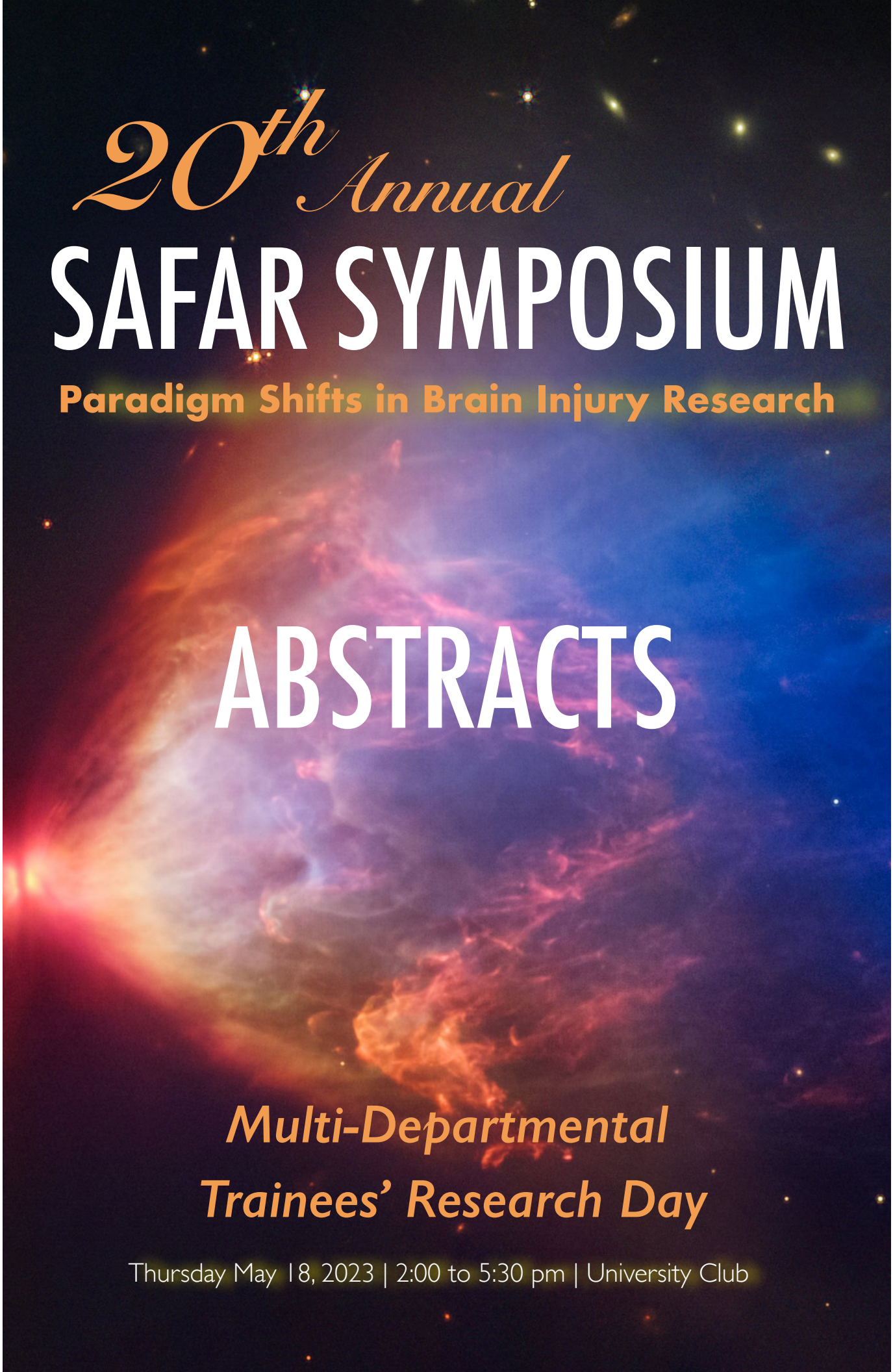

20th Annual SAFAR SYMPOSIUM

Paradigm Shifts in Brain Injury Research

ABSTRACTS

Multi-Departmental
Trainees' Research Day

Thursday May 18, 2023 | 2:00 to 5:30 pm | University Club

Table of Contents

DEPARTMENT OF ANESTHESIOLOGY AND PERIOPERATIVE MEDICINE

Oral Abstract O1

MODULATION OF NEUROPATHIC PAIN IN MULTIPLE SCLEROSIS BY SPINAL MICROGLIA AND SPHINGOSINE-1-PHOSPHATE RECEPTOR S1PR1

Lamerand SR,[1,2] Deshpande R,[1] Anumolu A,[1] 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 at University of Pittsburgh, Pittsburgh, PA

Introduction: ***1)*** *The sphingosine-1-phosphate receptor type 1 (S1PR1)* is an emerging target for the treatment of persistent pain. The S1PR agonist/functional antagonist fingolimod, an FDA-approved disease modifying agent for multiple sclerosis (MS), reduces pain-like behaviors in models of inflammatory and neuropathic pain. We reported that systemic fingolimod reduced mechanical allodynia in the EAE model of MS through an S1PR1-dependent mechanism. The current studies investigate the anatomical sites of action of S1PR1 agents. ***2)*** *Widespread microglia activation* is associated with MS. Numerous studies suggest that microglia mediate hypersensitivity in multiple models of peripheral neuropathic pain. The current studies investigate the contribution of microglia in the EAE model of central neuropathic pain.

Hypothesis: ***1)*** Spinal S1PR1 agonist actions of fingolimod and ***2)*** microglia mediate multiple sclerosis associated neuropathic pain (MSNP).

Methods: C57Bl/6 mice aged 10-12 weeks. **MSNP induction.** EAE induction included two subcutaneous injections on day 0 and day 7 -- one to each thigh, and each containing 100µg MOG_{35-55} plus 50µL of complete Freund's adjuvant (4mg MTB/mL; CFA), but neither containing pertussis toxin. **Behavioral testing.** Observer was blind to experimental treatment. *Mechanical sensitivity*: Hindpaw withdrawal threshold to plantar application of von Frey filaments. *Cool sensitivity*: Hindpaw withdrawal duration to plantar application of a drop of acetone. *Spontaneous /affective pain*: Three-chamber conditioned place preference paradigm. **Pharmacological intervention.** Random group assignments. Mice were injected with fingolimod (3nMol), SEW2871 (2nMol), NIBR0213 (3nMol), fingolimod + NIBR0213, or vehicle via the intrathecal route (5µL, i.t.). First injection was given on day 18/19, and then a second injection was given using a cross-over design on day 23/24. **Microglia Depletion:** *Localized microglia depletion*: i.t. injection of liposome-encapsulated clodronate (LEC,0.05mg/10µL) on day 15. *Global CNS depletion*: PLX3397 was administered either by oral gavage (92.5mg/kg/day x 7 days) from day 21-26 or in chow (600ppm x 10 days) from day 12-18.

Results: MOG+CFA injections caused mechanical and cold hypersensitivity that lasted weeks to months, while control CFA injection caused hypersensitivity that resolved within 15 days of the first MOG injection. Intrathecal fingolimod and SEW2871, but not NIBR0213, vehicle, or FTY720+NIBR0213 attenuated mechanical allodynia and cold hypersensitivity in EAE. Intrathecal fingolimod did not produce conditioned place preference, suggesting no effect on the spontaneous/affective components of pain. Spinal microglia depletion with i.t. LEC did not change cold hypersensitivity but did attenuate mechanical hypersensitivity with a peak effect at 72-hours post injection. Although global CNS depletion with PLX3397 (oral gavage) did not change hypersensitivity, PLX3397 (chow) prevented exacerbated cold hypersensitivity at day 21.

Conclusions: 1) Fingolimod engages spinal S1PR1 agonism to reduce MSNP. 2) Spinal microglia maintain mechanical allodynia in EAE. Global depletion of microglia may mask the antiallodynic effects of localized depletion due to depletion of anti-inflammatory microglia at supraspinal levels.

Significance: These results point to spinal S1PR1 and microglia as targets for future pharmacotherapy of multiple sclerosis-associated neuropathic pain.

Support: R01NS112632 and R01NS45954 (BKT), T32NS073548 and F31NS125974 (SRL)

Abstract 1

MAST CELL CONTRIBUTION TO PELVIC TACTILE ALLODYNIA AND SENSITIZATION OF PARABRACHIAL NUCLEUS NEURONS IN A MOUSE MODEL OF ENDOMETRIOSIS PAIN

Acharya S,[1] Prasoon P,[1] Taylor B,[1,2,3] Roman K[1]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine
[2]Pittsburgh Center for Pain Research, [3]Pittsburgh Project to end Opioid Misuse, Pittsburgh, PA

Introduction: Endometriosis (EM) impacts approximately 10% of women globally (Zondervan et al., 2020). Human EM lesions contain activated mast cells (MCs) (Matsuzaki et al., 1998) and elevated vascular endothelial growth factor (VEGF) (Rein et al., 2009). MCs in human lung tissue express VEGF (Marcella et al., 2021). Also, noxious colorectal stimulation increases neuronal activity in the parabrachial nucleus (PBN) (Dunckley, 2005); however, it is unclear how MCs and the PBN contribute to EM-associated chronic pelvic pain (EM-CPP)

Hypothesis: Mast cell activity and PBN sensitization drive pelvic tactile allodynia in EM mice.

Methods: C57BL/6J (B6) donor mice (6-weeks old) received a subcutaneous injection of estradiol benzoate (EB; 10µg) and 4 days later each uterine horn was excised, placed in Hank's Balanced Salt Solution (HBSS), and minced. Recipient mice received an intraperitoneal injection of either HBSS (500µl; "Shams") or HBSS+minced uterine horn (500µl; "EM mice") extracted from donors. To assess pelvic tactile allodynia in EM mice, von Frey (vF) was conducted before (baseline) and 7, 14, 21, and 28 days after tissue injection. We then administered the MC stabilizer ketotifen (Keto; 4.5mg/kg) or 0.9% saline on day 28 and vF thresholds were assessed at 3, 9, 18, and 36 hrs. In a dose response study of VEGF, we infused intrauterinely either saline (10µl) or VEGF (0.1pg, 1pg, 10pg, and 100pg) on days 1, 4, and 7 after baseline and vF tested from day 7 to 56. In a separate experiment, to determine whether EM sensitized the PBN to pelvic stimulation, the PBN from Shams and EM mice was intracranially injected with an AAV9-GCamp6s creindependent virus and implanted with a fiber photometry (FP) probe on day 14. Then we recorded changes to neuronal activity after repeated application of the 6g vF filament to the pelvic region on day 28.

Results*:* We evaluated the effect of Keto or saline on mechanical hypersensitivity in Sham control or EM mice. In Sham, neither saline (n=6) nor Keto (n=7) changed mechanical thresholds at any timepoint. In EM mice, Keto (n=8) but not saline (n=7) reversed hypersensitivity at the 9, 18, and 36 hr. timepoints ($p<0.05$). Mice that received infusion of VEGF at 0.1pg (n=4) developed mechanical hypersensitivity that lasted 21 days ($p<0.05$), while mice that received VEGF at 1pg (n=3), 10pg (n=4), or 100pg (n=3) developed pelvic tactile allodynia that lasted at least 4 weeks ($p<0.05$). Stimulus-evoked neuronal activity in the PBN remains unchanged in Sham control mice (n=3), but increased in EM mice (n=3, $p<0.05$).

Conclusions: 1) MC activity contributes to pelvic tactile allodynia in EM mice, 2) intrauterine infusion of VEGF induces pelvic tactile allodynia and 3) neuronal sensitization in the PBN is associated with pelvic tactile allodynia.

Significance: Mast cell stabilizers may serve as effective therapeutics to treat EM-CPP. Intrauterine infusions of VEGF may serve as a new model to study the development of pelvic tactile allodynia in mice.

Grant Support: NIDDK K01DK114395-06 (PI: Kenny Roman, PhD) and University of Pittsburgh Start-Up funds (PI: Kenny Roman, PhD and Co-PI: Bradley K. Taylor, PhD)

Abstract 2

SURGICAL INCISION ENGAGES ENDOGENOUS KAPPA OPIOID RECEPTOR (KOR) ACTIVITY IN SPINAL KOR-EXPRESSING NEURONS TO KEEP CHRONIC POSTSURGICAL PAIN IN REMISSION

Basu 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: Chronic postsurgical pain (CPSP) develops in millions of patients that undergo surgery. Prolonged opioid therapy is contraindicated, and other therapies lack sufficient analgesic efficacy. Clues for future approaches to treat CPSP may come from a better understanding of the endogenous mechanisms that cause and inhibit CPSP. To this end, our studies employ a latent sensitization (LS) model of CPSP. LS is a silent, long-lasting sensitization of nociceptive neurons that is tonically masked by compensatory activity of inhibitory G-protein coupled receptors (Taylor and Corder, 2014), including kappa opioid receptor (KOR) (Basu et al., 2021).

Hypothesis: Surgical incision induces LS at KOR-expressing neurons in the dorsal horn of the spinal cord that is kept in remission by tonic inhibition of KOR on sensory or spinal neurons.

Methods and Experimental Design: ***1)*** To determine whether endogenous KOR inhibition of LS occurs at sensory or spinal neurons, we crossed $Pirt^{cre}$ mice with $Oprk1^{lox/lox}$ mice to create $Oprk1^{DRG-/-}$ conditional knockouts. We performed plantar incision in $Oprk1^{lox/lox}$ controls and cKO mice. 21 days later, we injected either long-acting (LY2456302, 10µg, i.t.) or short-acting (BT-3761, 30mg/kg, i.p.) KOR antagonists, and measured mechanical and heat hypersensitivity. ***2)*** To investigate the contribution of KOR-expressing neurons to LS with a chemogenetics approach, $Oprk1^{Cre}$ mice received intraparenchymal injections of AAV8-hSyn-$hM4D_{Gi}$, or AAV8-hSyn-mCherry (control) into L3-L4 dorsal horn. Three weeks after AAV injection, clozapine N-oxide (3mg/kg, i.p.) was injected 15 minutes before LY2456302.

Results: ***1)*** Both KOR agents (BT-3761 and LY2456302) reinstated mechanical and heat hypersensitivity in $Oprk1^{lox/lox}$ control mice. Sensory neuron-specific deletion of KOR did not change reinstatement, suggesting a more likely contribution of endogenous KOR activity on spinal neurons to keep LS in remission. ***2)*** Chemogenetic inhibition of KOR-INs prevented both acute and LY2456302-induced reinstatement of mechanical and heat hypersensitivity, indicating that these neurons are necessary for both acute and chronic postsurgical pain.

Conclusions: Surgical incision engages endogenous KOR activity in spinal KOR-expressing INs to keep CPSP in remission.

Significance: Therapeutic goals to treat CPCP include prevention of the acute-to-chronic pain transition either by: ***A)*** maintaining tonic KOR analgesic activity thus restricting LS to the remission stage, or ***B)*** by improving the drugs or factors that inhibit LS at the maintenance stage, thereby arresting LS.

Current/Future Directions: Ongoing studies will: ***1)*** conditionally delete KOR in the spinal cord using Lbx1-Cre transgenic mice; and ***2)*** optogenetically inhibit spinal KOR-INs. These studies will lead to a better understanding of CPSP in terms of endogenous opioid analgesia and the neural circuits that underlie LS.

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

Abstract 3

MECHANICAL AND HEAT PAIN SENSITIVITY DURING EXTENDED WITHDRAWAL FOLLOWING CHRONIC INTERMITTENT ETHANOL VAPOR IN MALE AND FEMALE C57BL/6J MICE

Brandner AJ,[1,2,3] Taylor BK,[1,2,3] Farris SP[1,2,3]

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

Introduction: Individuals suffering from alcohol use disorder (AUD) experience a myriad of symptoms during withdrawal, with one of the most debilitating being chronic pain. AUD is characterized as a chronic, relapsing medical condition where many acute withdrawal symptoms, including moderate to severe pain that persists up to 2-3 months.

Hypothesis: To understand the relationship between alcohol and pain better, we utilized our established mouse model of alcohol withdrawal-induced mechanical and heat hyperalgesia to gauge pain thresholds throughout extended withdrawal.

Methods: Male and female C57BL/6J mice underwent chronic intermittent ethanol vapor exposure for 4 weeks to elicit chronic alcohol withdrawal-induced pain (CAWIP) sensitivity. Mechanical and heat sensitivities were assessed using von Frey and Hotplate behavioral assays at 1, 2, 3, 7, 14, 21, 28, and 35 days following cessation of chronic alcohol exposure.

Results: In male animals, we found mechanical hyperalgesia peaked at 1-2 days after the final exposure to alcohol vapor and subsided after 3 days into extended withdrawal. No heat sensitivity was observed in male animals. Females experienced peak mechanical and heat hyperalgesia at 2-3 days and subsided after 3 weeks and 3 days into extended withdrawal, respectively. Our male mechanical sensitivity results are consistent in other models of chronic alcohol withdrawal-induced pain. However, we are the first to report mechanical sensitivity lasting up to 3-4 weeks in male and female mice. Additionally, naltrexone (3mg/kg, i.p.) administered at day 35 after cessation of chronic alcohol reinstated mechanical sensitivity in female mice.

Conclusions: Our studies demonstrate a direct effect of chronic alcohol exposure and the importance of biological sex on pain-like behavior during withdrawal. Constitutive Mu-opioid receptor activity masks pain during extended withdrawal that can be blocked with naltrexone in female mice, suggesting a molecular mechanism of CAWIP. These results may provide a pharmacological approach to treating CAWIP with respect to biological sex.

Significance: Alcohol withdrawal-induced pain is a debilitating condition in individuals with AUD. Our findings will aid in defining pharmacological mechanisms in individuals with AUD and chronic pain.

Research/Grant Support: We gratefully acknowledge the support of NIH/NIAAA, NIH/NIDA, and NIH/NINDS grants AA024836 (SPF), DA037621 (BKT), NS045954 (BKT), NS112632 (BKT), and NS073548 (AJB). We would also like to acknowledge support from Bridging Connections in Addiction Research (BCAR) at the University of Pittsburgh and the Pittsburgh Foundation.

Abstract 4

THE USE OF INTRAOPERATIVE NEUROMONITORING TO PREDICT POST-OPERATIVE DELIRIUM IN PATIENTS UNDERGOING MAJOR CARDIAC SURGERY: A RETROSPECTIVE STUDY

Tallapaneni P,[1] Al Ta'ani O,[1] Subramaniam K,[1] Chen Y,[1] Gorijala V,[1] Al Qudah A,[1] Vogt K,[1] Nadkarni N,[1] Kiselevskaya V,[1] Thirumala P,[1] Sadhasivam S[1]

[1]Department of Anesthesiology and Perioperative Medicine
UPMC

Introduction: Postoperative delirium (POD) can occur in more than 50% of elderly patients undergoing cardiac surgery, resulting in hospitalization and significant morbidity and mortality[1]. Thus, early detection and intervention during surgery can be critical[2]. However, evidence is unavailable regarding the ability of somatosensory evoked potentials (SSEP) to predict POD and insufficient evidence is available regarding the ability of intraoperative electroencephalogram (EEG) to predict POD[3].

Hypothesis: The aim of the study was to determine the diagnostic accuracy of intraoperative neuromonitoring (IONM) such as EEG and SSEP in predicting POD in patients undergoing major cardiac surgery.

Methods: Clinical data from 578 patients undergoing cardiac surgery with IONM in 2019-2021 were retrospectively reviewed. Delirium was assessed multiple times using the Intensive Care Delirium Screening Checklist (ICDSC). Patients with an ICDSC score greater than or equal to 4 were considered to have delirium. Significant IONM changes (either SSEP or EEG changes) were evaluated based on a visual review of SSEP and EEG data and documentation of changes during surgery.

Results: Of the 578 patients undergoing cardiac surgery with IONM, 126 had delirium (21.8%). Significant IONM changes were noted in 134 patients, out of which 49 patients had delirium (36.6%). In contrast, 444 patients had no IONM changes during surgery, out of which 77 (17.3%) patients had delirium. Univariate analysis showed patients who had an IONM change are 2.75 times more likely to experience postoperative delirium 2.75 (95% CI 1.79-4.22, p <0.001). Upon multivariate analysis adjusting for age, gender, and baseline IONM, IONM changes were significantly associated with delirium, OR: 2.65 (95% CI 1.71-4.11, p <0.001). Furthermore, intraoperative EEG and SSEP changes were associated with significant postoperative delirium (p-value: 0.009 and <0.001, respectively).

Conclusions: Abnormal IONM changes during cardiac surgery are associated with an increased risk of delirium and severe delirium.

Significance: Our findings offer a strong basis for future research and analysis of SSEP and EEG monitoring to predict, detect, and prevent postoperative delirium.

References:

1. Best Pract Res Clin Anaesthesiol. 2012 Sep;26(3):277-87.
2. Anesth Analg. 2020 Jun;130(6):1572-1590.
3. Anesth Analg. 2020 Sep;131(3):712-719

Abstract 5

THERMOGRADIENT TEST FOR *IN VIVO* ASSESSMENT OF PAIN IN RODENTS

Deakin T, Wei S, Rhoades R, Tillman TS, Tang P, Xu Y

Department of Anesthesiology and Perioperative Medicine
University of Pittsburgh School of Medicine

Introduction: Frequent use of opioids for acute management of pain has partially contributed to the opioid-misuse epidemic in the US. Development of new and non-opioid analgesics requires robust assessments of both pain and treatment efficacies. Classical pain evaluation tools in the pre-clinical setting depend on animal reflexive responses to noxious stimulations. Such threshold approaches do not correlate well with human pain conditions, leading to misinterpretation of animals' supraspinal reflex as pain. Here we present a new Thermogradient Test (TGT) as a high-throughput pain evaluation tool for thermal hyperalgesia. The method depends on animals' self-reporting of thermal sensitivity and thus eliminates investigators' subjectivity altogether.

Hypothesis: We hypothesize that by giving rodents the freedom of choice in a TGT set-up, we will be able to assess more clinically relevant thermal pain based on animals' ambulatory behaviors on the surface of varying temperatures.

Methods: We constructed a thermal gradient runway using a partially thermally insulated ½" aluminum plate with two ends separately controlled by Peltier heating and cooling units. The plate is divided by ~24-cm high black walls into four lanes of 136.8 cm long and 10 cm wide, so that four rodents can be tested simultaneously. Animals are recorded via top down low-light video and analyzed with AnyMaze software over 10-15 min.

Results: With the cold end of the plate set at 4.1°C and the hot end at 57.3°C, we created a linear thermal gradient, which was measured and fitted by Y = 0.389X + 4.100, where X is the distance in centimeters from the cold end and Y is the temperature in Celsius. We first tested the sex difference in thermal sensitivity with naïve male (n=8) and female (n=7) mice. The duration at each temperature as a function of distance from the cold end shows a pseudo-Gaussian distribution for both male and female mice. The peak of the Gaussian distribution is ~26-32°C for male mice and ~32-38°C for female mice. While male and female mice walked the same total distance (~35 m in 15 min) and spent an equal amount of time in the intermediate temperature segment (26.2-35.1°C), the time spent in the 5.6-23.2°C and 38.1-55.8°C temperature segments are distinctly difference, with males and females spending 2-3 times longer in the cold and hot segments, respectively. We next tested if globally knocking out α3 glycine receptor, which is believed to control the pain gate in the spinal dorsal horn, will change the thermal nociceptive sensitivity in the TGT. We found that while the Gaussian distribution of thermal preference is similar among genotypes, the glyra3$^{+/-}$ (n=13) and glyra3$^{-/-}$ (n=9) mice have a narrower thermal preference distribution than the wildtype (n=11) mice. Interestingly, the heterozygous glyra3$^{+/-}$ females showed significantly fast escaping behavior from the cold segments than the wildtype and knockout females.

Conclusions: Our preliminary data suggest that thermal preferences on a thermal gradient as displayed in the TGT can provide noninvasive assessment of thermal sensitivity and pain based on animals' intrinsic behaviors.

Significance: The new TGT method is more clinically relevant, allowing different pain states to be objectively evaluated based on thermal sensitivity and hypersensitivity with and without analgesic treatments.

Research/Grant Support: This work is supported by R01NS122830 to YX.

Abstract 6

INTRAOPERATIVE MANAGEMENT OF PERIPHERAL NERVE BLOCKS AND NEURAXIAL ANESTHESIA IN TOTAL JOINT REPLACEMENT: A RETROSPECTIVE COHORT STUDY

DeWalt J

Department of Anesthesiology and Perioperative Medicine
University of Pittsburgh School of Medicine

Introduction: Local anesthesia and neuraxial anesthesia are two commonly used techniques to provide analgesia during total knee and total hip arthroplasty. There are benefits and limitations to both approaches. Our research compared a group that received a peripheral nerve block (PNB) for analgesia compared to a group that received neuraxial anesthesia with intrathecal morphine. We compared intraoperative vasopressor support, post-operative nausea and vomiting (PONV), and peak-pain post-operative day 1 scores.

Hypothesis: We hypothesized that the group receiving neuraxial anesthesia would require more intraoperative pressor support, have a greater incidence of PONV, and would have pain scores that were not worse than the group that received PNBs.

Methods: This study was designed as a retrospective cohort study. The control group received a PNB of the local anesthetic bupivacaine and the experimental group received intrathecal morphine. 10 patients in the control group (PNB) and 14 patients in the experimental group (neuraxial anesthesia) were randomly chosen by the investigator of the original trial. We performed a review of each patient's record and extracted the following: to assess peak pain scores, we recorded the highest pain score the patient reported on post-operative day one. To assess intraoperative pressor support, we recorded whether the patient was placed on a phenylephrine infusion during the case. To assess PONV, we recorded any complaints of PONV and if the patient received any anti-emetics or anti-nausea medication. We recorded this data in Microsoft Excel, deidentified it, and then transferred it to IBM SPSS. We then analyzed the data in SPSS to arrive at our results.

Results: 12/14 patients in the neuraxial anesthesia group and 4/10 patients in the PNB group were placed on a phenylephrine infusion; the difference was statistically significant. Median peak pain scores for the neuraxial anesthesia group and PNB group were 7.0 and 6.0, respectively. The difference was not statistically significant. 7/14 patients in the neuraxial anesthesia group and 2/10 patients in the PNB group experienced PONV; however, the difference was not statistically significant.

Conclusions: Patients that received neuraxial anesthesia were more likely to require intraoperative vasopressor support than patients that received PNB. There were no significant differences in post-operative pain scores or incidence of PONV.

Significance: This project is significant because it sought to further define the impacts that two different anesthetic approaches have on hemodynamic management in total joint replacement.

Research/Grant Support: n/a

Abstract 7

WIRELESS HIGH-FREQUENCY PERIPHERAL NERVE STIMULATION FOR CHRONIC REFRACTORY MERALGIA PARESTHETICA: A CASE REPORT

Evankovich M, Marshall T, Yin Z, Chauhan G

Department of Anesthesiology and Perioperative Medicine
UPMC, Pittsburgh, PA

Setting: Academic Medical Center

Patient: 75 y/o male with refractory meralgia paresthetica

Case Description: Patient reported 8-year history of "painful numbness [and] burning" along the right lateral thigh post- total hip arthroplasty. He rated his pain as 8 out of 10, which decreased to a rating of 2 out of 10 with gabapentin use, but unwanted medication side effects motivated him to seek alternative treatment. He was diagnosed with meralgia paresthetica and offered neurolysis; however, after seeing a pain specialist, he agreed to a trial of a STIMWAVE peripheral nerve stimulator. After the implantation procedure, his pain reduced to 0 out of 10 and his quality of life improved, with better sleep and less somnolence.

Assessment/Results: The patient reported more than 80% improvement in his pain and associated symptoms at 12-month interval.

Discussion: Meralgia paresthetica is a condition caused by entrapment of the lateral femoral cutaneous nerve that leads to paresthesia along the anterolateral portion of the thigh. Because of advancements in neuromodulation, peripheral nerve stimulation (PNS) has been considered a new treatment option for meralgia paresthetica. Newer PNS technology targets peripheral nerves directly yet in a minimally invasive manner. We report a case in which a PNS device provided more than 12 months of complete pain relief in a patient with meralgia paresthetica and helped the patient avoid a neurolysis procedure.

Conclusion: With recent advancements, PNS can be used to treat meralgia paresthetica in an effective yet minimally invasive manner. As newer PNS technology becomes more familiar to physicians and pain specialists, it is likely to be used as a mainstay treatment for meralgia paresthetica.

Level of Evidence: 4

Abstract 8

EVALUATING POSITIVE-ALLOSTERIC-MODULATOR-INDUCED CONFORMATIONAL CHANGES OF α3 GLYCINE RECEPTOR

Folmsbee D,[1] Mukherjee E,[3] Ramasubramanian V,[3] Schechter J,[3] Sesha D,[3] Chen Q,[1] Wells M,[1] Jiang L,[1] Tillman T,[1] Tang P,[1,2] Xu Y,[1] Koes D[2]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine
[2]Department of Computational & Systems Biology, University of Pittsburgh School of Medicine
[3]Dietrich School of Arts & Sciences, University of Pittsburgh

Introduction: About 11-40% of US adults suffer from chronic pain, which is often treated with opioids. Due to frequent opioid misuse, alternative approaches to pain management are desperately needed. Recent studies have suggested that positive allosteric modulators (PAMs) of glycine receptors (GlyRs) are potential non-opioid analgesic alternatives.

Hypothesis: Tetrahydrocannabinol (THC), an active ingredient in marijuana, has been identified as a GlyR PAM. Early experiments discovered a series of molecules with potential affinity for the THC binding site, but their potency and efficacy as GlyR PAMs have not been fully explored. This study aims to explore the potency and efficacy of this series of molecules to better understand allosteric modulation of GlyR and to identify new, potentially better GlyR PAMs for pain treatment.

Methods: Weighted ensemble (WE) simulations were used to study the impact of modulator binding (potency) to the THC binding site and the induced conformational changes to the ion channel function (efficacy). A hydrated membrane patch containing an α3 GlyR structure with THC in the experimentally suggested binding pocket was created to test THC binding stability and analyze the induced conformational changes in the system. Subsequent molecular dynamics (MD) simulations of the α3 GlyR with other PAM candidates were carried out to determine the optimal interactions for potency and efficacy.

Results: The study provides insight into the structural understanding of GlyR modulation, including the stability of THC binding site and the conformational changes induced by binding. The MD simulations of other PAM candidates provide insight into interactions of GlyR modulation, to try and establish a correlation between potency and efficacy.

Conclusions: We have developed a process to computationally analyze both potency and efficacy of potential PAMs in the THC binding site. A more thorough understanding of the structural characteristics of the THC binding site will enable the discovery of more effective PAMS for the α3 GlyR.

Significance: This work looks to provide a targeted approach to identifying new GlyR PAMs for pain treatment and contribute to the development of alternative pain management strategies.

Research/Grant Support: T32GM075770 (PI: Yan Xu, PhD)

Abstract 9

NEUROPEPTIDE Y1 INTERNEURONS IN INFLAMMATORY PAIN CIRCUITS

Hellman A,[1,2] Nelson TS,[3] Taylor BK[1,2]

[1]Department of Anesthesiology and Perioperative Medicine, Center for Neuroscience, Pittsburgh
[2]Center for Pain Research, Pittsburgh Project to end Opioid Misuse, University of Pittsburgh
[3]School of Medicine, Pittsburgh, PA, [3] Department of Molecular Pathology, New York University

Introduction: Neuropeptide Y1 acts at the GRP-expressing subpopulation of interneurons (Y1-INs) to inhibit behavioral symptoms of neuropathic pain (Nelson et al, submitted). However, the contribution of Y1-INs to postsurgical inflammatory pain remains unknown.

Hypothesis: We used chemogenetics and conditional knockout mice to test the hypothesis that Y1-INs are necessary for acute and chronic postsurgical pain (CPSP), and determine the subpopulations.

Methods: Male and female *Npy1r*^{cre}, *Npff*^{cre}, and *Grp*^{cre} mice received hemilaminectomy for intraparenchymal injections (333ml) of AAV8-hSyn-DIO-hM4D$_{Gi}$ or AAV8-hSyn-DIO-mCherry control. Conditional knockout (*Npff*^{cre}::*Npy1r*$^{loxP/loxP}$ or *Grp*^{cre}::*Npy1r*$^{loxP/loxP}$) and chemogenetic mice underwent plantar incision of the skin and underlying muscle (4mm). Mice were tested for hindpaw withdrawal responses to mechanical (von Frey) and thermal (hotplate) stimuli during acute PSP (2-3 days post-surgery) and CPSP (21 days post-surgery, followed by reinstatement of hyperalgesia with naltrexone, NTX, 3mg/kg, s.c.). Clozapine N-oxide (3mg/kg, i.p.) was administered for chemogenetic inhibition. Viral expression at L3-L4 was confirmed with *in situ* hybridization.

Results: Incision produced mechanical and heat hypersensitivity that peaked at 2-3 days and resolved within 21 days, after which NTX but not vehicle produced robust reinstatement of hypersensitivity. Acute PSP: Chemogenetic inhibition or transgenic knockout of Y1-INs and NPFF-INs, but not GRP-INs, alleviated mechanical and heat hypersensitivity compared to control ($p<0.05$, two-way ANOVA, Bonferroni correction; n=8/group). CPSP: Inhibition or knockout of NPY1r and GRP prevented reinstatement of hypersensitivity ($p<0.05$), while NPFF only partially prevented reinstatement ($p<0.05$). n=8/group.

Conclusion: Y1-INs are necessary for both acute and chronic postsurgical pain. At the acute timepoint the NPY interneurons functionally contributing to mechanical and thermal hypersensitivity are exclusively from the dorsal horn, but both dorsal horn and dorsal root ganglion receptors contribute to chronic reinstatement. Preliminary data suggests that the NPFF subpopulation of Y1-Ins are necessary for acute pain-like behavior, and the GRP subpopulation of Y1-INs are necessary for chronic reinstatement. Further experiments are necessary to confirm this working hypothesis.

Significance: Our studies promote NPY1r neurons as a promising pharmacological target for the development of agonists for the treatment of acute and chronic postsurgical pain. Understanding the subpopulations and anatomical location of the Y1-INs functionally contributing to pain behavior will significantly improve the effectiveness of future clinical treatments.

Research/Grant Support: R01DA37621, R01NS45954 and R01NS62306 (BKT); T32GM075770 (AH); and F31NS117054, and F99NS124190 (TSN). Virginia Kaufman Pain Challenge (AH, TSN, and BKT)

Abstract 10

PREDICTORS OF HERNIA RECURRENCE AFTER CONCOMITANT LAPAROSCOPIC VENTRAL HERNIA REPAIR AND BARIATRIC SURGERY

Hlavin C,[1,3] Donohue J,[2] Mayer N,[2] Lu L,[3] Bernardi K,[3] Brown J,[3] Neal M,[3] Courcoulas A[3]

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

Introduction: Evidence is compelling for the safety and acceptable short-term outcomes of concomitant bariatric surgery (BS) and laparoscopic ventral hernia repair (LVHR). No studies examine the association of weight regain patterns on ventral hernia recurrence after simultaneous laparoscopic Roux-en-Y (LRNY) or sleeve gastrectomy (LSG) and LVHR.

Hypotheses: 1. Weight regain will be associated with higher rates of hernia recurrence. 2. Patient with more complex, larger hernias, and primary tissue repair will have higher rates of recurrence.

Methods: We performed a retrospective analysis of patients with concomitant LRNY or LSG and LVHR from 2013-2015. The impact of demographics, comorbidities, hernia characteristics, repair technique, and post-operative weight trajectory was assessed on the outcome of recurrence, diagnosed clinically or radiographically.

Results: We included 92 patients, stratified by recurrence (yes: N=32, 34.8%, no: N=60, 65.2%). Median follow-up was equivalent (58.0 months vs. 55.5 months, p=0.396). The recurrence group was older (median age 52.4 years vs. 48.7 years, p=0.035) with more cardiovascular disease (CVD) (87.5% vs. 63.3%, p=0.027) and prior ventral hernia repairs (18.8% vs. 3.3%, p=0.035). There was no difference in pre-operative BMI, defect size, hernia reducibility or use of mesh. Most (78.1%) recurred prior to achieving nadir weight. Decision tree analysis found an age threshold of 45.3 years for hernia recurrence where frequency increases from 16.1% to 44.3%. Patients >45.3 years had more CVD (83.6% vs. 48.4%, p=0.001) and hypertension (82.0% vs. 48.4%, p=0.002), but lower pre-surgery BMI (45.5 vs. 52.1, p=0.01).

Conclusions: Hernia recurrence after concomitant BS and LVHR is not associated with weight regain as most patients recur prior to reaching nadir weight; however, older patients with CVD and prior ventral hernia repairs have higher rates of hernia recurrence.

Significance: Concomitant BS and LVHR is effective as most patients do not have hernia recurrence; however, patients as weight regain is a considerable problem post-bariatric surgery, older patients with prior ventral hernia repairs and more medical comorbidities should be considered for a staged procedure.

Research/Grant Support: NIH T32GM075770 (PI: Yan Xu, PhD)

Abstract 11

LOSS OF NEURONAL MD-1 LEADS TO INCREASED LY6G EXPRESSION IN AN IMIQUIMOD MODEL OF PSORIASIS

Keller M,[1] Jones JD,[2] Albers KM,[2] Ritter Jones M[1]

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

Introduction: The immune response to a cutaneous inflammatory challenge requires the interaction of many cell types, which include immune cells, keratinocytes and sensory neurons. While the role of peptidergic sensory afferents, those that release neuropeptides, i.e., CGRP and Substance P, has been well studied, the role of nonpeptidergic sensory afferents, which contain a limited quantity of neuropeptides, remains elusive. In a recent study, nonpeptidergic neurons that express Mrgprd receptors have recently been shown to suppress mast cell activation, suggesting an immune suppressor function. Transcriptomic studies of sensory neurons have shown that myeloid differentiation 1, MD-1, a molecule associated with the innate immune response, is produced almost exclusively by Mrgprd-expressing sensory neurons. Studies of MD-1 have demonstrated that decreased MD-1 mRNA expression is associated with proinflammatory disorders such as lupus and obesity, suggesting an anti-inflammatory function. Interestingly, neuronal MD-1 expression increases robustly in the presence of neurturin, a member of the glial cell line-derived neurotrophic factor family. Neurturin supports nonpeptidergic neuron development and function and is implicated in the skin pathogenesis observed in the inflammatory murine imiquimod model of psoriasis. Therefore, this model was selected to investigate the role of neuronally expressed MD-1 in the cutaneous immune response.

Hypothesis: The loss of neuronal MD-1 leads to increased inflammation in the imiquimod model of psoriasis.

Methods: Imiquimod was applied daily to the ear and dorsal skin of neuronal MD-1 conditional knockout mice as well as littermate controls for 6 days. At the end of the treatment, the skin was evaluated for the level of inflammation using ear thickness and the psoriasis area and severity index (PASI) score. mRNA expression of select genes in the skin was also evaluated.

Results: After 6 days of imiquimod treatment, the ear thickness of the neuronal MD-1 knockout mice was thicker than the littermate controls, and the PASI score was increased. RTPCR mRNA assays showed that Ly6G, a molecule expressed on neutrophils, was increased. Histological evaluation also demonstrated an increase in the number of neutrophils present in the inflamed skin of the MD-1 knockout mice as compared to controls. These data suggest that neuronal MD-1 expressed in nonpeptidergic sensory neurons has a suppressor role in the immune response.

Conclusions: Loss of neuronal MD-1 promotes cutaneous inflammation by increasing neutrophils in the imiquimod psoriasis model. Further studies will delineate the mechanism of this interaction.

Significance: These data suggest a role for nonpeptidergic neurons in the evolution of the inflammatory response and may lead to approaches to facilitate resolution of inflammation.

Research/Grant Support: Support was provided by UPP Foundation Research Grant (MRJ), VA VISN4 Competitive Career Development Fund (MRJ), and NIH grant #NS033730 (KMA).

Abstract 12

EFFECT OF DORSAL ROOT GANGLION STIMULATION ON ORTHODROMIC COMPOUND ACTION POTENTIALS

Loeza-Alcocer E,[1] Gold M[1]

[1]Department of Neurobiology
University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Dorsal root ganglion (DRG) stimulation is used to manage chronic pain, but the mechanism remains unknown. The DRG houses the cell bodies of sensory neurons which propagate action potentials from the periphery to CNS. DRG stimulation is thought to block the action potentials through the DRG, but the mechanisms underlying this block are still unclear.

Hypothesis: Given that GABA is released within the DRG, sensory neurons express GABAA receptors, and GABAA receptor activation blocks action potential propagation, one of the explanations for the efficacy of DRG stimulation, is that it is due to the activation of GABAA receptors within the DRG.

Methods: To test this hypothesis, we recorded compound action potentials (CAP) in sensory nerve central to the DRG, that were evoked with electrical stimulation of the nerve peripheral to the DRG before and after DRG stimulation. To test the role of GABAA receptors, we applied the same protocol in the presence of bicuculline, a GABAA receptor antagonist, to the DRG. Because sensory neurons can be classified into two groups those with rapidly conducting axons (A-fibers) and those with slowly conducting axons (C-fibers) and non-nociceptive information is carried by A-fibers, while nociceptive information is carried by C-fibers, we analyzed the A- and C-wave of the CAP.

Results: We found that DRG stimulation (21Hz for 2 min) inhibited both the A- and the C-wave of the CAP. Bicuculline increased both the A- and C-wave of the CAP and importantly, it reversed the DRG stimulation-induced block of the C-wave, and to a lesser extent, the A-wave. The results suggest that ambient GABA in the DRG is modulating the propagation of CAP and GABA via GABAA receptors is part of the mechanism underlying the efficacy of DRG stimulation.

Conclusion: Finally, these findings imply a better understanding of the mechanism of DRG stimulation to alleviate pain and the involvement of GABA in the sensory information processing at a peripheral level which may result in better strategies to manage chronic pain.

Research/Grant Support: RM1 NS128775 and R01 NS122784 (PI: Michel Gold, PhD).

Abstract 13

PSYCHOPHYSICAL PAIN CORRELATES AFTER KETAMINE FOR MAJOR DEPRESSIVE DISORDER

Makeen M,[1] Price R,[3] Wroble-Biglan M,[1] Lim G[1,2]

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

Introduction: Pain and depression are interrelated major sources of disability in the United States. However, we lack an understanding of the potential sensory neural basis for these relationships. The purpose of this study was to use quantitative sensory testing (QST) to evaluate changes in Pain Intensity (sensory pain) and Pain Unpleasantness (emotional pain) among subjects with treatment-refractory depression who were receiving subanesthetic ketamine therapy.

Hypothesis: Among patients with treatment-resistant depression, low dose intravenous ketamine will reduce residual pain, pain intensity and pain unpleasantness measured using mechanical temportal summation (mTS) by QST.

Methods: Subjects with treatment-refractory depression who planned to receive intravenous ketamine (0.5mg/kg for 40 minutes) were enrolled in this observational study. Baseline pain was measured using the PROMIS Pain Intensity and Pain Interference Short Form 3a, one hour prior to the start of ketamine infusion. QST by mechanical temporal summation (mTS) was conducted one hour prior to and within 1 hour after the completion of ketamine infusion. mTS protocols included measurement of pain using a standardized set of 7 custom-made weighted pinprick mechanical stimulators with fixed stimulus intensities. Stimuli were applied at an interstimulus interval (ISI) of 1-second on the dorsal surface of the third digit of both left and right hands. Probes were first serially applied using the lowest force (8mN) with subject rating pain on the 0-10 numeric rating scale (NRS) after each stimuli. The probe that created a pain score of 5 or higher (0-10 NRS) was used to complete a series of 10-stimuli applied at an ISI of 1-second with verbal NRS pain assessments reported by the subjects after each set of 10-stimuli. Subjects reported the residual pain from the stimulations 15-seconds after the last 10-probe stimulations and rated Pain Intensity and Pain Unpleasantness before and after the 10-probe stimulation experience on the 0-10 NRS.

Results: Twenty-two (22) subjects completed all study procedures. Pain scores obtained 15 seconds after QST testing were lower on average after ketamine infusion compared to baseline (left hand pain score mean difference 0.59±1.1, p=0.01; right hand pain score mean difference 0.86±0.89, p= 0.0001). Pain intensity scores were lower after ketamine infusion compared to baseline (left hand pain score mean difference 0.68±1.2, p=0.006; right hand pain score mean difference 0.82±0.85, p= 0.0001). Pain unpleasantness scores were inconsistent by hand with lower pain after ketamine infusion compared to baseline in the right M = 0.59±1.4, p= 0.03, but not left hand M= 0.32±1.2, p=0.12 hands.

Conclusions: Ketamine infusion at doses delivered for treatment of depression reduced mTS pain scores within an hour dosing.

Significance: These data provide direction for future research that queries the potential neural mechanisms of ketamine for people living with depression-augmented pain.

Research/Grant Support: This project was supported by NIH T32 GM075770 (PI: Yan Xu).

Abstract 14

MACHINE LEARNING APPLIED TO CHRONIC PAIN DRAWINGS IDENTIFIES UNDIAGNOSED FIBROMYALGIA: IMPLICATIONS FOR BUSY CLINICAL PRACTICE

Moses MA,[1] DeSensi R,[1] Jeong J,[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: Previous work applied a hierarchical clustering algorithm that sorted patients into distinct subgroups that differed in their distribution of pain and pain characteristics. One cluster in particular, Widespread-Heavy, was associated with a high number of body regions selected and worse pain characteristics overall, features characteristic of fibromyalgia (FM). Goals of the current study are to discern if hierarchical clustering can predict the diagnosis of fibromyalgia independently and to use bioinformatic data to give insight into which patients visiting a chronic pain practice receive a diagnosis of fibromyalgia.

Hypothesis: Patients in the Widespread-Heavy cluster are more likely to have a diagnosis of FM.

Methods: The study population consists of 21,423 patients from 3/2017-6/2019 who completed a CHOIR survey. FM cases were identified by ICD-10 code and were identified as Index (FM diagnosis at the time survey was completed) and Total (any FM diagnosis form 3/2017-11/2021). A bioinformatic proxy was developed that used 2016 FM diagnostic criteria consisting of Widespread Pain Index (WPI) and Symptom Severity Scale (SSS). CHOIR WPI was satisfied if >21 body regions were selected which is equivalent to the required 7/19 regions set out by the ACR criteria. ACR SSS was approximated through the use of PROMIS measures that were consistent with the symptoms described on the ACR diagnostic criteria. These included PROMIS physical function (PF), sleep disturbance, anxiety, depression, and global health mean. Logistic regression and summary statistics were used in analysis.

Results: Multivariate logistic regression analysis revealed a significant relationship between cluster membership and FM diagnosis (N =21344, LR Chi2(12) = 1970.69, $p<0.0001$, $R2 = 0.248$) with postestimation predictive margins indicating that patients in the Widespread-Heavy cluster are significantly more likely to receive a diagnosis of FM when controlling for age, sex, anxiety, and depression. A significantly greater percent of patients met bioinformatic criteria, 34.8%, than received a diagnosis of FM at the index visit, 22.4%. Lastly, in the entire sample significantly more males meet approximated FM criteria, 26.4%, than are diagnosed at the index visit with FM, 7%.

Conclusions: Patients belonging to the Widespread-Heavy cluster are more likely to receive a diagnosis of FM. Fibromyalgia appears to be underdiagnosed as more patients meet approximated diagnostic criteria than are diagnosed clinically at the same visit. A sex bias is present in the clinical diagnosis of FM that is contributing to underdiagnosis.

Significance: Patient reported information all obtained electronically in a matter of minutes can trigger further evaluation by the clinician if certain criteria are reported; criteria similar to that used in this study. With this approach, diagnosis may be less likely to be subject to sex bias and the time to diagnosis could decrease which could prompt better patient outcomes.

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

Abstract 15

CHRONIC PROSTATITIS PAIN IS ASSOCIATED WITH mTOR PHOSPHORYLATION IN THE BRAIN

Prasoon P,[1] Taylor BK,[1,2,3] Thumbikat P,[4,5] Roman K[1]

[1]Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh School of Medicine
[2]Pittsburgh Center for Pain Research
[3]Pittsburgh Project to End Opioid Misuse, Pittsburgh, PA,
Departments of [4]Urology and [5]Pathology, Northwestern University Feinberg School of Medicine

Introduction: Pain is a disabling feature of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). fMRI studies conducted in CP/CPPS patients showed decreased spontaneous activity in several brain regions such as the left anterior cingulate cortex (ACC) and the left and right insula. Rodents models of peripheral neuropathic pain are associated with increased mTOR phosphorylation (p-mTOR) in the ACC and insula. Also, a small nucleus accumbens (NAc) can predate the development of chronic pain. However, it is not known whether increased p-mTOR in relevant brain regions is associated with chronic pelvic pain in CP/CPPS. Thus, we used a validated mouse model of CP/CPPS known as experimental autoimmune prostatitis (EAP) to investigate whether mTOR signaling is increased or decreased in these brain regions.

Hypothesis: Chronic pelvic pain in EAP is associated with increased expression of p-mTOR and its downstream effector protein, p-S6(244), in the ACC, insula, and NAc.

Methods: We conducted experiments on male C57BL/6J (B6) mice (5-7 weeks old; n=3/group) with or without EAP. To induce EAP, mice received a subcutaneous injection of rat prostate antigen (1 mg/ml) mixed with an adjuvant (1:1). Control mice did not receive the antigen. Pelvic tactile sensitivity was measured from day 0 (baseline) to 30. On day 30, we euthanized EAP (n=3) and control mice (n=3), dissected the ACC, insula, and NAc, and conducted western blots on homogenates. In a separate cohort, we performed transcardial perfusions on day 30 and sectioned the brain from EAP (n=6) and control mice (n=7) to co-label for p-S6(244) and NeuN (neuronal marker). We used GA3 software (Nikon) to quantify the mean intensity of p-S6(244) immunofluorescence. Observers were blinded to experimental groups.

Results: Mice with EAP developed pelvic tactile allodynia from day 6 through day 30 ($p<0.05$). Compared to the control treatment, EAP increased p-mTOR in ACC, insula, and NAc immunoblots at day 30 ($p<0.05$). EAP increased p-S6(244) expression in the left hemisphere of the ACC and both hemispheres of the insula ($p<0.05$) but not in the left or right NAc.

Conclusions: Our data indicate that: 1) chronic pelvic pain in a mouse model of CP/CPPS is associated with increased p-mTOR in the ACC, insula, and NAc; and; 2) p-S6(244) expression is increased in the left ACC and the right and left insula. These results resemble the regional spontaneous activity changes in fMRI imaging studies conducted on patients.

Significance: Our findings suggest that mTOR inhibitors could be a promising approach for treating CP/CPPS.

Research/Financial Support: NIDDKK01DK114395-06 (PI: Kenny Roman, PhD) and the University of Pittsburgh Start-Up funds (PI: Kenny Roman, PhD, and Co-PI: Bradley K. Taylor, PhD)

Abstract 16

EFFECT OF TARGET TISSUE INNERVATION ON THE PHENOTYPE OF iPSC-DERIVED NOCICEPTIVE AFFERENTS

Preisegger M,[1] Nagarajan V,[1] Makarcyzk M,[2] Beckel J,[3] Lin H,[2] Gold M[1]

[1]Department of Neurobiology
[2]Department of Orthopaedic Surgery
[3]Department of Pharmacology and Chemical Biology
University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Pain is one of the most prevalent symptoms in medical settings and is associated with decreased quality of life and opioid addiction, making it one of the costliest health problems in the U.S. Induced pluripotent stem cells (iPSC) offer a promising solution to what has been a relatively slow translation of data from rodent models because iPSCs can be derived from human cells. Furthermore, subject-specific iPSCs for tissue derivation could serve as a platform for personalized medicine. However, despite significant advances in protocols for iPSC-derived sensory neuron generation, the phenotype of iPSC-derived nociceptive afferents (iPSC-NAs) lacks critical features of nociceptors in the intact organism. Developmental data and cross-anastomosis studies suggest that the target of innervation influences nociceptor properties. Hence, our objective is to determine whether providing a target tissue for iPSC-NA differentiation will facilitate the generation of sensory neurons with functional nociceptor properties.

Hypothesis: Limited expression of functional nociceptive proteins in iPSC-NAs is due to the absence of a target of innervation in vitro.

Methods: To drive the differentiation of the iPSC cell line to a nociceptor phenotype, the two small-molecule inhibitors of SMAD signaling combined with three small molecule inhibitors protocol was followed (Lampert, 2022). To confirm the phenotype of the differentiated neural cells, immunocytochemistry was performed using two nociceptive-specific markers (TRPV1 and NAV1.8). To provide a target of innervation, we used a two-chambered microfluidic polydimethylsiloxane system that allows differentiating iPSCs to innervate target tissues (urothelium, keratinocytes, and synovium) via microfluidic channels. Functional iPSC-NA properties were assessed using calcium imaging and microelectrode arrays.

Results: Our data shows that iPSC-NAs generated using a standard differentiation protocol (Lampert,2022) in the absence of target tissue express nociceptive markers (NaV1.8, TRPV1) detected by immunofluorescence. However, these proteins are non-functional.

Conclusions: While we have yet to fully test our hypothesis, our results are consistent with the suggestion that, in the absence of a target of innervation, nociceptive proteins present in iPSC-NAs are non-functional.

Significance: This platform may enable a viable strategy to address a major hurdle in the wider adoption of this potentially powerful technology.

Research/Grant Support: DoD - 416866

Abstract 17

TOUCH AND GO: ANALYSIS OF MOUSE GAIT FOR THE INVESTIGATION OF PAIN

Rhoades R,* Chen Q,* Deakin T, Tang P, Xu Y

Department of Anesthesiology and Perioperative Medicine
University of Pittsburgh School of Medicine

Introduction: Acute pain and chronic pain are a major public health concern. More than 116 million people in the U.S. are affected and this number is expected to increase as the population is aging. Preclinical research aimed at improving the reliability of diagnosis, evaluation, and treatment of pain is needed to address this growing problem. A knowledge gap exists for pain and treatment evaluation between humans and animals; the latter relies on experimenter's subjective judgements of elicited animal responses, rather than animals' own intrinsic behaviors.

Hypothesis: We hypothesize that by training the Long Short-Term Memory (LSTM) networks, a machine deep learning algorithm, we will be able to detect and diagnose animals experiencing differing degrees of inflammatory or neuropathic pain.

Methods: We developed a high-throughput gait recording and analysis device using the high spatial and temporal resolution touchpad technology. Spontaneous walking of mice in an open field is registered by individual touches as the animals explore the arena. Gait features are extracted from the touch data to train a LSTM model to differentiate between painful and non-painful conditions so that animals with and without pain or with and without analgesia treatment can be identified and quantified.

Results: Using naïve and complete Freund's adjuvant (CFA)-induced inflammatory pain as a test system, we extracted several features in the gait analyses showing behavioral dichotomy between naïve and CFA mice. Some of the promising features include the duration of individual steps, the distance and latency of touches in segments of predefined size, the distance and time between four adjacent touches, the time the animal spend in the center of the field vs. the periphery, angulation of animal steps, and speed of body movement. When using animals not used in the training, the model currently has a high prediction accuracy for pain but only marginal accuracy for non-painful states. We are refining the model to achieve > 95% accuracy in all predictions.

Conclusions: Further testing and verification will be required but we are close to fully developing an affordable high-throughput assay to assess pain behavior using the animal intrinsic behavior rather than relying on subjective analyses of elicited behaviors.

Significance: This work will contribute to the future development of the same artificial intelligence technology, combined with wearable sensors, for human pain and treatment evaluations.

Research/Grants support: R01NS122830 (PI: Xu). RR is supported by T32NS073548 (PI: Gold)

(* These authors have equal contributions.)

Abstract 18

CROSS-GENERATIONAL EFFECTS OF CHRONIC ETHANOL EXPOSURE ON THE NONCODING TRANSCRIPTOME: INTERSECTING HUMAN AND MOUSE DATA

Rice R,[1] Farris S,[1] Hill S,[2] Homanics G[1]

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

Introduction: Previous studies from our laboratory and others in rodents have demonstrated that paternal preconception ethanol exposure (PPE) confers intergenerational epigenetic, epitranscriptomic, and behavioral alterations. However, the effects of maternal preconception chronic ethanol exposure (MPE) as well as combinatorial effects of PPE/MPE are less characterized. In humans, we demonstrated that familial alcohol dependence was associated with changes in methylation and expression of cancer-related genes in subsequent generations. The current study seeks to characterize cross-generational transcriptomic effects of preconception chronic ethanol exposure in human and mouse. An emphasis is placed on noncoding genes due to their critical functions in epigenetics and transcriptional regulation.

Hypothesis: PPE and/or MPE result in robust cross-generational transcriptomic changes in mouse and human.

Methods: We exposed male and female C57BL/6J mice to five cycles of chronic intermittent vapor (CIEV) exposure and continuous-access two bottle choice (CA-2BC) to induce ethanol dependence and assay ethanol consumption behavior, respectively. Mice were cross bred to produce offspring with PPE, MPE, both, or neither. Whole blood and brain were collected from adult (6-8 weeks old) offspring for bulk RNA sequencing (RNA-Seq) analysis. Human frozen lymphocytes were banked from third-generation human participants (n=72) from multiplex families with or without AUD. Deep phenotyping of three generations allows for analyzing whether AUD is through the mother or father and whether the grandparent is similarly affected, controlling for maternal prenatal use of substances.

Results: Mouse CIEV exposure yielded pharmacologically relevant BECs averaging ~150 mg/dL and CA-2BC validated significant escalation of 15% (vol/vol) ethanol consumption. Ongoing bulk RNA-Seq data on blood samples will reveal effects on noncoding gene expression based on parental preconception chronic ethanol exposure. Our studies will also determine biomarkers of non-coding RNA in blood for cross-generational effects of chronic ethanol exposure in the brain.

Conclusions: Results from these ongoing studies are expected to provide novel insight in the cross-generational effects of alcohol exposure in rodents and humans and support the hypothesis that ancestral alcohol exposure can exert lasting effects that persist across generations.

Significance: These data will be the first to examine transcriptomic outcomes of both MPE and PPE across species and identify novel molecular targets for AUD.

Research/Grant Support: This work was supported by Bridging Connections in Addiction Research at the University of Pittsburgh as well as NIH T32 NS007433-21.

Abstract 19

IDENTIFYING PATIENT-CENTERED PSYCHOLOGICAL AND SOCIAL SUPPORT NEEDS AFTER TRAUMATIC BIRTH: A QUALITATIVE STUDY

Roberts T,[1] Nowakowski E,[1] Troyan T,[1] Kroh S,[1] Wanaselja A,[1] Gopalan P,[2] Shenai N,[3] Dalby P,[1] Romeo R,[1] Wroble Biglan M,[1] Lim G,[1, 2]

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

Introduction: Emergency deliveries increase risk for postpartum depression (PPD) and post-traumatic stress disorder (PTSD).1 2 3 Nearly one in three women describe their birth experience as traumatic,4 with higher incidence of PPD and PTSD (14% and 4%, respectively) in these cohorts.1 4 5 Patient-centered, post-birth support is limited for those who identify as having had a traumatic birth. We conducted a qualitative study of women with traumatic birth experiences to identify patient-centered priorities to optimize postpartum medical and psychosocial support.

Hypothesis: Women who experience traumatic birth would benefit from routine referrals to psychiatric and/or social work services for the reduction of PPD and PTSD post-delivery.

Methods: A qualitative study design with semi-structured interviews was chosen for study of women having emergent deliveries who self-identified as experiencing traumatic birth from March to May 2022. Participants completed the Stanford Acute Stress Reaction Questionnaire and the PTSD Checklist prior to responding to semi-structured interviews. Open-ended questions about labor and delivery events that improved or worsened their experience, perceptions of mental and physical support provided by staff, and patient attitudes about psychological referral after emergent deliveries.

Results: Eight women were enrolled. Three (37.5%) had a history of anxiety, depression, or PTSD prior to their birth. Most participants (75%) had symptoms of anxiety, depression, or PTSD at the time of the interview, with 60% meeting criteria for acute stress disorder and 50% meeting criteria for PTSD. Patient interviews revealed the following themes in patients experiencing the birth as traumatic: previous prenatal or peripartum medical concerns, prenatal expectations, and clinical staff communication style/quality during and after delivery. Women almost unanimously desired the offered psychosocial services after birth trauma, also noting they may not immediately accept the offered services.

Conclusions: Following a traumatic birth, women expect timely acknowledgement of the physical and mental toll of the experience by their obstetricians. Identified opportunities to enhance patient-centered care during emergencies include: 1) Prenatal education on alternative birth plans; 2) Improving patient-staff communication during emergent events; 3) Providing emotional support following emergent deliveries; 4) Including mental health conversations in postpartum period, prior to hospital discharge; 5) Providing psychiatric follow up for women with a history of traumatic prenatal or birth experiences or mental health disorders.

Significance: Many women experience trauma while giving birth under emergent circumstances placing patients at risk for postpartum depression, PTSD, and impaired mother-infant bonding. We desire to connect women to the resources they need.

Financial Support: Internal funding from the MWRI Staff Fund (PI: Grace Lim, MD MS).

References:

[1] Ertan D et al. (2021). *BMC Psychiatry*, 21(155)
[2] Khoramroudi R. (2018). *Journal of Family Medicine and Primary Care.* 7(1), 220-223
[3] Brummelte S, Galea LA. (2016). *Hormones and Behavior.* 77, 153-66
[4] Reed R et al. (2017). *BMC Pregnancy and Childbirth.* 17(21)
[5] Cook N et al. (2018). *Journal of Affective Disorders.* 1(224), 8-3.

DEPARTMENT OF CRITICAL CARE MEDICINE

Oral Abstract O2

SHORT CHAIN FATTY ACID SUPPLEMENTATION FOLLOWING TRAUMATIC BRAIN INJURY

Nelson BP,[1] Vagni V,[1] Mullet SJ,[3] Wendell SG,[3] Morowitz MJ,[4] Clark RS,[1,2,5,6] Simon DW[1,2,5,6]

[1]Safar Center for Resuscitation Research
[2]Children's Neuroscience Institute
Departments of [3]Pharmacology and Chemical Biology, [4]Surgery, [5]Critical Care Medicine, and [6]Pediatrics
University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Traumatic brain injury (TBI) induces chronic intestinal dysbiosis and depletion of short chain fatty acid (SCFA)-producing commensal gut bacteria. Gut-derived SCFAs promote microglial function, neurogenesis, and maintenance of the blood-brain-barrier. However, there is limited information about the role of SCFA repletion on long-term recovery following TBI.

Hypothesis: Repletion of the most abundant SCFAs in the gut, acetate and butyrate, will improve long-term cognitive recovery after TBI.

Methods: The controlled cortical impact (CCI) TBI model was utilized (6m/s, 2.2mm depth 50msec dwell) in adult male C57BL6/J mice. On the day of injury, mice were randomly assigned to a control diet of 15% high amylose maize starch (HAMS) or an intervention diet of 15% acetylated-butyrylated HAMS (HAMS-AB). Stool pellets were collected serially for quantification of acetate and butyrate by high performance liquid chromatography-mass spectrometry/mass spectrometry. Morris water maze (MWM) was conducted at 1-,3-, and 6-months post-injury. The MWM platform was placed in different quadrants for both the 3- and 6-month assessments to reassess spatial learning and memory retention. Analyses were performed with Kruskal-Wallis test and Dunn's multiple comparison.

Results: A total of 30 mice were randomized and 26 mice were used for final analysis (4 exclusions for death or requiring euthanasia). In MWM, probe swim speeds were statistically different at 1-month ($p<0.05$) but not by 6-months ($p=0.18$). MWM hidden platform latency at 6-months showed a treatment effect ($p=0.0034$) with differences observed between Sham+HAMS and CCI+HAMS at Day 3, 4, and 5 ($p= 0.0084$, $p=0.0117$, $p=0.0022$). Probe trials at 1- and 3-months indicated memory retention or a loss of injury effect with a trend continued to 6-months ($p=0.0203$, $p=0.0247$, $p=0.0863$). Acetate and butyrate fecal concentrations did not statistically differ between groups at baseline and 1-month; however, concentrations were increased in CCI+HAMS-AB when compared to both Sham+HAMS and CCI+HAMS at 6-months (acetate: 2.6 ± 2.1, 1.0 ± 0.1, 1.9 ± 0.6, $p=0.0066$; butyrate: 2.4 ± 1.8, 0.3 ± 0.2, 0.5 ± 0.2, $p=0.0002$).

Conclusions: Dietary supplementation with HAMS-AB increased intestinal availability of acetate and butyrate by month-6 post injury. CCI+HAMS-AB had intact fine motor function and improved cognitive outcome compared to CCI+HAMS.

Significance: Early post-injury SCFA dietary supplementation may promote cognitive benefits.

Research/Grant Support: R21NS115173

Abstract 20

VITAMIN C IS MANDATORY FOR THE TCA CYCLE PRODUCTION OF ANTI-INFLAMMATORY ITACONATE

Castellano E,[1,2,3] Korkmaz A,[1,2,3] Soysal E,[1,2] Gao Y,[1,2,3] Johnson C,[1,2,3] Scott J,[1,2,3] Epperly M,[4] Greenberger J,[4] Mullet ST,[5] Wendell SL,[5] Kagan VE,[1,3] Bayır H[1,2,3]

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

Introduction: Exposure to pro-oxidant disease conditions such as sepsis increases the demand for vitamin C (VitC) leading to its deficiency, which in turn can cause impaired immunity. This has awakened interest in VitC as a pharmacotherapy in severe sepsis, albeit with controversial results highlighting the lack of mechanistic knowledge on possible effects of ascorbate on inflammatory and immune responses. VitC's principal role as a reductant has been ascribed to mitochondria yet the specific link to the immune-modulating effects remains unknown. Tricarboxylic acid (TCA) cycle enzymes, especially citrate synthase (CS) and aconitase (ACO2), are highly prone to inactivation by pro-oxidants. ACO2 catalyzes the formation of cis-aconitate, the precursor of anti-inflammatory and antimicrobial metabolite, itaconate.

Hypothesis: VitC is essential for generation of itaconate and for maintaining its levels during systemic inflammation.

Methods: PND21 ODS rats, which lack functional L-gulonolactone oxidase (GULO) and therefore rely on diet for VitC like humans, were randomized to VitC supplemented (VitC+, 200 mg/100 mL) or regular (VitC-) water p.o. *ad libitum* for one week at which time plasma VitC levels (fluorescence assay), kidney cortical enzyme activity and TCA cycle metabolite (LC-MS) levels were measured. In separate experiments PND 28 ODS littermates were exposed to 6Gy of whole-body irradiation (WBI) or cecal ligation and puncture (CLP) to induce polymicrobial sepsis. Within 1h after WBI or CLP and at 24h after CLP the rats were randomized to 75 mg/kg VitC ip plus VitC+ water po (VitC+ group) or vehicle (NS) ip plus regular (VitC-) water po *ad libitum* for the duration of the experiment. All outcome assessments were performed by investigators blinded to treatment groups. $^{13}C_6$-glucose labeling was utilized as an isotopic tracer in HEK293 cells to further explore the role of VitC on the TCA cycle metabolites.

Results: VitC+ rats showed higher plasma VitC levels and increased kidney cortical CS and ACO2 activity vs. their VitC- counterparts. LC-MS revealed higher cis-aconitate and itaconate levels in renal cortices, liver and ileum of VitC+ vs VitC- rats. [$^{13}C_6$]-glucose labeling showed a large fraction of cis-aconitate [M+0] was not derived from labeled glucose in VitC+ cells. Fractional contribution of αKG to citrate was higher in VitC+ cells. LC-MS analysis using deuterium labelled itaconate and αKG showed increased itaconate and low αKG levels in VitC+ vs Vit C- cells. WBI and CLP studies showed higher murine sepsis severity (MSS) scores in VitC- rats indicating higher injury severity corraborating with lower cis-aconitate and itaconate levels in ileum and renal cortex vs Vit C+ rats. Expression of kidney injury marker NGAL was increased in kidney cortices of VitC- rats after CLP. Treatment of VitC- rats with itaconate attenuated CLP illness severity.

Conclusions: VitC is an obligatory nutrient in the production of itaconate in which carbons of cis-aconitate are mainly derived from glutamine via reverse TCA cycle. VitC plays a key role in itaconate generation via protection of cis-aconitase activity during systemic inflammation.

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

Research/Grant Support: NIH (T32HD040686, U01AI156923, S10OD023402, U01AI156924) and UPMC.

Abstract 21

HOSPITAL CONSOLIDATION IS ASSOCIATED WITH LOSS OF PEDIATRIC INPATIENT SERVICES: A LONGITUDINAL STUDY OF US HOSPITALS

Joseph AM, Davis BS, Kahn JM

CRISMA Center, Department of Critical Care Medicine
University of Pittsburgh

Introduction: Multi-hospital systems have grown in size and scope over the past two decades, contributing to concentration in hospital markets. In the same time period, pediatric inpatient services have become increasingly centralized. However, little is known about the relationship between these phenomena. We sought to characterize whether hospital consolidation is associated with the loss of inpatient pediatric services.

Hypothesis: We hypothesized that hospital consolidation is an independent risk factor for the subsequent loss of inpatient pediatric services.

Methods: We performed a longitudinal study of United States adult and children's general hospitals using the American Hospital Association (AHA) annual survey from 2011 to 2020. First, we constructed a binary variable reflecting whether hospitals provided inpatient pediatric services in a given year. We then analyzed the relationship between joining a health system and loss of pediatric inpatient services within five years after joining. We used logistic regression with generalized estimating equations to account for hospital-level panel data, controlling for hospital size and status as a critical access hospital. The final cohort included 2,150 unique hospitals and 16,497 hospital-years.

Results: Provision of inpatient pediatric services declined over time, from 840 of 1,887 hospitals (44.5%) in 2011 to 442 of 1,376 hospitals (32.1%) in 2020. Among hospitals with inpatient pediatric services not initially in a hospital system, joining a hospital system was associated with a loss of inpatient pediatric services within five years (adjusted odds ratio 1.57, 95% confidence interval 1.26-1.96, $p<0.001$). In secondary analyses varying the follow-up period, this association remained statistically significant at four years but not statistically significant at shorter follow-up lengths, although all point estimates were similar.

Conclusions: Hospital consolidation may be driving observed reductions in inpatient pediatric services, potentially leading to decreased access to care. More work is needed to better understand how these trends are affecting actual access as well as child health outcomes.

Significance: Policymakers should consider access to pediatric care when reviewing proposed mergers and acquisitions as part of antitrust review.

Research/Grant Support: NHLBI 5T32HL0076820 (PI: Jeremy Kahn, MD, MS)

Abstract 22

OUTCOME TRAJECTORIES IN ABUSIVE HEAD TRAUMA VERSUS ACCIDENTAL TRAUMATIC BRAIN INJURY

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

[1]Department of Critical Care Medicine
[2]Department of Pediatrics
[3]Department of Surgery
University of Pittsburgh School of Medicine

Introduction: Children who suffer abusive head trauma (AHT) are at high risk of morbidity and mortality.

Hypothesis: Children after AHT would have a higher incidence of new impairment compared to children after accidental traumatic brain injury (aTBI) at discharge, 1 y, and 5 y post-discharge.

Methods: Children < 3y old admitted with TBI to the Pediatric Intensive Care Unit (PICU) at a Level 1 Trauma Center between 2014-2019 were included. Patient and injury characteristics, TBI mechanism (abuse vs. accident) and Functional Status Scale (FSS) scores (pre-TBI, hospital discharge, and 1 and 5 y post-discharge) were abstracted from the electronic health record. New impairment was defined as change in FSS > 1 from baseline. Patients who died were assigned the highest FSS score.

Results: Four-hundred and sixty patients (170 AHT, 290 aTBI) were included in this study of which 13 children with AHT and 4 children with aTBI died. Children with AHT had higher frequency of severe GCS classification at admission (31/170 [18%] vs 33/290 [11%], p=0.04). Frequency of new impairment in AHT vs. aTBI patients were as follows: hospital discharge (55/170 [32%] vs. 31/270 [11%], p<.001), 1 year (43/154 [28%] vs. 26/259 [10%], p<.001), and 5 years (32/114 [28%] vs. 18/178 [10%], p<.001). All FSS subdomain scores were worse for AHT vs. aTBI patients at discharge. Sensory, communication, and motor domains were worse in AHT patients at 1 and 5 years. When stratified by GCS, more AHT patients with mild GCS at admission were discharged with impairment (25/130 [19%] vs. 10/244 [4%], p<.001) and continued to have impairment at 1 year (27/132 [20%] vs. 13/233 [6%], p<.001) and 5 years (21/99 [21%] vs. 12/150 [8%], p<.001) timepoints compared to aTBI. We isolated the patients with impairment at hospital discharge to look at the FSS trajectory and the only patients who continued to decline (8/66 at 1 year and 7/55 at 5 years) were AHT patients.

Conclusion: AHT patients had more longitudinal functional impairment than children with aTBI regardless of admission GCS. Children with TBI, especially AHT, require long-term care for recovery.

Significance: More research needs to be conducted on the prevention, treatment, and rehabilitation in AHT to improve outcomes.

Research/Grant Support: NIH 5T32HD040686-22

Abstract 23

END-ORGAN METABOLIC STRESS EVALUATION WITH MICRODIALYSIS DURING UNCONTROLLED HEMORRHAGIC SHOCK

Saraiva IE, Gordon L, Lagattuta T, Pinsky MR, Gomez H

Department of Critical Care Medicine
Center for Critical Care Nephrology
the CRISMA Center
University of Pittsburgh, Pittsburgh, PA

Introduction: Shock resuscitation strategies are usually based on macrohemodynamic data, with minimal information about specific microcirculatory and tissue energy metabolism. We have previously demonstrated associations between early resuscitation, microcirculation parameters, and markers of tissue metabolic stress in a porcine model of endotoxemic shock. We present preliminary data of metabolic stress markers in a porcine model of uncontrolled hemorrhage from liver laceration.

Hypothesis: We hypothesized that discordance will be seen between tissue markers and blood lactate in severe hemorrhagic shock resuscitation.

Methods: Four pigs were anesthetized and subjected to laparotomy and uncontrolled hemorrhagic shock by liver laceration. Once the mean arterial pressure (MAP) declined below 40mmHg for 3 minutes, we performed damage control surgery with packing and closure of the abdomen, and hemodynamic resuscitation according to an automated established protocol. Microdialysis catheters were inserted into both the left kidney cortex and the left hepatic lobe, continuously flushed and dialysates collected every 30 minutes during shock, resuscitation, and stabilization phases.

Results: Tissue lactate levels changes were found to be smaller than arterial blood lactate changes, except for 1 liver experiment which peaked at 4.60mmol/L, similar to blood. We found wide variation of L/P in different organs, ranging from elevations within normal range (from 8.76 increase to peak at 18.67 in the kidney) to severe increase that did not return to baseline after more than 1h of MAP >65 mmHg and decrease in arterial blood lactate level (44.75 at end of experiment from a peak of 67.88 in the liver). L/P changes were more sensitive than lactate levels alone to identify shock onset.

Conclusions: Our findings highlight that (1) metabolic stress markers vary by visceral organ studied and do not always reflect macrohemodynamic abnormalities and resuscitation, (2) like endotoxic shock, the discordance between tissue perfusion pressure and macrohemodynamic goals occurs during the resuscitation of hemorrhagic shock, (3) end-organ metabolic stress with hemorrhagic shock parallel changes seen in other conditions like endotoxemic shock, and (3) L/P changes have the potential to better reflect adequacy of resuscitation at the tissue level.

Grant Support: Partially funded from TRACIR grant: DoD W81XWH-19-C-0101 “Trauma care in a rucksack”

Abstract 24

YIELD OF COMPUTED TOMOGRAPHY AFTER NON-TRAUMATIC OUT-OF-HOSPITAL CARDIAC ARREST

Tam J,[1] Soufleris C,[2] Ratay C,[2] Frisch A,[2] Elmer J,[1,2,3] Flickinger KL,[2] Callaway C,[2] Coppler PJ[2]

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

Introduction: Computed tomography (CT) can identify the etiology of cardiac arrest and injuries related cardiopulmonary resuscitation (CPR). Diagnostic yield of CT has only been characterized in a few small cohort studies with varied imaging practices.

Hypothesis: Early comprehensive CT imaging (head-to-pelvis) of non-traumatic out-of-hospital cardiac arrest (OHCA) patients would yield clinically important findings.

Methods: We included non-traumatic OHCA patients treated at a single center from February 2019 to February 2021. Clinical practice was to obtain CT head in all comatose patients. Additionally, CT of the cervical spine, chest, abdomen, and pelvis were obtained if clinically indicated. We identified CT imaging obtained within 24 hours of arrival and summarized significant radiology findings. We used descriptive statistics to summarize population characteristics and imaging results and report their frequencies.

Results: We included 505 subjects. Mean age was 60 [SD 16] years and 144 (29%) had an initial shockable rhythm. Median CPR duration was 21 [IQR 12-35] minutes and 448 (89%) subjects were comatose on presentation. Almost all subjects had brain imaging (n=481, 95%), of which 33 (7% [95% CI 5-9%]) had intracranial hemorrhage and 157 (33% [95% CI 28-37%]) had cerebral edema. Fewer subjects had a cervical spine CT (n=202, 40%) though 4 (2% [95% CI 0-4%]) had vertebral fractures. Most subjects had a CT of the chest (n=396, 76%), and abdomen and pelvis (n= 347, 69%). Identified chest pathologies included rib or sternal fractures (222, 56% [95% CI 51-61%]), pneumothorax (27, 7% [95% CI 5-10%]), aspiration or pneumonia (300, 76% [95% CI 71-80%]), mediastinal hematoma (18, 5% [95% CI 3-7%]), and pulmonary embolism (13, 3% [95% CI 2-6%]). Significant abdomen and pelvis findings were bowel ischemia (23, 7% [95% CI 4-10%]), and splenic or liver laceration (7, 2% [95% CI 1-4%]).

Conclusions: CT identifies clinically important pathology after OHCA.

Significance: Given the prevalence of findings on CT after OHCA, providers should consider comprehensive imaging, especially in cases when details surrounding collapse are unclear.

Grant support: None

Abstract 25

ASSOCIATION BETWEEN PROPORTION OF NURSING WORKFORCE AGENCY STAFFING AND PEDIATRIC INTENSIVE CARE UNIT OUTCOMES

Taylor M,[1] Woeltje M,[1] Rooney S,[2] Au A,[1,3] Aldewereld Z,[1] Clark R,[1,3] Horvat C[1,3]

[1]Department of Critical Care Medicine, University of Pittsburgh School of Medicine
[2]Department of Pediatrics, UPMC Children's Hospital of Pittsburgh
[3]Safar Center for Resuscitation Research, University of Pittsburgh

Introduction: Children's hospitals are experiencing staffing strain secondary to increased patient volumes coupled with increased turnover of essential workers such as nurses. To bolster workforces, many hospitals have turned to temporary, agency workers to fill vacant positions.

Hypothesis: The objective of this study was to evaluate whether the proportion of agency staffing in pediatric intensive care units (PICUs) is associated with patient-centered outcomes.

Methods: This IRB-exempt, retrospective cohort study linked data from the Children's Hospital Association Pediatric Health Information Systems (PHIS) and PROSPECT Hospital Essentials (PROSPECT) databases from January 2018 to December 2022. PHIS harbors patient level data while PROSPECT harbors hospital and unit-level human resource metrics. Demographic and outcome data, including mortality, medical/surgical complications, readmission rate, vasoactive use, and measures of acuity were extracted from PHIS for all PICU admissions to hospitals providing data to both databases. The proportion of yearly agency staffing, total agency expense, salary, and revenue measures per PICU were extracted from PROSPECT. PICUs were sorted into quartiles based on the yearly proportion of agency staffing. Continuous data were summarized as medians with interquartile ranges, categorical data as counts with proportions. Categorical data were analyzed using the Pearson's chi-squared test and continuous data using the Kruskal-Wallis test.

Results: The analysis included 281,948 admissions across 21 hospitals ranging across all census regions of the United States. Agency staffing percentages were divided into quartiles (Q) by year; Q1 with 0% agency staff, Q2 with 1-5% agency staff, Q3 with 5.1-10% agency staff, and Q4 with >10% agency staff. Hospitals varied per year with their agency staffing percent. Average age for the overall cohort was 44 (9-136) months, 126,817 (45%) were female, and 182,458 (65%) had a complex chronic condition. There were significant differences across pooled (entire study length) agency quartiles in mortality, medical/surgical complications, ECMO cases and renal replacement therapy (all $P<0.001$). Overall, there were 8,994 (3.4%) medical complications in the cohort. There was a statistically significant difference across quartiles with 1,452 (2.4%) medical complications in Q1, 3,635 (3.2%) in Q2, 1,124 (2.3%) in Q3, and 2,782 (4.7%) in Q4 across the study period. ($P<0.001$). Length of stay and total days in the ICU decreased as the percent agency increased (both $P< 0.001$). The severity of illness and staff salary costs were significantly higher as the proportion of agency nurse staffing increased (both $P<0.001$).

Conclusions: The proportion of PICU agency nurses is associated with patient outcomes and overall illness severity. Notably, there was an association with higher medical complications in quartile 4 throughout the study dates. Further work that accounts for patient and unit-level confounders is necessary to better understand possible relationships between PICU nursing staffing metrics and patient outcomes.

Significance: This research aims to better understand patient outcome and cost-of-care changes associated with staffing strain at children's hospitals due to the COVID-19 pandemic.

Research/Grant Support: None

DEPARTMENT OF EMERGENCY MEDICINE

Oral Abstract O3

VALIDATION OF NON-INVASIVE SENSORS FOR MONITORING CORE TEMPERATURE

Thomas SS, Flickinger K, Callaway CW, Elmer J

Department of Emergency Medicine
University of Pittsburgh School of Medicine

Introduction: Accurate temperature measurement is critical to patient care. Core temperature monitoring is a gold standard but invasive.

Hypothesis: We hypothesized at least one of two non-invasive core temperature monitoring devices (BairHugger™ and CoreTemp (CORE)) would be accurate and unbiased in comparison to an invasive core temperature monitor.

Methods: We performed a single-center, feasibility trial of non-invasive core temperature monitors with patients who had invasive temperature monitoring as part of clinical care. Patients received either a BairHugger or CORE probe through convenience sampling based on sensor availability. We collected temperature data for each participant from both devices (non-invasive and invasive) in 10-minute intervals for a minimum of 2 readings. For each non-invasive device, we constructed Bland-Altman plots, determined mean difference between non-invasive and invasive temperature measurement and the 95% limits of agreement (LoA). We also calculated Spearman's Rank correlation coefficient.

Results: We enrolled 24 patients and collected 272 paired temperature values between October 2021 - December 2022, with an average of 9 observations per patients (2-18). A majority of patients had active temperature controlled via either a cooling catheter or gel adhesive pads. Bland-Altman bias was -1.1°C, with LoA -3.2°C to - 0.98°C for the BairHugger probe and a bias of 2.4°C with LoA -1.9°C to 6.6°C for the CORE probe. There was a strong correlation between the BairHugger and invasive temperatures ($r= 0.83$, $p<0.0001$); the CORE probe and invasive temperatures were only moderately correlated ($r=0.57$, $p<0.0001$).

Conclusion: Our study provides evidence for the use of non-invasive core temperature monitors in patient care. The BairHugger probe provided a biased but consistent measure compared to an invasive core probe. More data are required throughout mild hypothermic ranges to evaluate fully for bias across a range of temperatures encountered in clinical practice.

Significance: This study provides evidence for the use of non-invasive core temperature monitors in patient care, potentially reducing complications and improving patient comfort. The BairHugger Temperature probe provides a reasonable estimation of core temperature when invasive methods may not be assessable or available.

Abstract 26

ASSOCIATIONS BETWEEN HEART RATE VARIABILITY AND NEED FOR LIFESAVING INTERVENTION AND MORTALITY IN A LARGE HELICOPTER EMS SERVICE

Defilippi D,[1] Salcido D,[1] Zikmund C,[1] Weiss L,[1] Schoenling A,[2] Guyette F,[1] Poropatich R,[3] Pinsky M[4]

[1]University of Pittsburgh School of Medicine, Department of Emergency Medicine
[2]UPMC, Department of Emergency Medicine
[3]University of Pittsburgh, Center for Military Medicine Research
[4]University of Pittsburgh School of Medicine, Department of Critical Care Medicine

Introduction: Heart rate variability (HRV) measures give insight into the autonomic regulation of cardiac function in healthy and critically ill patients. Previous studies with relatively small sample sizes have shown that brief, early measurements of HR complexity in the prehospital setting can predict death before hospital discharge. The ease of measurement and predictive potential of HRV measures may make them valuable for the optimization of battlefield triage and care, although a deeper understanding of their utility is necessary.

Hypothesis: We hypothesized that HRV measures—specifically the HR complexity measure Sample Entropy (SampEn)—measured early in transport would be strongly predictive of the need for a prehospital lifesaving intervention (LSI) and the likelihood of death before hospital discharge ("mortality") for a large, heterogenous cohort of critically ill patients.

Methods: We obtained patient records from STAT MedEvac Air Medical Service. ECG data was retrieved from clinical monitors during the period immediately before ("pre-takeoff") and after helicopter takeoff ("post-takeoff"), modeling pretransport in-progress stabilization and intra-transport patient states in battlefield MEDEVAC. ECG signal data was batch processed using the PhysioNet Cardiovascular Toolbox (PNCT). Cases were rapidly screened via signal quality index (SQI), and those with a SQI ≥ 0.9 were included for analysis. Time, frequency, and complexity domain HRV measures were calculated using the PNCT analysis functions and averaged for the first 5 minutes of each ECG period. Patient outcomes were determined via review of flight crew activity logs and in-hospital discharge records of patients transported to UPMC hospitals. Multivariable logistic regression models were constructed, which analyzed the correlation between selected HRV measures and the outcomes of mortality and the need for a prehospital LSI, while adjusting for demography and case characteristics.

Results: Of the original sample size (n = 76,643), a total of 13,028 pretakeoff cases and 17,958 post-takeoff cases remained after being filtered for SQI or missing inhospital outcomes. A logistic regression model with the outcome of mortality yielded an odds ratio of 0.58 (95% CI [0.51,0.65]) for the pre-takeoff group and 0.57 (95% CI [0.51,0.62]) for the post-takeoff group, when correlated with SampEn. A second logistic regression model with the need for prehospital LSI as the outcome yielded an odds ratio of 0.45 (95% CI [0.42,0.49]) (pretakeoff) and 0.47 (95% CI [0.45,0.49]) (post-takeoff) when correlated with SampEn. $P > |z| = 0.00$ in each case, indicating a definite association.

Conclusions: There was an observed association between SampEn, as well as other HRV metrics, and the likelihood of a critically ill patient needing a prehospital LSI or surviving transport to a critical care facility. We expect that more work is needed to investigate this association.

Significance: HRV measures show predictive power across many prehospital conditions.

Research/Grant Support: NHLBI 1R01HL141916 (PI: Michael Pinsky, MD); US Army MRMC W81XWH-19-C-0101 (PI: Ronald Poropatich, MD)

Abstract 27

ASSOCIATION BETWEEN FINGER PLETHYSMOGRAPHIC FEATURES AND IMPEDANCE-BASED THORACIC FLUID CONTENT MEASUREMENT IN A LOWER BODY NEGATIVE PRESSURE MODEL OF HEMORRHAGIC SHOCK

Lane N,[2] Salcido D,[1] Zikmund W,[2] Weiss L,[1] Guyette F,[1] Poropatich R,[4] Weissman A,[1] Flickinger K,[1] DeMaio R,[1] Pinsky M[3]

[1]University of Pittsburgh School of Medicine, Department of Emergency Medicine, Pittsburgh, PA
[2]UPMC, Department of Emergency Medicine, Pittsburgh, PA
[3]University of Pittsburgh School of Medicine, Department of Critical Care Medicine, Pittsburgh, PA
[4]University of Pittsburgh School of Medicine, Center for Military Medicine Research, Pittsburgh, PA

Introduction: Early, non-invasive detection of hemorrhagic shock signatures may expedite lifesaving interventions on the battlefield. Finger photoplethysmography (PPG) holds some promise, but may require complementary signals to be effective, including transthoracic impedance (TTI). The objective of this study was to understand how PPG features correlate with TTI during hemorrhage

Hypothesis: We hypothesized that features of the PPG waveform would correlate with thoracic fluid content (TFC) as measured by TTI.

Methods: We obtained physiologic monitoring data from healthy adult subjects (n =19 mean (SD) age of 26 (8) years, 32% female) undergoing lower body negative pressure (LBNP) challenge. Instrumentation included a finger PPG sensor and a four-lead TTI monitor. We used a stepwise LBNP exposure program with decreases of -10 mmHg per 10 minutes and termination at the first sign of near syncope. TTI data was streamed to a custom program written in MATLAB (v2020b, Mathworks, Inc) and downsampled to 250Hz. We featurized each beat to include a systolic peak, diastolic/reflected peak, notch, beat length, beat area under the curve (AUC), absolute and relative peak amplitudes, peak-to-peak systolic/diastolic interval, and leading and trailing slopes. These were normalized to the instantaneous heart rate. The TFC signal was normalized to the subject's pre-LBNP baseline. We summarized all PPG/TFC features using descriptive statistics over five-minute epochs at the end of the baseline phase and each LBNP exposure step. Generalized estimating equations (GEE) models were used to examine the relationship between TFC and PPG features while controlling for LBNP stage and subject.

Results: PPG analysis demonstrated consistent trends in several measures across LBNP increased negativity stages, including increased systolic-diastolic peak interval, decreased diastolic peak height, and consequent decreased beat AUC. TFC analysis demonstrated a consistent decrease across stages, with end stage normalized TFC averaging 14.6% (range: 26.5% - 8.0%) of baseline fluid volume. The strongest average correlations between stage TFC and PPG features occurred in beat length (0.65), beat AUC (0.64), and systolic-diastolic interval (-0.72). In GEE models incorporating all stages, only beat length (i.e., heart rate; $p=0.02$) and notch height ($p = 0.002$) were significantly associated with TFC. However, in models considering only the final 2 LBNP stages, the systolic-diastolic interval ($p = 0.013$), notch height ($p < 0.046$), and beat length ($p < 0.001$) were significantly associated with TFC.

Conclusions: While both PPG features and impedance-based TFC trend congruently in the peri-shock state towards the end of LBNP exposure, these measures only partly track together, reflecting a lag between redistribution of thoracic fluid volume and peripheral pulse wave signals.

Significance: PPG alone may be most useful within 10 minutes of progression from compensated to decompensated hemorrhagic shock and may serve as a surrogate for TFC when direct thoracic sensing is not available.

Research/Grant Support: USAMRDC funded work - Contract W81XWH19C0101

DEPARTMENT OF NEUROLOGICAL SURGERY

Oral Abstract O4

PRE-INJURY FRAILTY BEST PREDICTS ONE YEAR MORTALITY IN OLDER ADULTS WITH TRAUMATIC SPINAL COLUMN FRACTURES

Varga G,[1] Fields DP,[1] Alattar A,[1] Eubanks J,[2] Agarwal N,[1] Hamilton DK,[1] Okonkwo DO,[1] Kanter AS,[3] Forsythe RM,[4] Weiner DK[5]

[1]University of Pittsburgh Department of Neurological Surgery
[2]University of Pittsburgh Department of Physical Medicine and Rehabilitation
[3]Hoag Neurosciences Institute
[4]University of Pittsburgh Department of Surgery
[5]University of Pittsburgh Department of Medicine (Geriatric Medicine), Psychiatry, Anesthesiology, and Clinical and Translational Sciences

Introduction: Americans aged 65 and older constitute the majority of trauma related hospital admissions and these individuals have worse one year outcomes than injury matched younger adults. Extant trauma grading scales do not incorporate measures of frailty (i.e., physiological vulnerability) and are of limited utility in predicting mortality in older adults.

Hypothesis: Frailty variables will predict one year mortality in adults aged 65 and older with isolated spinal column fractures.

Methods: We retrospectively reviewed over 35,000 trauma patients admitted to a level 1 trauma center from 2013-2018. Those included were age 65+ at the time of presentation with an acute traumatic spinal column fracture. Patients were excluded if they had a new or pre-existing neurological deficit, moderate-severe injury to another system (i.e., traumatic brain injury or hip fracture) or died during the index admission. Patient demographics, injury features, pre-injury medical conditions and pre-injury mobility status were evaluated using univariate and multivariate analyses.

Results: Of the 704 older adults included, 199 (28.3%) died within a year of the index trauma. No existing trauma grading scale (Glasgow Coma Scale, Abbreviated Injury Scale, Injury Severity Score, Trauma Score) was predictive of one year mortality using multivariable analysis. In addition, there was no difference in operative management ($p = 0.406$). Conversely, the pre-injury risk assessment index (RAI), a frailty scale incorporating age, functional independence, residence, and pre-existing health conditions, was independently predictive of one year mortality ($p < 0.001$). Serum albumin and prealbumin levels were inversely correlated with one year mortality ($p < 0.001$).

Conclusions: Isolated traumatic spine fractures are associated with increased mortality in older adults.

Significance: While current trauma indices fail to identify at-risk patients, pre-injury RAI as well as admission serum albumin and prealbumin levels represent important prognosticators of one year mortality. Further work is needed to explore the utility of targeting frailty variables to prevent poor outcomes in geriatric spine trauma patients.

Abstract 28

EARLY SURGERY IS ASSOCIATED WITH REDUCED OPIOID PRESCRIPTION USE IN PATIENTS WITH TLICS 4 SPINE FRACTURES

Adida S,[1] Casillo S,[1] Agarwal N,[1] Hamilton D,[1] Okonkwo D,[1] Fields D[1]

[1]Department of Neurosurgery
University of Pittsburgh School of Medicine

Introduction: The Thoracolumbar Injury Classification and Severity (TLICS) scale is a clinical tool for determining operative versus non-operative management of spinal fractures. The scale is a summation of scores from three factors: (1) the morphology of injury, (2) the integrity of the posterior ligamentous complex, and (3) the patient's neurological status. While TLICS score > 4 are managed with surgery, < 4 are managed with conservative (non-surgical) measures, there is management ambiguity for TLICS 4 patients. Some TLICS 4 patients are managed with early surgery, others undergo no surgery or delayed surgery if certain clinical sequelae occur (e.g. persistent pain). The impact of surgical timing on long-term pain outcomes is not understood.

Hypothesis: The purpose of this study is to test the hypothesis that surgical timing impacts long-term pain outcomes in patients with TLICS 4 fractures. Specifically, we hypothesize that early surgery for TLICS 4 patients leads to better long-term pain control when compared to delayed surgery or non-operative management.

Methods: A retrospective review of 59 patients was performed from January 2010 to January 2019. This study included neurologically intact patients with TLICS 4 fractures and severe back pain (requiring intravascular opioid pain medications). Patients with pre-existing spinal fractures, surgery or neurological deficits, confounding pain from non-spinal injuries, or recent (within 6-months) opioid pain prescriptions were excluded. Controlled medication use was determined through state Prescription Drug Monitoring Program records. Surgical management/timing and long-term outcomes were reviewed through the electronic health record. Univariate statistical analysis was used for data analysis and differences were considered significant if $p < 0.05$.

Results: Nearly 50% of TLICS 4 patients developed chronic pain; defined as opioid medication use > 6-months after injury. Patients who received early surgery (2.2 ± 1.1 days post injury) were less likely to develop chronic pain with fewer patients reporting disability ($p < 0.01$) or controlled medication use ($p < 0.0001$) when compared to patients undergoing delayed surgery (46.1 ± 10.3 days post injury) or no surgery.

Significance: Early surgery should be considered, and short-interval follow-up re-evaluation should become standardized for patients with new traumatic spinal fractures.

Grant Support: None

Abstract 29

THE RELATIONSHIP BETWEEN MORPHINE-3-GLUCURONIDE PLASMA CONCENTRATIONS AND HIPPOCAMPAL GFAP IMMUNOREACTIVITY FOLLOWING CONTROLLED CORTICAL IMPACT INJURY IN RATS

Birabaharan J,[1] Henchir J,[3] 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: In patients with severe traumatic brain injury (TBI), intravenous (IV) morphine is often administered for analgesia worldwide. Morphine's major metabolite, morphine-3-glucuronide (M3G) activates Toll-like Receptor 4 (TLR4) and exacerbate the neuroinflammatory response. Normally, M3G is unable to penetrate the blood-brain barrier (BBB), but in cases of a compromised BBB, including TBI, it can enter the brain and potentially activate TLR4 receptors on astrocytes.

Hypothesis: We hypothesize that acute IV M3G exposure exacerbates GFAP immunoreactivity 24hr after TBI.

Methods: To investigate this, we performed pharmacokinetic modelling to mimic an acute clinical exposure of M3G from a phase I study in healthy volunteers, and calculated a 2.5 mg/kg dose by intravenous injection for use in rats. To achieve this, a jugular catheter was placed in male Sprague Dawley rats (275-300g, n=6 per group) prior to controlled cortical impact (CCI) injury (2.5mm, 4m/s) or sham surgery. Rats were randomly assigned to receive 2.5 mg/kg M3G or vehicle. Blood was collected via the catheter at nine timepoints throughout the 24hr post-injury period.

Results: Systemic exposure of M3G was 19% higher in CCI rats compared to sham (Welch's t-test, p=0.02). Brains analyzed at 24hr post-injury for GFAP immunoreactivity revealed a potential trend in M3G exposure after CCI, with a modest increase in ipsilateral hippocampal GFAP immunoreactivity compared to sham (p=0.0679, two-way ANOVA).

Conclusions: Results suggest M3G exposure may increase reactive astrocytosis in the context of TBI.

Significance: There may be a potential mechanism triggered when administering morphine acutely in TBI patients, leading to exacerbated secondary injury effects. Further investigations are needed to elucidate this process.

Research/Grant Support: Walter L Copeland of The Pittsburgh Foundation (PI: Jonathan Birabaharan), NIH R01-NS124730(PI: Shaun Carlson) and NIH S10OD028540 (PI: Thomas Nolin)

Abstract 30

EVALUATION OF DHA PHARMACOLOGIC INTERVENTION ON NEUROBEHAVIORAL DEFICITS, HIPPOCAMPAL SYNAPTIC PROTEINS AND AXONAL INJURY MARKERS IN RATS AFTER REPEATED MILD TBI

Roberts A,[1] Fronczak K,[1] Henchir J,[1] Elsa K,[2] Wu Y,[2] Carlson SW,[1] Dixon CE[1]

[1]Department of Neurological Surgery, UPMC, Pittsburgh, PA
[2]Department of Developmental Biology, Rangos Research Center Animal Imaging Core, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Repetitive mild traumatic brain injury (rmTBI) accounts for a majority of all TBIs; neuroinflammation, synaptic dysfunction, and axonal injury are identified as contributors to impaired neurobehavioral function. Docosahexaenoic acid (DHA), an omega-3 fatty acid, is a promising therapeutic intervention assessed in severe TBI, but no research has been undertaken in a rmTBI preclinical model.

Hypothesis: This study aims to characterize and determine the efficacy of DHA pharmacological intervention after rmTBI in rats on functional outcomes, synaptic transmission, and axonal injury.

Methods: Adult male Sprague-Dawley rats (290-380g) received quadruple fluid percussion injury (qFPI, 1.25 atm, 24hr apart) or sham control surgery (qSham, anesthetic exposures 24hr apart). Animals were treated with 8, 16, or 40mg/kg DHA or vehicle (DMSO) intraperitoneally 5mins after the first surgical event, injections continued daily until sacrificed. Rats underwent motor and Morris water maze testing before sacrifice at 2wks post-injury. An additional cohort of animals was sacrificed at 1wk post-injury to assess synaptic impairment in the ipsilateral hippocampus.

Results: Repeated measures one-way ANOVAs demonstrated a significant injury effect on beam walking and spatial learning ability ($p<0.01$), with significantly reduced performance in injured groups, independent of treatment status. Two-way ANOVA analyses of immunoblots at 1wk post-injury (n=6/group) indicated significant injury effects on α-synuclein, CSP-α, Vamp2, synaptophysin, p-APP and LRRK2 ($p<0.05$), and significant treatment effects on synaptophysin, LRRK2, and p-LRRK2 ($p<0.05$). Analysis of microglia immunoreactivity demonstrated significant treatment and injury effects in the cortex ($p<0.05$). Ongoing work is assessing the effect of DHA on markers of axonal injury and high-definition fiber tractography with diffusion tensor imaging.

Significance: This study demonstrates that DHA resulted in significant treatment effects in the abundance of key synaptic proteins and attenuated microglia immunoreactivity. Further work is necessary to determine pleiotropic effects of DHA treatment after TBI.

Research/Grant Support: This work was supported by the Chuck Noll Foundation (CED, SWC).

Abstract 31

COMPARING CONTINUOUS VERSUS THRESHOLD DRAINAGE STRATEGIES FOR SPINAL PERFUSION PRESSURE OPTIMIZATION IN PATIENTS WITH ACUTE TRAUMATIC SPINAL CORD INJURIES

Shanahan R, Lavadi R, Puccio A, Buell T, Hamilton D, Agarwal N, Okonkwo D, Fields D

Department of Neurosurgery, UPMC, Pittsburgh, Pennsylvania

Introduction: Acute spinal cord hypoperfusion impairs motor recovery following traumatic spinal cord injury. Cerebral spinal fluid (CSF) diversion increases spinal cord perfusion pressure (SCPP); however, there is variability in this approach.

Hypothesis: This study was designed to compare the efficacy and potential complications of continuous versus threshold CSF drainage strategies in acute spinal cord injury patients.

Methods: A retrospective review of 19 acute spinal cord injury patients managed with intrathecal lumbar drains was performed. Six patients were managed with continuous drainage (CSF drained at regular intervals regardless of SCPP) while 13 patients had CSF drained only when SCPP fell below 65 mmHg (i.e. threshold drainage). Intrathecal pressure, SCPP, mean arterial pressure (MAP), and vasopressor utilization were compared using univariate statistical analysis. Differences were considered significant if $p < 0.05$.

Results: The cohort included over 1500 time points from 19 patients. While there was no difference in rates of sub-optimal SCPP (< 65 mmHg; $p = 0.166$), patients managed with threshold drainage were more likely to exhibit critically low SCPP (< 50 mmHg; $p = 0.003$) despite also having lower average intrathecal pressures ($p < 0.001$). There were no differences in average SCPP, MAP, or vasopressor utilization between the two groups ($p > 0.05$). There were no lumbar drain complications reported from either group.

Conclusions: Acute spinal cord injury patients managed with continuous drainage were more likely to avoid critically low SCPP when compared to patients managed with threshold drainage strategies.

Significance: These preliminary observations suggest the need for a prospective randomized controlled trial to validate these findings and understand its implications onto long-term motor recovery in acute spinal cord injury patients.

Research/Grant Support: N/A

Abstract 32

RESTORING NEUROGRANIN EXPRESSION AFTER CONTROLLED CORTICAL IMPACT: A NOVEL THERAPEUTIC APPROACH FOR ALTERED POST-SYNAPTIC SIGNALING AFTER TBI

Svirsky SE,[1,2] Henchir J,[2] Carlson SW,[2] Dixon CE[2,3]

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

Introduction: Neurogranin (Ng), an emerging TBI serum biomarker due to its association with cognition, is a calcium-sensitive calmodulin-binding protein essential for modulating synaptic plasticity. We previously demonstrated controlled cortical impact (CCI) significantly reduces hippocampal Ng up to 4 weeks post-injury. Conversely, increasing levels of Ng in a specific brain region facilitates synaptic plasticity, increases synaptogenesis and boosts cognitive abilities. Given the loss of Ng after injury, additional research is warranted to discern changes in post-synaptic signaling after TBI. Additionally, the therapeutic potential of increasing Ng for the treatment of TBI has yet to be investigated.

Hypothesis: We hypothesize decreased Ng will be associated with aberrant post-synaptic signaling after CCI.

Methods: Under isoflurane anesthesia, adult, male and female Sprague Dawley rats (250-350g, n=5-6 per injury/time-point/sex) received a sham/control or CCI injury (2.5mm depth, 4m/s). Ipsilateral hippocampal synaptosomes were isolated at 24 hours, 1, 2 and 4 weeks post-injury and western blot was used to evaluate protein expression of Ng-associated signaling proteins. Two-way ANOVA was used to evaluate main effects of injury and sex at each time-point. A dose response of AAV-Ng-GFP or AAV-GFP control was administered 30 minutes post-CCI (n=5/group) into the ipsilateral hippocampus. 4 weeks post-injury and AAV administration, brains were harvested for immunohistochemistry or for immunoblotting (as mentioned above).

Results: Two-way ANOVA showed significant main effects of injury and sex in a protein-dependent fashion for synaptic expression of Ng, phosphorylated-Ng (Ser36), phosphorylated-CaMKII (Thr286) and calmodulin across various time-points. Immunoblot and immunofluorescence showed viral-mediated restoration of Ng expression after CCI compared to control. Co-immunoprecipitation confirmed calcium-dependent interactions between calmodulin with exogenous Ng. A significant positive correlation was observed between exogenous Ng and phosphorylated-CaMKII expression (R^2=0.6122, p=0.0026).

Conclusions: Ng hippocampal post-synaptic signaling is altered after CCI. AAV administration restored Ng hippocampal expression after CCI and modulates downstream protein expression.

Significance: This study furthers our understanding of mechanisms of cognitive dysfunction within the synapse sub-acutely after TBI. Ongoing studies seek to determine if Ng gene therapy ameliorates TBI-induced post-synaptic signaling and behavioral outcomes.

Research / Grant Support: UPMC Children's Hospital of Pittsburgh (SES), VA-I01-BX005291 (CED), The Pittsburgh Foundation Walter L. Copeland Fund (SES)

Abstract 33

GAMMA KNIFE STEREOTACTIC RADIOSURGERY TO TREAT PATIENTS WITH BRAIN METASTASES FROM COLORECTAL PRIMARY CANCERS

Taori S, Wei Z, Deng H, Niranjan A, Lunsford D

Department of Neurosurgery, UPMC

Background: The role of stereotactic radiosurgery (SRS) for patients with brain metastases from colorectal cancers (CRC), has not been established. The authors present one of the largest single-institution experiences of CRC patients who underwent SRS with metastatic brain spread.

Methods: We retrospectively analyzed 112 metastatic CRC patients (64 female, 57.14%) with 450 brain metastases that were treated with Gamma Knife SRS between 2000-2022. The median age at SRS was 63 years (range: 28-86) and the median Karnofsky Performance Score (KPS) was 80 (range: 60-100). The primary sites were colon (86 patients, 76.79%) and rectal (26 patients, 23.21%). Kaplan-Meier and cox proportional-hazards model were utilized for univariate and multivariate analyses to identify prognostic factors.

Hypothesis: We hypothesized that patients treated with SRS for primary CRC will have adequate intracranial tumor control, low radiation-induced neurotoxicity, and comparable overall survival to other treatment modalities.

Results: The median patient survival after SRS was 7 months (range: 1-174). Ninety-eight (87.5%) patients expired at last follow up and 15 patients (15.31%) died related to progressive intracranial disease. KPS < 80 ($p=0.03$, HR: 0.63, 95% CI: 0.41-0.96) was associated with inferior overall survival using multivariate analysis. Seventeen patients (15.18%) had local tumor progression after SRS, at a median time of 7 months (range: 3-34) between SRS and progression. Twenty-six patients (23.21%) developed new brain metastases at a median of 5 months (range: 2-26) between SRS and new tumor detection. KPS ≥ 80 ($p=0.05$, HR: 0.96, 95% CI: 0.93-0.99), and < 3 brain metastases at SRS presentation ($p<0.01$, HR: 3.62, 95% CI: 1.53-8.56) were associated with better distant tumor control on multivariate analysis. The incidence of adverse radiation effects was 5.36%.

Conclusions: SRS effectively controls brain metastases from CRC with low risk of treatment related toxicity, thereby allowing patients to focus on primary disease management.

Significance: Current standard of care for patients with brain metastases from CRC cancers includes surgical resection and whole-brain radiation therapy (WBRT). Even then, median survival is low and varies between 2-8 months. Minimally invasive SRS circumvents surgical morbidities and common WBRT side-effects such as fatigue, while still providing effective intracranial tumor control and comparable overall survival rates. In this study, no difference in OS ($p=0.2$, HR: 1.28, 95% CI: 0.84-2.15) was found between patients treated with SRS and SRS + WBRT. We hope this study offers perspective and guidance to future physicians tasked with treating patients with brain metastases from CRC primary cancers.

Research/Grant Support: N/A

Abstract 34

THE ROLE OF STEREOTACTIC RADIOSURGERY IN PATIENTS WITH FORAMEN MAGNUM MENINGIOMAS

Taori S, Wei Z, Mehra M, Dasaka J, Mehta M, Rai K, Deng H, Hadjipanayis C, Niranjan A, Lunsford D

Department of Neurosurgery, UPMC

Background: Management of foramen magnum meningiomas (FMMs) varies widely and traditional treatment approaches have been associated with neurological morbidity and risks to the neurovasculature. The authors report the largest single institution experience of utilizing minimally invasive stereotactic radiosurgery (SRS) to treat FMMs.

Methods: Fifty-six patients (43 female, 76.8%) with FMMs treated with SRS between 1987 and 2022 were retrospectively reviewed. Median age at SRS was 61.5 (range: 30-83) years. Fourteen (25%) patients had pre-SRS surgical resection (3 gross total resection, 11 subtotal resection), while 6 (10.7%) had prior radiotherapy. Tumors were located at the anterior midline (10 patients, 17.9%), anterior lateral (31 patients, 55.4%), posterior midline (7 patients, 12.5%), and posterior lateral (8 patients, 14.3%) positions. The median prescription dose was 12.5 Gy (range: 10 – 16) and the median cumulative FMM tumor volume treated was 2.1 cc (range: 0.32 – 10.9). Kaplan-Meier, cox proportional-hazards models, and logistic regression were utilized for univariate and multivariate analyses to identify prognostic factors.

Hypothesis: We hypothesized that patients treated with SRS for FMMs will have effective tumor control, stable and/or improved neurological function post-SRS, and low radiation-induced neurotoxicity's.

Results: The median follow-up time was 35.5 months (range: 4-216). At last follow-up (n=51), complete imaging response was noted in 1 patient (1.96%), partial response was noted in 16 patients (31.4%), stability of disease was noted in 33 patients (64.7%), and progression was reported in 1 patient (1.96%) 9 months after initial SRS. The overall local tumor control rate was 98.04%. Patients who had prior surgery (p=0.04) and who were female (p=0.04) were more likely to have volumetric tumor regression on MRI post-SRS. Post-SRS (n=51), 15 patients (29.4%) had improved symptoms, 30 patients (58.8%) had stable symptoms, and 6 patients (11.8%) reported worsening symptoms as compared to pre-SRS. Margin dose > 11 Gy was significantly associated with improved neurological symptoms (p=0.04) post-SRS. The median overall survival was 111 months (range: 4-216). No patients reported adverse radiation effects (AREs).

Conclusions: SRS can effectively be used to control local tumor progression and improve neurological symptom in FMM patients while limiting AREs.

Significance: FMMs pose a clinical challenge due to their proximity to critical structures, such as the brainstem, lower cranial nerves, medulla, vertebral artery, and its branches. Current standard of care, surgical resection, is associated with significant rates of complications and morbidity. Gamma Knife SRS allows for minimally invasive tumor control, neurological symptom improvement, and low risks of toxicity. This study is the largest single-institution study in terms of patients and tumors to examine the long-term role of SRS in treating FMMs and determining prognostic factors for future clinical decision making.

Research/Grant Support: N/A

DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION

Oral Abstract O5

COMBINING A α7 NICOTINIC ACETYLCHOLINE RECEPTOR ALLOSTERIC MODULATOR AND ENVIRONMENTAL ENRICHMENT IMPROVES SUSTAINED ATTENTION AND CHOLINERGIC NEUROTRANSMISSION AFTER CONTROLLED CORTICAL IMPACT INJURY IN MALE AND FEMALE RATS

Annas EM, Moschonas EH, Rennerfeldt PL, Ranellone TS, Bertocchi MA, Race NS, Cheng JP, Kline AE, Bondi CO

Physical Medicine and Rehabilitation
Safar Center for Resuscitation Research
Center for Neuroscience
University of Pittsburgh, Pittsburgh, PA

Introduction: Traumatic brain injury (TBI) is a leading cause of disability and pharmacological strategies that enhance acetylcholine (ACh) transmission may ameliorate cognitive deficits. Enhancing acetylcholine (ACh) transmission after TBI may ameliorate cognitive deficits, especially combined with noninvasive rehabilitation, mirroring clinical approaches.

Hypothesis: We predicted that chronic NS-1738, a novel α7 nicotinic ACh receptor (α7-NAChR) positive allosteric modulator (PAM), will improve sustained attention post-TBI, alone and in combination with environmental enrichment (EE). Blocking α7-NAChRs with methylycaconitine (MLA) will attenuate the effects of NS-1738, confirming its mechanism of action.

Methods: Adult male and female rats were trained in the 3-choice serial reaction time task (3-CSRT) prior to right parietal controlled cortical impact (2.8 mm cortical deformation depth) or sham injury. Rats were randomized to NS-1738 (5 mg/kg) or vehicle (saline), as well as daily EE (6h) or standard housing for 28d starting post-injury day (PID) 1. Male subgroups also received daily α7-NAChRs blockade via MLA (3 mg/kg) injections. 3-CSRT retrials occurred on PID 14-24. Medial prefrontal cortex (mPFC) and basal forebrain 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 of both sexes exhibited impaired sustained attention ($p<0.05$) and ChAT disruptions in both mPFC and basal forebrain, which were improved by chronic NS-1738 ($p>0.05$). Moreover, NS-1738+EE rendered an additive effect on lowering omissions and improving inflammatory markers ($p<0.05$), including TREM-1 f(triggering receptor expressed on myeloid cells-1) and IL-1 RA (interleukin-1 receptor antagonist). TBI groups that received MLA demonstrated a reinstatement of performance deficits, as hypothesized.

Conclusions and Significance: Our findings support benefits of α7-NAChR PAM and/or EE treatment after experimental TBI on sustained attention and cholinergic neurotransmission.

Research/Grant Support: This work was supported in part by National Institute of Health grants NIH NS110609 (COB), NS084967, NS121037 (AEK), Research Advisory Committee, UPMC Children's Hospital of Pittsburgh (COB), as well as UPMC Children's Research Advisory Committee Dissertation Fellowship and Brain Injury Association of America Dissertation Grant (EHM).

Abstract 35

LONG-TERM PRETREATMENT OF ENVIRONMENTAL ENRICHMENT DOES NOT YIELD A PROPHYLACTIC EFFECT AGAINST TBI-INDUCED NEUROBEHAVIORAL DEFICITS FOLLOWING CONTROLLED CORTICAL IMPACT

Bittner RA,[1,2] Steber JA,[1,2] Tamura DM,[1,2] Capeci HE,[1,2] Rennerfeldt PI,[1,2] Vozzella VJ,[1,2] Moschonas EH,[1-3] Cheng JP,[1,2] Bondi CO,[1-4] Kline AE[1-3,5,6]

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

Introduction: Environmental enrichment (EE), a pre-clinical model of neurorehabilitation, confers significant behavioral and histological benefits when initiated *after* experimental traumatic brain injury (TBI). A recent study, however, revealed that EE pretreatment, when provided for 2-weeks *before* controlled cortical impact (CCI), does not produce a neuroprotective or prophylactic effect. Considering the well-supported therapeutic efficacy of EE for TBI-induced behavioral impairments, the current study utilized a 4-week EE pretreatment paradigm to determine whether the limited exposure provided by the previous 2-week EE pretreatment paradigm accounts for the observed lack of protection.

Hypothesis: Four weeks of EE pretreatment will exert a prophylactic effect against TBI-induced neurobehavioral deficits.

Methods: After 4 weeks of EE or standard (STD) housing, isoflurane-anesthetized adult male rats received a CCI of moderate severity (2.8mm deformation at 4 m/s) to the right hemisphere or sham injury and then were randomly assigned to post-operative EE or STD conditions. The group receiving EE *before* and *after* CCI was included to determine whether EE pretreatment affects the efficacy of post-TBI EE. Motor (beam-walking) and cognition (acquisition of spatial learning) were evaluated on post-operative days 1-5 and 14-19, respectively. The behavioral data were analyzed by two-way ANOVAs with repeated measures over testing days, followed by the Newman-Keuls post-hoc text, which corrects for multiple comparisons.

Results: The Post-TBI EE groups performed better than the Post-TBI STD groups ($p<0.05$) but did not differ from each other ($p>0.05$). However, despite 4 weeks of EE prior to TBI, no prophylactic effect was observed as there were no differences between the STD-housed TBI groups regardless of whether they received EE or STD housing before surgery ($p>0.05$).

Conclusions and Significance: Overall, no neuroprotective or prophylactic effect was observed with 4 weeks of EE pretreatment regardless of whether they received EE or STD post-operatively ($p>0.05$). These data do not support the hypothesis that a 4-week EE pretreatment paradigm would exert a prophylactic effect post-TBI. However, these data reproduce previous findings that post-TBI is effective and replicate a recent study claiming that EE pretreatment does not confer neuroprotection following TBI.

Research/Grant Support: This work was supported in part by National Institute of Health grants NS084967, NS121037 (AEK), NS110609 (COB).

Abstract 36

BREXPIPRAZOLE (REXULTI®), A THIRD-GENERATION ATYPICAL ANTIPSYCHOTIC DRUG, DOES NOT NEGATIVELY IMPACT BEHAVIORAL RECOVERY AFTER EXPERIMENTAL BRAIN TRAUMA

Capeci HE,[1,2] Tamura DM,[1,2] Bittner RA,[1,2] Steber JA,[1,2] Vellore AJ,[1,2] Eleti RR,[1,2] Carpio AN,[1,2] Victoria AM,[1,2] Connor BJ,[1,2] Moschonas EH,[1,2] Bondi CO,[1-4] Kline AE[1-3,5,6]

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

Introduction: Aggressive and agitative behaviors are common after traumatic brain injury (TBI). To manage these disruptive behaviors clinically, antipsychotic drugs (APDs) are frequently provided despite the risks on cognitive outcome after TBI. APDs that exhibit D_2 receptor antagonism have been shown to impede cognitive recovery after TBI. This it is important to evaluate APDs that may not be detrimental to the recovery process. Brexpiprazole (REXULTI®) is a third-generation atypical APD with partial D_2 and 5-HT_{1A} receptor agonist properties. Previous work from our laboratory has shown that pharmacotherapies with such actions improve motor and cognitive outcome after cortical impact injury.

Hypothesis: Brexpiprazole will improve motor and cognitive function after a controlled cortical impact (CCI) injury in adult male rats.

Methods: Isoflurane anesthetized adult male rats received either a CCI of moderate severity (2.8 mm tissue deformation at 4m/s) or sham injury (i.e., all surgical procedures minus the impact) and then were randomly assigned to receive brexpiprazole (1 mg/kg) or saline/DMSO vehicle (1 mL/kg), resulting in the following groups: TBI+brexpiprazole, TBI+vehicle, Sham+brexpiprazole, and Sham+vehicle. Treatments began 24h after TBI or sham injury and were administered intraperitoneally once daily for 21 days. Motor function was assessed on post-operative days 1-5 using beam-balance and beam-walk protocols and cognitive function was assessed on days 14-19 using a Morris water maze task with corresponding probe and visible trials. The behavioral data were analyzed by repeated measures ANOVA followed by the Newman-Keuls post-hoc test to determine group differences. Newman-Keuls was used as it controls for multiple comparisons.

Results: There were no differences in any endpoint measured between the brexpiprazole and vehicle-treated sham groups ($p > 0.05$), and thus the data were pooled. As expected, the sham group performed significantly better than both TBI groups in motor and cognitive function ($p < 0.05$). Moreover, there were no motor or cognitive differences between the TBI+brexpiprazole and TBI+vehicle groups ($p > 0.05$).

Conclusions and Significance: The dosing regimen of brexpiprazole utilized had no beneficial effects on behavioral outcomes after TBI, which does not support our hypothesis. Importantly, brexpiprazole did not exert deleterious effects on motor and cognition, which may make it a more viable option for managing clinical TBI-induced agitation without impairing recovery as has been reported with APDs with D_2 antagonistic properties, like haloperidol.

Research/Grant Support: This work was supported in part by National Institute of Health grants NS084967 and NS121037 (AEK).

Abstract 37

EXAMINING THE POTENTIAL EFFICACY OF CHRONIC GALANTAMINE ON ATTENTIONAL FUNCTIONING AND CHOLINERGIC NEUROTRANSMISSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Moschonas EH,[1,2] Annas EM,[1,2] Capeci HE,[1,2] Steber JA,[1,2] Bittner RA,[1,2] Ranellone TS,[1,2] Rennerfeldt PL,[1,2] Genkinger NR,[1,2] Domyslawski VB,[1,2] Vozzella VJ,[1,2] Cheng JP,[1,2] Kline AE,[1-3,5,6] Bondi CO[1-4]

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

Introduction: Cholinergic disruptions underlie attentional deficits following traumatic brain injury (TBI). Yet, acetylcholinesterase (AChE) inhibitors that solely increase intrasynaptic acetylcholine availability have produced mixed results.

Hypothesis: Chronic galantamine (GAL), a dual-action competitive AChE inhibitor and an α7 nAChR positive allosteric modulator that is FDA-approved for Alzheimer's disease will improve sustained attention post-TBI.

Methods: Adult male rats (n=10-15/group) trained in the 3-Choice-Serial-Reaction-Time-Task (3-CSRT) were randomly assigned to a cortical impact of moderate severity or sham injury and were administered GAL (0.5, 2.0, or 5.0 mg/kg) or saline vehicle (VEH; 1 mL/kg) i.p once daily beginning 24-h post-surgery until sacrifice (day-28). Measures of sustained attention (percent accuracy), distractibility (percent omissions), and impulsivity (premature responses) were assessed on post-operative days 21-27 with the 3-CSRT. On post-operative day-28, cortical lesion volume and basal forebrain cholinergic cells were quantified. The behavioral data were analyzed by repeated measures ANOVAs and the histological data were analyzed by ANOVA. When an overall statistical difference was revealed, the Newman-Keuls post hoc was utilized to determine specific group differences. A dose-dependent effect of GAL on erythrocyte AChE activity will be correlated to behavioral and histological outcomes (pending).

Results: All TBI groups regardless of treatment had decreased sustained attention vs. SHAM controls [*p*'s<0.05]. Moreover, the highest dose of GAL (5.0 mg/kg) exacerbated attentional deficits relative to the two lower doses of GAL and VEH [*p*'s<0.05] and increased lesion volume vs. the 2.0 mg/kg dose [*p*<0.05]. A significant reduction in cholinergic cells was observed in the TBI+VEH group vs. SHAM controls [*p*<0.05].

Conclusion and Significance: The lack of a GAL benefit in the 3-CSRT may suggest that a refined dosing paradigm that selectively modulates α7 nAChRs is necessary for more sophisticated cognitive tests.

Research/Grant Support: This work was supported in part by UPMC Children's Research Advisor Committee Dissertation Fellowship (EHM), Brain Injury Association of America Dissertation Grant (EHM), National Institute of Health grants NS110609 (COB), NS084967, NS121037 (AEK).

Abstract 38

METHODOLOGICAL CONSIDERATIONS FOR BEHAVIORAL AND STRESS PARADIGM DEVELOPMENT

Nasser L,[1,2] Convertino N,[1,2] Gober I,[1,2] Russell A,[1,2] Wagner A[1,2,3]

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

Introduction: Behavioral phenotyping in mice is complex and sensitive to environmental confounds. Reliability and reproducibility in behavioral phenotyping are critical for evaluating potential deficits after injury and for testing therapeutics. In developing behavioral and stress protocols, careful considerations are needed for variables such as environmental context (lighting, noise, time of day, etc.), and animal specifics (strain, age, sex, handling, etc.). In response to a recent program award, we aimed to develop and validate cognitive, motor, anxiety-like, anhedonia, and stress-inducing paradigms in adult male and female mice, taking into consideration and trialing environmental contexts to achieve replicable results based on previous literature.

Hypothesis: Methodological behavioral assay development is feasible to assess how the induction of a stress response and the effects of environmental cues influence behavioral phenotyping.

Methods: Young adult C57BL/6J male and female mice underwent behavioral tests to assess recognition memory (novel object recognition (NOR)), sociability (three chamber sociability test), anxiety-like behavior (elevated zero maze (EZM)), anhedonia (sucrose splash test (SST)), and exploratory behavior (cylinder test).Variations in habituation times (SST, three chamber sociability test), environmental lighting (cylinder test), protocol duration (NOR), and sex (three-chamber sociability, EZM) were assessed. A separate cohort of mice underwent a 2-week chronic variable stress (CVS) paradigm, where mice were exposed to unpredictable stressors twice daily (AM and PM). Daily body weight and coat assessment were recorded over the 2-week period, and adrenal gland and thymus weight were evaluated for stress related hypertrophy.

Results: We found that altering specific testing parameters and/or environmental cues impacted some behaviors. Increasing habituation time prior to SST testing from 20 to 60 minutes increased percent time grooming by 15.05% ($p<0.05$). Increasing habituation time by 10 minutes for the sociability test also increased interaction from an average of 58.1s to 117.9s ($p<0.05$) in males. In cylinder test, we found lighting had no impact on rearing behavior (25.75% in red-light, 27.71% in 80-lux, and 22.43% in 200-lux). Shortening the NOR testing protocol increased novel object interactions and increased discrimination ratio (DR) from –0.313 to 0.547 ($p<0.05$). Additionally, differing novel objects yielded different interactions. A brown box object had a DR of 0.547 and a red vase -0.026 ($p<0.05$) indicating increased interaction with the box. For EZM, exploration time differed by sex. Males had a greater preference for the closed arm (112.7s) vs open arm (65.3s), while females showed no preference. In developing the CVS paradigm, we found that 2 weeks may not be sufficient to induce a physiological stress response. CVS mice maintained or increased bodyweight over the study duration with no effect on grooming, adrenal weight (% of body weight), or thymus weight (% of body weight) compared to non-stressed, age-matched mice.

Conclusions: We found varying lighting, protocol, habituation, and sex can impact distinct behavioral tests. We also found that our proposed CVS paradigm may not elicit an appropriate stress response. Work will further develop these behavioral assays and will extend the CVS to ensure the accuracy and reproducibility of results.

Significance: These findings illustrate the necessity of controlling and minimizing environmental confounds when conducting mouse behavioral tests when incorporated into large-scale studies to test subtle behavioral deficits, physiological response, and therapeutic strategies.

Research/Grant Support: DoD CDMRP NEXUS and UPMC Rehabilitation Institute

Abstract 39

EFFECTS OF HIPPOCAMPAL VERSUS SYSTEMIC ADMINISTRATION OF sgp-130-Fc AFTER CONTROLLED CORTICAL IMPACT INJURY (CCI) ON LEARNING AND MEMORY AND NEURAL DAMAGE

Patel V,[1,2] Vadalia C,[1,2] Russell AL,[1,2] Gober I,[1,2] Wagner AK[1,2,3]

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

Introduction: Patients with traumatic brain injury (TBI) are at risk for poor neurologic outcomes likely linked to uncontrolled neuroinflammation. There are no post-acute pharmacologic therapies promoting neurorecovery after TBI. One promising therapeutic target is interleukin 6 (IL-6). IL-6 is elevated in TBI patients and IL-6 temporal profiles are linked to functional outcomes. IL-6 can signal through either classical signaling (neuroregulatory/beneficial) or via soluble IL-6 receptor (sIL-6R) mediated trans-signaling (inflammatory/pathologic). Soluble glycoprotein (sgp)130 inhibits IL-6/sIL-6R binding, selectively blocking IL-6 trans-signaling and possibly mitigating pathology. Our unpublished rodent work suggests that a chronic systemic intermittent dosing (every 3 days (D) starting D1 post-CCI) decreases injury-induced deficits in learning and memory (Morris water maze; MWM), reduces lesion volume and decreases IL-6 associated brain chemokines. However, others have suggested that sgp130-Fc effects may be dose and injection site-specific after TBI, with some studies showing a detrimental effect of sgp130-Fc with large single dose intrahippocampal infusion D3 post-injury.

Hypothesis: We hypothesized that treating mice with single dose sgp130-Fc 3 days post-CCI would have no to minimal effect on long-term learning, memory and neural pathology outcomes.

Methods: Male C57Bl/6J mice (12 weeks of age; n=8-14/group) received sham or CCI (6.0±0.2m/s; 2mm; 50-60ms). Three days post procedures animals received either sgp130-Fc or vehicle (PBS) via one of two routes: 1) intrahippocampal injection (1ug/1ul/10 min) or 2) intraperitoneal (10ug/100ul). Spatial learning/memory were assessed via MWM D14-19 post-CCI. Acquisition trials were assessed D14-18 (submerged platform). Mice were tested D19 on a retention probe trail (no platform) followed by visible platform tests. Cortical lesion volumes on D21 were quantified in cryosectioned and H&E stained tissue using image analysis software.

Results: We found sgp130-Fc treatment was safe, with no treatment-specific mortality nor loss of body weight. As expected, CCI mice had longer escape latencies than sham mice to the hidden and visible platforms ($p<0.05$). There was no effect of either hippocampal or systemic sgp130-Fc treatment as sgp130-Fc treatment did not improve latency to platform compared to vehicle-treated CCI mice. There was also no difference between vehicle-treated and sgp130-Fc treated shams in escape latency. There was no difference in distance to platform between treatment group. In probe trials, sham mice (vehicle and sgp130-Fc treatment) spent more time in the platform zone that CCI mice. There was no significant effect of either hippocampal or systemic sgp130-Fc treatment on zone time during probe trials compared to vehicle-treated CCI mice. Ongoing work is underway examining CCI contusion volume to compare the effect of hippocampal versus systemic sgp130-Fc to vehicle-treated mice.

Conclusions: Single subacute dosing of sgp130-Fc centrally or systemically did not improve learning and memory in our CCI model; however, importantly, it also was not detrimental to our behavioral outcomes. The lack of improvement with this dosing strategy may relate to dosing and timing, as well as the longitudinal nature of neuroinflammation following TBI.

Significance: By understanding the specific timing and dosing amount and route needed, we can better utilize for sgp130-Fc as a therapeutic for TBI.

Research/Grant Support: UPMC Rehabilitation Institute

Abstract 40

BDNF AND NEUROBEHAVIORAL RECOVERY IN PEDIATRIC TBI

Petersen B,[1] Conley Y,[2] Treble-Barna A[3]

[1]Department of Critical Care Medicine, University of Pittsburgh School of Medicine
[2]Department of Human Genetics, University of Pittsburgh School of Nursing
[3]Department of Physical Medicine & Rehabilitation, University of Pittsburgh School of Medicine

Introduction: Children with TBI can experience lasting neurobehavioral impairments; however, the biological factors affecting recovery are unclear. We aim to explore brain-derived neurotrophic factor (BDNF) as a potential biomarker of neurobehavioral recovery in children with TBI.

Hypothesis: We hypothesized that acute BDNF concentration would be positively associated with measures of neurobehavior in children 6 months after TBI.

Methods: We recruited 183 children (3-18 yrs) admitted to UPMC Children's Hospital after complicated mild to severe TBI. Plasma samples were collected acutely, and neurobehavioral measures (behavior, executive and adaptive function) were collected 6 months post-injury. Acute BDNF concentrations were compared by TBI severity. Linear regression was used to determine associations between BDNF and neurobehavioral recovery, adjusting for injury severity, age, and sex.

Results: Children with moderate-to-severe TBI (GCS 3-12, 17830.0+15060.8 pg/nL) had significantly lower BDNF post-injury than children with complicated mild TBI (GCS 13-15, 10735.1+7981.9 pg/nL, p=0.032). Acute BDNF was positively associated with fluid cognition at 6 months, indicating a greater capacity for learning and processing in novel environments (β=4.09, p=0.026). No significant associations were observed with other neurobehavioral measures.

Conclusions: Our study suggests BDNF has potential as a biomarker of pediatric TBI severity and predictor of long-term fluid cognition.

Significance: Determining biomarkers of neurobehavioral recovery is a critical aspect of precision medicine. These findings suggest BDNF may be useful to identify children at risk of poor recovery and guide future interventions.

Research/Grant Support: NICHD HD097030 (Treble-Barna)

Abstract 41

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

Race NS,[1,2] Bertocchi M,[2,3] Toader M,[2] Moschonas EH,[1-3] Rennerfeldt P,[1,2] Annas E,[1,2] Ranellone T,[1,2] Cheng JP,[1,2] Kline AE,[1-3,5,6] Bondi CO[1-4]

[1]Department of Physical Medicine & Rehabilitation
[2]Safar Center for Resuscitation Research
Centers for [3]Neuroscience, [4]Neurobiology, [5]Critical Care, [6]Psychology
University of Pittsburgh

Introduction: The most detrimental TBI sequelae negatively impacting quality of life and successful societal re-entry are those impeding healthy social behavior. Strategies to build healthy social behavior do not work reliably for individuals with TBI, due to poorly understood neural substrates, crude phenotypes, and lack of mechanism-based treatment targeting. Our previous work found unhealthy social behavior after blast TBI in rats was mediated by metabotropic glutamate receptor dysregulation in the orbitofrontal cortex.

Hypothesis: We explored longitudinal social behavior for the first time in rats exposed to one of two different controlled cortical impact (CCI) injuries (frontal or parietal), hypothesizing post-TBI unhealthy social behavior phenotypes would be present in both injury models.

Methods: Adult male Sprague-Dawley rats (n = 7-13/group) were randomized and exposed to TBI via moderate severity right parietal CCI, right frontal CCI, or sham injury (craniectomy + anesthesia without impact). After 14 day recovery, animals underwent serial social interaction (SI) sessions with novel then familiar naïve partners under dim (non-stressful) or bright (stressful) lighting conditions for 7 consecutive days (recording SI time, scored by blinded investigators). Subsequently, social preference between two naïve partners (one novel, one familiar) was assessed under both lighting conditions (recording total and relative SI times). Active and passive coping capacities were assessed in the shock probe defensive burying paradigm (SPDB) by measuring time spent burying and time immobile, respectively.

Results: We observed serially decreased SI time ($p<0.05$, rmANOVA) in frontal and parietal CCI groups, with differing deficit severity, onset latency, and behavior characteristics between models. Injured animals exhibited stress-dependent, aberrant social preferences and decreased active coping (decreased novelty-seeking and burying time, respectively; $p<0.05$, ANOVA).

Conclusions: Parietal and frontal CCI rats had impaired social safety learning and inability to healthily cope with stress through social familiarity. Non-social coping was also impaired. Phenotypic nuances indicate variability between injury types. Serial SI under stressful stimuli represents a useful preclinical paradigm for investigating post-TBI psychosocial impairments.

Significance: Phenotypic variation in psychosocial impairments between injury models suggests post-TBI stress management and unhealthy social behavior will require mechanism-based treatment targeting on an individual- and injury-specific basis.

Grant Support: Research Advisory Committee Grant, UPMC Children's Hospital of Pittsburgh

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
UPMC Children's Hospital of Pittsburgh
Safar Symposium Planning Committee

The 20th 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:

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