r^{lox/lox}$ controls but not in $Npy2R^{DRG-/-}$ mice. $Npy2R^{DRG-/-}$ mice exhibited less mechanical and thermal hypersensitivity as compared to $Npy2r^{lox/lox}$ controls in the SNI model of neuropathic pain and in a latent sensitization plantar incision model of persistent postsurgical pain.

Conclusions: Together, our findings suggest that Y2Rs on primary afferent neurons exert two functions: 1) tonic inhibition of nociception and itch in the absence of injury; and 2) facilitate persistent pain in the setting of nerve or tissue injury.

Significance: These results support continued investigation into Y2R antagonists as a promising therapeutic approach for the treatment of chronic neuropathic and/or postsurgical pain.

Research/Grant Support: This work was supported by NIH grants R01DA37621, R01NS45954 and R01NS62306 (PI: Bradley K. Taylor, PhD).

Abstract 5

THERMAL AND MECHANICAL HYPERALGESIA DURING WITHDRAWAL FROM CHRONIC INTERMITTENT ETHANOL VAPOR IN MICE

Brandner AJ,[1,2] Baratta AM,[1,2] Rathod R,[3] Ferguson C,[3] Taylor BK,[1,2,3] Farris SP[1,2,3]

[1]Center for Neuroscience, University of Pittsburgh SOM, Pittsburgh, PA USA
[2]Pittsburgh Center for Pain Research, University of Pittsburgh SOM, Pittsburgh, PA USA
[3]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh SOM, Pittsburgh, PA USA

Introduction: Approximately half of patients with alcohol use disorder (AUD) report presents itself with a myriad of symptoms during withdrawal, including severe pain, and this can be severe during abstinence. The relationship between AUD and pain has a circular causality; approximately half of AUD patients report chronic pain, while one quarter of chronic pain patients develop AUD. However, there is a lack of preclinical data showing that mice can elicit this pain – like phenotype resulting from chronic alcohol exposure in a sex – dependent manor.

Hypothesis: We wanted to explore the relationship of AUD and chronic pain by developing a mouse model of Chronic Alcohol withdrawal Induced Pain (CAIP).

Methods: Male and female C57BL/6J mice underwent chronic intermittent ethanol vapor (CIEV) exposure four days per week, for four weeks, to induce alcohol dependence. Hindpaw sensitivity to mechanical (with application of von Frey filaments) and heat stimuli were measured weekly at 1, 3, 5, 7, 24, and 48 hr following cessation of ethanol.

Results: Both males and females experienced increased mechanical sensitivity, while only females experienced an increase in thermal sensitivity. Mechanical hyperalgesia was prominent at the 24 – and 48 hr timepoints after two or four weeks of CIEV in males or females, respectively. Heat hyperalgesia developed after four weeks, but only in females.

Conclusions: CAIP develops in a sex – dependent manner in C57BL/6J mice as shown through increased heat and mechanical sensitivity to nociceptive stimuli during alcohol withdrawal.

Significance: These studies suggest a direct effect of multiple cycles of intoxication and withdrawal from alcohol on pain-related behavior and demonstrates important biological sex differences in the emergence of chronic alcohol-induced pain. Future studies identifying the molecular mechanisms contributing to these effects will be important for developing rational pharmacotherapies for treating these co-morbid conditions.

Research/Grant Support: We gratefully acknowledge the support of NIH/NIAAA, NIH/NIDA, and NIH/NINDS grants AA024836 (SPF), DA037621 (BKT), NS045954 (BKT), and NS112632 (BKT).

Abstract 6

ANESTHETIC-MEDIATED CHANGES IN FUNCTIONAL BRAIN ACTIVATION PREDICT BEHAVIORAL OUTCOMES

Burlew A,[1] Vogt K[1,2]

[1]University of Pittsburgh School of Medicine (UPSOM)
[2]Department of Anesthesiology and Perioperative Medicine, UPSOM

Introduction: Ketamine and midazolam are commonly used for anesthesia, but the neural correlates of their effects during pain are not well understood.

Hypothesis: Drug-mediated fMRI signal changes will correlate to changes in pain scores and memory scores in individuals.

Methods: Eighteen participants completed a pain and memory task while a fMRI scan was collected. The experiment involved an auditory memory encoding task which presented 90 words; 30 were paired with a 1s electric shock. Participants rated their pain throughout the trial, and memory was assessed the following day using a recognition test. Group average brain activation was computed using SPM12. Pain rating and memory performance data were plotted against changes in activation strength, comparing saline with ketamine and saline with midazolam. Brain Regions of Interest (ROIs) were defined using several methods.

Results: Generating ROIs using 5 mm spheres centered at the peak of group activation elucidated the greatest number of regions which correlate to behavioral measures of pain. Some regions demonstrate similar correlations using the activation-cluster ROIs, and few are replicated in the atlas-defined ROIs. Regions of the prefrontal cortex, default mode network, and pain processing centers show an association with participant-reported levels of pain under ketamine compared to saline. The following regions and their R^2 values compare the correlations using sphere ROIs and activation-cluster derived ROIs, respectively: Right posterior cingulate = 0.174 and 0.065; Right thalamus = 0.336 and 0.134; Right precuneus = 0.202 and 0.072. Scatter plots comparing ROI activation and pain scores indicate right thalamic activity increases as pain scores decrease, while activity in the right precuneus and right posterior cingulate decrease as pain scores decrease. Correlations between activation and changes in memory were not as strong in the midazolam condition. The greatest correlation existed in the right primary somatosensory region, with R^2 = 0.459 and 0.286 using sphere and activation-cluster ROIs, respectively.

Conclusions: The results of the pain-by-activation analyses demonstrate a potentially paradoxical relationship between brain activation and pain. While posterior components of the default mode network (posterior cingulate cortex and precuneus) show decreased activation during analgesia, thalamic activation increased. This could result from the decoupling of cortical association areas by ketamine, even as sub-cortical activity remains high due to continued afferent noxious stimulation. The results indicate smaller ROIs focused on areas of peak activation tend to show greater association to behavioral metrics.

Significance: Identifying regions implicated in these biologic processes can aid targeted pharmacologic research and clinical practice in the future. Individual variation in response to sedation contributes to sub-optimal pain control and memory ablation. Identifying more definitive measures of amnesia and analgesia have important implications for the management of pain and memory during surgery.

Research/Grant Support: Dean's Summer Research Project, K23GM132755

Abstract 7

UTILITY OF PERIPHERAL NERVE BLOCKS IN PEDIATRIC PATIENTS UNDERGOING EMBOLIZATION THERAPY FOR VASCULAR MALFORMATIONS

Evankovich M,[1] Kocher M,[2] Yilmaz S,[3] Visoiu M[2]

[1]University of Pittsburgh School of Medicine
[2]Department of Anesthesiology and Perioperative Medicine, UPMC
[3]Pediatric Interventional Radiology, Children's Hospital of Pittsburgh

Introduction: Congenital vascular malformations cause blood to bypass the capillary bed and directly drain into the venous system. Embolization is one type of treatment and has been used for several decades to treat these malformations. Treatment-related significant pain after the embolization is not uncommon (1), and peripheral nerve blocks could reduce such pain (2). However, there is limited data on the effect of peripheral nerve blocks on postoperative pain in patients undergoing embolization therapy. This study investigates the feasibility of peripheral nerve blocks for pain control following embolization therapy in patients with vascular malformations.

Hypothesis: We hypothesize that single-injection peripheral nerve blocks can be safely performed to provide perioperative analgesia for patients undergoing embolization therapy. They will reduce morphine milligram equivalents (MME) and postoperative pain scores in patients that receive peripheral nerve blocks.

Methods: We performed a retrospective study of patients that received embolization therapy for a vascular malformation at Children's Hospital of Pittsburgh from 2011 to 2020. Data collected included: demographics, nerve block characteristics, perioperative opioid and non-opioid analgesia, pain scores, and hospital stay.

Results: 882 patients aged one day to 61 years old met the inclusion criteria for our study. Peripheral nerve blocks were used in 4.6% of cases, with patients ranging in age from 5 to 49 years old. Our study suggests that peripheral nerve blocks decrease opioid consumption (average MME per kilogram was 0.173 for block patients vs. 0.280 for no blocks) (P = .01) but do not decrease pain scores (median 3). One patient from the sclerotherapy group reported sciatic nerve injury unrelated to the block.

Conclusion: The peripheral nerve blocks are feasible and can be used as a multimodal approach for postoperative pain control in patients undergoing embolization therapy.

Significance: This report is the first study investigating peripheral nerve blocks in patients undergoing embolization therapy.

References:

1. Ben-David B, Kaligozhin Z, Biderman D. Quadratus lumborum block in the management of severe pain after uterine artery embolization. Eur J Pain 22(6):1032-1034, 2018.
2. Murauski JD, Gonzalez KR. Peripheral nerve blocks for postoperative analgesia. AORN J 75(1):136-47, 2002; quiz 148-54.

Abstract 8

fMRI IMAGING CHANGES SEEN WITH THE APPLICATION OF AURICULOTHERAPY FOR CHRONIC PAIN

Gao T,[1] Vogt KM,[1] Ibinson JW[1]

[1]Department of Anesthesiology and Perioperative Medicine
University of Pittsburgh Medical Center

Introduction: Chronic pain is one of our nation's largest healthcare burdens, and anesthesiology research in recent years have focused on determining effective non-opioid management options. One potential such alternative method is auriculotherapy (AT), which has demonstrated analgesic effects lasting for weeks in surgical and chronic back pain patients at our tertiary-care center, although the mechanism of action remains unclear. fMRI imaging is a viable technique to elucidate brain functional connectivity changes before and after therapeutic interventions.

Hypothesis: Our group postulated that appreciable changes would be seen before and after AT in brain areas associated with acute and chronic pain recognition like the default mode network, insula, and the anterior cingulate cortex.

Methods: Patients with low back pain, > 3 months in duration, were recruited from chronic pain clinics in an academic medical center. This preliminary analysis includes 4 patients with 8-minutes of blood oxygen-level dependent fMRI data obtained before and 3 days after cryogenic AT treatment. Image analysis was performed in MATLAB using the CONN toolbox ver 20b, running on SPM12[3]. Image data were preprocessed within CONN, including motion correction and denoising. Seed-based functional connectivity was calculated using a region of interest (ROI) to ROI approach, using a standard brain atlas for ROI definition. Group average within-patient pre- vs. post- AT connectivity contrasts were calculated using a paired t-test framework, and results are displayed thresholded with $p<0.001$.

Results: Based on our exploratory analysis, connectivity changes were seen between the prefrontal cortex and important areas known to be involved in pain-processing (anterior cingulate), p-value = *0.0003*, and memory (hippocampus), p-value = *0.0010*. Interestingly, there were several changes in connectivity detected that involved the cerebellum with the insula (cognitive-evaluative components of pain), and the nucleus accumbens (receives nociceptive input). The role of the cerebellum in many cognitive functions, including the processing of pain is emerging, but still incompletely understood.

Conclusions: Our preliminary results, though from a very small cohort, showed significant changes in connectivity after cryo-AT treatment. Further research is needed to determine if these preliminary changes persist in the full study cohort. This also demonstrates the potential role fMRI can play in tracking brain changes over time, in response to treatments for chronic pain.

Significance: Finding viable alternatives to opioid use for chronic pain would provide a societal and cost-saving benefit to our national healthcare system. More research into alternative therapies can help elucidate their safety and efficacy and is warranted in order for physician-scientists to make appropriate recommendations for their patients and colleagues.

Abstract 9

THE NOVEL METHYLGLYOXAL SCAVENGER Cyck(Myr)R4E PREVENTS HYPERALGESIA IN THE db/db MODEL OF TYPE II DIABETES

Gralinski MR,[1] Santos DF,[1] Taylor BK[1]

[1]University of Pittsburgh, Department of Anesthesiology, Pittsburgh Center for Pain Research Pittsburgh, PA, USA

Introduction: Painful diabetic neuropathy (PDN) is a neurological complication of diabetes. The primary feature of both Type I and Type II diabetes (T2D) is hyperglycemia, or high blood glucose, which can cause peripheral neuropathy in the lower extremities known as diabetic neuropathy. T2D is associated with elevated blood levels of glucose and methylglyoxal (MG), a highly reactive byproduct of glycolysis thought to mediate hyperalgesia in PDN. Cyck(Myr)R4E is a novel peptide that acts as an MG scavenger to reduce the concentration of free MG in the blood.

Hypothesis: Cyck(Myr)R4E will reduce heat hypersensitivity in the db/db mouse model of T2D as well as reduce phosphorylated extracellular signal-regulated kinase (pERK) expression which serves as a marker for the sensitization of pain-related neurons in the spinal cord dorsal horn of db/db mice.

Methods: Our initial set of 14 mice [either db/db (38-41g) or db/+ heterozygous controls (20-25g)] were split among four treatment groups: db/db mice that received Cyck(Myr)R4E, db/db mice that received saline, db/+ mice that received Cyck(Myr)R4E, and db/+ mice that received saline. Beginning at 6 weeks of age, mice were treated 3 times a week for 6 weeks with intraperitoneal injection of either 0.125 mg/200 mL of Cyck(Myr)R4E or 5 mL/kg of saline. Heat hypersensitivity was assessed with the 52.5°C hot plate test. After the final injection of Cyck(Myr)R4E at 12 weeks of age, spinal pain responsive neurons in the spinal cord were activated with light touch stimulation in all 14 mice. The mice were then perfused, and laminectomies were performed to remove and preserve the spinal cords which were sliced and underwent immunohistochemistry to quantify pERK expression across the four groups.

Results: Cyck(Myr)R4E prevented the development of heat hypersensitivity in db/db mice at 12 weeks of age and significantly reduced pERK expression in the spinal cord dorsal horn.

Conclusion: MG is a relevant target for the prevention of PDN in T2D. Going forward, we will test the effects of Cyck(Myr)R4E injections up to 14 weeks of age on the development of hypersensitivity in Db/Db mice.

Abstract 10

CHEMOGENETIC ACTIVATION OF KAPPA OPIOID RECEPTOR-EXPRESSING NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA INHIBITS HYPERALGESIA IN A MOUSE MODEL OF ACUTE AND CHRONIC POSTSURGICAL PAIN

Hellman A,[1] Brandner AJ,[1] Taylor BK[1]

[1]Department of Anesthesiology and Perioperative Medicine, Center for Neuroscience
Pittsburgh Center for Pain Research, Pittsburgh Project to End Opioid Misuse
University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: The rostral ventral medulla (RVM) contains two neuronal populations that provide descending modulation of spinal nociceptive transmission: ON cells that begin to fire just prior to withdrawal from a heat stimulus and thought to be pronociceptive, and OFF cells which stop firing just prior to heat withdrawal and thought to be antinociceptive. A key neurochemical signature of OFF cells is expression of the kappa opioid receptor (KOR). Evidence from Dr. Ross' lab indicate that chemogenetic inhibition of RVM KOR neurons elicit pain-like behaviors in naïve mice. Furthermore, our lab found that latent postsurgical pain sensitization is kept in a state of remission by tonic activation of opioid receptors (Basu et al, 2021).

Hypothesis: Here, we to test the hypothesis that activation of RVM KOR neurons will inhibit behavioral signs of acute and chronic postsurgical pain.

Methods: Male and female Oprk1^{cre} mice (aged 8-12 weeks) received craniotomy for injection into the RVM of an AAV8-hSyn-DIO-hM3D$_{Gq}$ or control virus AAV8-hSyn-DIO-mCherry (8.6x10^10, 5 ul). After 21 days a 5mm incision was made through the skin and fascia of the left plantar hindpaw. The underlying muscle was raised, extended, and incised 4mm longitudinally leaving the muscle intact. Indices of acute (2-3 days post injury) and chronic (21 days post injury) pain included hindpaw withdrawal responses to mechanical and radiant heat stimuli applied to the plantar skin. Central sensitization facilitates protective aspects of acute pain and sensitization may continue in a latent state that is masked by an opposing inhibition mediated by inhibitory G-protein coupled receptors. Latent sensitization can be revealed as reinstatement of hyperalgesia upon administration of GPCR antagonists or inverse agonists such as naltrexone (3mg/kg, s.c.) or BT 3761 (short-acting KOR-selective antagonist, 0.3mg/kg, i.p.). Clozapine N-oxide (CNO) (3mg/kg, i.p.), followed by behavioral testing at 30, 60, 90, and 120 minutes. Following behavioral testing, viral expression and targeting in the RVM was confirmed post-fixation with immunohistochemistry (mCherry) and fluorescence *in situ* hybridization (KOR).

Results: Incision produced mechanical and heat hypersensitivity that peaked at 2-3 days and resolved within 21 days, after which BT3761 or NTX but not vehicle produce a robust reinstatement of hypersensitivity. Acute chemogenetic activation of RVM KOR with CNO reduced incision-induced mechanical and heat hypersensitivity compared to vehicle control ($p<0.05$, two-way ANOVA with Bonferroni correction; n=6/group). Chronically, CNO prevented the reinstatement of hypersensitivity triggered by either BT3761 ($p<0.05$ vs vehicle, n=8/group) or NTX ($p<0.05$ vs vehicle, n=8/group).

Conclusions: Our results indicate that chemogenetic activation of RVM KOR neurons inhibit behavioral signs of acute and chronic postsurgical pain.

Significance: Our studies build upon mounting evidence promoting RVM KOR neurons as a viable pharmacological target for the treatment of postsurgical pain.

Research/Grant Support: R01DA37621, R01NS45954 and R01NS62306 (PI: Bradley K Taylor, PhD) and T32GM075770 (Abigail Hellman, PhD).

Abstract 11

VENOVENOUS EXTRACORPOREAL MEMBRANE OXYGENATION TREATMENT PARAMETER DIFFERENCES IN OBSTETRIC PATIENTS

Hildreth B,[1] Nicholas A,[1] Petty K,[1] Grosse P,[2] Lim G[1]

[1]Department of Anesthesiology & Perioperative Medicine, University of Pittsburgh School of Medicine
[2]Department of Epidemiology, University of Pittsburgh

Introduction: As maternal mortality rates continue to rise due to increasing medical complexity, cardiac disease, and advanced maternal age, there is increased demand for advanced medical therapies such as extracorporeal membrane oxygenation (ECMO). Pregnant and postpartum women experience physiologic changes that lead to expected differences in ECMO treatment parameters, but few studies have identified key ECMO parameter adjustments for obstetric patients. Identifying key differences in ECMO settings can guide current and future therapies for critically ill pregnant women.

Hypothesis: We expect to find that ECMO parameters are different in pregnancy and post-partum, compared to non-pregnant controls.

Methods: This pre-pandemic retrospective case control study compared all pregnant and postpartum patients requiring venovenous (V-V) ECMO at a single institution between 2013-2020, with non-pregnant controls matched by age, body surface area, sex, and ECMO indication in a 1:1 ratio. Hourly vital signs, blood gas values, and ECMO variables for the first 72 hours after initiating ECMO were recorded. Descriptive statistics described indications for ECMO, survival rates, and timing of ECMO initiation. Means were summarized over 12-hour intervals and plotted for trends, and variability was accounted in the form of standard errors. Plots displayed means and the region within 2 standard errors of the mean for each ECMO variable, comparing differences between cases and controls while accounting for variability in the form of standard errors. The frequency of abnormal events in ECMO parameters were reported for each group. Analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC).

Results: A total of 10 records (5 cases and 5 controls) were identified; all 5 (100%) obstetric patients and 2 (40%) non-obstetric patients survived to hospital discharge. Indications for V-V ECMO in the cohort were viral pneumonia (4 of 10, 40%), aspiration pneumonia (3, 30%), status asthmaticus (1, 10%), and other acute respiratory illness (2, 20%). All pregnant cases had ECMO initiated on postpartum days 2.2 ± 2.7, with most initiated in the context of cesarean delivery (4 of 5, 80%). ECMO variables blood flow per kilogram per minute (BFPERKG) and carbon dioxide removal (sweep), were most notably different in pregnant vs. nonpregnant controls across the total treatment time (Figure 1). Pregnant cases had more frequent abnormal instances of ECMO variables than controls for low pH (case 64.6% vs. control 38.6%), respiratory rate (9.7% vs. 2.2%), and low PaO2 (case 63.3% vs. control 33%). Control cases had more frequent abnormal instances for heart rate (case 10% vs control 66.7%), oxygen saturation SpO2 (6.7% vs. 25.9%), high mean arterial pressure (MAP) (2.6% vs. 18.4%), and high PaCO2 (10.1 vs. 95.5%).

Conclusions: Obstetric patients requiring V-V ECMO receive treatment initiated in the postpartum period and are likely to survive to hospital discharge. ECMO sweep and BFPERKG parameters and resultant PaCO2 changes are notably different in obstetric patients compared to non-obstetric patients.

Significance: These results highlight potential improvement opportunities for mechanical hemodynamic support systems for critically ill pregnant women.

Research/Grant Support: NIH UL1-TR-001857

Abstract 12

REDUCING EXCESSIVE POST OPERATIVE OPIOID PRESCRIBING AT THE VA PITTSBURGH HEALTHCARE SYSTEM (VAPHS)

Hlavin C,[1,2] Muluk S,[3] Muluk V,[4] Ryan J,[4] Dhupar R[4,5]

[1]Department of Surgery, University of Pittsburgh
[2]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh
[3]University of Pittsburgh School of Medicine
[4]VA Pittsburgh Healthcare System, U.S. Department of Veterans Affairs
[5]Department of Cardiothoracic Surgery, University of Pittsburgh, University of Pittsburgh

Introduction: Drug overdose is the leading cause of injury-related death in the United States and is linked to opioid overprescribing. Variability in surgeon prescribing patterns is common in the post operative period and can be the nidus for dependence and addiction. This project aims to reduce opioid overprescribing at the Veteran's Affairs Pittsburgh Healthcare System (VAPHS).

Hypothesis: Creation and implementation of post operative opioid prescribing guidelines will decrease the amount, duration and total morphine milligram equivalents (MME) prescribed after surgical procedures.

Methods: The VAPHS Opioid Stewardship Committee collaborated with a multidisciplinary team to create prescribing guidelines for inpatient and outpatient general, thoracic, and vascular surgery procedures. Bundled order sets were incorporated into the provider workflow in the electronic medical system. Opioid prescription patterns were compared for Veterans who underwent any procedure for a three-month period pre- and post- guideline implementation.

Results: After implementation of opioid prescribing guidelines, MME, quantity of pills prescribed, and days prescribed were statistically significantly reduced for cholecystectomy (MME 140.8 v. 57.5, p=0.002; quantity 18.8 v 8, p=0.002; days 5.1 v. 2.8, p=0.021), inguinal hernia repair (MME 129.9 v. 45.3, p=0.002; quantity 17.3 v. 6.1, p=0.002; days 5.0 v 2.4, p=0.002), and umbilical hernia repair (MME 128.8 v. 53.8, p=0.002; quantity 17.1 v. 7.8, p=0.002; days 5.1 v. 2.5, p=0.022).

Conclusion: Including all patients, procedures with associated guidelines had a significant reduction in MME and days of opioids prescribed, but not quantity. Procedures without associated recommendations also demonstrated a decrease in overall opioid prescribing.

Significance: Post operative opioid prescribing guidelines can steer clinicians toward more conscientious narcotic disbursement. There may also be reductions in prescribing narcotics for procedures without guidelines as an indirect effect of practice change.

Research/Grant Support: T32GM075770 (PI: Xan Yu, PhD)

Abstract 13

DISTINCT KAPPA OPIOID RECEPTOR-EXPRESSING NEURAL PROJECTIONS IN THE VENTRAL TEGMENTAL AREA DISPLAY UNIQUE CONTRIBUTIONS TO PAIN AND OPIOID WITHDRAWAL

Holland R,[1,2] Chiang M,[1,2] Nguyen L,[2] Sherman S,[2] Zoltanski A,[2] Ross S[1,2,3]

[1]Pittsburgh Center for Pain Research, University of Pittsburgh School of Medicine
[2]Department of Neurobiology, University of Pittsburgh School of Medicine
[3]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine

Introduction: Opioid withdrawal is an excruciating and potentially life-threatening syndrome resulting from abrupt cessation of opioid use. Untreated opioid withdrawal frequently results in relapse and presents a major barrier to recovery from opioid use disorder (OUD). The kappa opioid receptor (KOR) has been shown to play complex roles in chronic pain and addiction through the modulation of dopamine neurons originating from the ventral tegmental area (VTA). However, the precise mechanisms through which KOR-expressing VTA (VTA^{KOR}) neurons contribute to the development of opioid withdrawal remains elusive.

Hypothesis: Chronic opioid use promotes withdrawal through VTA^{KOR} neural inhibition.

Methods: RNAScope FISH was performed to determine neurotransmitter expression in VTA^{KOR} neurons. The *Oprk1*^{cre} mouse was then utilized alongside cre-dependent viral approaches to genetically manipulate VTA^{KOR} neurons in morphine-naïve and dependent mice. Withdrawal was induced by escalating doses of morphine followed by naloxone; acute withdrawal behaviors, real-time place preference (RTPP), and conditioned place aversion (CPA) were assessed.

Results: VTA^{KOR} neurons express a diverse complement of neurotransmitters, with the highest proportion expressing tyrosine hydroxylase (Th), a marker for dopaminergic neurons, or coexpressing Th and Slc17a6, a marker for glutamatergic neurons. VTA^{KOR} neurons project to distinct regions throughout the medulla, midbrain, and forebrain, including the NTS, vlPAG, amygdala, BNST, and NAcC/NAcSh. In morphine-naïve mice, chemogenetic activation of VTA^{KOR} neurons increased thermal and mechanical withdrawal thresholds and attenuated capsaicin-induced mechanical hypersensitivity. In morphine-dependent mice, chemogenetic activation of VTA^{KOR} neurons reduced withdrawal-associated jumping, body weight loss, and CPA. Interestingly, while optogenetic activation of these neurons did not induce a RTPP in morphine-naïve mice, morphine-dependent mice expressing channelrhodopsin (ChR2) in VTA^{KOR} neurons spent more time in the light-paired chamber compared to eYFP controls.

Conclusions: VTA^{KOR} neurons are anatomically diverse, projecting throughout the brain to modulate downstream targets via multiple neurotransmitters. Furthermore, VTA^{KOR} neurons modulate acute and persistent pain responses. Importantly, VTA^{KOR} neural inhibition is necessary for the development of multiple dimensions of acute opioid withdrawal.

Significance: This study uses cutting-edge genetic approaches to demonstrate the effect of selective activation of KOR neurons in the VTA on opioid withdrawal in rodents. Taken together, this evidence strongly implicates KOR-mediated downregulation of VTA neurons in aversive states and identifies potential therapeutic targets in the treatment of opioid withdrawal.

Research/Grant Support: NINDS R01NS096705 (PI: Sarah E. Ross, PhD) NINDS F31NS116981 (PI: Ruby A. Holland, BA)

Abstract 14

THORACIC DORSAL ROOT GANGLION STIMULATION REDUCES ACUTE MYOCARDIAL ISCHEMIA INDUCED VENTRICULAR ARRHYTHMIAS BY ATTENUATION OF MYOCARDIAL EXCITABILITY

Kuwabara Y[1], Kimberly HQ[1,2], Siamak S[1], Yamaguchi T[1], and Aman M[1,2]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh
[2]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh Medical Center

Introduction: Ventricular arrhythmias induced by myocardial ischemia are the leading cause of sudden cardiac death (SCD). Dorsal root ganglion stimulation (DRGS) may serve as a novel neuromodulation strategy to reduce ischemia-induced cardiac sympathoexcitation and ventricular arrhythmias.

Hypothesis: DRGS can reduce ventricular arrhythmias and modulate cardiac autonomic imbalances caused by myocardial ischemia.

Methods: Yorkshire pigs were randomized into two groups. Myocardial ischemia was induced for one hour by ligating the left anterior descending coronary artery (LAD) in the LAD group (n =13). In the DRGS group (n =10), high-frequency stimulation (1 kHz) at the second thoracic level (T2) was initiated 30 minutes before LAD ligation and continued throughout one hour of ischemia. Cardiac electrophysiological mapping and Ventricular Arrhythmia Score (VAS) were assessed. An immunohistochemistry test was performed to investigate potential molecular mechanisms in the spinal cord and DRG.

Results: DRGS decreased the magnitude of activation recovery interval (ARI) shortening in the ischemic region (LAD: -202 $\pm$ 9.9 ms. DRGS: -162 $\pm$ 12 ms, p = 0.021) and decreased global dispersion of repolarization (DOR) at 30 minutes of myocardial ischemia (LAD: 9700 $\pm$ 861 ms^2. DRGS: 6672 $\pm$ 681 ms^2, p = 0.016). DRGS also decreased ventricular arrhythmias (VAS - LAD: 8.9 $\pm$ 1.1. DRGS: 6.3 $\pm$ 1.0, p = 0.038). Immunohistochemistry studies showed that DRGS decreased the apoptosis cells in the DRG (p = 0.0084) and % cFos with NeuN expressions in the T2 spinal cord (p = 0.048).

Conclusions: DRGS reduced the burden of myocardial ischemia-induced cardiac sympathoexcitation and has a potential to be a novel treatment option to reduce arrhythmogenesis.

Significance: DRGS can be a promising neuromodulation therapy for SCD.

Grant Support: NIH RO1 HL136836 and NIH R44 DA049630 (PI: Aman Mahajan, MD)

Abstract 15

S1PR1 AGONISM IN THE CENTRAL AMYGDALA REDUCES PERIPHERAL NEUROPATHIC PAIN

Lamerand SR,[1,2] Taylor BK[1,2]

[1]Department of Anesthesiology and Perioperative Medicine
Pittsburgh Center for Pain Research, and Pittsburgh Project to end Opioid Misuse
University of Pittsburgh School of Medicine, Pittsburgh, PA
[2]Center for Neurosciences University of Pittsburgh, Pittsburgh, PA

Introduction: Sphingosine-1-phosphate receptors (S1PRs) are an emerging target for the treatment of persistent pain. The S1PR1 agonist/functional antagonist fingolimod, a treatment for multiple sclerosis (MS), reduces pain-like behaviors in rodent models of inflammatory and neuropathic pain. We reported that fingolimod reduced mechanical allodynia in an experimental autoimmune encephalomyelitis (EAE) model of MS-associated neuropathic pain. This reduction in mechanical allodynia was mimicked by the S1PR1 agonist SEW2871, and blocked by the S1PR1 antagonist W146, indicative of an S1PR1-dependent mechanism. However, the anatomical site(s) of action remain unclear. To address this gap, we began by targeting the central nucleus of the amygdala (CeA), an important supraspinal center of pain.

Hypothesis: Pharmacological agonism at S1PR1 receptors in the CeA will inhibit mechanical and cold allodynia in the spared nerve injury (SNI) model of peripheral neuropathic pain.

Methods: SNI model of neuropathic pain: In C57BL/6 mice (7-9 weeks old), the common peroneal and tibial branches, but not sural branch, of the left sciatic nerve were ligated and cut. Intracranial cannula placement and injection: 4-7 days after SNI, mice were anesthetized and head-fixed in a stereotaxic apparatus. A guide cannula was implanted 1mm above the right CeA, followed by a minimum one-week recovery period. On day 14 post-SNI, an injection cannula was inserted and extended 1mm beyond the guide cannula. Mice received intra-CeA injection of fingolimod, CYM5442 (an S1PR1 agonist/functional antagonist), SEW2871, or vehicle followed by behavioral assessment from 10- to 120- minutes after injection. Behavioral testing: Hindpaw withdrawal responses to mechanical (von Frey) stimuli and, in the SEW2871 study, withdrawal response to cool (acetone) stimuli were assessed. Fluorescence *in situ* hybridization: 20-µm thick CeA sections were stained with an S1PR1 mRNA probe, and then imaged with a fluorescent microscope to evaluate the distribution of S1PR1.

Results: S1PR1 mRNA expression was ubiquitous throughout the CeA. All mice had developed mechanical and cold hypersensitivity within the sural innervation territory of the left lateral hindpaw when tested at 14 days post-surgery. Intra-CeA microinjection of fingolimod (0.01ng; 300nL) or CYM5442 (0.6ng; 300nL), but not saline, increased mechanical thresholds, peaking at 10 minutes, and lasting 30-45 minutes (n=5-7; 2-way RM ANOVA with Bonferroni post-test, $P< 0.05$ vs vehicle). Intra-CeA SEW2871 (0.1ug & 1.0ug; 0.2ul), but not saline, increased mechanical withdrawal thresholds, peaking at 15 minutes and a lasting 30-45 minutes and with no effect on cold withdrawal duration (n=8-9; 2-way RM ANOVA with Bonferroni post-test, $P<0.05$ vs vehicle).

Conclusions: Microinjection of the S1PR1 agonists/functional antagonists fingolimod or CYM5442 into the CeA reduces nerve injury-induced mechanical allodynia in male mice. Additionally, microinjection of the S1PR1 agonist SEW2871 reduced nerve injury-induced mechanical allodynia, but not cold allodynia, in both sexes. We conclude that S1PR1 agonism in the central amygdala reduces peripheral neuropathic pain.

Significance: These results point to S1PR1 in the CeA as a target for future pharmacotherapeutics for the treatment of neuropathic pain.

Research/Financial Support: R01NS112632 (B.K.T), T32NS073548 (S.R.L)

Abstract 16

ACUTE POSTPARTUM PAIN AND ANXIETY INFLUENCE LONG-TERM POSTPARTUM PAIN, MATERNAL-INFANT ATTACHMENT AND PARENTING SELF-EFFICACY

Makeen M,[1] Farrell L[1], Kenkre T[1], Lim G[1,2]

[1] Department of Anesthesiology & Perioperative Medicine, University of Pittsburgh
[2] Department of Obstetrics, Gynecology, & Reproductive Sciences, University of Pittsburgh

Introduction: Pain and depression are bi-directionally related in chronic pain settings, and worse labor pain has been linked to postpartum depression symptoms. These findings raise questions about whether improving pain and mood after delivery can improve maternal parenting function. However, few studies have examined relationships between postpartum pain and negative mood, and their effects on parent-infant relationship outcomes. We aimed to assess the relationships between postpartum pain, depression, parent-infant attachment, and parenting self-efficacy.

Hypothesis: New mothers who have lower pain intensity and unpleasantness during the labor and delivery period will have a reduced risk for postpartum depression, defined by Edinburgh Postnatal Depression Score (EPDS) score, and will have improved maternal-infant attachment, higher parenting self-efficacy, lower perceived stress, and improved child development.

Methods: This was a prospective longitudinal observational study of healthy, adult, nulliparous women at term gestation presenting for labor and delivery at ≥38 weeks gestational age. Baseline self-reported outcome assessments included validated inventories of depression (Edinburgh postnatal depression screen, EPDS), anxiety (state trait inventory, STAI), pain (brief pain inventory short, BPI). Demographic and labor variables were recorded. At 6 weeks and 3 months postpartum, self-reported assessments included EPDS, STAI, BPI, maternal infant attachment (MPAS), and parenting self-efficacy (PMPSE). Pain severity scores were calculated as the average of items 2-5 on the BPI and pain interference scores were averaged on items 8-14 from the BPI. Linear regression was used to estimate the effects of 6-week pain scores on 3-month pain scores. A *P*-value less than 0.05 was considered statistically significant.

Results: 187 subjects participated; 87 subjects had complete data on parent-infant attachment and 85 had complete parenting self-efficacy data. Worse postpartum anxiety scores were associated with lower parenting self-efficacy scores. Higher pain severity at 3 months was associated with lower parent-infant attachment and parenting self-efficacy scores Pain severity scores at 6 weeks postpartum were significantly associated with pain severity at 3 months (Parameter Estimate 0.25, 95% CI 0.07 to 0.43, *P*=0.01). The potential strength and dose-responsiveness of these relationships will be assessed and reported.

Conclusions: We observe a pattern of association between worse postpartum anxiety and pain with worse parenting outcomes. The potential relationships between postpartum anxiety, pain, and parenting self-efficacy deserve further investigation because reducing both postpartum pain and improving mood can potentially improve long-term postpartum parenting outcomes.

Significance: The deleterious consequences of postpartum depression, the intriguing data suggestive of a beneficial influence of labor epidural analgesia on postpartum depression, as well as the potential adverse consequences of this clinical intervention. Acute pain management may have potential repercussions on child rearing, maternal mood, infant neurocognitive development, and the parent-infant relationship. The paucity and weaknesses of existing data on the relationship between acute labor pain management and these outcomes make this an important topic to study.

Research/Grant Support: T32GM075770 (PI: Xan Yu, PhD)

Abstract 17

MOUSE STRAIN AND INJURY SEVERITY DETERMINES LATENT PAIN SENSITIZATION AFTER SURGERY, INFLAMMATION, OR NERVE INJURY

Martel-Matos A, Taylor B

Department of Anesthesiology and Perioperative Medicine, Pittsburgh, Center for Pain Research, and Pittsburgh Project to end Opioid Misuse, University of Pittsburgh, Pittsburgh, PA, USA

Introduction: *LS.* Latent pain sensitization (LS) and its opposing inhibition is an important mechanism for the transition from acute to chronic pain. LS has a characteristic temporal profile: injury → sensitization of dorsal horn neurons → initial hyperalgesia → development of mu-opioid receptor constitutive activity (MOR_{CA}) → masking of sensitization within a latent state → resolution of hyperalgesia (Taylor & Corder, 2014). When administered during the remission phase, pharmacological administration of a MOR inverse agonist disrupts the balance between LS and MOR_{CA} as manifested by a reinstatement of hyperalgesia (Corder et al., 2013). Despite the emerging recognition of LS as a robust and long-lasting model of chronic pain, little is known of key parametric determinants, including strain and injury severity. *Strain*. Genetic variability of acute and chronic pain is well recognized in both human and animal models. Nociception varies widely among inbred mouse strains (Mogil et al., 1999), but whether LS varies with strain has not been tested. We began with two commonly used mouse strains in pain research, C57Bl/6J (B6) and ICR (CD1) (Smith, 2019). *Injury severity*. The degree of tissue or nerve injury is an important determinant in the transition from acute to chronic clinical pain (Holmes et al., 2013), but has not been considered in LS; therefore, we asked to what degree of injury is required to trigger LS?

Hypothesis: LS will vary with mouse strain or injury severity after plantar incision, inflammation, or nerve injury.

Methods: Male B6 or ICR mice 6-8 weeks old were anesthetized with isoflurane and subjected to unilateral incision of the plantar hindpaw (1-5 mm in length plus the underlying plantaris muscle), intraplantar injection of the inflammogen complete Freund's adjuvant (CFA), or transection of the common peroneal and sural branches (CPxSx) of the sciatic nerve, leaving the tibial branch undisturbed. Each of these models is associated with mechanical hypersensitivity (evaluated with von Frey filaments ranging in gram force from 0.008g to 6g) and/or cold hypersensitivity (evaluated by application of 10-12 ul of acetone to the hind paw). After a waiting period of multiple weeks for hypersensitivity to subside, we administered the inverse agonists NTX by the subcutaneous (3mg/kg) route. We then repeated measurements of mechanical and cold sensitivity.

Results: *Plantar incision*. NTX dose-dependently reinstated hyperalgesia more potently in B6 than in ICR mice. Incision length was positively correlated with the degree of NTX induced reinstatement $P<0.05$. *Peripheral nerve injury*. CPxSx resulted in NTX-induced mechanical hypersensitivity and cold hypersensitivity that was greater in B6 as compared to ICR mice $P<0.05$. *Inflammation*. The CFA model of LS showed widespread hyperalgesia in both mouse strains.

Conclusions: First, we conclude that B6 mice exhibit greater latent postsurgical and neuropathic sensitization as compared to ICR mice. Second, we conclude that even small incisions produce some level of LS, and this gradually increases with increasing incision severity.

Significance: LS is a powerful animal model to study the mechanisms of genetic variability in the transition from acute to chronic pain.

Research/Grants support: The authors report no conflict of interest. Financial support for this project was provided by DA37621, NS45954, and NS62306 to BKT.

References:

Corder G, et al. Constitutive μ-opioid receptor activity leads to long-term endogenous analgesia and dependence. Science 341:1394, 2013.
Holmes A, et al. Determinants of chronic pain 3 years after moderate or serious injury. Pain Med 14(3), 336–344, 2013.
Marvizon JC, et al. Latent sensitization: A model for stress-sensitive chronic pain. Curr Protoc Neurosci 71:9.50.1-14, 2015.
Mogil JS, et al. Heritability of nociception I: Responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80:67-82, 1999.
Smith JC. A review of strain and sex differences in response to pain and analgesia in mice. Comp Med 69:490-500, 2019.
Taylor BK, et al. Endogenous analgesia, dependence, and latent pain sensitization. Curr Top Behav Neurosci 20:283-325, 2014.

Abstract 18

CORTICAL ACTIVITY DURING PAIN RELIEF: A FUNCTIONAL NEAR-INFRARED SPECTROSCOPY STUDY OF OFFSET ANALGESIA

Alter BJ,[1] Santosa H,[2] Maurer M,[1] Huppert TJ,[3] Wasan AD[1,4]

[1] Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh
[2] Department of Radiology, University of Pittsburgh
[3] Department of Electrical and Computer Engineering, University of Pittsburgh
[4] Department of Psychiatry, University of Pittsburgh

Introduction: Offset analgesia is defined by a dramatic drop in perceived pain intensity with a relatively small decrease in noxious input. Although functional magnetic resonance imaging studies implicate subcortical descending inhibitory circuits during offset analgesia, the role of cortical areas remains unclear. The current study performed task-based and connectivity analyses to elucidate the cortical correlates of offset analgesia using functional near infrared spectroscopy (fNIRS).

Hypothesis: Offset analgesia is associated with changes in cortical brain activity.

Methods: This study was approved by the University of Pittsburgh IRB. After providing informed consent, twenty-four healthy volunteers (18-50 years old) underwent fNIRS scanning at rest and then during offset (OS) and control (Con) heat stimuli applied to the forearm. NIRS data were collected with a head probe targeting bilateral frontal and somatomotor cortices (NIRx NIRSport2). Pain intensity was recorded continuously on a computerized visual analogue scale (COVAS, Medoc). NIRS data were preprocessed and analyzed in NIRS Toolbox.

Results: After controlling for physiological noise, widespread increases in cortical oxygenated hemoglobin concentration were observed, reflecting cortical activation during heat pain. OS – Con contrasts revealed deactivations in bilateral medial prefrontal cortex (mPFC) and bilateral somatosensory cortex (SSC) associated with offset analgesia. Right dorsolateral prefrontal cortex (dlPFC) showed activation only during OS. Functional connectivity analyses are ongoing.

Conclusions and Significance: These data demonstrate opposing cortical activation patterns during offset analgesia and support a model in which right dlPFC underlies ongoing evaluation of pain intensity change. With predictions of decreasing pain intensity, right dlPFC activation likely inhibits ascending noxious input via subcortical pathways resulting in SSC and mPFC deactivation. This study identifies cortical circuitry underlying offset analgesia and introduces the use of fNIRS to study pain modulation in an outpatient clinical environment.

Research/Grant Support: This study was supported by the University of Pittsburgh School of Medicine funds (ADW, BJA) and the International Anesthesia Research Society Mentored Research Award

Abstract 19

PATIENT SUBGROUPS IDENTIFIED SOLELY BY HIERARCHICAL CLUSTERING OF BODY MAP DATA PREDICT CHRONIC PAIN DIAGNOSES, INCLUDING FIBROMYALGIA WHICH MAY BE UNDERDIAGNOSED

Moses MA,[1] DeSensi R,[1] Anderson NP,[1] Jeong J-H,[2] Wasan AD,[1,3] Alter BJ,[1]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh
[2]Department of Biostatistics, University of Pittsburgh
[3]Department of Psychiatry, University of Pittsburgh

Introduction: Pain location and radiation are commonly used in pain diagnosis. The bodily distribution of pain can be measured in the clinic with pain drawings prompting the patient to mark areas of their pain on a "body map." We previously found that hierarchical clustering of patients by body map data alone leads to nine distinct subgroups that differ significantly from each other in pain intensity, quality, impact, and treatment outcomes. However, the relationship of body map cluster to pain diagnosis remains unknown.

Hypothesis: Body map cluster membership is associated with different patterns of pain diagnosis made by clinical providers.

Methods: In this study, chronic pain diagnosis data of 21,423 patients was collected. Data were extracted from a research registry which links patient-reported outcome data with electronic medical record data related to appointments at the University of Pittsburgh Pain Medicine Clinics from 3/17/2016–6/25/2019. ICD10 codes were grouped into diagnosis bins, nineteen in total, by pathophysiology and anatomic location. The frequency of diagnosis bins across the nine previously-identified body map clusters was examined with descriptive statistics. Based on this analysis and to facilitate hypothesis testing, the top nine diagnosis bins were included with the remaining bins grouped as "Other."

Results: Cluster assignment was associated with different diagnoses. We saw significant variation in the frequency of diagnosis bins by body-map cluster ($X2 = 2.2x10^3$, $df = 8$, $p < 0.0001$). Focusing on the diagnosis of fibromyalgia, multivariate regression analysis revealed that body map cluster independently predicted the likelihood of fibromyalgia diagnosis. Specifically, the "Widespread-Heavy" cluster was more likely to receive a diagnosis of fibromyalgia than other clusters after controlling for patient age, sex, anxiety, and depression. Forty-seven percent of fibromyalgia diagnoses belonged to this cluster. However, only 27% of all patients in the Widespread-Heavy cluster received a diagnosis of fibromyalgia.

Conclusions: The body map is able to identify diagnoses that are more prevalent amongst patients with certain cluster assignments. Fibromyalgia is significantly more prevalent in "Widespread-Heavy", a body map cluster previously shown to have worse outcomes and increased pain intensity. The seemingly low prevalence of fibromyalgia within this cluster, taken together with associated poor outcomes and increased pain intensity, suggest that fibromyalgia is potentially underdiagnosed.

Significance: This study highlights the utility of the pain body map in combination with algorithmic techniques, displaying potential utility for improved chronic pain diagnosis.

Financial Support: University of Pittsburgh School of Medicine funds (ADW, BJA) and the International Anesthesia Research Society Mentored Research Award (BJA).

Abstract 20

SPINAL NEUROPEPTIDE Y1 RECEPTOR-EXPRESSING INTERNEURONS REPRESENT A THERAPEUTIC TARGET FOR THE TREATMENT OF NEUROPATHIC PAIN

Nelson T[1] and Taylor B[1]

[1]Department of Anesthesiology and Perioperative Medicine
University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Neuropeptide Y (NPY) in the spinal cord dorsal horn exhibits long-lasting inhibitory control of nociceptive transmission after injury. The specific receptor targets of NPY in the spinal cord have not been well characterized, particularly in the setting of neuropathic pain. Previously we demonstrated that intrathecal administration of a selective NPY Y1 receptor agonist dose-dependently reduced both behavioral symptoms of pain and immunohistochemical expression of markers of central sensitization following peripheral nerve injury. Therefore, dorsal horn neurons that express the neuropeptide Y1 receptor (Y1-INs), an inhibitory G protein-coupled receptor, likely mediate the anti-nociceptive actions of NPY. The aims of this study were to test if Y1-INs are both necessary and sufficient for the manifestation of peripheral nerve injury-induced mechanical and cold hypersensitivities via *in vivo* chemogenetics.

Hypothesis: We hypothesize that Y1-INs are both necessary and sufficient for the behavioral expression of neuropathic pain.

Methods: AAV injections: Adult male and female $Npy1r^{Cre}$ mice received intraparenchymal injections of AAV8-hSyn-$hM3D_{Gq}$, AAV8-hSyn-$hM4D_{Gi}$, or AAV8-hSyn-mCherry (control) into the lumbar enlargement (L3-L4) of the left dorsal horn. Behavioral Testing: Mice were tested for hindpaw withdrawal responses to mechanical (von Frey up-down method), cool (acetone drop), and heat (52.5°C Hotplate) stimuli applied to the plantar skin by an experimenter blinded to drug or virus treatment. Conditioned Place Preference and Aversion testing included three days of counterbalanced chamber conditioning (morning vehicle pairing, afternoon CNO pairing) followed by the final testing day. The duration of time spent in CNO and vehicle paired chambers was compared before and after conditioning for each animal. Peripheral Nerve Injury: Mice underwent spared nerve injury (SNI), a neuropathic pain model in which the common peroneal and tibial branches of the sciatic nerve are ligated and cut, while leaving the sural nerve intact. Mice develop both mechanical and cold hypersensitivity. Drug Administration: clozapine N-oxide (CNO, 3mg/kg, i.p.)

Results: Chemogenetic inhibition of Y1-INs following SNI completely abolished mechanical and cold hypersensitivities ($p < 0.05$, n=7 mice/group). In uninjured mice, chemogenetic activation of Y1-INs induced multiple signs of pain including spontaneous nocifensive behaviors (lifting of hindpaw, licking, guarding, biting) ($p < 0.05$, n=3-5 mice/group) as well as heat, cold, and mechanical hypersensitivities ($p < 0.05$, n=7-8 mice/group). Additionally, chemogenetic activation of Y1-INs induced conditioned place aversion ($p < 0.05$, n=11 mice/group).

Conclusions: Our studies reveal the critical importance of DH Y1-INs to the manifestation of neuropathic pain-like behaviors and promote Y1-INs as a viable target for future pharmacotherapeutic interventions to treat neuropathic pain.

Significance: This study supports spinally directed NPY Y1 receptor selective agonists as a viable future pharmacotherapeutic target to treat neuropathic pain.

Grant Support: NINDS, NS45954 (PI: Bradley K. Taylor, PhD) & NINDS NS073548, NS117054, NS124190 (PI: Tyler S. Nelson)

Abstract 21

REPEATED OPIOID ADMINISTRATION CAUSES A LONG-LASTING LATENT PAIN SENSITIZATION THAT IS SUPPRESSED BY CONSTITUTIVE μ-OPIOID RECEPTOR ACTIVITY

Prasoon P, Basu P, Roman K, Taylor BK

Department of Anesthesiology and Perioperative Medicine
Center for Neuroscience, Pittsburgh Center for Pain Research
University of Pittsburgh School of Medicine

Introduction: Although morphine is commonly used for acute postsurgical pain, its repeated administration can sensitize nociceptive neurons in the dorsal horn of the spinal cord, leading to the phenomenon of opioid-induced hyperalgesia (OIH). It is unknown whether this sensitization persists after OIH resolves. To address this question, we applied the dual concept of latent pain sensitization (LS) and endogenous analgesia, as described in multiple models of inflammatory and neuropathic pain. First, inflammation or injury induces an initial hyperalgesia that resolves over time. Second, administration of a μ-opioid receptor (MOR) inverse agonist "rekindles," or reinstates hyperalgesia, even when delivered months after resolution. Here, we asked whether 1) repeated opioid administration causes a similar long-lasting phenomena of LS and endogenous opioid receptor analgesia; 2) whether this occurs in both males and females; 3) whether endogenous analgesia is mediated by MOR constitutive activity (MORCA); and 4) whether other G-protein coupled receptors (GPCRs), such as the neuropeptide Y Y1 receptor, maintains this "OIH-LS" in a state of remission.

Hypothesis: We hypothesize that MORCA maintains OIH-LS within remission in both sexes.

Methods: To induce OIH, male and female C56BL/6 mice received a daily single injection of morphine (20 mg/kg, S.C) for 7 days. On day 8, we confirmed the development of mechanical(vF) and heat (hotplate) hypersensitivity. After resolution of pain hypersensitivity on day 34, we intrathecally (i.t.) administered either a selective μ-opioid receptor inverse agonists (CTAP,100ng/5μl), a MOR neutral antagonist (6-β-Naltrexol, 10 μg/5μl)), or both, and them measured mechanical thresholds. Next, after an additional 7 days, we used an advanced hot plate assay to distinguish the sensory-discriminative and affective-motivational components of pain-like behavior. Then we waited an additional 7 days to quantify non-noxious mechanical stimulus-evoked Fos expression in the dorsal horn. In a separate study, to assess the contribution of the Y1-receptor to the suppression of OIH-LS at day 34, we administered a Y1 receptor antagonist (BIBO3304 5 μg/5μl, i.t) and then tested for reinstatement of mechanical hypersensitivity.

Results: CTAP reinstated mechanical hypersensitivity in both sexes, but to a greater degree in male mice. 6-β-naltrexone prevented CTAP reinstatement, indicating that ligand-independent MORCA, rather than opioid release, maintains OIH-LS in remission. CTAP did not change reflex withdrawals in the hotplate assay, but was associated with an increased duration of behavioral signs of affective pain, including paw attending, paw guarding, and escape jumping) in mice exposes to repeated morphine (but not saline control). CTAP increased non-noxious stimulus-evoked Fos expression in the dorsal horn. The Y1R antagonist BIBO reinstated hyperalgesia in our OIH-LS model.

Conclusions: We were able to establish a murine model of OIH-LS, indicating that repeated exposure to opioid leads to a long-lasting sensitization of nociceptive neurons in the dorsal horn. MORCA, rather than tonic ligand-dependent receptor activation, keeps OIH-LS within a state of remission in both male and female mice.

Significance: Our results indicate that repeated opioid exposure increases vulnerability to develop chronic pain. Our future studies aim to decipher the underlying molecular mechanisms that contribute to the development and maintenance of OIH-LS.

Research/Grant Support: NIH grants R01DA037621, R01NS45954, R01NS62306 and R01NS112321 (PI: Bradley K Taylor, PhD)

Abstract 22

SPINAL MU-OPIOID RECEPTOR EXPRESSING NEURONS CONTRIBUTE TO NEUROPATHIC PAIN

Qi Y, Nelson T, Prasoon P, Norris C, Taylor BK

Department of Anesthesiology and Perioperative Medicine
Center for Neuroscience, Pittsburgh Center for Pain Research
Pittsburgh Project to end Opioid Misuse, University of Pittsburgh School of Medicine

Introduction: Pharmacological administration of mu-opioid receptor (MOR) agonists by the intrathecal or epidural route produce profound inhibition of acute pain. These are mediated by inhibitory G-protein coupled MORs located on the central terminals of primary afferent nociceptive neurons as well as on MOR-expressing interneurons (MOR-INs) within the dorsal horn of the spinal cord. However, the contribution of MOR-INs to chronic pain is poorly understood. To fill this gap, we coupled the use of *cre* transgenic reporter mice with whole cell patch clamp electrophysiology to evaluate the neuronal activity of MOR-INs in a mouse model of traumatic nerve injury. We also used a chemogenetic approach to activate or inhibit MOR-INs, followed by the assessment of behavioral signs of neuropathic pain.

Hypothesis: Peripheral nerve injury sensitizes MOR-INs that then cause neuropathic pain.

Methods: MOR^{Cre} mice were bred with floxed tdTomato reporter mice (Ai14) to label MOR-INs for *ex vivo* whole cell patch clamp recording on spinal cord slices. The Spared Nerve Injury (SNI) model of neuropathic pain was induced by lesioning the tibial and common peroneal nerve branches of the left sciatic nerve, leaving the sural nerve intact. Intraspinal injection of AAV8-hSyn-DIO-hM3D(G_q)-mCherry or AAV8-hSyn-DIO-hM4D(G_i)-mCherry virus were conducted on MOR^{Cre} mice to excite or inhibit spinal MOR-INs, respectively. Three weeks after virus injection, behavioral testing included von Frey, acetone cold, and hotplate tests. Brush of the lateral surface of the left hindpaw by cotton swab was applied to assess Fos expression in MOR-INs after SNI.

Results: Immunochemistry revealed MOR-IN expression in both excitatory interneurons (TLX3+) and inhibitory interneurons (PAX2+). Electrophysiological studies of MOR-INs revealed four firing patterns in response to steady state depolarizing current injection: delayed, transient, phasic and tonic. These results indicate the heterogeneity of MOR-INs in the spinal dorsal horn. SNI reduced *oprm1* mRNA expression and DAMGO-induced outward currents in MOR-INs. SNI increased the intrinsic excitability and spontaneous synaptic activity of MORtdT neurons. SNI increased light brush-induced Fos expression in MOR-INs. These studies indicate that SNI increases the excitability of MOR-INs. Chemogenetic activation of MOR neurons in uninjured mice decreased withdrawal threshold to mechanical stimulation, while chemogenetic inhibition reduced SNI-associated mechanical and cold allodynia.

Conclusion: Our results using electrophysiology and Fos immunohistochemistry indicate that nerve injury sensitizes an important pro-nociceptive population of MOR-INs. Chemogenetics studies indicate that MOR-INs contribute to behavioral signs of neuropathic pain.

Significance: We speculate that MOR agonists inhibit acute pain by silencing these neurons; on the other hand, nerve injury causes neuroplasticity in these neurons, rendering them less responsive to MOR agonists. Our future studies aim to dissect spinal MOR interneurons populations (excitatory vs inhibitory) to better understand their contribution to dorsal horn pain circuitry and opioid analgesia.

Research/Grant Support: NIH grants R01DA037621, R01NS45954, R01NS62306 and R01NS112321 (PI: Bradley K Taylor, PhD)

Abstract 23

INTRAOPERATIVE TRIPLE LOW STATES, BUT NOT DOUBLE LOW STATES ARE ASSOCIATED WITH POST-OPERATIVE DELIRIUM

Reon B,[1] Schnetz MP,[1] Vogt KM[1,2,3,4]*

[1]Anesthesiology and Perioperative Medicine, School of Medicine, UPMC
[2]Clinical and Translational Science Institute UPMC
[3]Bioengineering, Swanson School of Engineering
[4]Center for the Neural Basis of Cognition UPMC

Introduction: Post-operative delirium (POD) is a common postoperative complication, occurring in up to 52% of noncardiac surgical patients, and is a significant cause of increased mortality and morbidity. The triple low state (TLS) is a composite metric of concurrent low bispectral index (BIS), MAC and MAP values, which has been associated with increased morbidity in postoperative patients and could represent a state of poor physiologic and neurologic reserve. Double low states (DLS) are simultaneously low values of two in these variables. This preliminary, retrospective study aimed to determine whether intraoperative TLS or DLS are associated with POD.

Methods: This retrospective study included non-cardiac and non-liver transplant surgical patients within a multi-hospital health system from 2016 – 2018 who had ICDS delirium scores measured during their hospitalization. Patients were included in our analysis if there was no evidence of pre-op delirium. The presence of a TLS was determined by patients simultaneously having a BIS value < 45, MAP < 75 and MAC < 0.7. Logistic regression was then performed to determine the effect of TLS events on the development of POD. Covariates included in our logistic regression model included age, gender, ASA physical status, whether the surgical case was an emergency, operative team surgical specialty, hospital site and the presence of a TLS/DLS during the procedure.

Results: Of the 259 patients that met our inclusion criteria, 95 patients had TLS events and 164 did not have a TLS event. Average TLS duration within the TLS cohort was 30.7 minutes. Interestingly, there was a higher incidence of POD (28.4%) in the TLS subgroup, compared to patients without TLS events (11.6%). These two patient cohorts had significant differences in demographic and clinical variables: including age, gender, and ASA physical status. Logistic regression analysis demonstrated that TLS events are associated with POD, OR 2.74 (1.26 – 5.69). Cox regression was performed using each combination of DLS events and TLS, which showed that only TLS events were associated with POD, HR 0.77 (0.01 – 1.52) p= 0.05.

Conclusions: In a retrospective, multihospital surgical population, we identified a population of patients who had TLS events intraoperatively and these patients were more likely to have post-operative delirium. This association was exclusive to TLS events, as each combination of DLS events were not associated with POD. This study lays the framework for larger studies to evaluate the potential role of TLS and other modifiable and nonmodifiable perioperative factors with the development of POD.

Significance: Post-operative delirium (POD) is a common post-operative complication, associated with increased healthcare cost, morbidity, and mortality. This preliminary work provides a potential framework for better risk stratifying patients at risk for POD, which could facilitate the implementation of more targeted clinical resources to combat POD, based on real-time intraoperative parameters.

Grant Support: MPS T32GM075770, KMV K23GM132755 and L30GM120759

Abstract 24

EFFECTS OF COMMONLY-USED INTRAVENOUS MEDICATIONS ON THE BISPECTRAL INDEX™: A RETROSPECTIVE QUANTITATIVE ANALYSIS OF ANESTHETIC RECORDS FROM TWO TERTIARY CARE HOSPITALS

Reon B,[1] Schnetz MP,[1] Vogt KM[1,2,3,4*]

[1]Anesthesiology and Perioperative Medicine, School of Medicine, UPMC
[2]Clinical and Translational Science Institute UPMC
[3]Bioengineering, Swanson School of Engineering
[4]Center for the Neural Basis of Cognition UPMC

Introduction: Patients undergoing anesthesia are increasingly being monitored for depth of consciousness using EEG based monitors such as Bispectral Index (BIS). Although some studies have demonstrated the effects of commonly used sedatives on BIS in very controlled settings, the effect of these medications in actual clinical practice is not as well characterized.

Hypothesis: Administration of amnestic or hypnotic medications will cause significant changes to intraoperative BIS measurements.

Methods: In a retrospective analysis of electronic anesthesia records from two hospitals within the UPMC system, we examined the effects of intravenous medications as administered in routine practice during primarily volatile agent-based anesthetics. To reduce bias from multiple medication administrations, discrete time windows were identified in which only a single drug bolus was administered, and subsequent changes in the BIS and concentration of volatile anesthetic (MAC) were analyzed for significant changes. Our primary outcome of interest was change in BIS, in response to intravenous anesthetics and analgesics. 95% confidence intervals were created for the resulting monitor values and compared to pre-determined thresholds for clinical significance. Secondary analyses explored the effects of different baseline volatile anesthetic concentrations and, dose of IV medication, and length of time window, as important parameters in the data and analysis methodology,

Results: To validate our methodology, we incorporated vasoactive agents, such as phenylephrine, ephedrine, and calcium chloride, which demonstrated a significant change in MAP but no change in BIS or MAC values. Surprisingly, administration of propofol at a median dose of 30 mg did not demonstrate a significant change in BIS values. Conversely, administration of midazolam and ketamine resulted in a significant decrease and increase in BIS values, with a net BIS change of 3 and 4 respectively. Interestingly, opioid administration (hydromorphone and fentanyl) was not associated with changes in BIS values. Furthermore, the changes in BIS seen with midazolam and ketamine demonstrated a dose dependent response, with high and low doses, further validating our methodology. The changes in BIS following ketamine and midazolam administration were clinically durable, even persisting at 20 minutes following drug administration.

Conclusions: In a retrospective, multihospital surgical population, we identified the effects of commonly used IV sedative medications on intraoperative awareness measurements. Our findings demonstrated that while there are clinically significant and durable changes in BIS values with the administration of midazolam and ketamine, the overall change is not as large as commonly believed in everyday clinical practice.

Significance: Our study demonstrated that administration of commonly used IV sedatives causes changes in BIS. However, these changes on average are not large and abnormally high or low intraoperative BIS values should not be solely ascribed to administration of IV medication and be viewed as clinically meaningful changes.

Grant Support: MPS T32GM075770, KMV K23GM132755 and L30GM120759

Abstract 25

AUTOMATED HOME-CAGE SIPPER DEVICES REVEAL AGE AND SEX DIFFERENCES IN ETHANOL CONSUMPTION PATTERNS

Rice RC,[1] Baratta AM,[1] Farris SP[1,2]

[1]University of Pittsburgh, Center for Neuroscience, Pittsburgh, PA
[2]University of Pittsburgh, Department of Anesthesiology & Perioperative Medicine, Pittsburgh, PA

Introduction: Free-choice ethanol consumption paradigms such as two-bottle choice (2BC) are commonly used to characterize ethanol consumption and preference of rodent models used to study alcohol use disorder (AUD). However, these assays are limited by low temporal resolution that misses finer ethanol consumption patterns, including circadian drinking patterns that play critical roles in AUD pathogenesis. Circadian rhythms vary among age and sex groups as well as individuals, but details of age differences in ethanol consumption patterns in particular remain poorly characterized. Modern, cost-effective tools are becoming widely available that could elucidate these patterns, including recently described, Arduino-based home cage sipper devices, which we sought to validate in extended ethanol consumption paradigms.

Hypothesis: We hypothesized that adaptation of these devices would uncover distinct age- and sex-related differences in temporal drinking patterns not detectable through experimenter measurements.

Methods: To test this hypothesis, we used these home-cage sipper devices in a 14-day continuous 2BC paradigm where male and female adolescent (3-week), young adult (6-week), and mature adult (18-week) C57BL/6J mice were given access to water and 20% ethanol (vol/vol) in water. Daily grams of fluid consumption were manually recorded just prior to dark cycle onset, while devices continuously recorded sip number and timing.

Results: We were able to validate the use of these devices in this paradigm as well as for the determination of individual ethanol consumption timing; correlation of manually-recorded fluid consumption versus daily total sipper data revealed statistically significant prediction of fluid consumption across all experimental groups, and blood ethanol concentrations at predicted peak from 14-day sipper data were significantly correlated with ethanol sip counts at sampling time. Manual and sipper device data were able to quantify ethanol drinking patterns among age- and sex subgroups as well as variation among individual animals.

Conclusions: Consistent with prior studies, females consumed more ethanol than males, and adolescent mice consumed the most ethanol overall. Sipper data revealed distinct circadian features of drinking patterns between age and sex groups. Phase advancement was observed as age increased for female mice, while phase delay was evident with age in male mice. Additionally, female mice were found to consume more of their daily fluid intake during the light cycle.

Significance: Overall, our findings suggest new implications in the study of age- and sex-related differences in ethanol consumption patterns. Future studies that augment the 2BC drinking paradigm with automated home-cage sipper devices will be useful for testing pharmacotherapies and genetically engineered mice to identify novel avenues of treatment for AUD.

Research/Grant Support: We gratefully acknowledge the support of NIH/NIAAA grants F31AA029942, R00AA024836, and U01AA020889.

Abstract 26

OUTCOMES FOLLOWING SUPRASCAPULAR NERVE BLOCK FOR REVERSE DELTA SHOULDER ARTHROPLASTY

Anyama B, Rosario B, Orebaugh S

Department of Anesthesiology and Perioperative Medicine, UPMC

Introduction: Reverse Delta Shoulder Arthroplasty (RDSA) mandates preservation of muscle tone to reduce the risk of postoperative dislocation. Studies have shown a 4.7% incidence of dislocation with this surgical technique (1). Orthopedists at our institution typically request that no brachial plexus block be performed, to reduce the chance of dislocation during the postoperative phase. We utilize suprascapular nerve block (SSNB) to provide analgesia while attempting to avoid significant muscle relaxation for RDSA (2,3). In this pilot study, we evaluated the surgical and analgesic outcomes with the use of SSNB with RDSA.

Hypothesis: We hypothesized that integration of SSNB for RDSA cases would not result in shoulder dislocations, while also reducing postoperative pain scores and opioid use. Our primary outcome was incidence of dislocation during inpatient hospital stay. Secondary outcomes included NRS pain scores after surgery and intraoperative and postoperative opioid (OME) use.

Methods: Chart review was conducted for this IRB-approved study of patients undergoing RDSA, with or without SSNB, from 2018-2020. We compared frequency of glenohumeral dislocation after surgery, NRS postoperative pain scores, and OME (up to 24 hours). We sought a difference in pain scores of more than 1.7 (out of 10) in the postoperative period up. Additionally, we compared opioid use in OR and over first 24 hours. Student's t test was used to compare the pain scores and opioid doses between the two groups.

Results: 21 cases of RDSA were evaluated, 13 who received SSNB along with 8 who did not. Patient demographics are presented in Table 1. No shoulder dislocations occurred in either group. Pain scores were not significantly different between the two groups (Table 2). We also found no difference in opioid requirements: Intraoperative OME 44.6 (20.6) for SSNB group versus 46 (17.3) for non-block group ($p = 0.87$), and Postoperative OME 95 (62.5) for SSNB group versus 128 (155.8) for non-block group ($p = 0.50$). No surgical complications were reported.

Conclusion: The use of SSNB in this pilot study did not lead to shoulder dislocation. However, pain scores and opioid requirements were not different between the two groups, suggesting that, while the relative preservation of muscle tone around the glenohumoral joint with SSNB is beneficial, this nerve block was insufficient to provide significant postoperative analgesia in RDSA.

References:

1. Kohan EM, Chalmers PN, Salazar D, et al. Dislocation following reverse total shoulder arthroplasty. J Shoulder Elbow Surg 2017 Jul;26(7):1238-1245.
2. Auyong DB, Hanson NA, Joseph RS, et al. Comparison of Anterior suprascapular, supraclavicular, and interscalene nerve block approaches for major outpatient arthroscopic shoulder surgery: A randomized, double-blind, noninferiority trial. Anesthesiology 2018 Jul;129(1):47-57.
3. Cho N, Kang RS, McCartney CJL, et al. Analgesic benefits and clinical role of the posterior suprascapular nerve block in shoulder surgery: a systematic review, meta-analysis and trial sequential analysis. Anaesthesia 2020 Mar;75(3):386-394.

Abstract 27

IDENTIFICATION OF SPINAL ITCH NETWORKS AND THEIR INHIBITION BY KAPPA OPIOID SIGNALING

Sheahan T,[1,2] Manalo A,[1,2] Perry V,[1.2] Fanien L,[1,2] Ross S[1,2]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine
[2]Pittsburgh Center for Pain Research and Department of Neurobiology, University of Pittsburgh School of Medicine

Introduction: Itch is a prevalent health problem in need of safe, effective treatments, yet our limited understanding of how itch stimuli are transmitted within the nervous system has left it poorly managed. In particular, little is known about how itch stimuli are integrated in the spinal cord.

Hypothesis: Thus, we sought to identify which neurons in the spinal cord convey itch for the first time, and to test the hypothesis that compounds that cause itch behavior in mice activate a common spinal neuron network.

Methods: We are addressing this fundamental question using a combination of multiphoton imaging, behavioral pharmacology, genetics, and molecular approaches.

Results: First, we leveraged an *ex vivo* spinal cord preparation in combination with two-photon (2P) Ca^{2+} imaging to visualize activity of excitatory spinal cord dorsal horn neurons in response to centrally acting pruritogens. We show that while pruritogens (e.g. gastrin-releasing peptide, somatostatin, substance P) act directly on different spinal neurons, they engage a common downstream spinal neuron network. Next, we investigated whether kappa opioid receptor (KOR) agonists, which are currently in the development for the treatment of chronic itch, act on this itch spinal neuron network to inhibit itch. We demonstrate that spinal delivery of KOR agonists blocks scratching evoked by the same centrally acting pruritogens. Moreover, using 2P Ca^{2+} imaging of the spinal cord, we visualize where KOR agonists act within spinal itch networks to inhibit the spinal transmission of itch. Finally, we present a molecular characterization of KOR-expressing spinal neurons using a combination of viral tracing and fluorescent in situ hybridization.

Conclusions: In sum, these data provide both novel basic insights into the spinal coding of itch, as well as mechanistic understanding of KOR agonists that are in clinical development for the treatment of chronic itch.

Significance: This study sheds lights on how itch is transmitted within the nervous system under normal conditions, which will allow us to study how this processing is changed in chronic itch conditions. Moreover, this work provides a potential mechanism of action for the kappa opioid receptor agonists that are currently in therapeutic development for chronic itch.

Grant Support: Supported by NIH grants F32NS110155 (TDS), R01NS0967905 (SER).

Abstract 28

GENICULAR NERVE RADIOFREQUENCY ABLATION REDUCES CHRONIC KNEE PAIN IN A REAL-WORLD PATIENT POPULATION

Tieu R, Nguyen Q, DeSensi R, Wasan A, Alter B

Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

Introduction: Radiofrequency ablation (RFA) of the genicular nerves has been shown to reduce osteoarthritis knee pain in randomized-controlled trials with uniformly selected patients. However, there is limited knowledge of RFA outcomes in heterogeneous populations. Real-world data is needed to fill this gap.

Hypothesis: Genicular RFA decreases pain intensity for patients experiencing chronic knee pain.

Methods: The University of Pittsburgh Division of Pain Medicine Patient Outcomes Repository for Treatment (PORT), which incorporates patient-reported outcomes collected with the Collaborative Health Outcomes Information Registry software (CHOIR), was queried to identify patients who underwent genicular RFA and had completed PROMIS surveys capturing pain, function, mood, and other relevant domains prior to the procedure and at follow up visits. Putative cases were identified by CPT code and confirmed with chart review. A comparison group was identified in PORT as having knee osteoarthritis but lacking the RFA CPT code. Baseline and follow up data were extracted for 149 patients treated with RFA and 232 OA patients who did not receive RFA. Pain intensity and impression of change were the primary outcomes.

Results: At baseline, there was no significant difference in pain intensity between RFA-treated and OA-comparator patients (RFA: 6.5 ± 1.8; OA: 6.7 ± 1.9; $p = 0.213$). However, RFA-treated patients had lower symptomatic scores and higher functionality scores as assessed by PROMIS measures. At 3 months, RFA-treated patients reported decreased pain intensity compared to OA patients (RFA: 5.9 ± 2.3; OA: 6.4 ± 2.1; $p = 0.049$). However, there was no significant change in pain intensity (baseline – follow-up) between groups (RFA delta: -0.5 ± 2.3; OA delta: -0.3 ± 1.0; $p = 0.314$). Among RFA-treated patients, treatment decreased pain intensity as reported at the 3-month follow-up visit. Moreover, over half of RFA-treated patients reported meaningful change in their pain.

Conclusions: RFA benefits patients with chronic knee pain by decreasing their pain. It is of significant interest to see how long treatment effects last; thus, longer follow-up periods are being pursued.

Significance: Chronic knee pain can be debilitating for geriatric patients. RFA represents a minimally invasive therapeutic procedure that can treat chronic knee pain.

Grant/Research Support: This study was supported by the University of Pittsburgh School of Medicine funds (ADW, BJA) and the International Anesthesia Research Society Mentored Research Award (BJA). RT was funded by T32 grant GM008208 from the NIH.

Abstract 29

DORSAL ROOT GANGLION APPLICATION OF RESINIFERATOXIN REDUCES MYOCARDIAL ISCHEMIA INDUCED VENTRICULAR ARRHYTHMIA

Yamaguchi T,[1] Kuwabara Y,[1] Wong B,[1] Salavatian S,[1,2] Howard-Quijano K,[1,3] Mahajan A[1,3]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh
[2]Department of Medicine, Division of Cardiology, University of Pittsburgh Medical Center
[3]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh Medical Center

Introduction: Sympathetic activation after myocardial ischemia causes ventricular arrhythmias. Nociceptive nerves expressing transient receptor potential vanilloid 1 (TRPV1) are involved in cardiac afferent sympathetic hyperexcitation caused by myocardial ischemia.

Hypothesis: We investigated whether the dorsal root ganglion (DRG) application of resiniferatoxin (RTX) would reduce myocardial ischemia-reperfusion (IR) induced ventricular arrhythmia through suppressing spinal afferent sympathetic hyperexcitation in the preclinical model.

Methods: 21 Yorkshire pigs were assigned into the Sham, IR, and IR+RTX groups. Anesthetized pigs underwent laminectomy to expose the left thoracic 2 (T2)-T4 DRGs and a sternotomy to expose the heart for performing an IR intervention. RTX (50ug) was administered into the left T2-T4 DRGs through the epidural space. Cardiac excitability was assessed by cardiac electrophysiology mapping and arrhythmias counting. To elucidate the mechanism, we used immunofluorescence to compare TRPV1 and calcitonin gene-related peptide (CGRP) expression in the DRGs and spinal cord.

Results: Reduction of activation recovery interval (ARI), as a surrogate for action potential duration, during IR intervention was significantly attenuated in the IR+RTX (IR: -110ms ± 18ms vs. IR+RTX: -65ms ± 11ms, $P = 0.031$). The arrhythmia score in ischemia was decreased in the IR+RTX (IR: 6.8 ± 1.4 vs. IR+RTX: 2.4 ± 0.7, $P = 0.009$). The number of VTs during myocardial ischemia was reduced in the IR+RTX (IR: 5 ± 1.9 vs. IR+RTX: 0 ± 0.3, $P = 0.045$). CGRP expression in the DRGs and spinal cord was attenuated in the IR+RTX (DRGs: 2.6% ± 0.8% in IR vs. 0.1% ± 0.1% in IR+RTX, $P = 0.008$. Spinal cord: 12.8% ± 2.3% in IR vs. 4.6% ± 0.6% in IR+RTX, $P = 0.021$).

Conclusions: Ipsilateral thoracic DRG application of RTX reduced myocardial ischemia-induced arrhythmia in a porcine model. Immunofluorescence analysis demonstrated that depleting the spinal afferent sympathetic neuronal pathway could be the underlying mechanism.

Significance: The present study suggests a new neuromodulation approach to the use of RTX to reduce cardiac arrhythmias.

Research/Grant Support: National Heart, Lung, and Blood Institute, National Institutes of Health Research Project Grant (R01) HL136836 (PI: Aman Mahajan, MD, PhD)

Abstract 30

EXPLICIT MEMORY REDUCTION DURING PAIN BY SEDATION WITH DIVERSE ANESTHETICS

Zhang L,[1,2] Vogt K[1]

[1]Department of Anesthesiology and Perioperative Medicine, UPSOM
[2]Department of Psychology, Carnegie Mellon University

Introduction: Aversive experiences while under anesthesia during surgery can result in a learned sympathetic response to pain despite no explicit recollection of the event. This study investigates the conditions necessary for amnesia, analgesia, and inhibition of fear conditioning while under sedation by three intravenous anesthetics.

Hypothesis: Fentanyl, dexmedetomidine, and propofol, would result in decreased explicit memory performance compared to the control, saline.

Methods: Using a randomized within-subject crossover design, 36 adults received both saline and a sedative dose of fentanyl, dexmedetomidine, or propofol during separate visits. They performed an experimental memory paradigm in which they heard a series of words and generated a mental picture or story surrounding the word. Words were played in either a male or female voice, and shocks were associated with only one of these voices (75% reinforcement). Recognition memory performance was quantified with d', the z-scores for accurate recognition minus false positives.

Results: Explicit memory recollection was reduced under propofol (d'=1.84 [1.11-1.58], mean [±SE]) and dexmedetomidine (d'=1.34 [1.65-2.03]) compared to saline (d'=2.04 [1.85-2.23]) but increased under fentanyl (d'=2.37 [1.84-2.91]). Under saline, subjects displayed explicit memory for 17% of words paired with pain and mis-attributed pain-pairing to 8% new words. Subjects showed increased generalization, to 21% of previously-heard words not paired with pain under propofol. Under fentanyl, subjects accurately identified only 5% of previously pain-paired words.

Conclusions: These preliminary results show an increase in recollection of specific words items under fentanyl, while decreasing recollection of the pain-pairing. We speculate that analgesia, could allow a shift of attentional focus away from painful stimuli and thus improve overall memory encoding, while weakening the associative memory of the pain itself. Additionally, receiving propofol may cause the generalization of memory of pain to different stimuli not previously-paired with pain. Both results are preliminary and warrant further verification in the full study cohort.

Significance: Understanding the interactions between anesthetic sedation, pain processing, and the formation of memory and conditioned responses may help prevent memory-related sequelae after otherwise painful procedures.

Support: NIGMS K23GM132755 (PI: Keith Vogt)

DEPARTMENT OF CRITICAL CARE MEDICINE

Abstract 31

EFFECT OF VITAMIN C IN JUVENILE ACUTE RADIATION SYNDROME

Castellano E,[1,2,3] Korkmaz A,[1,2,3] Gao Y,[1,2,3] Johnson C,[1,2,3] Scott J,[1,2,3] Epperly M,[4] Greenberger J,[4] Kagan VE,[1,3] Bayır H[1,2,3]

[1]Children's Neuroscience Institute, University of Pittsburgh
[2]Safar Center for Resuscitation Research, Department of Critical Care Medicine, University of Pittsburgh
[3]Center for Free Radical and Antioxidant Health, Department of Environmental and Occupational Health, University of Pittsburgh
[4]Department of Radiation Oncology, University of Pittsburgh

Introduction: Today's threat of nuclear attack is greater than ever. Exposure to ionizing radiation triggers sterile and non-sterile inflammatory responses particularly in the intestinal system. Vitamin C has emerged as a potential treatment in inflammatory conditions such as sepsis. The exact mechanisms how vitamin C dampens inflammatory response is poorly understood. Vitamin C is a highly effective reductant and essential for enzymes that regulate gene expression. It has been shown to protect the TCA cycle enzyme aconitase from inactivation. Aconitase catalyzes the formation of cis-aconitate, the precursor of itaconate which plays an important role in inflammatory response as it inhibits NLRP3 inflammasome activation via itaconylation. NLRP3 inflammasome activation results in the cleavage of gasdermin D and IL1b fueling systemic inflammation.

Hypothesis: Vitamin C is essential to maintain itaconate levels after whole body irradiation (WBI).

Methods: Effect of WBI was investigated using juvenile wild type Wistar and osteogenic disorder Shionogi (ODS) rats. ODS rats, like humans, lack functional L-gulonolactone oxidase (GULO) and therefore rely on dietary ascorbate. Rats were irradiated at a dose rate of 310cGy/minute and monitored for clinical illness severity using a validated murine sepsis score. Plasma and ileal tissue were collected for quantification of vitamin C by a fluorescence assay and TCA cycle metabolites by LC-MS respectively. HEK293 cells were utilized to investigate the effect of vitamin C supplementation on itaconate levels. Given that itaconate is synthesized from cis-aconitate by IRG1, RNA sequencing was performed to assess possible transcriptional regulators of IRG1.

Results: Male PND28 Wistar rats had a 100%, 70% and 20% survival at 28 days after exposure to 4, 5 and 6Gy TBI, respectively (n=5/grp, $p<0.05$). Survival after 6 Gy TBI was decreased in ODS rats with 100% mortality by day 18. ODS rats randomized to vitamin C supplementation by i.p 75 mg/kg at 1h after 6 Gy WBI and in the drinking water had increased plasma ascorbate levels and improved acute radiation illness severity vs ODS rats randomized to saline injection at 1h after 6 Gy WBI and regular water thereafter (n= 9-11/grp, $p<0.05$). Cis-aconitate and itaconate levels were higher in ileum of vitamin C + naïve and irradiated rats compared to vitamin C - naive and irradiated rats (n=6/group, $p<0.05$). Time course and dose response studies showed that HEK293 cells grown in DMEM media containing 1% penicillin/streptomycin and 10% FBS required 8 hours treatment 15 mM vitamin C to achieve intracellular Vitamin C concentration of ~20 nmol/mg protein. DMEM media had negligibly low Vitamin C levels. Itaconate levels were markedly higher in vitamin C vs media only supplemented cells. RNA sequencing showed upregulation of several transcription factor genes- Irf3, Aco2, Irf 9 and Cebpb- in naïve vitamin C - ODS rats compared to naïve vitamin C + ODS rats.

Conclusions: Vitamin C plays a key role in outcome after radiation and in the synthesis of itaconate which may be in part due to epigenetic regulation. Further studies are required to evaluate whether itaconate depletion plays a key role increased mortality seen in Vitamin C- ODS rats upon WBI.

Significance: Our studies suggest an important role in the synthesis of immunomodulatory molecule itaconate by vitamin C.

Research/Grant Support: T32HD040686, U01AI156923

Abstract 32

RISK FACTORS FOR THE DEVELOPMENT OF PERSISTENT ACUTE KIDNEY INJURY IN CRITICALLY ILL PATIENTS. A RETROSPECTIVE ANALYSIS

Hamahata N, Toro J, Priyanka P, Sakhuja A, Saraiva IE, Kellum JA, Gomez H

Center for Critical Care Nephrology, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) Center
Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Introduction: Acute kidney injury (AKI) is a frequent complication in critically ill patients. Failure to recover beyond 72h (persistent AKI) is associated with worse outcomes.

Hypothesis: This study aimed to describe the occurrence of persistent AKI and to identify its risk factors as well as its association between mortality.

Methods: This is a retrospective study using the High-Density Intensive Care (HiDenIC) database, which includes patients admitted to the ICU from October 2008 to December 2014. We defined severe AKI as KDIGO stage 2 or 3, transient AKI as AKI that recovers within the first 72h, and persistent AKI as persistence at stage 3 for ≥72h. Primary and secondary outcomes were 90-day mortality and major adverse kidney events at 90 days (MAKE90), a composite of death, need for renal replacement therapy or persistent AKI. The occurrence of severe AKI, transient and persistent AKI was estimated using descriptive statistics. The association between persistent AKI and 90-day mortality was reported using multivariate logistic regression (MVL) and the Kaplan-Meier curve.

Results: We identified 119,783 critically ill patients out of a source population of 287,495. Of these, 38,107 (31.8%) developed severe AKI. Of patients with severe AKI, 36,424 (95.5%) had transient AKI, while 1,683 (4.4%) had persistent AKI, which accounts for 1.4% of the total population. Persistent AKI was associated with 90-day mortality and it was only second to a Charlson score ≥6 as the most important risk factor for 90-day mortality (OR 2.65, 95%CI 2.33-3.00), and was the most important risk factor for MAKE90 (OR 19.44, 95%CI 15.44-24.5). Baseline creatinine and Charlson score were the highest risk factors for persistent AKI.

Conclusions: Persistent AKI occurred in 1.4% of all critically ill patients, was associated with a 3-fold increase in 90-day mortality and nearly a 20-fold increase in MAKE90.

Significance: Our results suggest that the first 72h of AKI may be analogous to the 'golden hour', wherein preventing persistent AKI could decrease morbidity and mortality, particularly critically ill patients with worse baseline creatinine and multiple comorbidities.

Abstract 33

MYELOPEROXIDASE DEFICIENCY LIMITS THE GENERATION OF PRO-INFLAMMATORY PUFA-CONTAINING LYSOPHOSPHOLIPIDS AFTER CONTROLLED CORTICAL IMPACT

Korkmaz A,[1,2,3] Samovich S,[4] Tyurina YY,[4] Kagan VE,[3,4] Bayır H[1,3,4]

[1]Department of Critical Care Medicine, University of Pittsburgh
[2]Interdisciplinary Neuroscience PhD Program, Ankara University
[3]Children's Neuroscience Institute, UPMC Children's Hospital of Pittsburgh
[4]Department of Environmental and Occupational Health, University of Pittsburgh

Introduction: Lysophospholipids (LPLs) are known as pivotal signaling lipids acting as regulators of inflammation. They are canonically generated via phospholipase A-driven hydrolysis of membrane phospholipids (PLs) but also via a non-canonical pathway - acting on plasmalogen phospholipids - by myeloperoxidase (MPO).

Hypothesis: Unlike phospholipases A1 and A2 which attack sn-1 and sn-2 positions in PLs, MPO reacts with vinyl-ether bond at the *sn*-1 position of plasmalogens and generates 2-sn-polyunsaturated Lyso-PLs (2-sn-PUFA-LPLs) which may act as a pro-inflammatory mediator via G-coupled-receptors in the brain after TBI.

Methods: To explore the effects of the MPO-driven mechanisms, wild type (wt) and MPO knock-out (KO) mice (8-12 wk) underwent CCI (5 m/sec velocity, 1.2mm depth, n=5/group). Naive mice were used as control. The enzymatic activity was measured in pericontusional area at different time points and liquid chromatography-mass-spectrometry (LC-MS/MS) was performed to characterize the phospholipidome.

Results: MPO activity began to increase at 6 h and peaked at 12h after CCI vs control. We found that CCI caused accumulation of LPLs formed via canonical PLA2-catalyzed reaction(s) in the brain of both wt and MPO-KO mice. These LPL species were identified as 1-sn-16:0-2-sn-0:0-LPE, 1-sn-18:0-2-sn-0:0-LPE, 1-sn-18:1-2-sn-0:0-LPE, 1-sn-16:0-2-sn-0:0-LPC and 1-sn-18:0-2-sn-0:0-LPC. We also discovered the PUFA-LPL produced via a non-canonical MPO driven hydrolysis reactions of phospholipid plasmalogens pathway in the brain of wt mice. They were represented by 1-sn-0:0-2-sn-20:4-LPE, 1-sn-0:0-2-sn-22:4-LPE and 1-sn-0:0-2-sn-20:4-LPC. No increase in the contents of PUFA-LPL was detected in MPO-KO mice.

Conclusions: This study demonstrates for the first-time the presence of non-canonically MPO-generated PUFA-LPLs in the brain of CCI exposed mice.

Significance: Given the high susceptibility of PUFA-LPL to enzymatic and non-enzymatic oxygenation reaction, their pathogenic role and mechanisms of action as regulators of inflammatory responses should be further investigated.

Research/Grant Support: NS117000, NS061817 (PI: Hülya Bayır, MD)

Abstract 34

MULTIORGAN DYSFUNCTION AND NEW IMPAIRMENT IN PEDIATRIC ABUSIVE AND ACCIDENTAL TRAUMATIC BRAIN INJURY: AN INTERIM REPORT

McNamara C,[1] Kalinowski A,[2] Menchaca C,[1] Horvat C,[1] Simon D,[1] Even K,[3] Gaines B,[4] Richardson W,[4] Berger R,[5] Fink E[1]

[1]Department of Pediatric Critical Care Medicine, UPMC Children's Hospital of Pittsburgh
[2]Department of Pediatrics, UPMC Children's Hospital of Pittsburgh
[3]Department of Pediatric Critical Care Medicine, Penn State Health Milton Hershey Medical Center
[4]Department of Pediatric Surgery, UPMC Children's Hospital of Pittsburgh
[5]Department of Child Advocacy, UPMC Children's Hospital of Pittsburgh

Introduction: Abusive head trauma (AHT) is a leading cause of death in children with traumatic brain injury (TBI). Survivors acquire significant morbidities such as seizures, developmental delay, and blindness at higher rates than accidental TBI (aTBI). Multi-organ dysfunction syndrome (MODS), defined as organ dysfunction in two or more organ systems, is also associated with morbidity and mortality in pediatric critically ill children, including TBI. Our objective was to analyze the occurrence of early MODS and its association with new impairment at hospital discharge by AHT or aTBI etiology.

Hypothesis: We hypothesize that MODS occurs at higher rates in AHT and is associated with worse outcomes at hospital discharge.

Methods: IRB approval was obtained. Children <3 y old admitted to the PICU with TBI between 2014-2021 were eligible for inclusion. Patient and injury characteristics, MODS status on PICU day 1, and new impairment status (Functional Status Scale score change from pre-ICU to hospital discharge > 1) were abstracted from the Benedum Trauma Center database and electronic medical record. Multivariable logistic regression was performed to examine the association between MODS on d1 and TBI etiology with new impairment status.

Results: In an interim analysis (n=147/597), 68 children had AHT and 79 children had aTBI. The AHT group was younger (median 4 [2, 10] vs. 10 [2, 22] mos), had worse Injury Severity Scores (median 18 [11, 27] vs 10 [10, 17]), longer hospital length of stay (median 6 [4, 15] vs 2 [2, 3] days), and more frequently had new impairment at hospital discharge (35% vs 14%), $p<0.05$. More children with AHT had MODS on d1 compared to those with aTBI (37% vs. 24%), $p<0.05$. Among children with MODS, the most frequent organ dysfunctions for both AHT and aTBI patients were neurologic (37% vs. 19%), cardiovascular (49% vs. 56%), and pulmonary (35% vs. 19%), respectively. In multiple logistic regression analysis, MODS on day 1 (odds ratio 48.90, 95% confidence interval [13.89, 172.07]) and AHT mechanism (5.69, [1.62, 19.99]) were associated with new impairment at hospital discharge, $p<0.05$.

Conclusions: Interim data suggest a higher frequency of MODS on day 1 in a cohort of critically ill children with AHT compared to those with aTBI, which was associated with new impairment at hospital discharge.

Significance: Children with TBI, especially due to AHT, may benefit from innovative strategies to prevent and treat MODS and longitudinal care for functional impairment.

Abstract 35

ASSOCIATION BETWEEN MASK RECOMMENDATIONS AND BRONCHIOLITIS HOSPITAL ADMISSIONS IN THE UNITED STATES

Pelletier JH, Rakkar J, Au AK, Fuhrman DY, Aldewereld Z, Clark RSB, Kochanek PM, Horvat CM

Department Critical Care Medicine
University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Introduction: Bronchiolitis represents 18% of hospital admissions in children under two years old. The US CDC issued two recommendations for masking in public during 2020-2022. The effects of community masking on bronchiolitis hospitalizations are unknown.

Hypothesis: We hypothesized there would be a temporal association between mask recommendations and decreased bronchiolitis admissions in the United States during the COVID-19 pandemic.

Methods: Retrospective cross-sectional study of 33 US hospitals contributing to the Pediatric Health Information Systems (PHIS) database between 1/1/2010 and 12/31/2021. Inpatient admissions with a principal diagnosis of bronchiolitis were transformed into time series and ensemble forecasting models were used to analyze monthly admissions in comparison to United States Centers for Disease Control and Prevention mask recommendations and state-level mask mandates.

Results: There were 217,208 admissions for bronchiolitis among 197,380 patients across 33 hospitals in 21 states. Before the first CDC mask recommendation, there were 16,298 admissions for bronchiolitis compared to 17,764 (95% confidence interval [95% CI]: 11,390-26,692) predicted admissions. During the first CDC mask recommendation, there were 1,508 admissions for bronchiolitis, compared to 19,426 (95% CI: 11,500-31,047) predicted admissions (92.2% reduction). After the CDC masking recommendation expired, bronchiolitis admissions increased leading to an out-of-season peak in August 2021 with 2,641 admissions compared to 578 (95% CI: 267-1,067) predicted. After masking recommendations resumed, admissions decreased such that there were 2,257 admissions in December 2021 compared to 4,155 (95% CI: 2,668-6,041) predicted. Of 15 states that instituted a mask mandate with data available, 15/15 (100%) had fewer-than-forecasted admissions during their mandate period. 8/15 (53.3%) states had higher-than-forecasted bronchiolitis admissions after mask mandates expired ($p = 0.013$).

Conclusions: In this cross-sectional database analysis, there was a marked reduction in bronchiolitis admissions during periods of mask recommendations, followed by an out-of-season peak.

Significance: Masking recommendations may be useful in increasing hospital surge capacity in future epidemics, but with attention to the possibility of out-of-season Palivizumab dosing.

Research/Grant Support: NICHD 5T32HD040686-20 (JHP and JR), NINDS 5K23NS104133 (AKA), NIDDK 5K23DK116973(DYF), NICHD 1K23HD099331-01A1 (CMH)

Abstract 36

INHIBITION OF LSD1 SERVES AS A BETTER EPIGENETIC TARGET FOR ENHANCEMENT OF CELL DEATH THAN JMJD3

Richter B,[1,2] Souryavong A,[2] Dar H,[2] Kagan VE,[2] Bayır H[1,2]

[1]Department of Pediatric Critical Care Medicine, Children's Hospital of Pittsburgh
[2]Department of Environmental and Occupational Health, University of Pittsburgh

Background: Iron-dependent ferroptosis, caspase-deficient necroptosis, and inflammatory pyroptosis are novel cell death pathways implicated in several disease states including cardiovascular events, pancreatitis, inflammatory bowel disease, acute kidney injury, and neurodegeneration. As cell death pathways are genetically encoded, disentangling the epigenetic regulation of necroptosis using histone demethylase inhibitors may lead to the identification of key genes and regulator proteins that can be therapeutically targeted to attenuate cell death. Previously, our lab identified GSK J1 (demethylase JMJD3 specific) as a weak epigenetic enhancer of necroptosis as well as ciclopirox (pan-histone demethylase inhibitor) as a potent inhibitor of cell death. The methylase, EZH2, performs the opposing function of JMJD3 leading to a potential target as well for inhibition of cell death. Further work needs to be done to identify a more potent necroptosis enhancer, so demethylase LSD1 serves a primary interest for this study as preliminary work identified it as one of the necroptosis inducing targets.

Hypothesis: Inhibition of LSD1 causes a more potent and reliable increase in necroptotic cell death than inhibition of JMJD3.

Methods: To investigate the epigenetic regulation of necroptosis, we pre-incubated HT-22 cells for 2 hours with demethylase inhibitors, GSK J1 (JMJD3) and RN1 dihydrochloride (LSD1). Pre-incubation with EZH2 specific inhibitor, GSK343 was also performed. Cells in the positive control group were treated with necrostatin-1s (20 μM), a reagent that specifically inhibits necroptosis. Necroptosis was induced using a mixture of zVAD.fmk (20 μM), SM-164 (40 nM), and mTNF (20 ng/mL) for approximately 20 hours. Cell death was then analyzed via a commercial LDH kit (ProMega) or with propidium iodide staining followed by flow cytometry.

Results: Our necroptosis assay looking at EZH2 inhibition with GSK343 at 2.5 μM led to an unexpected small increase in necroptotic cell death in several of the assays. Furthermore, GSK J1 at 20 μM was unreliable for causing any significant enhancement of cell death with many experiments yielding at most a 5% increase on average. On the other hand, inhibition of LSD1 with RN1 dihydrochloride at 40 μM was able to show significant enhancement of necroptotic cell death of 15-20% ($p<0.01$) using both LDH and flow cytometry.

Conclusions: By investigating the epigenetic control of cell death using histone demethylase inhibitors, we identified that LSD1 serves as the more potent target for inhibition compared to JMJD3 to promote necroptosis in HT-22 cells. Furthermore, we determined that EZH2 was a suboptimal target for lowering cell death levels in our experiments thus turning our attention away from JMJD3 as a prime regulator of cell death to LSD1. Therefore, an epigenetic level of control in necroptosis could be determined in the future as we continue to investigate the exact biochemical mechanisms at play using siRNA interference and RNAseq.

Significance: This research sheds important insight on epigenetic regulation of necroptosis. The genes and regulatory proteins affected by these inhibitors may potentially be therapeutic targets that could decrease or enhance cell death in various disease states affected by necroptosis. In the case of our experiment, the enhancement of necroptosis could serve a role in the treatment of cancers. Other disease states such as cardiovascular disease and traumatic brain injury could also be treated with inhibitors of necroptosis such as ciclopirox as well.

Research/Grant Support: U01 Radiation (PI: Hülya Bayır, MD)

Abstract 37

KIDNEY TUBULAR AMP-ACTIVATED PROTEIN KINASE (AMPK) INDUCES CELL DIFFERENTIATION DURING SEPSIS-ASSOCIATED ACUTE KIDNEY INJURY

Toro J,[1] Mutchler S,[2] Manrique-Caballero C,[1,2] Saraiva IE,[1] Hamahata N,[1] Baty C,[2] Frank AM,[1] Subramanya A,[2] Byersdorfer CA,[3] Gómez H[1]

[1]Program for Critical Care Nephrology, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) Center, Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, USA
[2]Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA
[3]Department of Pediatrics, Division of Blood and Marrow Transplantation and Cellular Therapies, University of Pittsburgh, Pittsburgh, PA, USA

Introduction: Sepsis-associated acute kidney injury (S-AKI) is a life-threatening complication. Sepsis induces a shift in kidney tubular epithelial cell (TEC) metabolism, from oxidative phosphorylation (OXPHOS) toward glycolysis. We and others have shown that activation of promoters of OXPHOS, like the AMPK, decreases the development of S-AKI and mortality. A potential driver of this harmful metabolic shift may be the evolutionary conserved defense mechanism of de-differentiation.

Hypothesis: We hypothesize that sepsis induces de-differentiation in the kidney leading to more severe AKI, and that AMPK prevents this by maintaining TEC differentiation.

Methods: We first exposed wild type (WT) animals to either sham or cecal ligation and puncture (CLP) and assessed the development of de-differentiation in TEC by two immunofluorescence (IF) methods: (i) internalization of the Na^+K^+ATPase pump, (ii) the expression of the de-differentiation marker Sox9. Then, we created a doxycycline-induced kidney tubule-specific AMPK knockout system (AMPK KO). We exposed AMPK KO and WT littermates to doxycycline for 3 weeks to induce KO of AMPK in TEC, and then all mice were subjected to CLP. We compared the expression of Sox9 in TEC using IF, TEC injury by monitoring the expression of NGAL, and the development of S-AKI using plasma creatinine (Cr) 24h after CLP.

Results: Sepsis induced the internalization of the Na^+K^+ATPase toward the cytoplasm, and the expression of Sox9. AMPK KO animals had a higher expression of Sox9 in TEC than WT animals, regardless of TEC injury measured by NGAL. AMPK KO animals had a two-fold increase in S-AKI severity as compared to WT 24h after CLP.

Conclusion: Sepsis induces TEC de-differentiation, which may explain the known metabolic shift toward glycolysis. Importantly, in the absence of TEC AMPK, sepsis induces a higher expression of de-differentiation markers, and a higher severity of S-AKI.

Significance: These data suggest that AMPK expression is an adaptive mechanism that limits kidney injury and severity and supports the hypothesis that such protection may be mediated by preventing TEC de-differentiation.

DEPARTMENT OF EMERGENCY MEDICINE

Abstract 38

PREHOSPITAL ELECTROENCEPHALOGRAPHY IN TRAUMA DURING HELICOPTER TRANSPORT: A PILOT OBSERVATIONAL COHORT STUDY

Barton DJ,[1] Coppler PJ,[1] Talia NN,[1] Charalambides A,[2] Stancil B,[2] Callaway CW,[1] Guyette FX,[1] Elmer J[1]

[1]Department of Emergency Medicine, University of Pittsburgh School of Medicine
[2]Lifeware Labs, Pittsburgh, PA

Introduction: Early recognition of traumatic brain injury (TBI) is important to optimize patient care. Electroencephalography (EEG) can identify TBI, but feasibility of EEG has not been evaluated in the prehospital setting.

Hypotheses: Our primary objective was to assess the feasibility of obtaining EEG waveform data using a one-channel device during air medical transport after trauma. Additionally, we tested the association of quantitative EEG measures with presence of TBI on initial head computerized tomographic (CT) imaging.

Methods: We performed a pilot prospective, observational study of consecutive patients transported by critical care helicopter ambulance from the scene of trauma to a single level 1 trauma center. During transport, prehospital crews placed a novel, proprietary small (~5 cm) electrographic monitor ("Apollo" by Lifeware Labs, Pittsburgh, PA, USA) on the forehead to record 200 Hz EEG. We collected clinical characteristics and head CT reports. EEG waveform post-processing included bandpass (0.1-30 Hz) and notch filtering to minimize motion artifacts, and fast Fourier transform to summarize component frequencies. We reviewed waveforms and selected 90 seconds of recording for quantitative EEG analysis, including delta-range (0-4 Hz), theta-range (4-8 Hz), and alpha-range (8-13 Hz) power. Measures were compared between individuals with and without TBI findings on CT scan using Mann-Whitney tests.

Results: Forty patients were enrolled in whom the EEG monitor was placed by ambulance personnel. EEG recordings were successfully obtained in 34 patients (85%). Reasons for failure included uncharged battery (n=5) and user error (n=1). Data was lost during file transfer in 3 cases. Of 31 patients with available data, satisfactory EEG signal was recorded in 26 patients (84%). Patients were a mean (SD) age of 48 (16) years, 79% male, and the most frequent mechanism of injury was motor vehicle accident (50%). Eight patients (24%) had TBI on CT scan; median Glasgow Coma Scale was 15, and one patient required delayed neurosurgical decompression. Patients with and without TBI had similar median values of total delta power (458.4 vs. 329.0 mV; p=0.79), alpha power (63.0 vs. 72.1 mV; p=0.74), and theta power (97.5 vs. 88.8 mV; p=0.41).

Conclusions: Prehospital EEG is feasible during helicopter transport of trauma patients. Further study is needed to assess utility of quantitative EEG metrics for brain injury detection.

Significance: Quantitative EEG is measurable in prehospital trauma patients during air medical transport. This could be used as a tool in future studies of TBI diagnosis in the prehospital setting.

Research/Grant Support: NHLBI T32HL134615 (PI: Clifton Callaway, MD, PhD and Donald Yealy, MD) and K23NS097629 (PI: Jonathan Elmer, MD). Lifeware Labs provided research EEG devices but had no role in study design, data analysis, or reporting.

Abstract 39

SIMPLE AND INTERPRETABLE METHODS FOR AUTOMATED QUANTIFICATION OF CEREBRAL EDEMA SEVERITY

Case N,[1] Coppler P,[1] Callaway C,[1] Elmer J[1,2,3]

[1] Department of Emergency Medicine, University of Pittsburgh School of Medicine
[2] Department of Critical Care Medicine, University of Pittsburgh School of Medicine
[3] Department of Neurology, University of Pittsburgh School of Medicine

Introduction: After cardiac arrest, hypoxic-ischemic brain injury can cause cerebral edema. Severity of edema estimated as the ratio of grey matter to white matter density (GWR) on brain computerized tomography (CT) predicts recovery. Edema lowers tissue radiodensity primarily in grey matter and so reduces GWR. GWR is typically determined through manual selection of regions of interest, which is laborious and unreliable.

Hypothesis: We tested the hypothesis that computational approaches to quantifying the distribution of x-ray attenuation in the entire brain CT could detect edema.

Methods: From a registry of post-arrest patients, we identified 7 cases with severe edema and 10 cases with no edema based on expert interpretation and manual GWR. We extracted demographic data from our registry and Digital Imaging and Communication in Medicine (DICOM) files for the first post-arrest CT. CTs were obtained using a GE LightSpeed VCT 64-channel scanner (120 kVp, 225 mA, 5mm slice thickness, 32 slices). We analyzed the 17th slice of each DICOM, corresponding to the level of the basal ganglia. We extracted the 512x512 pixel array, which we converted to Hounsfield units (HU). We excluded HU >40 or <10 to focus on non-bone tissue densities. We summarized each patient's distribution of HU and fit two-group Gaussian finite mixture models, then used Rank-Sum tests to compare summary statistics between patients with and without edema. We performed all analyses using Python.

Results: Of 17 included patients, median [IQR] age was 51 [44- 74] years and 24% survived to hospital discharge. Edema patients had lower variance in HU compared to non-edema patients (34.04 [32.67 – 36.06] vs 50.12 [45.39 – 51.47], P = 0.001). In finite mixture models, distribution means were closer together in the edema vs non-edema patients (median difference in means: 7.06 [6.75 – 8.26] vs 10.66 [9.91 – 11.46], P = 0.001).

Conclusions: Exploring HU distributions on brain CT may be useful to quantify edema severity in applications where high reproducibility is more important than expert interpretation.

Research/Grant Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Abstract 40

INCIDENCE OF ABUSIVE HEAD TRAUMA DURING THE COVID-19 PANDEMIC IN WESTERN PENNSYLVANIA

Cercone D,[1] Berger R,[2] Manole M,[1] Soung J,[1] Noorbakhsh K[1]

[1]Division of Pediatric Emergency Medicine, University of Pittsburgh School of Medicine
[2]Division of Child Advocacy, University of Pittsburgh School of Medicine

Introduction: Abusive head trauma (AHT) is the leading cause of death from non-accidental trauma in children. Lifestyle disruptions during the COVID-19 pandemic added to family stressors increasing the risk for family violence. Reports regarding the impact of the COVID-19 pandemic on rates of AHT are limited and with conflicting results. There have been no reports thus far describing the incidence of AHT during the entire first year of the COVID-19 pandemic.

Hypothesis/Objective: To determine the incidence and clinical characteristics of AHT during the first year of the COVID-19 pandemic compared to the two prior years.

Methods: We performed a retrospective cohort study of all patients with AHT <5 years old presenting to a single pediatric tertiary care center from March 11, 2018 to March 10, 2021. AHT was defined as any intracranial injury due to blunt impact or shaking or an isolated skull fracture with additional findings consistent with physical abuse. Injuries were deemed highly concerning or definitive for physical abuse by the UPMC Children's Hospital of Pittsburgh Child Protection Team. Data obtained included: patient demographics, length of stay, physical examination findings, retinal exam findings, radiologic results, EEG results, and mortality. Data were compared for the two years before the pandemic (March 2018 to March 2020) and the first year of the pandemic (March 2020 to March 2021). Statistical comparisons of patient characteristics were performed using Mann-Whitney U, Chi-square, or Fisher's exact tests.

Results: There were 27 AHT cases during the pandemic year and 55 combined AHT cases during the two pre-pandemic years. Patients were similar in regard to age, sex, and race during both time periods. The mortality rate was 29.6% (n=8) during the pandemic vs. 3.6% (n=2) prepandemic (p=.002). The pandemic saw a significant increase in patients with retinal hemorrhages (p<.001) and abnormalities seen on cervical spine imaging (p=.020). A similar incidence of dermatologic abnormalities, abnormal EEGs, and abnormal skeletal surveys were noted for AHT patients during both time periods.

Conclusions: This study showed a similar number of yearly AHT cases during the COVID-19 pandemic compared to the two previous years. There was a significant increase in mortality along with retinal hemorrhages and abnormalities seen on cervical spine imaging for AHT patients during the pandemic. These data suggest an increased severity of AHT encounters during the pandemic.

Significance: Given this concern for increased severity of AHT, future studies could focus on identifying families or patients at increased risk for violence during health crises in order to try and identify potential areas of targeted interventions.

Abstract 41

ASSOCIATION OF NONEMERGENT PEDIATRIC EMERGENCY DEPARTMENT VISIT RATE AND CHILDHOOD OPPORTUNITY INDEX, BY CENSUS TRACT

Hincapie MR,[1] Corbera-Hincapie MA,[2] Suresh S,[1,3] Butler G,[3] Ray KN[4]

[1]Division of Pediatric Emergency Medicine, UPMC Children's Hospital of Pittsburgh
[2]Division of Pediatric Gastroenterology, UPMC Children's Hospital of Pittsburgh
[3]Division of Health Informatics, UPMC Children's Hospital of Pittsburgh
[4]Division of General Academic Pediatrics, UPMC Children's Hospital of Pittsburgh

Introduction: Over half of pediatric emergency department (ED) visits are deemed nonemergent. With the increasing rates of nonemergent ED visits becoming a major contributor to increasing health care costs, strategies have been proposed to redirect some visits to primary care providers. Prior approaches to reduce nonemergent ED visits have often focused on individual patient factors and interventions, however, emerging data are identifying important associations between ED visits and neighborhood factors, warranting further examination.

Hypothesis: Census tracts with higher rates of nonemergent Pediatric ED visits will have lower COI scores, indicative of fewer neighborhood resources.

Methods: We identified nonemergent PED visits using the Emergency Severity Index 4 and 5 during a 3-year period (01/01/18-12/31/20) at a tertiary children's hospital. Patient encounters with a residence outside of the hospital's county were excluded. Using GIS software (ArcGIS Pro 2.7), 93% of patient residence were successfully geocoded and assigned to census tracts. We used the Childhood Opportunity Index 2.0 (COI), a metric for assessing neighborhood resources and conditions that play a role in child health, to examine the association of neighborhood factors on nonemergent ED visits. The COI provides a score between 0 and 100 for each census tract, using 2015 US Census Bureau and the American Community Survey data, with the lower scores suggestive of less opportunity. We examined the association between census tract ED visits per 100 children and census tract COI score by using Pearson correlation coefficient. Census tract nonemergent ED visits per 100 children ranged from 0 (lowest quartile) to 102 (highest quartile) with a higher value indicative of a higher child population by census tract presenting to the ED. We compared COI across quartiles using one-way ANOVA.

Results: 75,372 nonemergent PED visits occurred across 393 census tracts in Allegheny County during the study period. Many of the high quartile visits, concentrated around the center of the city, which has higher rates of very low and low COI scores across COI domains. Census tract nonemergent ED visits per 100 children and census tract COI were negatively correlated at -0.6 ($p<0.001$ for all years). Overall COI score and its three COI domain scores each declined across increasing quartiles.

Conclusion: Census tracts with higher rates of nonemergent ED visits had fewer neighborhood resources, and this was consistent across all study years. This study is one of the first to apply Geographic Information System (GIS) methodology, a novel technique to perform spatial analysis, in the evaluation of nonemergent ED visits and its relationship to geographic resources.

Significance: Data from this study will provide critical health information relevant to how we approach social determinants of health in the pediatric ED setting, assess outpatient enhanced access services, and elucidate reasons to expand resources in resource-limited neighborhoods. These results will aide in the design and planning of future investigations with targeted interventions, such as public health informatics and advocacy efforts to address the impact of neighborhood factors on child health. These study results indicate the potential for many structural community-based approaches to address nonemergent ED visits.

Research/Grant Support: Not Applicable

Abstract 42

THE PREVALENCE OF ECG FINDINGS ENCOUNTERED BY PARAMEDIC DURING AMBULANCE TRANSPORT

Kates L,[1] Faramand Z,[1,2] Al-Zaiti S[1,2]

[1]University of Pittsburgh, Emergency Medicine
[2]University of Pittsburgh School of Nursing

Introduction: Emergency medical services (EMS) are often required to acquire and interpret an electrocardiogram (ECG) for patients during transport. The ECG can identify a wide array of findings corresponding to different pathologies, however teaching all these findings for EMS personnel can be cumbersome and an inefficient undertaking. Implementing a teaching method that focuses on the most common findings in a prehospital ECG is the most efficient method, however, the most common findings remain unknown. In this study, we aimed to identify the most common ECG findings encountered by EMS personnel during transport.

Methods: We enrolled consecutive patients with non-traumatic chest pain transported via ambulance. The ECG findings were automatically documented by the Philips algorithm(XX). An independent reviewer reviewed and collected the automated ECG documentation.

Results: The sample included 5092 patients transported by EMS. The most common finding documented by the ECG algorithm was non-specific T-wave abnormalities, which was present in 22% of the population. This was followed by sinus tachycardia (19%), left ventricular hypertrophy (13%), ST-elevation (13%), left atrial enlargement (12%), ventricular premature complexes (11%), and prolonged QT intervals (10%). Meanwhile, acute myocardial infarction was documented in 7% and acute ischemia was documented in 1% of the population. The least common findings were second degree heart block and sinus pause (<1%).

Conclusions and significance: The most common findings encountered by EMS personnel are non-specific T-wave abnormalities and sinus tachycardia. The optimal EMS training curriculum for ECG reading should primarily focus on identifying the most common findings encountered during prehospital transport.

Grant support: This study was funded by grant from NIH / NHLBI R01HL 137761

Abstract 43

PATIENT CHARACTERISTICS ASSOCIATED WITH HOSPITAL ADMISSION OR ANTIARRHYTHMIC MEDICATION CHANGES AFTER EMERGENCY DEPARTMENT EVALUATION OF SUPRAVENTRICULAR TACHYCARDIA

Schmucker KA,[1] Morris CS,[2] Tisherman RT,[3] Manole M,[1] Arora G,[4] Dunnick J[1]

[1]UPMC Children's Hospital of Pittsburgh, Department of Pediatrics, Division of Pediatric Emergency Medicine
[2]UPMC Children's Hospital of Pittsburgh, Department of Pediatrics
[3]UPMC Department of Orthopedics
[4]UPMC Children's Hospital of Pittsburgh, Department of Pediatrics, Division of Cardiology

Introduction: Supraventricular tachycardia (SVT) is a relatively frequent diagnosis in the pediatric emergency department (ED). However, currently there are no consensus guidelines for ED disposition. Better understanding of those who are admitted or have antiarrhythmic medication changes may avoid potentially unnecessary transfers.

Objective: Identify patient factors associated with hospital admission or cardiac medication changes upon discharge in patients evaluated for SVT in a pediatric ED.

Design/Methods: This study was conducted at a tertiary children's hospital affiliated with an academic university that serves as the primary referral center in the area. A retrospective review of children aged 0-18 years seen in the pediatric ED for SVT was conducted using electronic medical record data over a seven-year period. Patients with congenital cardiac disease or prior cardiac surgeries were excluded. Variables of interest included age, prior diagnosis of SVT, current antiarrhythmic medications, history of Wolff-Parkinson-White syndrome, other medical comorbidities, intercurrent illness, transfer, number adenosine doses given, electric cardioversion, duration of SVT, evaluated in person by cardiology, and presence of preexcitation. Multivariable logistic regression analysis was used to determine the association between the variables of interest and the primary outcome of admission or change of antiarrhythmic medications.

Results: We analyzed 197 patient encounters. The mean age was 7.3 years. Of these, 52.7% were admitted to the hospital or discharged with antiarrhythmic medication changes. This primary outcome was associated with younger age (OR 0.77, CI 0.67-0.86, $p<0.0001$), increased number of adenosine doses (OR 5.45, CI 1.55-22.3, $p<0.0125$), history of preexcitation (OR 5.82, CI 2.01-18.83, $p=0.0016$), intercurrent illness (OR 3.75, CI 1.27-12.07, $p=0.0205$), evaluated in ED by cardiology (OR 6.42, CI 2.43-19.37, $p<0.0004$).

Conclusions: Patient factors associated with admission or change in cardiac medication include age, history of preexcitation, intercurrent illness, number of adenosine doses prior to arrival, and cardiology evaluation. These findings help to identify patients who benefit from tertiary care evaluation for SVT. Future work should use these criteria to identify potentially avoidable transfers from community centers to tertiary care hospitals.

Abstract 44

CAPILLARY STASIS FOLLOWING MOUSE CARDIAC ARREST: PERFORMANCE OF THE FLUX VARIANCE METHOD

Nelson BP,[1,2] Gunasekaran V,[1] Toader A,[3] Stezoski J,[2] Wang P,[3] Vazquez AL,[3] Manole MD[1,2]

[1]Department of Pediatrics
[2]Safar Center for Resuscitation Research
[3]Department of Radiology
University of Pittsburgh

Introduction: Cerebral hypoperfusion early after resuscitation may worsen neurological injury after cardiac arrest (CA). At the level of microcirculation, vasoconstriction and capillary stasis (no-reflow) were observed *in vivo* in a pediatric rat CA model. Capillary stasis was present in 15-25% of capillaries and was observed to be a dynamic phenomenon, with some capillaries regaining flow post-CA. Capillary stasis has not been characterized to date in adult models of CA. Moreover, current methods to evaluate capillary stasis are subjective and time-consuming.

Hypothesis/Objective: To characterize cortical capillary stasis in adult mice post-CA and to develop an objective method of volumetric assessment of capillary stasis.

Methods: Mice underwent tracheal intubation, mechanical ventilation, arterial and venous femoral catheterization. A 4 mm cranial window was placed over the parietal cortex. The vessels were delineated with fluorescent dextran administered IV. We induced CA for 5 minutes, and then resuscitated with chest compressions and epinephrine (IV). We imaged the cortex pre-CA and serially post-CA for 1 hour using *in vivo* multiphoton microscopy. Capillary patency was quantified via the black signals of red blood cells (RBC) traversing the fluorescent plasma. We assessed capillary stasis using two methods: a qualitative assessment of capillary RBC flow and a quantitative assessment of capillary RBC flux variance. The capillary RBC flow was visually categorized by an observer as normal flow, slow flow, and no flow. To assess capillary flux variance, we processed and analyzed the flow images in MATLAB. We generated colorimetric flow maps and calculated the coefficient of variance to assess RBC signal fluctuations in capillaries. The RBC flux variance for the capillaries was grouped into 3 categories: high variance (normal flow), medium variance (slow flow), low variance (no flow). We compared RBC flow with RBC flux variance using linear regression.

Results: We assessed flow in 574 capillaries pre-CA and 419 capillaries post-CA. At baseline, we observed normal flow in most capillaries (94±3% and 90±6%, flow and flux variance method, respectively), while capillary slow and no flow were rare (6±2% and 10±3%, flow and flux variance method, respectively). Post-CA, slow and no flow increased to 23.1±7% for the capillary flow method and 21±7% for the capillary flux variance method, with no difference between methods (p=0.9). There was a strong association between the capillary flow qualitative assessment and the capillary flux variance quantitative assessment (correlation coefficient=0.99).

Conclusions: We observed and quantified capillary stasis in the adult mouse CA model. There was a high degree of correlation between the RBC flow and RBC flux variance methods. Significance: The observed capillary stasis in the mouse CA model can serve as a platform to assess the contribution of vascular mural cells (smooth muscle cells, pericytes) to capillary stasis using transgenic mice. The RBC flux method is a valuable tool for the quantification of capillary stasis, essential for the evaluation of blood flow directed therapies.

Grant support: R33NS107785 (MDM), R01HD075760 (MDM), R21NS12150, R01NS094404, Children's Neuroscience Institute (MDM, ALV)

Abstract 45

SODIUM BICARBONATE ADMINISTRATION IS ASSOCIATED WITH IMPROVED SURVIVAL IN ASYSTOLIC AND PEA OUT-OF-HOSPITAL CARDIAC ARREST

Niederberger S,[1] Crowe R,[2] Salcido D,[3] Menegazzi J[3]

[1]University of Pittsburgh School of Medicine
[2]ESO
[3]Department of Emergency Medicine, University of Pittsburgh School of Medicine

Introduction: Sodium bicarbonate ("bicarb") administration in out-of-hospital cardiac arrest (OHCA) is intended to counteract acidosis, although there is limited clinical evidence to support its routine administration. Even so, bicarb is commonly used in resuscitation. We sought to analyze the association of bicarb with resuscitation outcomes in non-traumatic OHCA.

Hypothesis: In adults experiencing non-traumatic OHCA, prehospital bicarb administration by EMS improves survival to hospital discharge.

Methods: Records were obtained from the 2019-2020 ESO Data Collaborative prehospital electronic health record database, which spanned 1,322 agencies in 50 states. We included all adult patients experiencing cardiac arrest in non-hospital settings with resuscitation attempted. We restricted analyses to cases with resuscitation lengths between five and forty minutes. Analyses were stratified by presenting rhythm: VF/VT, PEA, and asystole. The outcomes of ROSC at any time during the prehospital encounter and survival to hospital discharge were compared by bicarb status using propensity score matching and logistic regression analyses with and without adjustment.

Results: We analyzed 23,567 records, 28.3% (6,663) of which included bicarb administration. Most patients had a presenting rhythm of asystole (67.4%), followed by PEA (16.6%), and VF/VT (15.1%). In the propensity-matched cohort, ROSC rates were higher in the bicarb group than the control group among patients presenting in asystole (bicarb 10.6% vs control 8.8%; p=0.013). There were no differences in ROSC rates by bicarb status in the PEA or VF/VT presenting rhythm groups. Survival was higher in the bicarb group than the control group for asystole (bicarb 3.3% vs control 2.4%; p=0.020) and for PEA (bicarb 8.1% vs control 5.4%; p=0.034). There was no difference in survival by bicarb status in the VF/VT group. These results were consistent across adjusted and unadjusted logistic regression analyses; bicarb was associated with improved ROSC and survival in asystole [unadjusted odds ratios (95% CI): ROSC 1.23 (1.04-1.44), survival 1.40 (1.05-1.87)], and bicarb was associated with improved survival in PEA (1.54 (1.03-2.31). Adjusting did not substantially alter the results.

Conclusions: Overall, bicarb administration was associated with survival in non-shockable rhythms and was associated with increased ROSC in asystole. Findings from this limited observational study should be corroborated with future prospective work that includes neurologically intact survival as an outcome.

Significance: As there is currently no treatment indicated for patients in non-shockable rhythms other than continued CPR and repeat doses of epinephrine, these findings suggest a need to reconsider current AHA guidelines which do not support the routine use of bicarb in OHCA.

Grant Support: University of Pittsburgh School of Medicine T35 Grant Award (5T35DK065521-17) from the National Institutes of Health (PI: Thomas R. Kleyman, MD)

DEPARTMENT OF NEUROLOGICAL SURGERY

Abstract 46

FLUID PERCUSSION INJURY LEADS TO AN IMBALANCED EVOKED RELEASE BETWEEN HIPPOCAMPAL GLUTAMATE AND GAMMA-AMINOBUTYRIC ACID ALONG WITH COGNITIVE IMPAIRMENT

Birabaharan J,[1] Henchir J,[3] West R,[2] Nolin T,[1,2] Empey P,[1,2] Carlson S[3]

[1]Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy
[2]Department of Pharmacy & Therapeutics, University of Pittsburgh School of Pharmacy
[3]Department of Neurosurgery, Safar Center for Resuscitation Research
University of Pittsburgh School of Medicine

Introduction: Cognitive impairment from traumatic brain injury (TBI) is a substantial source of morbidity after injury. Impaired cognitive abilities have been well observed both clinically and in animal models after TBI. In a healthy hippocampus, glutamate (excitatory) and GABA (inhibitory) amino acid neurotransmitters release is dictated by a coordinated response integral to hippocampal dependent learning and memory. From within hours to months after TBI, neurotransmitters release imbalances have been associated with neuroexcitoxicity and synaptic dysfunction, that can manifest to chronic post traumatic epilepsy. Simultaneous assessment of these neurotransmitters is informative to understanding these imbalances.

Hypothesis: We hypothesized that evoked glutamate and GABA release is altered after fluid percussion injury (FPI), thus contributing to cognitive impairment.

Methods: Male Sprague Dawley rats (SHAM = 9, Lateral FPI = 9) were tested for motor and cognitive function for 2wks after FPI. A microdialysis probe was implanted in the ipsilateral hippocampus. Cerebrospinal Fluid (aCSF) was pumped through the probe. High potassium aCSF was administered to measure evoked release. Microdialysis samples were collected every 20min. Using a novel LC/MS approach, we measured glutamate (50-1500ng/mL) and GABA (5-150ng/mL) concentrations simultaneously.

Results: Results show that in FPI rats glutamate release was significantly lower ($p=0.05$) but GABA release was unchanged compared to sham.

Significance: Simultaneous measurements of glutamate and GABA can be implemented and ongoing work is aimed at understanding mechanisms contributing to impaired synaptic function and for testing the efficacy of therapeutic interventions to promote recovery of synaptic function after TBI.

Grant Support: NIH R21 NS111099 (PI: Shaun Carlson), Chuck Noll Foundation, Walter L Copeland of The Pittsburgh Foundation, NIH TL1 TR001858 (JB, PI: Kevin Kramer), NIH S10OD028540 (PI: Thomas Nolin)

Abstract 47

CHARACTERIZATION OF A RAT MODEL OF REPETITIVE MILD FLUID PERCUSSION INJURY AT 2 WEEKS POST-INJURY

Fronczak K,[1] Roberts A,[1] Parry M,[1] Holets E,[1] Henchir J,[1] Dixon CE,[1,2] Carlson SW[1]

[1]Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, USA
[2]VA Pittsburgh Healthcare System, Pittsburgh, PA, USA

Introduction: Repetitive mild traumatic brain injury (rmTBI) is a prominent public health concern, with linkage to debilitating chronic sequalae. Developing reliable and well characterized preclinical models of rmTBI is imperative in the investigation of the underlying injury mechanism. The lateral fluid percussion injury (FPI) model is a reliable method of rmTBI replication, though it is currently underutilized.

Hypothesis: We aim to perform a novel characterization of a variation of the lateral repetitive mild FPI (rmFPI) model.

Methods: Adult male rats received one, two or four mild FPI (1.25 atm) or sham surgeries, implemented 24h apart (n=12 per status). Acute behavioral impairment was assessed with beam balance and Morris water maze tasks. Histopathology was assessed with a qualitative analysis of tissue damage via Cresyl violet staining, and with quantitative measurements Iba-1 and synaptophysin immunoreactivity (n=6).

Results: Performances in behavioral tasks revealed significant differences in impairment between injured groups. Qualitive analysis of Cresyl violet staining revealed visible tissue damage following four FPI only, which led us to further characterize the subacute pathophysiological outcomes of the dual FPI (dFPI), as this injury was the most clinically relevant. Immunoreactivity measures showed that dFPI led to no significant changes in synaptic density two weeks post-injury, as measured by synaptophysin abundance, but did lead to a striking increase in Iba-1-positive microglia in several brain regions.

Conclusions: We have provided a novel account of the subacute post injury outcomes occurring in response to this variation of rmFPI.

Significance: With this, we have demonstrated the reliability of the lateral FPI model in rmTBI replication.

Research/Grant Support: NIH 1R21NS111099, Chuck Noll Foundation and the Walter L. Copeland Fund of The Pittsburgh Foundation (SWC)

Abstract 48

EXPRESSION OF KEY RETINOIC ACID SIGNALING PROTEINS FOLLOWING CONTROLLED CORTICAL IMPACT IN RATS

Ranellone N,[1] Svirsky SE,[1,2] Henchir J,[1] Li Y,[1] Carlson SW,[1] Dixon CE[1,3]

[1]Department of Neurological Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
[2]Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA
[3]V.A. Pittsburgh Healthcare System, Pittsburgh, PA, USA

Introduction: Traumatic brain injury (TBI) has been shown to cause numerous deficits that can affect motor skills, cognition, and memory. Retinoic Acid (RA) signaling facilitates neuronal development, modulates neuronal plasticity, and is involved in neurogenesis in the adult hippocampus. This study aims to understand the effect of experimental TBI on RA signaling by looking at specific proteins at key points of the RA pathway. ALDH1A1 is involved in RA synthesis; CYP26A1 and CYP26B1 are involved in RA degradation. This study also investigates a dose-response effect of exogenous RA treatment on rats after experimental TBI on RA signaling protein expression.

Hypothesis: We hypothesized that CCI would replicate a neurodevelopmental stage where RA signaling would be activated after injury. This would increase ALDH1A1 and CYP26B1 expression as a compensatory signal. CYP26A1 expression would not significantly change after injury, but may demonstrate a response to exogenous RA, as previously described.

Methods: Adult, male Sprague-Dawley rats underwent either controlled cortical impact (CCI) or sham control surgery. Post-surgery, rats were given daily intraperitoneal injects of either vehicle (DMSO) or retinoic acid (0.5, 1, 5, or 10mg/ml in DMSO) for 2 weeks. Protein expressions were evaluated by Western blot in ipsilateral hippocampus whole cell lysates 2 weeks after injury.

Results: A one way ANOVA showed no group effect in whole cell hippocampal expression of ALDH1A1 and CYP26B1 ($p>0.05$). One way ANOVA showed a significant group effect for CYP26A1 expression ($p=0.0304$). Tukey's post-hoc test showed significantly higher CYP26A1 protein expression in the 1mg dose group compared to the 10mg group ($p=0.0145$). No other significant differences were observed.

Conclusions: ALDH1A1 and CYP26B1 protein expression level did not significantly change after CCI or exogenous RA which may suggest a compensatory recovery in expression. The increase in CYP26A1 expression in the 1mg dose group compared to the 10mg group suggests the 1mg has a proper homeostatic response to exogenous RA compared to the 10mg group.

Significance: This is the first study to our knowledge to investigate the effects of CCI injury or exogenous RA administration on the expression of these proteins and provides some insight into the RA signaling pathway after TBI. Further understanding the RA signaling pathway after TBI can explicate pathological mechanisms that contribute to deficits and lead to the development of novel treatments.

Research/Grant Support: Children's Hospital of Pittsburgh of the UPMC Health System (SES), NIH R01-NS106925, NIH R21-NS115440, and PA Department of Health grant 4100077083 (CED).

Abstract 49

CHARACTERIZATION OF HIPPOCAMPAL CA1 DENDRITIC SPINE DENSITY AND MORPHOLOGY AND GLUA1 EXPRESSION 2 WEEKS AFTER CONTROLLED CORTICAL IMPACT IN RATS

Svirsky SE,[1,2] Yoon H,[2] Henchir J,[2] Li Y,[2] Carlson SW,[2] Dixon CE[2,3]

[1]Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA
[2]Department of Neurological Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA
[3]V.A. Pittsburgh Healthcare System, Pittsburgh, PA

Introduction: Extensive effort has been made to study the role of synaptic deficits in cognitive impairment after traumatic brain injury (TBI). The dendritic spine is a dynamic structure, which functions as the anatomical locus of synaptic plasticity and underlies learning and memory. His study examined the effect of controlled cortical impact (CCI) on hippocampal CA1 dendritic spine density and morphology, along with protein expression of hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 sub-unit, a protein integral to synaptic plasticity.

Hypothesis: We hypothesized that experimental TBI decreases anatomical metrics of synaptic plasticity. This will result in decreased spine density and altered spine morphology 2 weeks after CCI. Synaptic protein expression of the AMPA GluA1 receptor is also predicted to decrease.

Methods: Adult, male Sprague Dawley rats (275-300g, 3 animals per group, 2-3 neurons per animal) received either CCI (2.5mm deformation, 4m/s) or control/Sham surgery. 2 weeks postinjury, brains were processed for Golgi staining, z-stacks were imaged on a Nikon confocal microscope and spines were counted and classified using Neurolucida 360 software.

Results: No change in spine density was observed between groups. However, there was a significant decrease in the number of “mushroom” spines on apical dendrites and a trending decrease on basal dendrites after CCI compared to Sham (Student’s t-test, $p=0.0227$, $p=0.0533$, respectively). There was a trending decrease in number of “thin” spines on apical dendrites after CCI ($p=0.0533$). Protein expression of GluA1 was measured by western blot in hippocampal synaptosomes. There was a significant decrease in GluA1 expression after CCI compared to Sham ($p<0.0001$).

Conclusions: In conclusion, CCI significantly alters CA1 dendritic spine morphology and GluA1 expression 2 weeks post-injury, reflective of cognitive deficits previously observed at this timepoint.

Significance: Changes in dendritic anatomy correspond to previous studies observing behavioral and electrophysiological cognitive deficits after experimental TBI. Dendritic density and morphology may be used to in tandem with these metrics to evaluate future therapeutic studies targeting synaptic plasticity deficits after TBI.

Research/Grant Support: Children’s Hospital of Pittsburgh of the UPMC Health System (SES), NIH R01 NS106925, NIH R21-NS115440.

DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION

Abstract 50

BRIDGING ENVIRONMENTAL ENRICHMENT WITH AMANTADINE AFTER CONTROLLED CORTICAL IMPACT INJURY IN ADULT MALE RATS

Bittner R,[1,2] Vozzella V,[1,2] Brennan C,[1,2] Eleti R,[1,2] Moschonas E,[1-4] Cheng J,[1,2] Bondi C,[1-4] Kline A[1,2,4]

[1]Physical Medicine & Rehabilitation
[2]Safar Center for Resuscitation Research
[3]Neurobiology
[4]Center for Neuroscience
University of Pittsburgh, Pittsburgh, PA

Introduction: Environmental enrichment (EE) facilitates recovery after traumatic brain injury (TBI). Historically, EE has been provided immediately after TBI, but this approach is not clinically relevant as strenuous rehabilitation is typically not initiated early after TBI to critical care patients because of injury limitations and the need to focus on life saving measures. Yet, treating TBI early is vital for optimal recovery. Hence, we sought to administer amantadine (AMT) as a bridge therapy before commencing EE a week later, which was also presented in a manner to mimic clinical rehabilitation (e.g., 6 h/day).

Hypothesis: Bridging EE with AMT will result in better neurobehavioral benefits than AMT or EE alone.

Methods: Isoflurane-anesthetized male rats received a cortical impact of moderate severity (2.8 mm deformation at 4 m/s) or sham injury and then housed in standard (STD) conditions for one week and administered either AMT (10 mg/kg or 20 mg/kg) or saline vehicle (1 mL/kg) intraperitoneally for 7 days (bridge). EE began on day 8 for the AMT bridge and continuous EE groups. Motor (beam-walking) and cognition (acquisition of spatial learning) were evaluated on days 7-11 and 21-25, respectively. The behavioral data were analyzed by two-way ANOVAs with repeated measures over testing days, followed by the Newman-Keuls post-hoc test, which corrects for multiple comparisons.

Results: The data showed that delayed and abbreviated EE, whether provided alone or in combination with AMT benefitted both behavioral outcomes ($p<0.05$) vs. STD housing. The 20 mg/kg AMT bridge + EE group performed better than the EE alone group in the acquisition of spatial learning in the water maze ($p<0.05$) but did not differ from EE alone or 10 mg/kg AMT bridge + EE in recovery of motor function ($p>0.05$).

Conclusions and Significance: That the 20 mg/kg AMT bridge group performed better than the EE alone group in the cognitive test ($p<0.05$), supports the hypothesis that adding the treatments would provide better benefit. Also, despite not exhibiting an additive benefit in the motor task, these data show that EE does not have to be implemented immediately after TBI and does not have to be provided continuously once initiated to confer benefits after TBI, indicating that our current model of neurorehabilitation can be used to evaluate the potential efficacy of various treatments in a rehabilitation paradigm.

Research/Grant Support: Supported in part by NIH grants 1 R56 NS113810, 5 R01 NS084967, 1 R01 NS121037 (AEK) and NS094950, NS099683, NS110609 (COB)

Abstract 51

EXPLORING ETANERCEPT TO TREAT ASPHYXIAL CARDIAC ARREST-RELATED NEURO-DISABILITY

Dimeo EG,*[1,2] Russell AL,*[1,2] Kline KE,[1,2] Braverman A,[1,2] Stezoski J,[1,3] Drabek T,[1,4,5] Kochanek PM,[1,3] Wagner AK[1,2,4]

[1]Safar Center for Resuscitation Research
[2]Department of Physical Medicine & Rehabilitation
[3]Department of Critical Care Medicine
[4]Center for Neuroscience
[5]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

Introduction: Cardiac arrest (CA) survival has increased over time due to improved resuscitation techniques, but the resulting hypoxic ischemic brain injury (HIBI) leaves survivors with lasting neurological disability. While we have demonstrated cell death in the hippocampus as a marker of dysfunction, the nigrostriatal pathway is also affected by asphyxial (A)CA. In a rat ACA model, there is a marked increase in tumor necrosis factor alpha (TNF-α) in the first few hours after injury, which is most pronounced in the striatum. TNF-α is secreted by microglia, and elevated TNF-α expression can exert deleterious effects in the brain including cell death. Blocking the TNF-α signaling cascade may prevent the induction of programmed cell death and control the inflammatory response to HIBI in the striatum.

Hypothesis: Reducing TNF-α levels via Etanercept (ETN) will improve behavioral outcomes and reduce striatal neuroinflammation and cell death after ACA.

Methods: Adult male Sprague-Dawley rats (N=14) underwent either sham or 5-min no flow ACA. One-hour post-surgery rats received either vehicle (VEH) or ETN (5 mg/kg). Rats then underwent a series of neurological and behavioral testing over a 14-day recovery period. Neurological deficit scores (NDS) were quantified on days (D)1, 2, 3 and 14 post-injury. Motor coordination (beam walk) and balance (beam balance) were tested on D0, 5, 8, 11, and 13 postinjury. Sensorimotor function [acoustic startle response (ASR); D3 and 11 post-injury] and myoclonus (D2 and 11 post-injury) were also measured. On D14, brains were collected, and the striatum was immunohistochemically labeled to measure microglial infiltration and activation.

Results: We found no differences in 14-day survival between ACA VEH vs. ACA ETN groups. ETN resulted in a trend ($p=0.08$) toward improved NDS on D2 post-ACA (ACA VEH=30%; ACA ETN=18%). Overall, ACA rats had worse motor coordination ($p<0.05$) and balance ($p<0.05$) than shams. Although not significant, ACA ETN rats had improved beam balance times vs. ACA VEH rats. ACA increased startle response (D3; $p<0.05$) and myoclonic events (D2; $p<0.05$) vs. sham counterparts. Interestingly, on D11, ETN decreased ACA-induced myoclonic events vs. ACA VEH ($p<0.05$). ETN also reduced the ACA-induced elevation in startle responses vs. VEH rats ($p<0.05$). ACA males had increased striatal microglia vs. shams ($p<0.05$). There was a reduction in total microglia in the striatum of ACA ETN rats vs. ACA VEH rats($p<0.05$). Ongoing studies are analyzing morphological differences in striatal microglia.

Conclusions: We report that ACA results in lasting behavioral deficits in motor coordination, startle response and myoclonus that are associated with both cell death in the hippocampus and striatal inflammation. Treatment with ETN, a TNF-α inhibitor, promotes neurobehavioral recovery and decreases striatal inflammation. Future studies will aim to investigate the extent of striatal damage and determine the efficacy of ETN treatment post-ACA in females.

Significance: Targeting therapeutics to vulnerable brain regions is a potential treatment opportunity that could show substantial benefit in the post-acute recovery phase after ACA.

Research/Grant Support: AHA 18TPA34170553; NS108386-01A1; Lloyd Reback Family Gift (PI: A.K. Wagner, MD)

Abstract 52

PSYCHOSOCIAL RISK IN PEDIATRIC TBI: RELIABILITY OF THE PSYCHOSOCIAL ASSESSMENT TOOL

Kesbhat A, Treble-Barna A

Department of Physical Medicine and Rehabilitation
University of Pittsburgh School of Medicine

Introduction: Pediatric traumatic brain injury (TBI) is a leading cause of death and disability in children. Previous studies link greater childhood adversity to poorer neurobehavioral outcomes after TBI. However, a reliable clinical or research tool to comprehensively measure adversity in children with TBI is lacking. The Psychosocial Assessment Tool (PAT) is a caregiver-report measure of psychosocial risk in pediatric patients. The PAT has demonstrated reliability and validity in various pediatric populations, including cancer, sickle cell disease, irritable bowel disease, and headache, but has not yet been studied in TBI. We evaluated the reliability of the PAT in a sample of children with TBI and characterized their psychosocial risk relative to children with orthopedic injuries (OI).

Hypotheses: The PAT will demonstrate high internal consistency and test-retest reliability in children with TBI during the acute period after injury 2; Children with TBI will have higher PAT scores at 6-months post-injury relative to their acute PAT scores 3; Children with TBI will have higher PAT scores at 6-months post-injury relative to children with OI.

Methods: Children aged 3-18 years who were hospitalized overnight for complicated mild to severe TBI (n=96) or OI (n=36) participated in a longitudinal observational study. Caregivers completed the PAT at the time of injury (baseline) and 6-months post-injury. We compared injury groups on the total PAT scores at 6-months using *t*-tests. We also compared the PAT scores from baseline to 6-months within the TBI group using a paired *t*-test. We categorized participants into the Pediatric Psychosocial Preventative Health Model (PPPHM) tri-level risk system based on total scores. We compared the TBI and OI groups on their risk levels at 6-months using a chi-square test. Lastly, we used the Kuder-Richardson Formula 20 and Pearson's correlation coefficient to examine internal consistency and test-retest reliability, respectively, within the TBI group.

Results: There were no significant group differences in PAT total scores (TBI=0.85±0.81; OI=0.59±0.49; t=1.56; p=0.123; Cohen's d=0.37) or risk level distributions (TBI [universal risk n=35, targeted risk n=9, clinical risk n=5]; OI [universal risk n=25, targeted risk n=2, clinical risk n=1]; χ^2=3.307; p=0.191; Cramer's V=0.21) at 6-months. There were also no significant differences between the TBI PAT scores from baseline (0.78±0.57) to 6-months (t=1.06; p=0.297; Cohen's d=0.19). Internal consistency was strong for the total PAT score (KR-20=0.83) and moderate-strong for all subscale scores (KR-20=0.66-0.81), except family structure (KR-20=0.51) and family beliefs (KR-20=0.39), which were weak. Test-retest reliability was strong (r=0.87; p<0.001) between the PAT scores at baseline and 6-months within the TBI group.

Conclusions and Significance: The results show small, non-significant differences between TBI and OI PAT scores and risk level distributions 6-months post-injury. The results require replication in larger samples to better assess the effect that injury group may have on psychosocial risk. With demonstrated internal consistency and test-retest reliability, the PAT is a reliable clinical and research tool to characterize psychosocial risk in pediatric TBI. Upon testing its validity against other established measures related to adversity in a larger sample, the PAT can be used to identify children with TBI at risk for psychosocial problems.

Research/Grant Support: This study was supported by the National Institute for Child Health and Human Development (NICHD) grant K01HD097030 and R03HD101746, the UPMC Rehabilitation Institute Pilot Award, and the CHP Scientific Program funds.

Abstract 53

SUBTLE DIFFERENCES IN TARGETED TEMPERATURE MANAGEMENT IMPACT BEHAVIORAL AND NEUROPATHOLOGICAL OUTCOMES AFTER ASPHYXIAL CARDIAC ARREST: THE ROLE OF SEX

Kline K,*[1,2] Russell A,*[1,2] Akkineni S,[1,2] Dimeo E,[1,2] Gober I,[1,2] Stezoski J,[1,3] Drabek T,[1,4,5] Kochanek P,[1,3] Wagner A[1,2,4]

[1]Safar Center for Resuscitation Research
[2]Department of Physical Medicine & Rehabilitation
[3]Department of Critical Care Medicine
[4]Center for Neuroscience
[5]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

Introduction: Cardiac arrest (CA) survivors have lasting neurological impairments due to anoxic brain damage. To date, targeted temperature management (TTM) is the only recommended intervention to improve neurological outcomes after out-of-hospital CA. According to clinical guidelines, TTM with a target of 32°C-36°C is advocated for all post-resuscitation CA patients. In experimental male rats, a 1°C decrease from normothermia (NT) decreases cell death and neuroinflammation. While TTM is extensively used clinically, preclinical studies are needed to investigate its impact on lasting behavioral outcomes. There is also a large gap in our understanding of the differences between male and female responses post-CA. We aim to investigate how TTM impacts behavioral outcomes and hippocampal pathology after ACA including assessment of sex differences.

Hypothesis: TTM post-ACA will improve survivorship and promote behavioral recovery in a sex-dependent manner compared to maintenance at NT post-ACA.

Methods: Adult Sprague-Dawley rats underwent either sham or 5-min no-flow ACA. Temperature was controlled for 18 hours post-ACA. NT males (n=26) and females (n=36) were held at 37-38°C. TTM males (n=8) and females (n=3) were held at 36-37°C. All rats underwent neurological deficit (ND) assessment and behavioral testing over 14-days. Motor behaviors including coordination (beam walk), balance, and gross motor testing (open field; OF) were assessed. Hippocampal CA1 cell death was quantified using H&E and Fluorojade C (FJ) labeling. Immunohistochemistry for microglia was quantified in CA1 to measure inflammation.

Results: TTM males had higher survival rates than NT males (88% vs 23%). 2 days post-ACA, NT males had worse ND scores than TTM males (NT=148%; TTM=79%; $p<0.05$), with no differences at any other timepoint. ACA rats had poorer balance ($p<0.05$), traversed a beam more slowly ($p<0.05$) and traveled less distance in the OF assay ($p<0.05$) than shams. TTM males balanced longer and traversed the beam more quickly than NT males, suggesting improved balance and coordination. There was no difference between NT and TTM males in OF distance. CA1 cell death in shams was negligible (H&E and FJ), as expected. There was a reduction in cell death (H&E) in TTM males (30%) vs. NT males (43%). There was also a decrease in FJ+ cells (NT=0.030cells/μm; TTM=0.026cells/μm). Studies are ongoing for similar behavioral and pathological comparisons of NT and TTM in females post-ACA. Our preliminary data show that TTM females have higher survival rates than NT females (63% vs 37%).

Conclusions: Overall, we identified small improvements in motor performance with TTM post-ACA in males. This is accompanied by a reduction in CA1 cell death with TTM after ACA. Ongoing data collection will allow us to examine if these patterns hold true in females. Our data suggest that TTM promotes modest neurorecovery post-ACA.

Significance: This study provides a better preclinical understanding of how TTM may be used to model behavioral recovery post-ACA, allows is to explore sex differences, and should be a feature of translational survivor-based studies that focus on rehabilitation-relevant treatments.

Research/Grant Support: AHA 18TPA34170553; Lloyd Reback Family Gift; NS108386 (PI: A.K. Wagner, MD)

Abstract 54

ENVIRONMENTAL ENRICHMENT IMPROVES TRAUMATIC BRAIN INJURY-INDUCED BEHAVIORAL PHENOTYPE AND ASSOCIATED NEURODEGENERATION

Moschonas EH,[1,2,3] Vozzella VJ,[1,3] Cheng JP,[1,3] Bondi CO,[1,3] Kline AE[1,3]

[1]Physical Medicine & Rehabilitation
[2]Center for Neuroscience
[3]Safar Center for Resuscitation Research
University of Pittsburgh, Pittsburgh, PA

Introduction: Environmental enrichment (EE) after traumatic brain injury (TBI) facilitates the recovery of neurobehavior and cognition but how it does so is not well defined. A reasonable explanation is that EE attenuates deleterious secondary sequelae like neuroinflammation and oxidative stress.

Hypothesis: EE lessens the TBI-induced upregulation of N-methyl-D-aspartate receptors, subtype-1 (NMDAR1), which in turn reduces neuroinflammation and oxidative stress markers like ionized calcium-binding adapter molecule 1 (Iba1), 3-nitrotyrosine (3-NT), and 4-hydroxynonenal (4-HNE), respectively, vs. standard (STD) housing and leads to improved motor and cognitive performance.

Methods: Forty-eight adult male rats received either a cortical impact of moderate-to-severe injury (2.8 mm deformation at 4 m/s) or sham surgery and then were housed in either EE or STD conditions. Motor (beam-balance/walk) and cognition (spatial learning and memory retention) were assessed using well-validated beam and Morris water maze tasks on post-surgery days 1-5 and 14-20, respectively. Immunohistochemistry was performed on tissue obtained at post-surgery day 21.

Results: EE decreased hippocampal NMDAR1, Iba1, 3-NT, and 4-HNE, and facilitated motor recovery and the acquisition of spatial learning and memory relative to STD-housed controls ($p<0.05$).

Conclusions and Significance: These novel findings support the hypothesis and elucidate potential mechanisms for the benefits conferred by EE after TBI.

Research/Grant Support: Supported in part by NIH grants 1 R56 NS113810, 5 R01 NS084967, 1 R01 NS121037 (AEK) and NS094950, NS099683, NS110609 (COB).

Abstract 55

SEX-LINKED DIFFERENCES IN STRIATAL PROTEIN EXPRESSION FOLLOWING EXPERIMENTAL ASPHYXIAL CARDIAC ARREST

Padamati S,*[1,2] Gober I,*[1,2] Russell A,*[1,2] Kline K,[1,2] Stezoski J,[1,3] Drabek T,[1,4,5] Kochanek P,[1,3] Wagner A[1,2,4]

[1]Safar Center for Resuscitation Research
[2]Department of Physical Medicine & Rehabilitation
[3]Department of Critical Care Medicine
[4]Center for Neuroscience
[5]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

Introduction: Cardiac arrest (CA) is a leading cause of death in the United States. Although survival rates have increased recently due to improved resuscitation techniques, survivors still face chronic neurological disabilities resulting from hypoxic-ischemic brain injury (HIBI). The neurobehavioral deficits after CA have been understudied, and current treatments are often borrowed from traumatic (T)BI studies with no specificity to injury type or the sex of the patient. The nigrostriatal pathway is a largely over-looked neural network vulnerable to HIBI that relays information to the striatum via dopamine (DA) signaling. This DA release, and associated striatal synaptic plasticity in medium spiny neuron (MSN) connections, aids the striatum with control of consciousness, voluntary movement, and cognitive function. It is important to understand how these functions are disrupted after experimental asphyxial (A)CA.

Hypothesis: There will be differences in chronic (2-weeks post-ACA) striatal DAergic, GABAergic, and synaptic plasticity protein expression after ACA, and these differences will be associated with sex-specific behavioral deficits.

Methods: Adult male and female rats (N=12) received either sham or a 5-min no flow ACA. Acoustic startle response (ASR) and myoclonus were assessed throughout the 14-day study duration given their link to striatal-related behaviors and as a measure of sensorimotor function. Two weeks after surgery, brain tissue was harvested and the striatum was processed to examine pre-synaptic DA [DA Transporter (DAT)], post-synaptic DA [catechol-O-methyltransferase (COMT) and DA and cAMP-regulated phosphoprotein-32 (DARPP-32)], synaptic plasticity [post-synaptic density-95 (PSD-95)], and medium spiny neurons (GABA) [glutamate decarboxylase 65/67 (GAD65/67)]-relevant protein expression using western blot analysis.

Results: ACA males and females had an increased startle response ($p<0.05$) and more myoclonic events ($p<0.05$) vs. shams. At later time points post-injury (day 11 and 13), ACA females had a decreased startle response and number of myoclonic events ($p<0.05$) vs. ACA males, although deficits in females did not return to sham performance levels. Our preliminary data showed that DAT expression, a protein that removes DA from the synapse, was higher in sham females than sham males, and ACA increases DAT expression in both sexes. There was a more robust increase in DAT expression in ACA females than ACA males vs. sham counterparts. COMT, an enzyme that degrades catecholamines, increased in males after ACA; however, there was no change in ACA females. There was no effect of ACA or sex on DARPP-32 expression, a key DA dependent cell signaling molecule regulating GABAergic outflow. PSD-95 was also higher in sham females than sham males. In males, ACA decreased PSD-95 expression vs. shams ($p<0.05$). GAD65/67 expression did not change after ACA in both sexes.

Conclusions: We found that females had better behavioral neurorecovery after ACA than males. We also identified innate and injury-induced sex-linked differences in striatal protein expression. Further work will explore how sex-linked differences in protein expression may associate with behavioral recovery after ACA.

Significance: Our findings support the need to develop medically precise ACA-targeted rehabilitation and treatments that account for sex differences in the striatum.

Research/Grant Support: AHA 18TPA34170553; Lloyd Reback Family Gift; NS108386-01A1(PI: A.K. Wagner, MD)

Abstract 56

NEUROBEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF TRAUMATIC BRAIN INJURY IN SPONTANEOUSLY HYPERTENSIVE RATS

Ranellone T,[1] Race N,[1] Moschonas E,[1] Reddy R,[1] Rennerfeldt P,[1] Toader M,[1] Wehrmeyer B,[1] Cheng J,[1] Stezoski J,[2] Kochanek P,[2] Kline A,[1] Bondi C[1]

[1]Department of Physical Medicine and Rehabilitation
[2]Department of Critical Care Medicine
Safar Center for Resuscitation Research, University of Pittsburgh

Introduction: Approximately 2.8 million people sustain a traumatic brain injury (TBI) yearly, with many experiencing long-term disabilities often exacerbated by pre-existing comorbidities. In the US, an estimated 50% of adults suffer from hypertension, which may lead to heart attacks, strokes, and premature death. There is a critical need to investigate TBI in hypertensive rats to better characterize neurological, physiological, and cognitive impairments, and to enhance clinical translatability. This study explores the effects of TBI on Spontaneously Hypertensive Rats (SHR) via a battery of behavioral assays, such as motor coordination/balance, hippocampal-dependent learning, sustained attention, and anxiety-like symptoms.

Hypothesis: Hypertension will exacerbate TBI-induced neurobehavioral deficits.

Methods: A pathophysiological study was conducted on Spontaneously Hypertensive Rats (SHR) compared to normotensive Wistar Kyoto (WKY) rats. Rats were assigned to receive a controlled cortical impact (CCI; 2.8mm cortical deformation depth, 4 m/s) or a sham injury. Both sham and TBI rats underwent the Beam Walking Task (motor) as well as the Morris Water Maze (MWM; spatial learning). Open field testing (OFT) was performed to examine anxiety, while Shock Probe Defensive Burying Task (SPDB) inspected passive/active coping behavior. 3-Choice Serial Reaction Time Task (3-CSRT) was used in a separate cohort of SHR rats to examine sustained attention and distractibility. Before surgery, rats underwent 3-CSRT training for 3-5 weeks to a 2 s cue in operant chambers. Starting on post-op day 14, rats underwent 10 days of 3-CSRT re-testing. Data were analyzed using ANOVAs followed by Newman Keuls post hoc tests.

Results: Adult male SHR TBI rats exhibit 10% higher heart rate and 30% higher mean arterial pressure than injured WKY counterparts. Moreover, injured SHR rats display impaired beam-walking capability, as well as reduced spatial learning compared to SHR shams ($p<0.05$). SHR TBI rats presented more immobility and anxiety-like behavior in comparison to SHR shams, seen as reduced center area exploration in OFT and less time approaching and burying the shock probe in SPDB ($p<0.05$). SHR TBI rats also displayed markedly reduced percent accuracy and increased omissions during 3-CSRT suggesting impairments in sustained attention ($p<0.05$).

Conclusions: Results indicate that TBI in rats with a hypertensive phenotype renders neurobehavioral deficits across a variety of behavioral tasks.

Significance: Understanding the impact that underlying conditions such as hypertension may have on TBI pre-clinically is critical to further developing clinically-relevant therapies.

Grant Support: NIH NS110609 and Children's Hospital of Pittsburgh (COB)

Abstract 57

POSITIVE EFFECTS OF α7 NICOTINIC ACETYLCHOLINERGIC RECEPTOR MODULATION AND ENVIRONMENTAL ENRICHMENT ON SUSTAINED ATTENTION, CHOLINERGIC NEUROTRANSMISSION, AND SYSTEMIC INFLAMMATION AFTER CONTROLLED CORTICAL IMPACT INJURY

Reddy R,[1] Race N,[1] Moschonas E,[1] Rennerfeldt P,[1] Ranellone T,[1] Annas E,[1] Bertocchi M,[1] Cheng J,[1] Carlson S,[2] Dixon E,[2] Kline A,[1] Bondi C[1]

[1]Department of Physical Medicine and Rehabilitation
[2]Department of Neurological Surgery
Safar Center for Resuscitation Research, University of Pittsburgh

Introduction: Traumatic brain injury (TBI) is a leading cause of cognitive disability worldwide. Post-TBI attentional impairments lack proven treatments and can result from cholinergic dysregulation, which suggests that pharmacological strategies that amplify activation of acetylcholine (ACh) receptors may ameliorate behavioral deficits. To more closely mimic the clinical setting, combining a pharmacological therapy with noninvasive rehabilitation (i.e., enriched environment, EE, housing) may prove to be an efficient approach for cognitive recovery.

Hypothesis: Chronic administration of NS-1738, a novel α7 nicotinic ACh receptor (α7-NAChR) and type-I positive allosteric modulator (PAM), will improve sustained attention post-TBI, alone and in combination with EE. Blocking α7-NAChRs with methylycaconitine (MLA) will attenuate the beneficial effects of NS-1738, further confirming its mechanism of action.

Methods: Adult male rats were trained in the 3-choice serial reaction time task (3-CSRT), reaching stable pre-injury baselines prior to moderate severity right parietal controlled cortical impact (CCI) or sham injury. Rats were randomized to NS-1738 (3 mg/kg) or vehicle (1 mL/kg saline) starting post-injury day (PID) 1 and continued daily [subacute (7d); chronic (28d)]. The chronic paradigm co-investigated with daily environmental enrichment (EE; 6h/d), and subgroups were also subjected to daily α7-NAChRs blockade via MLA (3 mg/kg) injections. 3-CSRT retrials occurred on PID 14-24 with sacrifice PID 30. Medial prefrontal cortex (mPFC) Western blots assessed cholinergic markers [acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and α7-NAChR]. Microarray analysis examined serum inflammatory gene expression. Statistical analysis utilized ANOVAs with Newman-Keuls post hoc tests.

Results: TBI rats exhibited impaired 3-CSRT sustained attention versus shams ($p < 0.05$), which was improved by chronic NS-1738 ($p<0.05$) but not by subacute NS-1738 ($p > 0.05$) treatment. Moreover, NS-1738+EE rendered an additive effect on lowering omissions and improved inflammatory markers ($p < 0.05$) including TREM-1 (triggering receptor expressed on myeloid cells-1) and IL-1 RA (interleukin-1 receptor antagonist). TBI decreased mPFC ChAT and AChE ($p < 0.05$) with partial restoration by subacute NS-1738. TBI groups that also received MLA demonstrated a reinstatement of performance deficits, as hypothesized.

Conclusions: Our findings support benefits of α7-NAChR type-I PAM and/or EE treatment after experimental TBI in sustained attention, cholinergic neurotransmission, and systemic inflammation.

Significance: Enhancing cholinergic transmission after TBI may be beneficial for neurobehavioral recovery.

Grant Support: NIH NS110609 and Children's Hospital of Pittsburgh (COB)

Abstract 58

sgp130-FC AS A TREATMENT FOR BEHAVIORAL AND NEUROLOGICAL DEFICITS FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

Ujhazy J,*[1,2] Russell A,*[1,2] Mandava A,[1,2] Gober I,[1,2] Nasser L,[1,2] Kochanek P,[1,4] Wagner A[1,2,3]

[1]Safar Center for Resuscitation Research
[2]Department of Physical Medicine & Rehabilitation
[3]Center for Neuroscience
[4]Department of Critical Care Medicine
University of Pittsburgh

Introduction: Traumatic brain injury (TBI) poses a significant risk of permanent disability or death. Following TBI, a systemic cellular immune response triggers inflammatory cascades in the brain. There currently exist no definitive post-acute therapeutics to improve TBI recovery. Many clinical and preclinical studies have found increased interleukin 6 (IL-6) levels post-TBI, making it a potential therapeutic target. IL-6 can signal through either a *classical*-signaling cascade, which leads to anti-inflammatory effects, or through *trans*-signaling, which promotes inflammation and contributes to various CNS pathologies. sgp130-Fc inhibits the *trans*-signaling pathway and thus has the potential to be used as an anti-inflammatory therapeutic.

Hypothesis: Treatment with recombinant sgp130-Fc chimera protein (sgp130-Fc) will decrease CCI-induced behavioral deficits and neural damage after TBI in rats.

Methods: Adult male Sprague-Dawley rats (n=40) were randomly assigned to four experimental groups: 1) Sham + vehicle (VEH) (n=9); 2) Sham + sgp130-Fc (n=9); 3) CCI + VEH (n=9); and 4) CCI + sgp130-Fc (n=10). CCI was delivered to the right hemisphere using a 6mm flat impactor (2.8 mm impact depth at 4 m/s); sham rats underwent all surgical procedures minus the CCI. sgp130-Fc (R&D Systems) was administered intraperitoneally (i.p.) on days 1, 4, 7, 10, 13, 16, and 19 post-injury (DPI) during the 21-day study period; VEH-treated rats received i.p. phosphate buffered saline injection on the same schedule. Beam walk and beam balance behavioral tests assessed motor function. The Morris Water Maze (MWM) assessed spatial learning and memory. Cortical lesion volumes were quantified using image analysis at 21 DPI.

Results: CCI impaired motor function on the beam walk and beam balance assays in that CCI animals balanced for shorter periods of time ($p<0.05$) and took longer to traverse the beam walk ($p<0.05$) vs. shams. sgp130-Fc treatment did not improve these motor deficits after CCI. CCI also impaired spatial learning acquisition in the MWM, wherein CCI animals displayed inefficient non-spatial and thigmotaxic swim patterns more frequently than shams likely resulting in increased latency to platform ($p<0.05$). sgp130-Fc treatment reduced day 18 platform acquisition latency vs. CCI+VEH ($p<0.05$). CCI+sgp130-Fc rats also showed increased spatially-oriented swim strategies vs. CCI+VEH. Lastly, at 21 DPI, sgp130-Fc-treated rats also exhibited decreased lesion volume vs. CCI+VEH rats ($p<0.05$).

Conclusions: We report that sgp130-Fc is an effective treatment for cognitive deficits after CCI in rats. The improvements in spatial learning were associated with decreased neural damage (lesion volume) after sgp130-Fc treatment. By inhibiting the IL-6 *trans*-signaling pathway associated with immune response and inflammation, sgp130-Fc may reduce neuroinflammation and thus counteract a central mechanism of CNS pathology.

Significance: sgp130-Fc shows clinical potential in reducing behavioral and neurological deficits after TBI. Further studies should examine dose, timing, and confirm mechanism.

Research/Grant Support: UPMC Rehabilitation Institute (PI: A.K. Wagner, MD) and Summer Undergraduate Research Award (Anisha Mandava)

Abstract 59

MUSIC AS A NOVEL AND NON-INVASIVE REHABILITATIVE PARADIGM TO PROMOTE MOTOR, AFFECT, AND COGNITIVE RECOVERY AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Vozzella V,[1,3] Moschonas E,[1,3] Cooper I,[1,3] Rennerfeldt P,[1,3] Reddy R,[1,3] Ranellone T,[1,3] Cheng J,[1,3] Jarvis J,[1,3] Fink E,[2,3] Bondi C,[1,3] Kline A[1,3]

[1]Physical Medicine & Rehabilitation
[2]Pediatric Critical Care Medicine
[3]Safar Center for Resuscitation Research
University of Pittsburgh, Pittsburgh, PA

Introduction: Traumatic brain injury (TBI) is a leading cause of disability worldwide. Pharmacological interventions to treat TBI have not successfully translated to the hospital, thus strongly advocating for the need to assess novel, safe, nonpharmacological strategies. Music may hold an untapped potential for improving neurobehavior after TBI as clinical reports in CNS-injured adults showed that music-based interventions improved cognitive and emotional functioning. Basic science data in non-TBI rats also show that music enhances cognition.

Hypothesis: Providing classical music to adult rats after TBI will ameliorate TBI-induced deficits in cognition and neurobehavior.

Methods: Adult male rats (n=6 per group) received a controlled cortical impact of moderate severity (2.8 mm impact at 4 m/s) or sham injury and 24 h later were randomized to classical music or ambient room noise for 3 h/day from 19:00-22:00 for 32 days (last day of behavior). Motor (beam-walk), cognitive (acquisition of spatial learning and executive function), anxiety-like behavior (evaluated via open field and shock probe defensive burying tasks), as well as histopathology (lesion volume), neuroplasticity (BDNF) and neuroinflammation/plasticity (Iba1, CD-68, and CD-163) were assessed. The data were analyzed by ANOVAs and Newman-Keuls post-hoc tests.

Results: Music improved motor, cognitive, and anxiety-like behavior vs. no music. Music also reduced cortical lesion volume and increased hippocampal BDNF expression and markers of neuroplasticity vs. no-music.

Conclusions and Significance: These preliminary findings support the hypothesis and provide strong support for music as a potential rehabilitative therapy for experimental TBI and perhaps clinically as well. On-going studies are filling in the current groups and evaluating the potential efficacy of music intervention in pediatrics of both sexes.

Research/Grant support: Supported in part by NIH grants 1 R56 NS113810, 5 R01 NS084967, 1 R01 NS121037 (AEK) and NS094950, NS099683, NS110609 (COB)

The Safar Center for Resuscitation Research gratefully acknowledges the support of our sponsors:

Dr. Ake N. Grenvik Endowed Chair in Critical Care Medicine
Department of Anesthesiology & Perioperative Medicine
Department of Critical Care Medicine
Department of Emergency Medicine
Department of Neurological Surgery
Department of Physical Medicine & Rehabilitation
University of Pittsburgh School of Medicine
UPMC
Peter M. Winter Institute for Simulation, Education and Research
S. William Stezoski Family
Allan Braslow and Virginia Klunder
Children's Neuroscience Institute | UPMC Children's Hospital of Pittsburgh

Safar Symposium Planning Committee

The 19th Annual Safar Symposium is hosted by Patrick M. Kochanek, MD, MCCM, director of the Safar Center for Resuscitation Research, and Aman Mahajan, MD, PhD, MBA, chair of the Department of Anesthesiology and Perioperative Medicine. Committee members are:

Christa Brown, MA
Clifton W. Callaway, MD, PhD
Robert S.B. Clark, MD
C. Edward Dixon, PhD
Anthony E. Kline, PhD
John O'Donnell, DrPH, CRNA
Paul E. Phrampus, MD, CPE
Amy K. Wagner, MD
Yan Xu, PhD

University of Pittsburgh

UPMC | CHILDREN'S HOSPITAL OF PITTSBURGH

SAFAR CENTER FOR RESUSCITATION RESEARCH
UNIVERSITY OF PITTSBURGH

Established in 1994
WISER
Education and Simulation Improving Healthcare

www.Safar.Pitt.edu