

Abstracts from
The 35th Annual
National Neurotrauma Symposium

July 7–12, 2017
Snowbird, Utah

Trainee Competition Finalists

T01-01

SMART HUMAN NEURAL STEM CELLS TO MODIFY SCAR AND OPTIMIZE REGENERATION AFTER TRAUMATIC CERVICAL SPINAL CORD INJURY

Christopher Ahuja^{1,2}, Mohamad Khazaei², Sara Baig², Jainetri Merchant², Priscilla Chan², Michael Fehlings^{1,2}

¹University of Toronto, Neurosurgery, Toronto, Canada

²Genetics and Development, University Health Network, Toronto, Canada

Human induced pluripotent stem cell-derived neural stem cells (hiPS-NSCs) represent an exciting therapeutic strategy for traumatic spinal cord injury (SCI) as they can replace lost neural circuits, remyelinate denuded axons and provide local trophic support. Unfortunately, most patients are in the chronic phase of their injuries where dense chondroitin sulfate proteoglycan (CSPG) scarring significantly impairs neurite outgrowth and regenerative cell migration. Several scar-modifying enzymes have been shown to synergistically enhance NSC-mediated recovery, however, nonspecific intrathecal administration can produce off-target effects. We aimed to generate a genetically-engineered line of hiPS-NSCs, termed Spinal Microenvironment Modifying and Regenerative Therapeutic (SMaRT) cells, uniquely capable of expressing a scar-modifying enzyme within the host environment to enhance functional recovery. A proprietary enzyme was non-virally integrated into hiPS-NSCs and a monoclonal line of SMaRT cells was generated and extensively characterized. The expressed enzyme rapidly degrades CSPGs on biochemical assays and allows neurons to extend into CSPG-rich regions *in vitro*. Furthermore, unlike wild-type hiPS-NSC media, conditioned SMaRT cell media can degrade post-injury rodent CSPGs in *ex vivo* injured cord cryosections. T-cell deficient rats (N=60) with translationally-relevant chronic C6-7 clip-contusion injuries have been randomized to receive: (1) vehicle, (2) hiPS-NSCs, (3) SMaRT cells, or (4) sham surgery (laminectomy). While blinded sensorimotor behavioural assessments and rehabilitation are ongoing with a long-term 32-week endpoint, interim histologic analysis shows that grafted human cells are extending remarkably long ($\geq 20,000 \mu\text{m}$) axons along host white matter tracts in the rostral and caudal directions. This work provides exciting proof-of-concept data that genetically-engineered SMaRT cells can degrade CSPGs *in vitro* and that human NSC transplants can grow long axons in chronic cervical SCI to potentially form a bridge for sensorimotor signal transmission. This work is generously supported by the Canadian Institutes of Health Research, Phillip and Peggy DeZwirek, OIRM, and the Krembil Foundation.

Keywords: Chronic, Glial scar, Neural precursor cell, Rehabilitation

T01-02

QUANTIFYING 3D WHOLE BRAIN DEFORMATION USING SONOMICROMETRY DURING DYNAMIC HEAD ROTATIONAL LOADING

Ahmed Alshareef, J. Sebastian Giudice, Jason Forman, Matthew B. Panzer
University of Virginia, Center for Applied Biomechanics, Charlottesville, USA

Understanding the mechanical response of the human brain during impact is crucial to predicting injury severity and elucidating injury mechanisms. Currently, finite element models of the human brain are the state-of-the-art technique for assessing brain injury risk, investigating potential TBI mechanisms, and developing preventative mechanisms. However, they have been developed using limited experimental data quantifying relative brain-skull motion during head impacts. The objective of this study was to develop a methodology to quantify the 3D deformation of cadaveric brain specimens *in situ* during high-rate rotational head motion. An array of neutrally-dense sonomicrometry crystals were implanted into the brain of a single cadaveric head specimen (male, 53 years, 116 kg, 173 cm). These crystals are capable of transmitting and receiving ultrasonic pulses where the ultrasonic time-of-flight is used to determine the distance between each crystal pair. Eight transmitting crystals were affixed to the inner skull, and 24 receiving crystals were implanted in the brain. Dynamic 3D spatial time-history data for each receiving crystal was calculated using trilateration and reported as brain tissue motion relative to the skull. Four purely rotational motion conditions, ranging from a peak angular velocity of 20–40 rad/s with a duration of 30–60 ms, were applied to the same cadaveric head/brain specimen in the three anatomic planes. This is the first study to quantify 3D brain deformation in one specimen in response to varying severity rotations in all three anatomical planes using sonomicrometry. Sonomicrometry provided highly repeatable 3D displacement data of the dynamic motion of the brain. Brain deformation response was dependent on peak angular velocity, duration, and loading direction – axial rotation resulted in the greatest deformation. Peak-to-peak displacement reached as high as 23 mm in the most severe case. The transient response of the brain lasted between 100–200 ms. The natural frequency of the brain markers was found to be 12–20 Hz. Funding was provided under NHTSA contract number DTNH221500022/0002.

Keywords: mTBI/Concussion, Brain Deformation, Sonomicrometry, Finite Element Validation

T01-03

EARLY SURGICAL INTERVENTION AMONG PATIENTS WITH ACUTE CENTRAL CORD SYNDROME IS NOT ASSOCIATED WITH HIGHER MORTALITY

Jake Godzik¹, Jay Dalton², Rohit Mauria¹, Kristina Chapple¹, Alan Cook³, Jay Turner¹

¹Barrow Neurological Institute, Department of Neurosurgery, Phoenix, USA

²Washington University School Of Medicine, Department of Neurosurgery, St. Louis, USA

³Chandler Regional Hospital, Department of Trauma, Chandler, USA

Introduction: Conflicting reports exist regarding mortality and morbidity of early surgical intervention for Acute Central Cord Syndrome (ACS) in multisystem trauma. As a result, the optimal timing of decompression in acute central cord syndrome (ACS) in patients with multisystem trauma remains controversial.

Methods: We performed a retrospective cohort study using the National Trauma Data Bank (NTDB, years 2011–2014), including

patients >18, with ACS (identified using ICD-9). Information was collected on demographics, mechanism of injury, timing of surgery (≤ 24 hours, >24 hours), Charlson Comorbidity index (CCI), and injury severity index (ISS). Logistic regression and propensity were used to evaluate relationship between hospital mortality and surgical timing.

Results: 2383 patients with ACS following trauma were identified. The average age was 56 ± 15 , and 79.3% were male, with an average ISS of 19.5 ± 9.0 , and mortality rate of 3.0% (72). A total of 731 (30.6%) patients underwent surgery for ACS within 24 hours. Univariate analysis did not demonstrate significantly higher mortality rate in the early surgery group ($p=0.127$), although the early surgery group demonstrated significantly higher ISS (20.1 vs 19.2, $p=0.04$), lower CCI (2.1 vs 2.6, $p<0.001$), and younger age (53.1 vs 57.7, $p<0.001$). Binary logistic regression demonstrated higher ISS (OR 1.05, $p<0.001$), higher CCI (OR 1.84, $p<0.001$), and days to surgery (OR 0.89, $p=0.027$) as significant predictors of mortality. Propensity score weighting demonstrated no significant relationship between days to surgery and in-hospital mortality ($P=0.138$).

Conclusions: Delayed surgical intervention does not appear to reduce mortality among patients with acute central cord syndrome. We theorize that survival in the NTDB is confounded by multisystem trauma and existing comorbidities, rather than choice of surgical timing. Delaying definitive surgical care may predispose patients to worsened disposition and greater neurological morbidity.

Keywords: central cord syndrome, national trauma data bank, mortality

T01-04

HEAD-TO-HEAD COMPARISON OF POPULAR CLINICAL ASSESSMENTS TOOLS USED IN THE MANAGEMENT OF SPORT-RELATED CONCUSSION (SRC)

Nicholas Guzowski, Michael A. McCrea, Lindsay D. Nelson
Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

Formal clinical testing has become widespread in the assessment and management of athletes with sport-related concussion (SRC). A variety of assessments tools are available to assess the symptoms, cognitive, postural stability, and other impairments that can arise after injury. Yet there are limited data directly comparing the performance of differing assessment tools from which clinicians can identify the most sensitive measures for clinical use. The aims of this study were to compare the sensitivity of an array of clinical assessment tools to SRC and, secondarily, to explore patterns of heterogeneity across athletes in clinical domains affected by SRC in order to inform recommendations about the multidimensional clinical assessment of SRC. Male football players ($N=917$) enrolled in Project Head-to-Head II at preseason examinations, where they completed assessments of symptoms, cognitive performance, oculomotor functioning, and postural stability. Participants who sustained SRCs ($n=60$) and matched non-injured controls ($n=64$) underwent repeat assessments at 24–48 hours and at days 8, 15, and 45 post-injury. Measures of oculomotor and vestibular functioning were the most sensitive performance metrics to SRC (King-Devick Test and Balance Error Scoring System area under the ROC curve, AUCs, at 24–48 hours = .75 and .73, respectively, $p's < .01$). Additionally, memory performance as measured by the computerized Immediate Post-Concussion and Cognitive Testing (ImPACT) battery was sensitive to SRC to a lesser degree (AUC = .61, $p = .036$), while computerized and paper-and-pencil measures of attention, processing speed, and executive functioning (e.g., Standardized Assessment of Concussion, Trail Making Test, Wechsler Adult Intelligence Scale-IV Processing Speed Index) did not significantly distinguish SRC from control subjects. Evaluation of patterns of impairment among subjects across clinical domains

revealed significant heterogeneity in the clinical manifestations of SRC. The data help to identify the assessment tools with the strongest overall sensitivity to SRC while also supporting the multidimensional assessment of athletes with SRC.

Keywords: Sport-related Concussion, Neuropsychological Testing, Multidimensional Assessment, Oculomotor Functioning

T01-05

IN VIVO CALCIUM IMAGING OF HIPPOCAMPAL CA1 NEURONS REVEALS A FUNCTIONAL INJURY SIGNATURE OF PRIMARY BLAST NEUROTRAUMA

Matthew Hemphill, Shanti Tummala, David Meaney
University of Pennsylvania, School of Engineering and Applied Sciences, Philadelphia, USA

The extent of neuronal dysfunction following mild traumatic brain injury (mTBI) is largely unknown, and is a major limitation to the development of treatment strategies. A key unknown is how the neural circuits in brain areas important for cognition change after mTBI, and how these changes affect the neural computations during behavior. Recent reports indicate that the blast overpressure component of an explosion alone can produce some pathology and behavioral deficits common amongst other forms of TBI. We used a miniaturized endoscope implanted in awake behaving mice, in combination with a genetically encoded calcium indicator in the hippocampus, to (1) identify a functional neural circuit 'signature' of blast induced TBI (bTBI) and (2) to track the progression of this circuit signature over time after blast exposure to elucidate the trajectory of recovery at the single cell/network level. We transduced CA1 Hippocampal neurons with GCaMP6f, a genetically encoded calcium indicator, and utilized a miniaturized, head-mounted microscope to detect transients of intracellular calcium – serving as a proxy of action potential events - in awake, unconstrained mice. We measured changes in calcium transient morphology from multiple mice ($N=6$) and utilized a regression model to identify factors that best distinguished injured and sham groups. We found that changes in variation in magnitude and duration were statistically significant in predicting injured and sham cells. We then scored each cell immediately post blast and tracked its progression at 1 and 5 days. Cells with higher initial scores exhibited larger deviations in Calcium signals at day 5 compared to cells with lower scores, indicating a predictive capacity to identify cells that exhibit sustained responses. These results indicate that primary blast overpressure bTBI alters Calcium signaling at the level of single neurons, with sustained effects of at least 5 days. This platform for tracking the response of individual cells *in vivo* in time will facilitate the future interrogation of functional recovery and the effects of therapeutic strategies on its trajectory. (Funding Source: MURI W911MF-10-1-0526).

Keywords: Blast Induced TBI, in vivo Imaging, Functional Injury, Hippocampus CA1, Calcium Imaging, Mild TBI

T01-06

INSIGHTS INTO THE PBT02 TREATMENT THRESHOLD: EXPLORING A TREATMENT WINDOW SUGGESTED BY 'BIG DATA'

Ryan Hirschi¹, Gregory Hawryluk¹, Jessica Nielson², J. Russell Huie², Lara Zimmermann³, Rajiv Saigal⁴, Quan Ding⁵, Adam Ferguson², Geoffrey Manley²

¹University of Utah, Neurosurgery, Salt Lake City, USA

²University of California San Francisco, Neurosurgery, San Francisco, USA

³University of California at Davis, Neurosurgery, Sacramento, USA

⁴University of Washington, Neurosurgery, Seattle, USA

⁵University of California at San Francisco, Physiologic Nursing, San Francisco, USA

Brain tissue hypoxia is common after traumatic brain injury (TBI). Technology exists to detect hypoxia and guide corrective therapy. Current guidelines for the management of severe TBI recommend maintaining $P_{bt}O_2 > 15\text{--}20\text{ mmHg}$, however uncertainty persists as to the optimal treatment threshold. $P_{bt}O_2$ measures were prospectively and automatically collected every minute from consecutive patients admitted to the San Francisco General Hospital (SFGH) intensive care unit over a 6-year period. We analyzed mean $P_{bt}O_2$ values in TBI patients and the proportion of $P_{bt}O_2$ values below each of 75 thresholds between 0 mmHg to 75 mmHg over various epochs up to 30 days from time of admission. Patient outcomes were calculated using the Glasgow Outcome Scale. We explored putative treatment thresholds by generating 675 separate receiver operator curves (ROC) and 675 generalized linear models (GLM) to examine each 1 mmHg threshold for various epochs. A total of 1,380,841 $P_{bt}O_2$ values were recorded from 190 patients recovering from TBI. A high proportion of $P_{bt}O_2$ measures were below 20 mmHg irrespective of examined epoch. Time below treatment thresholds was more strongly associated with outcome than mean $P_{bt}O_2$. A treatment window was suggested; a threshold of 19 mmHg most robustly distinguished patients by outcome, especially from days 3–5. Benefit to maintaining values at least as high as 33 mmHg was suggested, however. Our “big data” analysis substantially informs the relationship between $P_{bt}O_2$ values and outcome. We were able to discern a therapeutic window for $P_{bt}O_2$ in TBI patients along with minimum and preferred $P_{bt}O_2$ treatment thresholds. Traditional treatment thresholds which have the strongest association with outcome may not be optimal.

Keywords: Brain oxygenation, $P_{bt}O_2$, Outcome, Threshold, Treatment window

T01-07

MICROGLIAL INFLAMMASOME ACTIVATION IN PENETRATING BALLISTIC-LIKE BRAIN INJURY

Stephanie Lee¹, Shyam Gajavelli¹, Markus Spurlock¹, Karla Rivera¹, Cody Androni¹, Juan Pablo de Rivero Vaccari¹, Robert Keane^{1,2}, Ross Bulock¹, Dalton Dietrich¹

¹Miami Project to Cure Paralysis, Department of Neurological Surgery, Miami, USA

²Physiology and Biophysics, University of Miami Miller School of Medicine, Miami, USA

Penetrating traumatic brain injury (PTBI) remains a significant cause of death and disability in the United States without effective therapies. A rodent PTBI model, penetrating ballistic-like brain injury (PBBI) has uncovered several secondary pathophysiological mechanisms such as reduced glucose uptake, neurodegeneration, inflammation, and apoptosis that magnify the primary injury. Targeting components of these mechanisms may help improve PTBI outcomes. Activators of innate immunity contribute to secondary injury mechanisms following traumatic brain injury (TBI). Inflammasomes are the key regulators of IL-1 β mediated inflammation after TBI and present as clinically relevant targets for therapy. The role of inflammasomes in PBBI pathophysiology has yet to be determined. Towards this, adult male Sprague-Dawley rats were subjected to unilateral sham or PBBI surgery and

sacrificed at various time points. Tissues were assessed for expression of cytokines IL-1 β , IL-18 and components of the inflammasome, ASC (apoptosis-associated speck like protein containing a caspase activation and recruitment domain), caspase-1, NLRP3 (NOD-like receptor protein 3) and GSDMD (gasdermin-D) by immunoblot analysis and assessed for ASC cell-type expression by immunohistochemistry. Cortical IL-1 β and IL-18 expression increased 4h–48h and 48h–72h, respectively after injury. PBBI also increased caspase-1, ASC, NLRP3, and GSDMD expression from 24h–48h. Compared to sham and contralateral cortex, microglial numbers significantly increased 48h post-injury in the ipsilateral cortex. ASC expression was predominantly increased in activated microglia, permeating the enlarged cell bodies and into the processes. Taken together, this is the first report of inflammasome activation after PBBI and suggests that these activators of inflammation lead to an exacerbation of the pro-inflammatory state post-injury which could underlie the long-term sequelae of PBBI. Inhibition of the inflammasome in PBBI will evaluate its therapeutic potential for PTBI. Supported by NIH NINDS award R01NS089443.

Keywords: PBBI, Penetrating Ballistic-like Brain Injury, Inflammasome, Microglia, Inflammation

T01-08

TEMPORAL PROFILE OF REGIONAL BLOOD-BRAIN BARRIER DISRUPTION IN A MOUSE MODEL OF BLAST-INDUCED MILD TRAUMATIC BRAIN INJURY

Aric Logsdon^{1,2}, Kole Meeker¹, Kristin Bullock¹, William Banks^{1,2}, David Cook^{1,2}

¹Veterans Affairs Puget Sound Health Care System, Geriatric Research Education and Clinical Center, Seattle, USA

²University of Washington School of Medicine, Division of Gerontology and Geriatric Medicine Department of Medicine, Seattle, USA

A number of groups, including ours, indicate that mild blast-induced traumatic brain injury (mTBI) can cause transient blood-brain barrier (BBB) disruption. Whether short-lived BBB repair processes afford prolonged neuroprotection to mTBI, or can subsequently promote chronic pathologic and functional impairment is not well understood. To begin addressing this issue we mapped the temporal and anatomical profile of BBB disruption following blast exposure. C57/BL6 mice received two consecutive blasts that recapitulate detonation of high explosives in the open field. To assess blast-induced BBB disruption, we co-injected radiolabeled [¹⁴C]-sucrose and [^{99m}Tc]-albumin, which do not readily cross the intact BBB. We quantified [¹⁴C]-sucrose and [^{99m}Tc]-albumin in ten brain regions at 0.25, 3, 24, 72, and 168 hours post-blast. A significant increase in [¹⁴C]-sucrose was observed in all brain regions, except striatum, at 0.25 h post-mTBI ($p < 0.01$) compared to sham controls. A similar effect was also observed at 3, 24 and 72 h post-mTBI ($p < 0.05$). Results suggest an immediate, and sustained BBB opening after mTBI. A significant increase in [^{99m}Tc]-albumin was also observed at 0.25 h post-mTBI ($p < 0.01$), but only in frontal cortex and brain stem. No significant differences in [^{99m}Tc]-albumin were found among any brain region at 3 and 24 h post-mTBI. However, a significant increase in BBB disruption was measured in the frontal cortex and brain stem at 72 h post-mTBI ($p < 0.05$). These results argue that large openings of the BBB are restored within 24 h post-mTBI, followed by a secondary BBB disruption at 72 h. We hypothesize that the secondary BBB opening could be targeted to potentially reduce the chronic pathophysiology associated with neurotrauma.

Funding: T32AG05235401 (A.F.L.)

Keywords: Blast injury, Blood-brain barrier, Radioactivity, Neuropathology

T01-09

APOLIPOPROTEIN-E4 (APOE4) IMPAIRS BLOOD BRAIN BARRIER FUNCTION AND STABILIZATION FOLLOWING TRAUMATIC BRAIN INJURY

Bevan Main, Sonia Villapol, David Barton, Stephanie Sloley, Kathryn Stefos, Mark Burns

Georgetown University, Neuroscience, Washington, USA

Background: Blood Brain Barrier (BBB) dysfunction occurs in human TBI patients, yet the molecular mechanisms underlying this pathology remain unclear. The APOE4 gene polymorphism is associated with unfavorable outcomes after TBI including prolonged coma, poor prognosis and enhanced risk of late-onset Alzheimer's disease. Recent evidence implicates APOE in regulating BBB integrity in an isoform dependent manner, via suppression of Cyclophilin A (CypA)-Matrix metalloproteinase-9 (MMP-9) signaling at the Neurovascular Unit (NVU); however, the contribution of apoE to TBI-induced BBB permeability has not been investigated.

Methods/Results: Wildtype (C57Bl/6) and humanized APOE3/APOE4 targeted replacement mice were subject to a controlled cortical impact model of TBI, before NVU and BBB permeability responses characterized at 1, 3, 7, and 10 days post-injury. In wildtype mice, an inverse relationship between soluble apoE and BBB permeability is observed, such that BBB permeability decreases as apoE levels increase over time post-TBI ($n=5$, $**p<0.01$). In APOE3 and APOE4 mice, acute pericyte loss is observed in both genotypes; however, APOE4 mice exhibit delayed pericyte recruitment back to the ipsilateral cortex 7 days post-TBI ($n=4-5$, $***p<0.001$). Furthermore, QPCR analysis of microvessels revealed increased MMP9 expression in APOE4 mice at 1, 3 and 7-days post-TBI ($n=5-6$, $**p<0.01$), in parallel with reduced expression of tight junction proteins Zonula Occludens-1, Occludin and Claudin-5 compared to APOE3 counterparts ($n=5-6$, $*p<0.05$). Significantly, at 10 days post-injury, BBB leakage remains in APOE4 but not APOE3 mice ($n=4-5$, $**p<0.05$), suggesting that the E4 isoform impairs BBB stabilization following TBI. This prolonged elevation of BBB permeability in APOE4-TR mice may contribute to deleterious secondary injury processes and indeed T2-weighted MRI shows APOE4 mice have 78% increased lesion volume compared to APOE3-TR mice, 28-days post-injury ($n=8-11$, $**p<0.01$).

Conclusion: These results identify the key role of APOE in mediating BBB permeability and stabilization following TBI. Future studies investigating genotype-specific therapies targeting the BBB may prove beneficial in improving outcomes after TBI.

Keywords: Blood Brain Barrier, Apolipoprotein-E4, Neurovascular Unit, Pericyte signaling, BBB permeability and stabilization

T01-10

THE STEALTH PATHOLOGIES OF MILD TBI AND DEMONSTRATION THAT ADVANCED NEUROIMAGING MAY NOT BE AS SENSITIVE AS ADVERTISED

Arka Mallela, Randel Swanson, Victoria Johnson, Andrew Parker, Mark Elliott, Maura Weber, Nimish Acharya, John Cagnetti, Kacy Cullen, Ragini Verma, Douglas Smith

University of Pennsylvania, Department of Neurosurgery, Philadelphia, USA

Background: Diffuse axonal injury (DAI), a signature injury of mild TBI (mTBI), is a 'stealth' pathology, invisible on conventional neuroimaging. Diffusion tensor imaging (DTI) has been used to identify DAI in clinical populations. However, studies demonstrate conflicting changes

such as increases, decreases, or no change in fractional anisotropy (FA) or mean diffusivity (MD) following injury. Multiple pathologies, including axonal injury, BBB disruption, or ionic/fluid shifts, can change diffusion properties. Further validation in preclinical models is required.

Objectives: To determine if diffusion imaging can identify histologic axonal injury in a translational, clinically-relevant, closed-head rotational injury model.

Methods: Nine female swine underwent closed-head rotational injury. Animals were imaged before and 72 h following injury. Amyloid precursor protein (APP) IHC was performed to identify damaged axonal profiles. The change in diffusion metric (FA, MD) and number of APP(+) axonal profiles was correlated across all WM and in specific tracts-internal capsule (IC), fornix, corpus callosum, etc.

Results: Mean FA/MD was not significantly different after injury in any tract ($p>0.05$) despite the presence of APP(+) profiles. However, the proportion of voxels with high FA was significantly lower post-injury in the L, R fornix and R IC ($p<0.05$). The change in proportion correlated with the number of APP(+) axons ($p<0.05$). In whole brain analysis, in voxels with APP(+) axons, the degree of APP pathology correlated with diffusion change ($p<0.05$). However, diffusion changes were also observed in voxels not containing APP(+) axons.

Conclusion: Based on this preliminary translational study, changes in DTI metrics may be sensitive but not specific to histologic axonal injury. Averaging metrics along tracts may average out diffuse/multifocal changes, so changes along each tract must be analyzed. Further comparison of diffusion imaging and other pathologies and clinical correlation in larger human studies is required.

Support: Supported by DOD grant, PT110785 and NIH grants, NS38104, NS092389 and EB021293

Keywords: Mild TBI, Concussion, Neuroimaging, Diffuse Axonal Injury

T01-11

CANNABIDIOL ADMINISTRATION AFTER SPINAL CORD INJURY REDUCES ALLODYNIA IN MALE AND FEMALE RATS, WITH MOST ROBUST EFFECTS IN FEMALES

Amanda Mohaimany-Aponte¹, Betty Pat¹, Sean McAllister², Candace Floyd¹

¹*University of Alabama at Birmingham, Physical Medicine and Rehabilitation, Birmingham, USA*

²*California Pacific Medical Center Research Institute, San Francisco, USA*

Over 60% of persons with spinal cord injury (SCI) experience neuropathic pain (SCI-NP), which frequently is listed as a top concern due to few treatment options. Clinically, cannabidiol (CBD), a non-psychoactive constituent of *Cannabis sativa*, has shown efficacy in treating some chronic pain disorders. However, effectiveness of CBD in treating SCI-NP has not been examined. The central hypothesis of this study is that acute post-SCI administration of CBD will ameliorate SCI-NP development and that these effects are sexually dimorphic. We recorded baseline motor and sensory function measures from adult male and female Sprague Dawley rats. Rats then received either laminectomy or SCI with the SCI groups receiving a unilateral 300 kdyn impact at the 5th cervical level. SCI groups were randomized to receive vehicle or CBD (100 mg/kg) beginning 30 minutes post-SCI, then once daily for 7 consecutive days. Weekly assessments of motor and sensory function were taken for 6 weeks. We found SCI reduced motor function in both sexes and CBD treatment conferred modest improvement in motor function in females. Regarding sensory tests, SCI induced an increase in sensitivity to mechanical stimuli in males and CBD reduced

this tactile allodynia in males. Robust cold allodynia was observed in both sexes after SCI and CBD reduced development of cold allodynia in both males and females, with more pronounced reduction in females. Additionally, over-grooming of the affected hindpaw was observed in both sexes following SCI and the percentage of rats exhibiting over-grooming was reduced in CBD treated males and females. In conclusion, CBD administration post-SCI conferred modest reduction in motor deficits in females and significant effects in ameliorating cold allodynia and over-grooming in both sexes, with more robust effects in females. Support: UAB TJ Atchinson SCI Research Program and Conquer Paralysis Now Grant (CF, SM).

Keywords: spinal cord injury, neuropathic pain, cannabidiol

T01-12

REBALANCING OF BRAIN CIRCUITS BY TEMPORARY CORTICAL SILENCING SHOWS POST-INJURY TEMPORAL DEPENDENCE AFTER RAT TBI

Afshin Paydar, Neil G. Harris

UCLA, Neurosurgery, Brain Injury Research Center, Los Angeles, USA

We have previously shown that the injured brain is both functionally disconnected as well as hyperconnected, and in a region-dependent manner after controlled cortical impact (CCI) injury in the adult rat. Our recent data shows that remote, homotopic cortical regions become hyperexcitable with time post-injury, and we hypothesized that this results in increased trans-hemispheric cortical inhibition, preventing reorganization of the primary injured hemisphere. In support of this, other data from our lab show that temporary silencing the contralesional sensorimotor cortex at 1wk after CCI injury normalizes affected forelimb use, but not when conducted at 4 wks. To determine whether this occurs due to restoration of the afferent pathway connectivity, or to reorganization of brain-centric circuits, we acquired forelimb-evoked-fMRI and resting state fMRI data at 1 and 4 wks post-injury after contralateral silencing with intraparenchymal injection of muscimol, and compared data to a vehicle-injected-injured (n=9 vs 3) as well as sham rats (n=3). Data were analyzed by statistical parametric mapping for the forelimb-evoked cortical map, and by graph theory for network-based functional connectivity (fc). As predicted, contralesional silencing at 1wk and 4 wks post-injury decimated the unaffected-limb-evoked contralesional cortical map, compared to vehicle-injected ($P < 0.05$, $z = 1.7$ cluster-corrected), but this did not restore the affected-limb-evoked cortical map to the ipsilesional hemisphere at either post-injury time-points. However, contrary to this, while fc analysis also demonstrated that silencing led to the expected contralateral cortical disconnection (reduction in strength of connections, $P < 0.05$, FDR-corrected $q = 0.05$) at both time-points, it also showed that silencing significantly lessened the injury-induced ipsilateral disconnection at 1wk that was present in vehicle-injected-injured rats, so that connection strength was more similar to sham rats. This effect occurred coincident with a normalization of hyperconnectivity in remote regions, likely indicating a causal effect and in agreement with prior data showing behavioral gains due to silencing at this time post-injury. Crucially, this cortical silencing-induced balancing of cortical circuits was much reduced or absent in the same rats at 28 days, also in agreement with the absence of behavioral gains in our prior data.

Acknowledgments: UCLA BIRC; NIH NINDS R01NS091222; NGH is a fellow of The Center for Neuroskills, Bakersfield, California

Keywords: Traumatic Brain Injury, Reorganization, contralesional cortex, cortical silencing, resting state fMRI, functional connectivity, forelimb stimulation-induced fMRI

T01-13

INFLAMMATORY RESPONSE TO PERIPHERAL PAIN INPUT AFTER SCI IS BLOCKED BY A SPINAL TRANSECTION

Joshua Reynolds, Joel Turtle, Gizelle Leal, Yung-Jen Huang, Misty Strain, James Grau

Texas A&M University, Psychology, College Station, USA

When communication with the brain is disrupted by means of a spinal transection, noxious stimulation applied below the injury induces a state of over-excitation (central sensitization) that impairs adaptive plasticity. When this same stimulation is applied after a contusion injury, it increases the extent of secondary injury and impairs long-term recovery (reviewed in Grau et al., *J Neurotrauma*, 2017). The present study examined how spared fibers regulate the effect of nociceptive stimulation after a contusion injury. Sprague-Dawley rats (~350 g) received a spinal contusion at T12. Eighteen hours later, subjects were scored on the BBB locomotor scale before receiving either a spinal transection or a sham surgery at T2. Six hours following the second surgery, subjects were exposed to 6 min of nociceptive stimulation (intermittent shock applied via tail electrodes). As previously reported, nociceptive stimulation increased the area of secondary injury and hemorrhage. Western blotting revealed that this effect of stimulation was correlated with increased expression of IL-18, IL-1b, TNF, Caspase 1, Caspase 3, Caspase 8, IGG, and Hemoglobin. These effects were most evident at the contusion site. All of these effects were eliminated when communication with the brain was blocked by means of a spinal transection. To explore the generality of these effects, we have also examined how the application of a peripheral irritant (capsaicin) affects tissue sparing after a contusion injury. Here too, nociceptive input increased the extent of hemorrhage and this effect was eliminated by a spinal transection. These findings suggest that the adverse effect nociceptive stimulation has at the site of injury depends upon communication with rostral neural systems. We are currently exploring the processes that underlie this interaction.

Supported by the Craig H Neilsen Foundation and the National Institute of Health award number R21465891-00001 to JW

Keywords: SCI, Hemorrhage, C-fibers, Pyroptosis

T01-14

ENRICHED ENVIRONMENT REARING DIFFERENTIALLY AFFECTS DENTATE SPINES AFTER REPEAT CONCUSSIONS IN ADOLESCENT RAT

Patrick Riley, Tiffany Greco, Christopher Giza, Mayumi Prins, Michael Folkerts

UCLA, Neurosurgery, Los Angeles, USA

The greatest incidence of concussive injuries occurs during adolescence and young adulthood, during the dynamic maturational period of ongoing myelination and synaptic pruning. A single mild TBI during adolescence has been shown to interfere with normal pruning (Mychasiuk 2015), behavioral impairments and transient metabolic alterations (Prins). Repeat mTBI at short intervals has been shown to exacerbate outcomes in adolescent rats (Prins 2013) and it is hypothesized that it will decrease synaptic density, contributing to behavioral impairments. It is also hypothesized that enriched environment (EE)-induced plasticity can improve anatomy and function. Postnatal day 35 rats (n=3/group) were given sham or 2 closed head concussion (24 h interval) and immediately placed in single or EE housing for 30 days, followed by Novel object recognition testing. Golgi-Cox staining was analyzed for spine density and type in the dentate granule cells of the hippocampus. Three neurons per animal were selected and both the proximal and distal segments were

isolated for synaptic density and synaptic type analysis with RE-CONSTRUCT. Sham single housed and EE housed rats interacted with the novel object 67% and 84% of the time. rTBI single housed showed decrease in novel object recognition (53%), which improved to 61% among rTBI EE animals. Sham EE reared showed 20–24% greater spine density on segments proximal and distal to granule cell bodies, with greatest increases in long-thin, filopodia and thin synapses, reflecting newer synapses. Following rTBI in single housing there was a 15–19% decrease in synaptic density at proximal and distal segments, with thin and mushroom synapses predominant lost. rTBI EE animals showed significant decrease in synaptic density proximally with thin and mushroom synapses lost, but distally segments did not show loss of mushroom synapses with EE. RTBI during development affects EE-induced plasticity as measured by reductions in working memory and decreases in hippocampal synaptic connections.

Marilyn & Austin Anderson, UCLA BIRC, NFL Charities, UCLA Easton Neurotrauma Laboratories, UCLA Steve Tisch BrainSPORT program

Keywords: adolescent, repeat concussion, environment enrichment, synaptic spines

T01-15

RECORDINGS OF THE HIPPOCAMPUS IN BEHAVING RODENTS AFTER TRAUMATIC BRAIN INJURY REVEAL FIELD AND NEURONAL CODING DISRUPTIONS

Robin Russo¹, Paul Koch¹, Christopher Adam^{1,2}, Maura Weber¹, Victoria Johnson¹, John Wolf^{1,2}

¹University of Pennsylvania, Department of Neurosurgery, Philadelphia, USA

²Corporal Michael J. Crescenzo Veterans Affairs Medical Center, Philadelphia, PA

Persistent memory deficits are a major sequela of TBI, yet network level mechanisms of memory impairment are poorly understood. Studies using the fluid percussion injury (FPI) model have reported reduced theta power in the hippocampus (HC), but how this change influences memory-relevant HC networks is unknown. We have previously examined hippocampal circuitry post-injury acutely, demonstrating robust changes in CA1 neuronal entrainment to HC theta. To overcome limitations due to anesthesia, we chronically implanted electrodes in rodents, allowing examination of HC networks engaged in relevant awake behavior after injury. Experimental TBI was induced in male Sprague Dawley rats by FPI at mild to moderate (1.6–1.8 atm) severity. Implantation of multi-shank silicon probes with 32–64 electrodes on a high-resolution microdrive allowed for high-quality recordings of single units and simultaneous laminar field potentials. These recordings were also obtained while the animal was freely moving in an open field and a radial arm maze using 64-channel wireless telemetry. We demonstrate robust extracellular field potentials and stable unit recordings post-implant following FPI out to 14 days post-injury. Preliminary results from acute and chronic recordings indicate that CA1 neurons remain entrained to theta in non-injured and injured animals. However, distribution of preferred phase in injured CA1 neurons revealed two sharp peaks compared to a wide distribution with one peak in non-injured animals. These results indicate abnormal theta entrainment which could cause failure to coordinate neuronal ensembles that represent place during memory tasks. Distribution of axonal injury (revealed with amyloid precursor protein) suggests that loss of inputs from entorhinal cortex and medial septum may contribute to these changes, and reduction in performance on the Morris Water maze. Ongoing work to characterize memory deficits in the radial arm maze combined with awake recordings during this task may reveal mechanisms of trauma-induced network dysfunction.

Keywords: electrophysiology, behavior, learning and memory, hippocampus

T01-16

CHRONIC TREATMENT WITH NMDAR AGONIST ENHANCES PRO-PLASTICITY AND PREVENTS PRO-DEATH PATHWAYS AFTER DEVELOPMENTAL TBI

Saman Sargolzaei, Yan Cai, Shauna Perigo, Srijia Bhaduri, Philip Rosenbaum, Neil G. Harris, Christopher C. Giza
UCLA, Neurosurgery, Los Angeles, USA

Very little is known about the role of N-Methyl-D-aspartate receptors (NMDAR) and downstream pro-plasticity and pro-death molecular pathways following developmental TBI. Recent evidence indicates beneficial effects of D-Cycloserine (DCS), a partial NMDAR agonist, to treat cognitive and memory deficits following TBI, and this may occur partly by alteration of neuroplasticity-associated molecular pathways. We tested the hypothesis that sub-acute treatment with DCS following injury results in an enhancement of hippocampal pro-plasticity pathways and prevents upregulation of pro-death pathways after lateral fluid percussion injury (LFPI). 32 postnatal day 19 male Sprague Dawley rats were randomized into 4 groups (sham-saline, sham-DCS, LFPI-saline, LFPI-DCS, 8/group). All received 5 i.p. injections of 30 mg/kg DCS or saline every 12 hours until post-injury day (PID) 3. Ipsilateral hippocampal tissues were harvested on PID14 and processed for immunoblotting of pro-plasticity (pAkt, pCamKII, NR1, and NR2B) and pro-death markers (nNOS and p38). Results indicate that after injury there was a significant effect of DCS treatment on pro-plasticity and pro-death pathways compared to saline-treated injured rats in ipsilateral hippocampus: an upregulation of pAkt (8.8%, $P < 0.05$), pCamKII (29.18%, $P < 0.05$), NR1 (10.79%, $P < 0.05$), NR2B (10.81%, $P < 0.05$), and a significant decrease in nNOS (11.29%, $P < 0.05$) and p38 (3.8%, trend). These findings support the hypothesis that NMDAR agonist administration enhances neuroplasticity-related molecular pathways following developmental TBI. Mechanism-based treatments have particular promise for translation to improved TBI recovery. Supported by: R01NS27544, R01NS091222 UCLA Easton Labs for Brain Injury, UCLA Steve Tisch BrainSPORT program, UCLA BIRC.

Keywords: NMDAR agonist, Chronic treatment, plasticity, developmental TBI, D-cycloserine

T01-17

CHRONIC CORTISOL TRAJECTORIES MEDIATE SIL6R EFFECTS ON GLOBAL OUTCOME AFTER SEVERE TBI

Alexandria Schuster¹, Raj Kumar¹, Prerna Ranganathan¹, Byung-Mo Oh¹, Amy Wagner^{1,2}

¹University of Pittsburgh, Department of Physical Medicine and Rehabilitation, Pittsburgh, USA

²University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

Severe traumatic brain injury (TBI) elicits a stress response due to stimulation of the hypothalamic-pituitary-adrenal (HPA) axis that is associated with poor recovery. Distinct inflammatory signaling mechanisms associated with autonomic injury underlie HPA axis stimulation. With brief exposure, cortisol can have anti-inflammatory properties. However, prolonged elevated cortisol levels perpetuate pathological immune responses. Interleukin 6 (IL-6) is an important

pro-inflammatory mediator associated with 6-month outcome, that when bound to its soluble receptor (sIL-6R), stimulates the HPA-axis into a state of sustained stress. Relationships between post-acute serum cortisol and inflammatory cytokines have not been extensively studied in TBI. Thus, we investigated a possible regulatory role for cortisol in mediating IL6R effects on 6-month outcome for N=89 individuals with severe TBI. Individual mean serum cortisol and sIL-6R levels from samples collected over the first 3 months post-injury were assessed, and sIL-6R levels for the cohort were quartiled. Group based trajectory analysis (TRAJ) on cortisol levels measured in the same samples identified two subgroups—a stable-high group (N=50) and a decliner (N=39) group. 6-month Glasgow Outcome Scale (GOS) scores were dichotomized as poor (GOS=2/3) or good (GOS=4/5) outcome. Logistic regression showed sIL-6R quartiles predicted 6-month GOS (OR=1.82, p=0.014), where higher quartiles were associated with poor outcome. Cortisol TRAJ also predicted GOS (OR=5.53, p=0.0004), where the stable-high group had greater odds for poor outcome. sIL-6R quartiles also predicted cortisol TRAJ group membership (OR=0.639, p=0.024). Mediation analysis considering cortisol TRAJ as the mediator variable showed that cortisol TRAJ attenuated the relationship of sIL6R to GOS (OR=1.5, p=0.087). These results indicate IL-6 signaling through the sIL6R is influenced by cortisol and may represent an important post-acute contribution to global outcome post-injury. Bi-directional communication between the HPA axis and IL-6 family proteins provides novel insight onto the propagation of chronic inflammation and the long-term stress response post-TBI. Support: DODW81XWH-071-0701, NIDILRR-90DP0041.

Keywords: Chronic cortisol, Chronic inflammation, Soluble interleukin 6 receptor, Traumatic brain injury

T01-18

KINESIN KIF2A EXPRESSION IN THE INJURED SPINAL CORD

Oscar Seira^{1,2}, Jie Liu¹, Peggy Assinck^{1,3}, Wolfram Tetzlaff^{1,2,4}

¹ICORD-University of British Columbia (UBC), Vancouver, Canada

²Department of Zoology, Vancouver, Canada

³Graduate Program in Neuroscience, Vancouver, Canada

⁴Department of Surgery, Vancouver, Canada

Axons in the central nervous system (CNS) typically fail to regenerate after SCI. Mechanistically, this failure to regenerate is due to an inhibitory environment as well as a diminished intrinsic capacity of CNS neurons for axon growth including a disruption of the cytoskeleton. The cytoskeleton, and in particular microtubules (MT), plays a critical role in axonal transport and axon growth during development. In this regard, members of the kinesin superfamily of proteins (KIFs) have been demonstrated to regulate the extension of axons and growth of collateral branches. Some KIFs have been shown to promote axonal regeneration while in contrast the kinesin KIF2A has been found to negatively control axonal and collateral branches growth by depolymerizing microtubules. Using dorsolateral funiculus crush (DLF) and contusion (Infinite Horizon (IH) impactor) models to induce SCI in adult rats, we examined the temporal and cellular expression of KIF2A in the injured spinal cord. By Western blot analysis we observed an increase of KIF2A expression with a maximal level at 10 days to 8 weeks post injury. KIF2A immunoreactivity was localized in cultured cortical neurons, as well as in tissue sections of injured spinal cords in neurons and mature oligodendrocytes at the vicinities of the injury. Importantly, we observed increased KIF2A at the injured axonal tips as well as in Calbindin and Parvalbumin positive propriospinal neurons. We also show the inhibition of KIF2A in vitro with siRNA promoting neurite extension and therefore propose that the expression in the injured axons may contribute to neurite

outgrowth inhibition after injury. It remains to be shown whether the expression of KIF2A in Calbindin and Parvalbumin positive neurons might contribute to a prevent random neurite outgrowth and play a role in neuropathic pain after SCI. Further studies will determine whether the KIF2A becomes a viable target for the treatment of SCI.

Keywords: cytoskeleton, kinesin, spinal cord, neuropathic pain, axonal regeneration

T01-19

WITHDRAWN FROM COMPETITION

T01-20

BLOCKADE OF BOTH IL-1 α AND IL-1 β ARE NECESSARY FOR MAXIMAL ANTI-INFLAMMATORY AND COGNITIVE BENEFIT FOLLOWING TBI

Brittany Todd, Jo Mahoney, Polly Ferguson, Alex Bassuk, Elizabeth Newell

University of Iowa Carver College of Medicine, Pediatrics, Iowa City, USA

IL-1 is a central regulator of the immune response to acute brain injury. IL-1 α and IL-1 β are the best-characterized cytokines of this family and signal through IL-1 receptor (IL-1RI). An understanding of the contribution of individual IL-1 pathway molecules following TBI is lacking and is of vital importance in developing potential anti-IL-1 therapies. We use lateral fluid percussion injury adapted to mice targeting moderate-severe TBI. C57BL/6J and global IL-1 KO mice (on C57BL/6J background) were used. Gene expression was evaluated by qPCR. Barnes maze testing was used to evaluate cognitive function 2 weeks post-TBI. FPI resulted in increased inflammatory cytokine expression (IL-1 α , IL-1 β , TNF and IL-6) in both focal (left parietal cortex, hippocampus) and remote regions (brainstem, cerebellum). At 6 hours, IL-1RI ablation decreased the spread of IL-1 β and IL-6 expression to remote regions (brainstem, cerebellum). At 24hrs, IL-1RI ablation improved resolution of IL-1 β and IL-6 in both focal (parietal cortex) and remote (brainstem) regions. Compared to WT littermates, IL-1RI KO mice performed better in the Barnes maze probe trial whereas IL-1 α and IL-1 β KO mice showed no significant improvement.

Conclusions: FPI results in a diffuse inflammatory response with increased expression of pro-inflammatory cytokines at the injury epicenter, and in areas remote from impact. Combined, but not individual IL-1 α and IL-1 β blockade, prevented spread and hastened resolution of inflammatory cytokine expression. Furthermore, ablation of IL-1 α or IL-1 β alone was insufficient for cognitive protection following TBI, whereas combined blockade via IL-1RI ablation did result in cognitive rescue. Pharmacologic IL-1RI blockade appears to be a superior therapeutic strategy over individual IL-1 molecule blockade following TBI.

Support: K12 HD27748-11

Keywords: Interleukin-1, fluid percussion injury, cytokine

T01-21

PERSISTENT DECREASE OF CEREBRAL BLOOD FLOW IN TRAUMATIC BRAIN INJURY AND SPORTS-RELATED CONCUSSION

Fredrik Vedung¹, Gunnar Antoni³, Anders Wall³, Markus Fahlström², Elna-Marie Larsson², Niklas Marklund¹

¹Uppsala university, Institution of neurosciences, Dep of neurosurgery, Uppsala, Sweden

²Uppsala university, Department of Radiology, Uppsala, Sweden

³Uppsala university, PET-centre, Uppsala, Sweden

We hypothesized that persisting cerebrovascular abnormalities contribute to chronic cognitive problems commonly observed in traumatic brain injury (TBI) and sports-related concussions (SRC). To test this hypothesis, we used arterial spin labeling (ASL) magnetic resonance imaging (MRI) to evaluate cerebral blood flow (CBF) in patients with moderate to severe TBI and in symptomatic athletes with SRCs.

Seven healthy controls (age 29.7 ± 6.4) with no prior history of concussion/TBI, 8 athletes (age 27.6 ± 6.8) with previous SRC, fulfilling the criteria for post concussive syndrome, sustained in median 23 (6-132) month previously and 6 patients (age 28.8 ± 7.5) with a moderate-severe TBI sustained in median 18 (10-29) months prior to the investigations were included. ASL and structural imaging was performed on a 3.0 T integrated PET/MR-system. Neuropsychological evaluation using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) cognitive screening tool was performed at time of MRI evaluation.

A globally decreased CBF was observed in grey and white matter, in TBI patients (55 ± 13 ml/100g/min and 41 ± 8 ml/100g/min, respectively) and in SRC 59 ± 5 ml/100g/min and 43 ± 3 ml/100g/min, respectively, compared to controls 72 ± 12 ml/100g/min and 54 ± 11 ml/100g/min respectively. There were no CBF differences between the TBI and SRC groups. The RBANS test showed that both TBI patients (75 ± 24) and SRC athletes (76 ± 12) performed worse than controls (102 ± 16).

Our results suggest that ASL using 3T MRI is a suitable method for evaluating CBF following TBI and SRC and that cerebrovascular abnormalities persist chronically following both moderate-severe TBI and in symptomatic athletes with SRC. It is plausible that CBF decrease contributes to persisting cognitive and behavioral deficits observed in both post-concussive syndrome and TBI.

Acknowledgment of support: Swedish Brain Foundation, Swedish Research Council under the framework of EU-ERA-NET NEURON CnsAFlame, Bissen Brain Walk and Selander Foundation

Keywords: Cerebral perfusion, Concussion, ASL, athletes

T01-22

METHYLPREDNISOLONE TREATMENT ENHANCES EARLY RECOVERY FOLLOWING SURGICAL DECOMPRESSION FOR DEGENERATIVE CERVICAL MYELOPATHY

Pia Vidal¹, Antigona Ulndreaj^{1,2}, Michael Fehlings¹⁻³

¹Toronto Western Research Institute and Spine Program, Krembil Neuroscience Center, University Health Network, Genetics & Development, Toronto, Canada

²Institute of Medical Science, University of Toronto, Toronto, Canada

³University of Toronto, Division of Neurosurgery and Spine Program, Surgery, Toronto, Canada

Degenerative cervical myelopathy (DCM) is caused by age-related degeneration of the cervical spine, leading to chronic compression of the spinal cord. The current treatment consists of surgical decompression. Our laboratory has shown that after decompression, increased neuroinflammation and spinal cord reperfusion contribute to the development of a secondary injury. Methylprednisolone (MPSS) has been used to moderate inflammation in the spinal cord following

spinal cord injury, but has been shown to compromise the peripheral immune system. The primary objective of this study was to assess the effectiveness of MPSS in enhancing neurological recovery following decompression and evaluate whether it compromises the composition of circulating immune cells. Gradual compression of the spinal cord was induced in C57B/L mice by inserting a biomaterial underneath the C5-6 lamina. After 12 weeks of DCM, animals received one dose of MPSS (30mg/kg) or vehicle 30 minutes before decompression and two weeks after decompression. Composition of circulating white blood cells was analyzed by flow cytometry and locomotor recovery was assessed with the Catwalk system. One single dose of MPSS before surgical decompression significantly enhanced the beneficial effects of surgical decompression for two weeks, by further improving locomotion ($*p < 0.05$). In addition, MPSS treatment reduced IL-1b production without inducing changes in the composition of circulating white blood cells compared to the vehicle-treated group. Our data suggest that MPSS has an early protective effect in improving locomotion following decompression for degenerative cervical myelopathy without compromising the composition of circulating immune cells.

Keywords: decompression, degenerative cervical myelopathy, gait assessment

T01-23

OPTOGENETIC MODULATED CORTICOSPINAL TRACT SPROUTING AND MOTOR RECOVERY FOLLOWING A CERVICAL LATERAL SPINAL CORD HEMISECTION

Wei Wu¹, Josue Ordaz¹, Tyler Nguyen¹, Yiping Zhang², Christopher B. Shields², Xiaoming Jin¹, Xiao-Ming Xu¹

¹IUPUI, Neurological Surgery, Indianapolis, USA

²Norton Neuroscience Institute, Neurosurgery, Louisville, USA

The corticospinal tract (CST), the major tract for voluntary control of dexterous movement, fails to regenerate in adult mammals after spinal cord injury (SCI). Furthermore, after an incomplete SCI, spared CST axons lack sufficient plasticity to induce meaningful functional improvement. Previous studies have shown that augmenting the activity of cortical neurons produces some effects after electrical or chemical stimulations. However, the lack of spatial and temporal resolutions hinders an understanding of the mechanisms of therapeutic effects, which is important for developing activity-dependent therapies for SCI. In the present study, we hypothesized that augmentation of CST neural activity would be sufficient to promote its plasticity, and subsequently, functional recovery. We used an optogenetic stimulation approach to selectively stimulate the motor cortex in which axons were spared in the CST contralateral to a cervical 5 (C5) right-sided hemisection, thus mimicking human Brown-Séquard syndrome. Our results showed that optogenetic stimulation for 1 week promoted robust sprouting of spared CST axons across the midline to innervate the denervated side of the gray matter. Such a "cross the midline" phenomenon was not found in the non-stimulated group. Notably, the optogenetic-mediated CST plasticity correlated well with improved functional recoveries, measured by pellet retrieval, grid-walking, cylinder, and rotarod tests. Brain mapping further revealed that optogenetic stimulation enhanced the spot number and amplitude of electrophysiological recordings, as compared to non-stimulation. Finally, optogenetic stimulation significantly increased the production of brain-derived neurotrophic factor (BDNF), a critical factor for synaptic plasticity, in both motor cortex and spinal cord. In conclusion, selective

augmentation of the pyramidal neurons in the motor cortex using optogenetic stimulation is sufficient to initiate CST axonal sprouting in the spinal cord, leading to forelimb functional improvement after a cervical spinal hemisection.

Keywords: Spinal cord injury, optogenetics, CST, Plasticity, locomotor recovery

T01-24

INHIBITION OF NADPH OXIDASE ATTENUATES FUNCTIONAL DEFICITS IN MIDDLE-AGED MICE AFTER SPINAL CORD INJURY

Bei Zhang, William Bailey, Anna McVicar, Amy Veldhorst, John Gensel

University of Kentucky, Spinal Cord and Brain Injury Research Center, Lexington, USA

The average age at the time of spinal cord injury (SCI) has steadily increased from the average age of 29 years old in the 1970's to the current age of 42 years old. Recent findings from our group and others demonstrate that middle-aged mice exhibit worse functional deficits and exacerbated tissue damage after SCI. This is associated with age-dependent increases of reactive oxygen species (ROS) production, NADPH oxidase (NOX) activity and pro-inflammatory macrophage activation. Despite these age-specific differences, clinical therapies are being examined in individuals regardless of age and are based upon preclinical data generated almost exclusively using young animals. The purpose of the current study is to test the extent to which age affects the efficacy of SCI treatment. Specifically, we hypothesize that the effectiveness of apocynin, a NOX inhibitor for SCI, is age-specific. We applied mild-to-moderate contusion injury at the thoracic level (T9 laminectomy, 50 kdyn Infinite Horizons) to 4-month-old (4 MO) and 14 MO mice. We treated mice with apocynin (5 mg/kg, intraperitoneal injection) or vehicle (1% DMSO) at 1 and 6 hours post injury, then daily for 1 week. We examined the effect of apocynin treatment on functional and anatomical recovery from SCI. Our results show that apocynin effectively improves functional recovery and decreases lesion volume in 14 MO but not in 4 MO SCI mice. This suggests that apocynin may exhibit age-dependent neuroprotection by blocking excessive NOX-mediated ROS production. To the best of our knowledge, our data is the first to identify age as a critical regulator for SCI treatment efficacy. Age therefore needs to be considered as an important clinical variable to tailor therapeutic interventions and best serve the diverse SCI community.

Acknowledgments: this work is supported by Craig H. Neilsen Foundation and NIH NINDS R01NS091582

Keywords: Aging, Apocynin, Functional recovery, Reactive oxygen species

T01-25

PRIMARY TRAUMATIC AXONOPATHY AFTER IMPACT ACCELERATION: CHARACTERIZATION OF PATHOLOGY WITH 3D HIGH-RESOLUTION METHODS

Nikolaos Ziogas, Leyan Xu, Jiwon Ryu, William Gilliam, Tariq Omer, Vassilis Koliatsos

Johns Hopkins University School of Medicine, Department of Pathology / Division of Neuropathology, Baltimore, USA

Diffuse axonal injury (DAI), a primary axonopathy encountered in traumatic brain injury (TBI) of various types and degrees of severity, is a common neuropathological entity with mechanisms that are not well understood and no effective treatments. In experimental settings, it is often grouped together with axonal degeneration secondary to perikaryal injury. Animal models of primary traumatic axonopathy that replicate the conditions of human DAI are very important for the understanding of the basic cellular and molecular mechanisms of this condition. In these experiments we used the impact acceleration (IA) model of diffuse axonal injury that we modified for use in the mouse and explored the advantages of novel, high-resolution neuroanatomical methodologies. To characterize axonal pathology, we used the transgenic mouse line Thy1-eYFP-H expressing YFP in distinct populations of neurons, including layer V pyramidal neurons. Using CLARITY to render the whole mouse brain transparent and two-photon microscopy coupled with high-working-distance objective with sub-micron resolution, we visualized in 3D the whole corticospinal tract (CST) from the cerebral peduncles to pyramidal decussation in single-axon resolution. Individual axons and classical axonal abnormalities were identified in exceptionally high detail and quantified at 3 h and 24 h post injury. CLARITY-based immunohistochemistry for amyloid precursor protein (APP) was used to compare the sensitivity of conventional markers of axonal injury with that of high-resolution methods. We found that CLARITY is extraordinarily revealing of the location, type, and magnitude of axonal abnormalities that appear to cluster in lower pyramids and pyramidal decussation. In parallel, we found that the cell bodies of CTB-filled, layer V pyramidal neurons whose axons were injured in the CST (based on perikaryal p-c-JUN immunoreactivity) atrophied, but did not die. In addition, in experiments using extravasation of IgG from LEA-labeled endothelial cells as a marker of blood-brain-barrier disruption, we found positive signal in brain stem very early post injury. Furthermore, we established the role of endogenous and blood-borne inflammatory response involving activated or transformed microglia and blood macrophages. Together, we present a high-resolution multivariate model that can be used for molecular interrogation of specific mechanisms and serve as potential platform for the development of novel therapeutics.

Keywords: Diffuse Axonal Injury, Impact Acceleration, CLARITY, 2-Photon Imaging, Corticospinal Tract, 3D Neuropathology

Poster Abstracts

A01 AGING

A01-01

AGING EXACERBATES FUNCTIONAL DEFICITS BUT NOT GROSS TISSUE DAMAGE IN A RAT MODEL OF TBI

Janna Harris^{1,2}, Raeesa Gupte², Morgan Brucks², Sarah Christian², Paul Keselman², William Brooks^{2,3}

¹University of Kansas Medical Center, Anatomy and Cell Biology, Kansas City, USA

²University of Kansas Medical Center, Hoglund Brain Imaging Center, Kansas City, USA

³University of Kansas Medical Center, Neurology, Kansas City, USA

Adults over the age of 65 have a higher incidence of, and worse outcomes from, traumatic brain injury (TBI) than younger adults. Developing effective treatments to improve outcomes in this vulnerable population will require preclinical studies in aged animal models to better understand the effects of aging on TBI pathophysiology. In this study we sought to characterize functional and tissue effects in adult (3 month) and aged (20 month) male F344 rats that had received identical controlled cortical impact (CCI) injuries targeting the sensorimotor cortex. Sensorimotor outcomes were assessed with beam walk and adhesive removal tests from day 1–14 after TBI. On day 14, rats were scanned with T2-weighted MRI at 9.4 Tesla to assess lesion volumes. We found that acute functional impairment after TBI was worse in the aged rats than in younger adults, and that subsequent spontaneous recovery of function was diminished. However, brain lesion volumes measured at day 14 did not differ across age groups ($21.8 \pm 8.0 \text{ mm}^3$ aged vs. $21.9 \pm 8.1 \text{ mm}^3$ adult), similar to our previous findings after CCI in mice (Onyszchuk et al. 2008). These findings confirm that the effects of older age on TBI outcomes in humans can be reproduced in the CCI model in aged rats. The similar lesion size in adult and aged rats suggests that age-related differences in the plasticity or function of spared brain tissue may be more important than lesion size in determining functional outcomes after TBI. Future studies to understand aging effects on the metabolic function of spared perilesional brain areas after TBI are warranted.

Supported by: NINDS (R21 NS091920) and a University of Kansas Lied Basic Science grant to Dr. Harris.

Keywords: TBI, Aging, Behavioral outcome, Animal models, Magnetic resonance imaging

A01-02

DOES USAGE OF PLATELET OR DDAVP PREVENT PROGRESSION OF TRAUMATIC INTRACRANIAL HEMORRHAGE IN PATIENTS ON ANTI-PLATELET MEDICATION?

Jamie Ullman¹, **George Koutsouras**², Amrit Chiluwal¹, Katherine Wagner¹, David LeDoux¹, Omar Bholat¹

¹North Shore University Hospital, Department of Neurosurgery, Manhasset, USA

²New York College of Osteopathic Medicine, Old Westbury, USA

Introduction: Anti-platelet agents (APA) are some of the most commonly used medications in the general population. When these patients suffer traumatic brain injury resulting in an intracranial hemorrhage (ICH), there is a concern for hemorrhage expansion due to their depressed platelet function. In post-traumatic ICH (PTICH), the degree of volume expansion with pre-injury APA use is not well delineated, as studies have shown variable results. Current management may involve platelet and/or desmopressin (DDAVP) administration; however, clear clinical evidence for their use is lacking. In this study, we have explored the utility of prophylactic administration of platelet and DDAVP administration in preventing the expansion of PTICH.

Methods: Medical and radiological records of patients receiving treatment at North Shore University Hospital between January 2015–June 2016 for PTICH were reviewed. Patients were included if they were on pre-injury APA (aspirin and/or Plavix), but not on anticoagulation therapy. Subjects must have received a non-contrast CT scan between 6–24 hours following the initial scan, without undergoing a surgical intervention between the two scans. The use of DDAVP and platelets was collected. Chi-square and ANOVA analysis performed to calculate group and mean differences, respectively.

Results: Of 131 subjects, 46.6% had at least two distinct regional hemorrhages and 28.2% were on dual-antiplatelet therapy. 55% received both DDAVP and platelet transfusion prior to follow up CT scan. On follow up, 83% had a stable or decreased hemorrhage, while 17% had an increased hemorrhage. While adjusting for the type of APA used, there was no significant improvement in hemorrhage when comparing subjects who received platelets and/or DDAVP to those who did not receive therapy ($p > 0.05$).

Conclusion: The prophylactic use of platelets and DDAVP does not seem to prevent the progression of acute PTICH in patients exposed to pre-injury APA. Therefore, judicious use of these agents is warranted in the acute management of PTICH.

Keywords: coagulation, platelets, Aspirin

A01-03

CHALLENGES IN GERIATRIC TRAUMATIC BRAIN INJURY RESEARCH RECRUITMENT AT A LEVEL I TRAUMA CENTER

Caitlin Robinson¹, John Yue¹, Sabrina Taylor¹, Molly Rose Morrissey¹, Geoffrey Manley¹, Raquel Gardner²

¹University of California, San Francisco, Neurosurgery, San Francisco, USA

²University of California, San Francisco, Neurology, San Francisco, USA

The incidence of traumatic brain injury (TBI)-related hospitalizations among older adults is four to 12 times that of younger age groups. Yet, older adults are underrepresented in TBI clinical research, thus limiting evidence-based management.

To elucidate this age-related disparity in research participation, we compared rates of exclusion criteria between older (≥ 65 y) vs. younger (< 65 y) adults screened 04/2014-03/2016 at the Zuckerberg San Francisco General Hospital (ZSFG) for participation in the ongoing 18-site

Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study. TRACK-TBI aims to enroll 3,000 patients with acute TBI across the spectrum of age and TBI-severity.

Of 9471 screened patients, 2656 (28%) were older adults. 88% of older adults vs. 78% of younger adults met ≥ 1 exclusion criteria ($p < 0.001$). Overall, the highest number of patients were excluded due to non-English speaking (18%), substance abuse (14%), homelessness (14%), significant past medical history (PMH; 10%), neurological disorder (8%), polytrauma (5%), psychiatric disorder (4%), in custody (4%), unsuitable home location (3%), and psychiatric hold (1%). Exclusion criteria with highest age-related discrepancy (older vs. younger, $p < 0.001$) included: non-English speaking (27%-vs.14%), significant PMH (16%-vs.-8%), neurological disorder (15%-vs.5%), substance abuse (6%-vs.-18%), homelessness (4%-vs.-17%), psychiatric disorder (3%-vs.-5%), and in custody (0.4%-vs.-6%).

In this single center study, older adults with TBI were more likely than younger adults to screen out of TBI research due to lack of English ability or pre-existing medical or neurological disorders. These findings suggest that exclusion criteria pertaining to English ability and pre-existing conditions serve to preferentially exclude older adults from TBI clinical research, effectively functioning as age cut-offs. As the global population ages and grows and numbers of older adults with TBI continues to rise, it will become increasingly important to consider broadening standard inclusion/exclusion criteria in TBI clinical research studies in order to ensure generalizability of results.

Keywords: Traumatic Brain Injury, Aging, Recruitment

A01-04

AGING WITH TRAUMATIC BRAIN INJURY: AGE-AT-INJURY EFFECTS ON PATHOPHYSIOLOGY

Rachel Rowe^{1,2}, Helena Morrison³, James Vickers⁴, Jonathan Lifshitz^{1,2}, Jenna Ziebell⁴

¹Barrow Neurological Institute at Phoenix Children's Hospital, Child Health, Phoenix, USA

²University of Arizona, COM-Phx, Phoenix, USA

³University of Arizona, Nursing, Tucson, USA

⁴University of Tasmania, Hobart, Tasmania

Traumatic brain injury (TBI) is more than a singular event, such that a disease state with evolving pathophysiology ensues over an extended timeframe following the initial insult. Effective recovery from injury and enduring/remaining pathology may depend on many parameters, including the biological age at the time of injury. Throughout "normal" aging, neuroinflammatory status is in constant flux and homeostatic processes oscillate. TBI activates inflammatory processes, such that the sustained inflammatory state disrupts neurovascular units, including microglia, that can contribute to injury-induced behavioral morbidities. Given the comparable inflammatory profiles of the aged and the injured brain, we hypothesize that TBI accelerates pathological hallmarks of aging in the brain. A single cohort of rats was received at post-natal day (PND)10 and subgroups of this cohort ($n = 11-12/\text{group}$) were subjected to a moderate midline fluid percussion injury at PND17, PND35, 2 mo, 4 mo, or 6 mo or were naïve. Rats were assessed for motor function, anxiety-like behavior, cognitive performance, and depression-like behavior across their lifespan. These published data indicated TBI can negatively impact neurological function during discrete stages of development and aging. Tissue was collected at 10 mo for histopathology. A skeleton analysis method was used to quantify microglial ramification in Iba-1 stained cortical tissue. There was no overall difference in number of microglia endpoints per cell or process length per cell among treatment groups at 10 mo of age. For the first time, rod microglia, an

activated morphology identified in brain tissue following diffuse brain injury, Alzheimer's disease, and aging, were identified in rats injured at PND17, PND35, and 6 mo. The interplay of age-at-injury and aging with an injury are translationally important factors and continued investigation of injury-induced neuropathology, including astrocytes and neurons are ongoing. More complete understanding of these factors can direct therapeutic interventions and personalized medicine for TBI survivors. Funding: AZ-Alzheimer-Consortium; WiNTR-VISA

Keywords: pediatric, age-at-injury, concussion, neurovascular unit

A01-05

AGE-AT-INJURY AFFECTS CHRONIC DIFFUSE TRAUMATIC BRAIN INJURY AND ALZHEIMER'S DISEASE RELATED BEHAVIORS AND PATHOLOGIES

Maha Saber^{1,2}, Caterina Branca^{3,4}, Salvatore Oddo^{3,4}, Jonathan Lifshitz^{1,2}, Rachel K. Rowe^{1,2}

¹Phoenix Children's Hospital, Child Health, Phoenix, USA

²University of Arizona- COM-Phoenix, Neurotrauma, Phoenix, USA

³ASU, Banner Research center, Tempe, USA

⁴ASU, Life Sciences, Tempe, USA

Alzheimer's disease (AD) affects over 5.3 million Americans and cost the US \$226 billion dollars in the year 2015 alone (Alzheimer's Association). Traumatic brain injury (TBI) can increase the risk of developing multiple age-related neurological disorders and neurodegenerative diseases, including AD. However, chronic consequences of a single diffuse TBI remain poorly understood. Many patients that suffer from TBI exhibit similar neurological pathologies to AD patients including deposition of amyloid plaques, formation of neurofibrillary tau tangles, and increased neuroinflammation. However, it is unknown how biological age at time of injury and severity of TBI contribute to these pathologies. The aim of this study is to determine whether a chronic diffuse TBI exacerbates AD-like pathology, neuroinflammation, and cognitive deficits and if this effect is dependent on age-at-injury. For this study we used 3 \times Tg (B6;129-Psen1 Tg[APP^{Swe}, tauP301L]1Lfa) mice, a commonly used model of AD, and wild-type controls. Mice received a midline fluid percussion injury (mFPI) or sham injury at 2 mo of age or 6 mo of age and assessed for cognitive function at 10 mo of age. Behavior analyses included Morris water maze and radial arm maze to evaluate reference memory; novel object recognition to evaluate recognition memory; and contextual fear conditioning to evaluate associative learning. Sensorimotor function was assessed using rotarod. At 10 mo of age (4-8 mos post-injury), brains were collected for histopathological analyses. F4/80 and Iba1 immunostaining determined the persisting inflammatory burden in chronic stages of TBI. Ongoing analyses correlating inflammation in chronic stages of TBI with behavioral deficits will allow us to determine effects of age-at-injury on TBI outcomes. Data from this study will provide much needed evidence for who is at greater risk for developing AD-like pathologies and cognitive deficits after a diffuse TBI.

Funding: Arizona Alzheimer's Consortium

Keywords: Diffuse injury, 3 \times Tg mouse model

A01-06

FRAILITY OR AGE? MORTALITY AND DISPOSITION AFTER TRAUMATIC SPINAL CORD INJURY IN THE ELDERLY

Brian Schnettgoecke, Timothy Choi, Charles Wade, John Holcomb, Sasha Adams

McGovern Medical School, Trauma, Houston, USA

Objectives: Approximately 12,500 spinal cord injuries (SCI) occur annually in the USA, and elderly comprise the largest number of trauma center admissions. Predicting outcome and discharge disposition in geriatric patients with traumatic SCI is imperative to appropriately direct hospital resources and coordinate extensive post-injury needs. While age is commonly used to predict mortality and disposition in geriatric patients, it does not adjust for altered physiologic reserve or level of frailty. The modified Frailty Index (mFI) assesses pre-injury deficits and is predictive of outcome and disposition, but has not been studied in geriatric traumatic SCI. We hypothesized that mFI is more reliable than age when predicting in-hospital mortality or unfavorable discharge disposition (UFD) in elderly SCI.

Methods: The trauma registry of our urban Level I trauma center was queried over 5 years (1/1/11–12/31/15) for patients (≥ 55 years) with blunt SCI. Pre-admission comorbidity data were collected from clinical records and mFI scores calculated. In-hospital mortality and UFD were determined and logistic regression assessed their relationship with age, injury severity score (ISS), and mFI. ROC curve analysis evaluated the relative performance of age and mFI in predicting mortality and UFD. SCI was defined as complete/incomplete cord injury, including cord contusions. Patients lacking adequate documentation were excluded.

Results: Of 807 with blunt SCI patients meeting criteria, 299 were age ≥ 55 years, of which 281 had sufficient data for inclusion. Median age was 68 years (IQR 61–76), 72% were male, 79% had incomplete SCI, median ISS was 18 (IQR 14–26), and median mFI score was 0.09 (IQR 0–0.18). Frailty and age were not correlated ($R^2 = 0.06$). Employing multiple logistic regression analysis, mFI was not significantly associated with UFD, while age ($p < 0.001$) and ISS ($p < 0.001$) were. Upon ROC curve analysis, mFI did not add significant predictive value to a model including age and ISS (AUC 0.7617 vs. 0.7616).

Conclusions: Age and ISS predict in-hospital mortality and UFD in elderly patients with traumatic SCI better than mFI.

Keywords: Traumatic SCI, Frailty, Outcomes

A01-07

GERIATRIC TRAUMATIC BRAIN INJURY: UNIQUE CONSIDERATIONS FOR PRESENTATION AND OUTCOME

Sourabh Sharma¹, John Yue¹, Allison Kaup², Geoffrey Manley¹, Raquel Gardner²

¹UCSF, Neurosurgery, San Francisco, USA

²UCSF/SFVAMC, Neurology, San Francisco, USA

Older adults have the highest rates of traumatic brain injury (TBI)-related emergency department visits, hospitalizations, and deaths. Few studies have investigated age-related differences in presentation and neurobehavioral recovery following acute TBI. We compared injury characteristics and clinical outcomes between young (18–39 years), middle-aged (40–59) and older (≥ 60) adults suffering acute TBI from the prospective multicenter Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) study. Outcomes included Glasgow Outcome Scale-Extended (GOS-E), Rivermead Postconcussional Symptoms Questionnaire (RPQ), Post-Traumatic Stress Disorder (PTSD) Checklist-Civilian Version (PCL-C) scored using the Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) criteria. Categorical variables were compared across age groups using the Pearson's chi-squared test, and continuous variables were compared using analysis of variance for continuous variables followed by Tukey's post hoc test. In 572 patients, (older-21%/middle-aged-35%/young-44%) falls predominated in older compared to young and middle-aged adults (71%-vs.-47%/34%; $p < 0.001$). TBI severity was worse among older adults by Abbreviated

Injury Scale-Head (72%-vs.-50%/44%; $p < 0.001$) but not by Glasgow Coma Scale ($p = 0.238$). Compared to middle-aged and young-adults, older adults had lower rates of loss of consciousness (64%-vs.-83%/80%; $p = 0.001$) and post-traumatic amnesia (64%-vs.-68%/77%; $p = 0.023$), and higher rates of intracranial pathology on computed tomography scan (64%-vs.-41%/39%; $p < 0.001$) intensive care unit admissions (51%-vs.-29%/36%; $p < 0.001$), and six-month mortality (18%-vs.-7%/1%). Fewer older and middle-aged adults achieved good functional recovery compared to young-adults (GOSE ≥ 7 : 53%/51%-vs.-66%; $p = 0.017$). Older adults reported less postconcussional symptoms (RPQ: 9.6 ± 10.9 -points-vs.- 15.0 ± 13.8 -points/ 9.7 ± 11.1 ; $p < 0.001$) and had lower rates of screening positive for PTSD (12%-vs.-31%/23%; $p = 0.016$). Older TBI patients presented with lower rates of loss of consciousness and amnesia, but suffered increased intracranial pathology, intensive care admissions, and overall mortality compared to younger patients. At six-months, however, greater than half of older adults had good outcome per GOSE and were less likely to have PCS and PTSD compared to middle-aged patients. Critical avenues for future research will be to determine predictors and mechanisms of favorable versus unfavorable recovery in geriatric TBI.

Keywords: Geriatric, Traumatic brain injury, Postconcussional symptoms, Glasgow outcome scale-extended

A01-08

AGE AND INJURY ALTERS GLUCOSE TRANSPORTER GENE EXPRESSION AND NEURONAL VIABILITY IN THE RODENT SPINAL CORD

Ramona Von Leden, Kasey Moritz, Byrnes Kimberly

Uniformed Services University of the Health Sciences, Bethesda, USA

Aging spinal cord tissue shows increased inflammatory activity and oxidative stress, resulting in reduced neuronal viability and contributing to worsened outcome following spinal cord injury (SCI). Previously, we have demonstrated using positron emission tomography (PET)-based measurements of glucose uptake that 12 month (middle-aged) rats show an altered glucose uptake profile compared to 3 month (young adult) rats. To explore the potential effects and mechanism behind these findings, we aimed to determine how age affects glucose transporter (GLUT) gene expression and neuronal health. Briefly, spinal cord tissue was obtained from groups of naïve or injured (1 or 30 days post-injury (dpi)) young adult and middle-aged male Sprague Dawley rats. Tissue was processed for quantitative-PCR analysis, protein quantification or immunostaining of NeuN, a neuronal specific marker of nuclear proteins. In naïve tissue, middle-aged rats showed significant increases in GLUT3 and GLUT4 gene expression compared to young adults. After SCI, neuron specific GLUT3 was significantly decreased and non-specific GLUT4 significantly increased at all time-points post injury in young adult rats, likely due to decreased neuronal viability and increased inflammatory activity. In middle-aged rats, however, while GLUT3 was significantly decreased at all time points, GLUT4 was significantly increased only at 1 dpi; the reduction in GLUT3 was significantly greater than that observed in young adult rats at 30 dpi. Further, in middle-aged rats, GLUT1 (expressed by endothelial cells) was significantly increased at 1 dpi and significantly decreased by 30 dpi. Although protein quantification of NeuN revealed no significant difference between young adult and middle-aged rats, immunostaining revealed an altered staining pattern with NeuN localized outside of the neuronal nucleus, suggesting compromised neuronal function. Overall, these findings show an inverse relationship between GLUT gene expression and glucose uptake patterns, as middle-aged rats show significantly reduced basal glucose uptake that was increased at chronic post-injury time-points compared to

young rats. Our findings suggest that alterations in glucose uptake with age, resulting from compromised neuronal viability and increased inflammatory activity, may influence GLUT gene expression profiles.

Keywords: glucose uptake, glucose transporters, neuronal viability, contusion injury, rodent models

A02 ASTROCYTE

A02-01

ASTROCYTIC EXOSOMAL MIRNAS UNDER INFLAMMATORY CONDITIONS: IMPLICATION AS BIOMARKERS

Manish Bhomia, Manoshi Gayen, Nagaraja Balakathiresan, Barbara Knollmann-Ritschel

Uniformed Services University of the Health Sciences, Pathology, Bethesda, USA

Astrocytes are the most abundant glial cell population in the brain which are critical to maintenance of central nervous system. Astrocytes mediate the inflammatory cascade following traumatic brain injury (TBI). MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules that regulate gene expression. Exosomes are extracellular membrane vesicles secreted by mammalian cells containing proteins, lipids and miRNAs. The cargo of exosomes may reflect the physiological state of the cells and hence, they have been studied as mediators of cell signaling and biomarkers.

In this study, we evaluated the exosomal miRNAs released from astrocytes under inflammatory conditions and studied their potential role in mediating inflammation and potential for an inflammatory biomarker for TBI. Human primary fetal astrocytes were treated with interleukin 1beta (IL-1b). The exosomes from the astrocytes were pelleted from the cell free supernatant by ultracentrifugation. MiRNA profiling was performed on the total RNA extracted from the exosomes. Ingenuity pathway analysis (IPA) was used to identify the role of the secretory miRNAs.

Treatment with IL-1b induced an increased expression of GFAP. The presence of exosomes was confirmed by immunoblotting of tetraspanin membrane markers (Alix and CD9). MiRNA profiling of the exosomal RNA identified 5 miRNAs which were exclusively released only by activated astrocytes whereas, 23 miRNAs were differentially secreted on IL-1b stimulation. Furthermore, IPA analysis indicated that most of these miRNAs are involved in the regulation of the neuroinflammatory pathways. Moreover, some of these exosomal miRNAs were also reported as biomarkers of human TBI. Therefore, we conclude that inflammation lead to a release of astrocytic miRNAs which may be involved in cellular crosstalk for mediating inflammation during TBI. The miRNAs can be used as astrocyte injury biomarker and can help in differentiating neuronal injury from inflammation during TBI.

The opinions expressed herein are those of authors and are not necessarily representative of those of the Uniformed Services University, Department of Defense or, the United States Army, Navy, or Air Force and DMRDP.

Keywords: Inflammation, Astrocyte, Exosome, Biomarker, TBI

A02-02

ENHANCING INTEGRATION OF SCHWANN CELL TRANSPLANTS FOLLOWING SPINAL CORD INJURY

Susana R. Cerqueira¹, Robert C. Dauser¹, Martin Oudega¹⁻³, Mary Bartlett Bunge^{1,3,4}

¹Miller School of Medicine, University of Miami, Miami Project to Cure Paralysis, Miami, USA

²Miller School of Medicine, University of Miami, Bruce W. Carter Department of Veterans Affairs Medical Center, Miami, USA

³Miller School of Medicine, University of Miami, Department of Neurological Surgery, Miami, USA

⁴Miller School of Medicine, University of Miami, Department of Cell Biology, Miami, USA

Schwann cell (SC) transplantation has been extensively studied for spinal cord injury (SCI) repair. SCs are neuroprotective and promote axon regeneration and myelination in the lesion site. However, glial scar tissue surrounding the lesion site is inhibitory for both SC integration and axon growth into the host cord. Astrocytes contribute to this inhibition by secreting chondroitin sulfate proteoglycans (CSPGs), expressing ephrins, and forming an intricate network of astrocytic processes. The goal of this study was to investigate a drug that can transiently suppress astrocyte function to enable SCs to integrate and axons to grow across the implant-host spinal cord interface. 6-Aminonicotinamide (6-AN) disrupts the metabolic pathway in glial cells, and in the proper concentration can be used to transiently decrease their inhibitory actions following injury. Our in vitro data shows that 6-AN acts in a time and dose-dependent manner, causing retraction of astrocyte processes and decreasing their metabolic rates. The metabolic rate in SCs is only marginally decreased by 6-AN. Importantly, using an in vitro SC/astrocyte confrontation assay, we found that 6-AN treatment caused SC migration into the astrocyte terrain. In a rat model of spinal cord contusion, extensive migration of SCs into the adjacent spinal cord tissue was seen 3 weeks after SC transplantation and 6-AN treatment. No additional damage was observed in the injection site, suggesting that the dose used was not harmful. We suggest that 6-AN treatment is a tool that can transiently alter the glial scar, resulting in improved SC-astrocyte integration. Future studies will investigate axon growth, motor function, and immune cell responses in response to 6-AN treatment.

Keywords: Glial Scar

A02-03

DECIPHERING MECHANOBIOLOGICAL MECHANISMS OF ASTROCYTE REACTIVITY TO SHOCK WAVE

Nora Hlavac¹, Pamela VandeVord^{1,2}

¹Virginia Tech, Biomedical Engineering and Mechanics, Blacksburg, USA

²Salem Veterans Affairs Medical Center, Salem, USA

Astrocytes contribute to brain function through both active and homeostatic support to neurons. They play an equally integral role in pathological sequelae associated with traumatic brain injury and other CNS insults. Much evidence exists to suggest that astrocyte response to injury is governed by impulse mechanics, however, mechanisms of their mechanosensitivity remain largely uncharacterized. This study aimed to understand mechanobiological roles in reactive astrogliosis in response to high rate mechanical impulses. We have previously shown that environmental factors influence astrocyte reactivity to shock wave. Together, these evidences lend to the hypothesis that astrocyte reactivity is at least in part governed by mechanical disturbances and associated mechanotransduction. In this study, primary rat astrocytes were exposed to a single high rate overpressure using an underwater shock wave generator. Structural reactivity was assessed by quantifying alterations in gene and protein expression of intermediate filament proteins up to 48 hours post-insult. Inflammatory potential was evaluated through

alterations in cytokine expression. In order to determine potential correlative mechanotransduction pathway activation, key transcription factors in the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and mitogen-activated protein kinase (MAPK) pathways were quantified for cells exposed to mild severity overpressure at the same time points following insult. Results indicate that reactivity occurs within the 48 hour timeframe, as marked by gene and protein upregulation of glial fibrillary acidic protein. Fluctuations in gene expression of several cytokines, including interleukin-1β and interleukin-6, were also observed when compared to sham. Moreover, differential activation of NFκB and MAPK pathways occurred parallel to these phenotypic changes. Specifically, phosphorylated p65 and p38 were significantly altered as compared to sham. Increased levels of phosphorylated focal adhesion kinase suggests that these changes may be the direct result of integrin activation following mechanical stimulation. Further characterization of integrin clustering and mechanotransduction signaling as a result of shock wave exposure will be necessary to establish definitive links in the mechanisms associated with astrocyte reactivity to isolated mechanical stimulus.

Keywords: in-vitro injury, mechanotransduction, reactivity, shock wave

A03 AXONAL INJURY

A03-01

NEURAL STEM CELL TRANSPLANTATION IN TRAUMATIC BRAIN INJURY

Farhana Akter, Xinming Yu

University of Cambridge, Neuroscience/Neurosurgery, Cambridge, United Kingdom

Traumatic brain injury (TBI) is a leading cause of death worldwide. It can also lead to chronic impairment of function and causes a significant socioeconomic burden. Current therapies are limited and no intervention can alter the underlying pathological process. Over the past two decades a number of studies have investigated the potential for neural stem cell (NSC) transplantation in TBI models.

Purpose: To systematically review the literature to determine how many papers investigate the use of NSC transplantation in TBI. Our primary aim is to determine whether the studies are robust and can form functional neural cells.

Materials and Methods: A total of 406 published articles were identified in our systematic search of electronic databases (PUBMED, WEB OF SCIENCE). After screening for eligibility, a total of 30 studies investigating the use of NSCs in animal models of TBI were found and underwent comprehensive review, quality assessment, data extraction and statistical analysis. Two independent reviewers evaluated articles by using the The Animals in Research: Reporting in vivo Experiments (ARRIVE) guidelines.

Results: Thirty studies investigated the use of NSC in TBI. All papers described the migration of NSCs within 1 week to 12 weeks of implantation. Ten papers showed evidence of increased survival and migration in the presence of trophic factors. The degree of TBI ap-

pears to be important in cell survival and transplanted NSCs have shown to survive better after mild injury than after severe injury. All studies provided evidence of neuron formation after transplantation. However, only five studies showed evidence of NSCs establishing neural connections and ability to generate action potentials.

Conclusions: Despite some promising results, there have only been a limited number of studies, which have provided evidence of transplanted cells forming functional connections. We recommend all studies investigate whether NSCs can form mature functional neural cells and correlate this with behavioral function and cognition.

Keywords: traumatic brain injury, neural stem cells, stem cells, functional neurons, systematic review, metanalysis

A03-02

ATTENUATION OF AXONAL DEGENERATION RELATIVE TO MYELIN PATHOLOGY AFTER MILD TRAUMATIC BRAIN INJURY IN MICE LACKING SARM1

Christina Marion^{1,2,3}, Kryslaine Radomski^{1,2}, **Regina Armstrong**^{1,2,3}

¹USUHS, Anatomy, Physiology & Genetics, Bethesda, USA

²USUHS, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

³USUHS, Program in Neuroscience, Bethesda, USA

Traumatic brain injury (TBI) results in traumatic axonal injury (TAI) in white matter tracts. Myelin pathology may contribute to deficits experienced after TBI. Myelin pathology may be due to secondary effects along degenerating axons or may occur as primary pathology of white matter injury. The current studies investigate the relationship between axonal degeneration and myelin pathology. Sarm1 is essential for execution of the conserved axon death pathway in injured axons. *Sarm1*^{-/-} mice exhibit suppressed Wallerian degeneration and long-term survival of injured axons. Using a mild TBI (mTBI) model that results in TAI in the corpus callosum, we assessed axon degeneration and myelin pathology in *Sarm1*^{-/-} and wild type mice. The corpus callosum was sampled using electron microscopy 3 days post-mTBI or sham procedure. In wild type mice, TBI increased axon degeneration and produced two forms of myelin pathology - demyelination of intact axons and excessive myelin extending out from axons. In *Sarm1*^{-/-} mice, axon degeneration and demyelination were both suppressed after TBI. However, formation of excessive myelin figures after mTBI was similar in wild type and *Sarm1*^{-/-} mice. We generated myelin reporter mice to label myelin with green fluorescent protein (GFP) to visualize these excessive myelin figures by confocal microscopy. *PLPcreER*^{T2}; *mTmG* mice were given a single low-dose of tamoxifen prior to mTBI/sham procedure, to induce recombination in sparse oligodendrocytes. At 3 days post-injury, aberrant myelin formations were observed along GFP-labeled internodes in mTBI animals as compared to sham controls. These myelin abnormalities may represent dynamic myelin remodeling in a compromised axon-myelin unit. These results indicate that Sarm1 may be an important therapeutic target to interrupt processes that produce axon degeneration and demyelination after mTBI. In addition, mTBI also produces excessive myelin that was not mitigated by suppressing axon

degeneration. Funded by the Center for Neuroscience and Regenerative Medicine.

Keywords: myelin, demyelination, neuropathology, plasticity

A03-03

CHANGES IN SODIUM CHANNELS AFTER CONCUSSION IN SWINE

Edgardo Arroyo, Maura Weber, Victoria Johnson, Douglas Smith
University of Pennsylvania, Neurosurgery, Philadelphia, USA

Diffuse axonal injury (DAI) has emerged as a primary pathological substrate of mild traumatic brain injury (mTBI) or concussion. DAI includes swollen axonal profiles resulting from mechanical and chemical damage to the cytoskeleton with interruption of axonal transport. In addition, dysregulation of axonal sodium channels with substantial influx of sodium has been observed in an in vitro model of axon trauma. In turn, these high sodium levels trigger increases in intra-axonal calcium levels in a feed-forward pathway of protease activation and axon degeneration. Although there is indirect evidence that this same process occurs in TBI in vivo, little is known about specific changes to axonal sodium channels in the injured brain.

Here, we used a model of head rotational acceleration in swine that mimics the biomechanics of concussion in humans to examine potential post-injury changes in specific subtypes of voltage gated sodium channels. Seventy-two hours after injury the brain was fixed and immunohistochemical analyses were performed on brain sections that included the corpus callosum. For sham animals, typical $\text{Na}_v1.6$ positive nodal clusters were found throughout the white matter region. However, for injured animals, $\text{Na}_v1.6$ labeling appeared to be split into heminodal-like profiles in APP co-labelled axonal swellings. In addition, patchy loss of $\text{Na}_v1.2$ was observed in the white matter, suggesting proteolysis of these channels as is seen in vitro after trauma. These data suggest that the two main sodium channel subtypes found on both myelinated and unmyelinated axons in the brain are modified after trauma. These findings support examination of potential therapies targeting sodium channels to combat progressing DAI. This work was supported by DOD grant, PT110785 and NIH grants NS092389, NS038104 and EB021293.

Keywords: Sodium Channels, Diffuse Axonal Injury, APP, Neurodegeneration

A03-04

DTI VOXEL-WISE ANALYSIS OF MILD TBI IN NEONATAL PIGS FOLLOWING NON-IMPACT HEAD ROTATION

Brittany Coats¹, Boston Terry², Greg Scott¹, Osama Abdullah³
¹*University of Utah, Mechanical Engineering, SALT LAKE CITY, USA*

²*University of Utah, Electrical and Computer Engineering, SALT LAKE CITY, USA*

³*University of Utah, Bioengineering, SALT LAKE CITY, USA*

The objective of this study was to develop a robust voxel-wise image analysis routine to detect subtle changes in axonal diffusion metrics following repetitive head trauma in a neonatal pig model. To achieve this objective, diffusion weighted images of anesthetized piglets (3–5 days old) were acquired before and after a repetitive, non-impact inertial head rotation. Animals were divided into three groups. Group 1 experienced cyclic head rotation and was imaged 24 hours post-

injury. Groups 2 and 3 experienced thoracic compressions designed to increase cerebral blood volume (CBV) and ICP to levels reported for intense infant crying, and then experienced the cyclic head rotation. Group 2 was imaged immediately post-injury (acute) and Group 3 was imaged 24 hours post-injury. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were computed. A weighted integral measure (WIM) for each metric was created to account for the cluster size and magnitude of the statistical differences. Significant differences from baseline imaging were detected in all groups. WIM levels in Group 1 were lower than in Groups 2 and 3, suggesting there is overall less injury in Group 1 compared to Groups 2 and 3. Significant clusters in Group 1 were observed primarily in the internal capsule and corona radiata, correlating well with locations of positive β APP staining. Groups 2 and 3, which had the same injury induced, had different responses depending on the timing of imaging. Acute imaging (Group 2) resulted in significant increases in MD and decreases in AD, while longer term imaging (24 hours in Group 3) resulted in significant decreases in FA and increases in RD. These data suggest increased CBV and ICP prior to mechanical loading can increase axonal injury. Further, DTI biomarkers of axonal injury will be highly dependent on the timing of the imaging.

Keywords: Diffusion Tensor Imaging, Repetitive Head Trauma, Pediatric, Voxel-based imaging

A03-05

CELL AND NETWORK HEALTH FAR REMOVED FROM TRAUMATIC AXONAL INJURY REGION AFFECTED BY RELEASE OF MOLECULES ABOVE 3KDA

Jean-Pierre Dolle, H. William Covert, Douglas Smith
University of Pennsylvania, Neurosurgery, Philadelphia, USA

Traumatic axonal injury (TAI), a common consequence of traumatic brain injury (TBI), results in both axonal degeneration and cell death in regions far removed from the site of injury. Here, we examined health and calcium network activity changes in axons and cell soma networks adjacent and far away from axonal tracts after exposure to media taken from axons experiencing TAI. Briefly, primary rat cortical neurons were grown on micropatterned deformable membranes, whereby a series of parallel 2 mm-long lanes containing only axons span two populations of neuronal soma. The axon-only region was rapidly stretched via mechanical parameters based on clinical TBI. Thirty minutes following injury, media from injured axons was removed and either placed on uninjured cultures or separated into <3KDa or >3KDa and then placed on uninjured cultures. Calcium levels and network activity was assessed at 24, 48 and 72 hrs later using the genetically encoded calcium indicator GCaMP6. Significant axonal degeneration was observed within 24 hrs following addition of injured media where numerous calcium accumulations along the length of the axons is observed which progressively worsens over 72 hrs. Cells adjacent to the axon-only region start to degenerate within 24 hrs exposure and are all dead within 72 hrs. Delayed degeneration in cells far away from the axon-only region starts around 48 hrs, with a few surviving cells observed at 72 hrs. By 72 hrs, there is no associated calcium network activity observed. When media was separated into <3KDa, >3KDa injured media showed increased cell death and decreased calcium activity compared to <3KDa injured media. These findings may shed some light on potential molecules responsible for cell death far removed from regions of traumatic axonal injury. This work was supported by DOD grant, PT110785 and NIH grants NS056202 and NS038104.

Keywords: Traumatic axonal injury, Cell death, Calcium activity, Secondary injury

LINEAR DISCRIMINANT ANALYSIS FOR HIGH CONTENT SCREENING IN NEUROTRAUMA

Jack Phillips¹, Sydney Sherman¹, Ying Wu², **John Finan¹**

¹NorthShore University HealthSystem, Neurosurgery, Evanston, USA

²Northwestern University, Electrical Engineering and Computer Science, Evanston, USA

The molecular pathology of neurotrauma is multi-modal so target-based drug screening is difficult. Therefore, phenotypic screening is attractive. Phenotypic screening requires an in vitro model and a phenotypic parameter with a high z' , indicating low signal-to-noise ratio. Conventionally, $z' > 0$ is required and $z' > 0.5$ is preferred. High content image analysis generates many phenotypic parameters. Typically, the parameter with the highest z' is used for screening. This study hypothesized that performance could be further improved by combining several injury parameters into a composite parameter using linear discriminant analysis (LDA). 19 quantitative measures of cell viability and neurite morphology were calculated from images of an in vitro neurotrauma experiment using MetaXpress software. In the model, circular silicone cell culture membranes were indented from underneath with cylindrical posts to induce trauma. Indentations 1, 2, 3, 4 and 5 mm deep were used. The midpoint of the dose response was 3 mm. 4mm was defined as negative control. 2mm was defined as positive control. The highest z' for any single parameter was 0.121. A LDA transform was trained across all parameters to optimally discriminate injured and uninjured neurons using the 1mm and 5mm data. This transform was then applied to the 2 mm and 4 mm data (that were not included in the training of LDA) and the z' was 0.261. In addition to image parameters (i.e. parameters with a single value for each image), image analysis returned several cell parameters (i.e. parameters with a single value for each cell). For a given image, each of these is characterized by its density distribution. Specifically, we used a 10-bin histogram for each individual cell parameter. This process expanded the number of image parameters to 160. LDA on this dataset produced $z' = 0.582$, which renders a significant improvement. Therefore, a high content neurotrauma screen was elevated from marginally viable to high performing using LDA. Supported by NIH R21NS098129.

Keywords: high content screening, computer vision, multi-parametric methods, drug discovery

A03-07

NEUROIMAGING OF DIFFUSE AXONAL AND VASCULAR INJURY IN CHRONIC TRAUMATIC BRAIN INJURY

Margalit Haber¹, Franck Amyot^{2,3,4}, Kelly Flesher², Kimbra Kenney^{2,3,4}, Carol Moore³, Erika Silverman¹, Yi-yu Chou⁴, Dzung Pham⁴, Eric Wasserman⁴, Ramon Diaz-Arrastia¹

¹University of Pennsylvania, Neurology, Philadelphia, USA

²USUHS, Neurology, Bethesda, USA

³HJF, CNRM, Bethesda, USA

⁴NIH, NINDS, Bethesda, USA

Traumatic Brain Injury (TBI) results in diffuse axonal injury (DAI) and diffuse vascular injury (DVI). Both DAI and DVI result from inertial shearing forces, and the two terms are often used interchangeably, the spatial relationships between DAI and DVI have not been carefully studied. Multimodal magnetic resonance imaging (MRI) can help to distinguish these injury mechanisms: diffusion tensor imaging (DTI) provides information about axonal integrity,

while arterial spin labeling (ASL) and functional Blood Oxygen Level Dependent imaging (BOLD) with hypercapnia challenge, reflect cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) respectively. Chronic TBI participants (n=27) and age- and education-matched healthy controls (n=15) underwent multimodality MRI. The Freesurfer image analysis suite (MGH, Harvard, MA) was used to segment each MP-RAGE image into regions of interest (ROIs). Mean values of mean diffusivity (MD), fractional anisotropy (FA), CBF, and CVR were extracted for each ROI. Additionally, maps were normalized into a common space (MNI Atlas) and z-score maps were generated based on a pool of healthy controls. Normality of an ROI/voxel was determined based on z-score (abnormal MD: z-score >2.5; abnormal FA, CBF, and CVR: z-score <-2.5). Abnormal ROIs in one modality were not predictive of abnormalities in another modality. Approximately 8–10% of abnormal voxels for CVR and CBF also show an abnormal voxel value for MD, while only 1% of abnormal CVR and CBF voxels show a concomitant abnormal FA value. These data indicate that chronic TBI patients display two distinct endophenotypes: microstructural tissue/axonal injury and vascular injury that are spatially independent.

Keywords: Diffusion Tensor Imaging, Cerebral Vascular Reactivity, Chronic TBI, MRI

A03-08

USE OF HUMAN IPSC NEURONS TO STUDY TRAUMATIC AXONAL INJURY IN VITRO

Gisele Hansel, Jean-Pierre Dolle, Stewart Anderson, Sean Ryan, Jianping Li, Douglas H Smith
Upenn, Neurosurgery, Philadelphia, USA

Potentially due to their unique ultrastructure and anisotropic arrangement in fascicles, axons are particularly vulnerable to damage during mechanical loading from traumatic brain injury. This has been extensively demonstrated via in vitro models of traumatic axonal injury, where mechanical disruption of the axon cytoskeleton has been shown to trigger pathophysiological cascades. However, since most of the in-vitro studies used rodent neurons, corroboration using human neurons is important to determine the clinical relevance. To use human iPSC in an established in vitro model to examine the effects of dynamic stretch injury of micropatterned human axon fascicles in comparison to injured rat axon fascicles. Micropatterning of human iPSC neurons on deformable membranes created two populations of neuron soma spanned by parallel lanes of axon fascicles. Cultures were placed in a pressure-controlled device that injects a controlled air pulse into the pressure chamber. This cause dynamic selective stretch limited to the axon region. Three different pressures were used to cause injury. Over three days post-injury the cultures were examined for morphological and physiological changes and viability. After stretch injury, undulations were observed in a periodic arrangement along axons, which slowly relaxed back to the pre-stretch straight orientation. This reflects immediate mechanical damage to axonal microtubules that then buckle causing delayed relaxation of the axon, previously been characterized with rat axons. Varicose swellings began to form along the injured axons and by three days post injury, some axons had degenerated and disappeared. The extent of this damage and degeneration was greatly reduced compared to the sequence of rat axon degeneration at the same levels of injury. These data demonstrate that dynamic stretch injury in human iPSC axons induces mechanical microtubule damage leading to transport interruption and degeneration, similar to rat axons. The human axons appeared more resilient to the same level of injury in rat axons. This may be due to a larger diameter

and more microtubules in human axons, which could provide more protection against mechanical disruption.

Keywords: Axonal injured, iPSC derived neuron, Calcium influx, neurodegeneration

A03-09

RBM5 INCREASES NEURONAL DAMAGE IN A MODEL OF MECHANICAL STRETCH-INJURY

Travis Jackson, Shawn Kotermanski, Patrick Kochanek
University of Pittsburgh, Critical Care Medicine & Pharmacology and Chemical Biology, Pittsburgh, USA

RNA binding motif 5 (RBM5) induces apoptosis in cancer cells. Little is known concerning its pro-death function(s) in the CNS. We reported that lentivirus mediated RBM5 knockdown (KO) decreases the activation of caspases following staurosporine (STS) injury in human neuronal SHSY5Y cells. Others have shown that RBM5 inhibition prevents caspase-3 activation, and the upregulation of pro-death p53 expression, in rat PC12 cells after being subjected to hydrogen peroxide injury. Taken together the evidence supports our hypothesis that RBM5 is toxic to CNS cells. However, RBM5 mediated neurotoxicity has not been established directly in primary neurons. Here we tested if lentivirus mediated RBM5 overexpression augments damage to primary cortical rat neurons after a mechanical stretch-injury. Cortical tissue was harvested from E16 rat embryonic brains. Cells were cultured on Silastic membrane plates (BioFlex). Day in vitro (DIV) 9 neurons were subjected to a gas-induced deformation using the cell injury controller (CIC-II) device (50 ms; 54% bilateral stretch). The following day (24 h post-injury), media was collected to assay lactate dehydrogenase (LDH) levels for quantification of cell death, and subsequently cultures were harvested for protein analysis. Mechanical stretch-injury significantly increased LDH release at 24 h post-injury compared to uninjured controls. Stretch-injured neurons overexpressing RBM5 had significantly higher LDH levels vs. empty-vector controls. In non-viral characterization studies, spectrin breakdown products (SBDPs) were also higher at 24 h post-injury in neurons subjected to stretch. Stretch induced SBDP levels were further augmented by RBM5 overexpression. Finally, an auxiliary study was done to test if RBM5 overexpression exacerbates neuronal injury by other insult mechanisms. RBM5 overexpressing neurons had significantly lower 24 h viability (measured by CellTiter-Blue®) in response to 100 nM STS. Results confirm that RBM5 promotes damage in primary neurons, and in a TBI-relevant injury model. This work was supported by NIH grant R21NS088145. Key words: stretch-injury, neuron, TBI, RNA binding motif 5.

Keywords: Mechanical stretch-injury, RNA binding motif 5 (RBM5), Lentivirus

A03-10

DECREASED EFFECTIVENESS OF THE AXON INITIAL SEGMENT IN GENERATING AN ACTION POTENTIAL AFTER MILD CENTRAL FLUID PERCUSSION INJURY

Kimberle Jacobs, Jianli Sun, Da Lee, Matthew Baer, Michal Vascak
Virginia Commonwealth University, Anatomy & Neurobiology, Richmond, USA

Recently there has been an increased recognition that structural and physiological alterations within the neocortical gray matter likely contribute to long term morbidity after mild traumatic brain injury (mTBI). We have previously shown that within 2 days after mild central fluid percussion injury, layer V neocortical pyramidal neurons (LVpyr) have abnormal intrinsic properties. Here we have refined our previous approach, with a second derivative (2ndDeriv) analysis of the action potential (AP), separating the peak current amplitude at the axon initial segment (AIS) from that at the soma. Using whole cell patch clamp, a series of depolarizing pulses were injected to produce APs. The AP was smoothed (7 point boxcar filter, repeated 3 times) prior to calculation of the 2nd Deriv. In shams, this always produced two peaks, the first corresponding to the AP generated at the AIS and the second to that initiated at the soma. At 1 and 2 days after mTBI fewer than 40% of axotomized LVpyr neurons (AX) showed the AIS peak. Surprisingly, the intact LVpyr neurons (InT) also showed a loss of the AIS peak 1 day after injury (<30% with 2 peaks). This recovered quickly, however with all InT neurons having two peaks by 2 days after injury. In addition, the somal 2ndDeriv peak was significantly decreased for all AX and for the 1day InT, perhaps reflecting a reduced density of sodium channels. When this study was repeated in Cyclophilin-D (CypD) knock-out mice, all groups had two 2ndDeriv peaks, suggesting that the AIS was capable of producing APs in these mice. The reduced somal peak was partially ameliorated in CypD knock-out mice. Together these results suggest that the AIS is functionally affected by mTBI, even for structurally intact neurons, and that mitochondria protection is important for maintenance of AIS AP firing capabilities. Supported by NIH grant NS077675. CypDKO mouse strain originally supplied by Michael Forte, Vollum Institute, OHSU.

Keywords: Axon Initial Segment, Cyclophilin-D, Axotomized, Neocortex, Intact Neurons

A04 AXONAL INJURY

A04-01

THE BURDEN OF TRAUMATICALLY INDUCED AXONAL INJURY IS NOT INCREASED FOLLOWING REPETITIVE TRAUMATIC BRAIN INJURY

Yasuaki Ogino, John Povlishock
Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

Recently, interest has increased in the damaging consequences of repetitive traumatic brain injury (rTBI). We and others have confirmed that the injury severity, together with the time between insults are important variables, with the suggestion that rTBI can exacerbate both vascular and axonal injury (AI). In assessing AI, most have relied on the visualization of amyloid precursor protein (APP), although this typically underestimates the total burden of AI. We previously reported the increased sensitivity and utility of antibodies to phosphorylated c-Jun (p-c-Jun) in detecting both acute and long term AI. Because of this, we now reevaluate the burden of AI following rTBI using the quantitative assessment of neuronal p-c-Jun. Adult mice were subjected to central fluid percussion injury (CFPI) at an intensity of 1.40 atm 2x with a 3-hour interval between injuries. Controls employed an initial CFPI followed by a sham injury 3 h later. All animals were euthanized 24 hour after second insult and were perfused with 4% paraformaldehyde. Brain regions incident to the craniotomy were sectioned at 40 μ m followed by immunofluorescent labeling for NeuN and p-c-Jun. Images of layer V in neocortical gray matter were captured using Zeiss LSM710 confocal microscope and

quantitatively analyzed using ImageJ software. Statistical analyses were performed by R software. No animal showed any evidence of contusion or hematoma formation. All animals revealed significantly longer and consistent righting reflex times after injury compared to the sham injury alone. Among the layer V pyramidal neuron population, neither NeuN+ cells nor p-c-Jun+ cells revealed significant differences in number. The proportion of those neurons revealing p-c-Jun positive immunoreactivity was approximately 4%. These analyses suggest that the rTBI did not cause a significant increase in the number of axotomized neurons nor did it impact on the recovery of righting reflex times. This unanticipated finding may be related to the low intensity injury used. Thus, this issue mandates reevaluation with repetitive injuries of increased intensity. This study was funded by NIH grant NS077675.

Keywords: fluid percussion injury, c-Jun, repetitive TBI

A04-02

OPTOGENETICALLY ENGINEERED HUMAN NEURONAL PRECURSORS FOR TRANSPLANTATION IN THE NORMAL AND INJURED RODENT BRAINS

Jiwon Ryu¹, Leyan Xu¹, Philippe Vincent², John Curtin³, Shirin Sadeghpour¹, Elisabeth Glowatzki², Vassilis Koliatsos¹

¹JHMI SOM, Neuropathology, Baltimore, USA

²JHMI SOM, Otolaryngology Head and Neck Surgery, Baltimore, USA

³JHU, Behavioral Biology, Baltimore, USA

Objectives: Optogenetically engineered human neuronal precursors (hNPs) have great potential as tools in regenerative neuroscience: their fate can be tracked in vivo by their fluorescence reporter gene (survival and differentiation) and the functionality of their differentiated neuronal progenies can be assessed as response to light stimulation or inhibition.

Methods: We transfected H9 embryonic stem cell-derived hNPs with lentivirus harboring human channelrhodopsin (hChR2). We differentiated H9 into the cortical lineage. Transfected hNPs were transplanted into the motor cortex of sham and impact acceleration (IA)-injured rodents a week after injury. Two months after transplantation, the survival and differentiation of the optogenetically engineered hNPs were analyzed by immunohistochemistry (SC121, HuNu, Tuj1 and YFP antibodies) and subsequent stereological analysis to explore degree of neuronal/synaptic maturation of the transplanted neurons and their structural integration with the host system. To affirm the fate disposition of transplanted cells in vitro, an aliquot of cells destined for transplantation was differentiated in vitro and their fate specificity and optogenetic functionality of ChR2 (+) neurons were characterized with electrophysiology and immunocytochemistry.

Results: Transfected NPs survived well in IA-injured animals. 60–65% of survived cells displayed high ChR2-YFP expression in ventricle, pia and parenchyma, and 93% at the graft site. 57–72% of HuNu (+) cells were Tuj1 (+) neurons extending axons over corpus callosum to reach remote targets (mostly piriform cortex). In vitro differentiated cells were mature enough to produce spontaneous action potentials (AP) 100 days after initiation of differentiation; AP was blocked by tetrodotoxin. Optogene positive neurons were responsive to 470 nm light and produced inward currents. We are presently investigating the optogenetic functionality of transplant-derived neurons in live animals.

Conclusions: Optogenetically engineered hNPs with cortical fate preference survive and differentiate well in brains subjected to diffuse TBI and lentivirally introduced hChR2 is functional in vitro. We propose that thus engineered cells hold great promise as tools to explore new circuit formation in the injured CNS.

Keywords: Optogenetics, TBI, Stem cells, Transplantation, Electrophysiology

A04-03

COMPLETE NERVE TRANSECTION DUE TO FRACTURE: A REPORT OF TWO CASES AND LITERATURE REVIEW

Emily Sieg, Russell Payne, Nathan Patrick, Michael Darowish, Shelly Timmons, Elias Rizk, Kimberly Harbaugh

Penn State University Milton S Hershey Medical Center, Vice Chair for Administration, Director of Neurotrauma, Hershey, USA

Introduction: Peripheral nerve trauma often affects young patients and can lead to a significant and long lasting disability and loss of productivity. Annual incidence is approximately 45 cases per 100,000 and severe peripheral nerve injury occurs in 3–5% of polytrauma cases. Case reports, case series and case control studies have looked at the incidence of complete nerve transection in the setting of fracture and the need for surgical exploration dating back to the 1920s. We present two cases of nerve laceration accompanying traumatic fracture along with a thorough review of the literature on this topic.

Methods: Search terms “sciatic nerve” OR “ulnar nerve” OR “median nerve” OR “peroneal nerve” OR “tibial nerve” OR “radial nerve” AND “laceration” OR “transection” AND “fracture.” Articles were reviewed by two study authors and were included for discussion if they specifically reported nerve laceration accompanying traumatic fracture. We also included two cases of nerve laceration from fracture that were treated at our medical center.

Results: Our initial search yielded 254 articles. Out of these, we found 32 papers that specifically reported nerve lacerations accompanying traumatic fractures. We also present two unique cases from our institution along with operative photographs. The first is a patient with a humerus fracture and complete ulnar nerve transection. The second case is a patient who suffered a femur fracture and a resultant complete transection of the sciatic nerve.

Conclusion: Nerve laceration accompanying traumatic fracture is rare. The most well-known example of this is radial nerve injury associated with humeral shaft fractures. We review the reported cases of nerve laceration in the literature and report two cases treated at our institution. Though rare, nerve laceration should be considered in the setting of traumatic fracture with neurological injury. These cases should receive early imaging and there should be a low threshold for early surgical exploration.

Keywords: Traumatic Fracture, Nerve Laceration, Nerve Transection, Nerve Injury, Nerve Palsy

A05 BALLISTIC INJURY

A05-01

LONGITUDINAL PROFILE OF GFAP, ALPHA-II-SPECTRIN, AND THEIR BREAKDOWN PRODUCTS IN RAT CSF AFTER PROBE INSERTION OR PENETRATING TBI

Kristen DeDominicis, Casandra Cartagena, Hye Hwang, Deborah Shear, Angela Boutte

Walter Reed Army Institute of Research, Silver Spring, USA

Biofluid based biomarkers hold great potential for tracking severe TBI progression. Increased CSF levels of glial fibrillary acidic protein

(GFAP), alpha-II-spectrin, and their break-down products (BDPs) have been described acutely after TBI. However, their use as subacute-chronic biomarkers is unclear. This work conducted longitudinal analyses of GFAP, alpha-II-spectrin, and their BDPs following severe TBI. Penetrating ballistic-like brain injury (PBBI) was induced by inserting a probe through the right frontal pole followed by rapid balloon inflation to create a temporary cavity. Additional rats sustained a less severe probe insertion without balloon inflation (probe) or were exposed to craniotomy alone (sham). CSF was collected 24 h, 3 d, 7 d, 1 m, or 3 m after injury and concentrated with centrifugal spin-filters. GFAP, alpha-II-spectrin, and BDPs were assessed by Western blot and/or ELISA. Based on western blots, alpha-II-spectrin and its BDPs were not elevated following probe injury but PBBI led to widespread elevation compared to sham. Full length protein (280kDa) was significantly elevated at 24 h and 3 d following PBBI. SBDP145-150 were robustly increased at 24 h-1 m. SBDP-120 was detected at 3 d. ELISAs indicated that GFAP levels were not significantly upregulated at 24 h or 3 d. At 7 d-3 m, GFAP was not detected in the majority of samples. To confirm these results, western blots were performed to determine if full length GFAP or its BDPs could be ascertained. These data indicate that GFAP-BDPs were significantly increased at 24 h following PBBI. In accordance with ELISA quantitation, GFAP and its BDP were not detectable in most samples collected at 7 d-3 m. However, nearly half of PBBI rats at 1 m and 10% of sham rats at 3 m did display the characteristic banding of GFAP-BDPs. These data indicate that elevation in injury-induced biomarkers remain detectable in CSF as late as 1 m following PBBI. Alpha-II-spectrin and its SBDP145-150, as opposed to GFAP, may have greater utility as acute-chronic CSF biomarkers.

Keywords: GFAP, alpha-II-spectrin, chronic, rat

A05-02

INITIAL OBSERVATIONS ON A BALLISTIC MODEL OF BLUNT INJURY IN THE GYRENCERPHALIC BRAIN: BEHIND HELMET BLUNT TRAUMA IN PIGS

Nikolaos Ziogas¹, James Gurganus², **Hamasa Ebad**¹, Michael Horsmon³, Karen Pizzolato-Heine², William Mermagen², Vassilis Koliatsos¹

¹Johns Hopkins University School of Medicine, Department of Pathology/Division of Neuropathology, Baltimore, USA

²Army Research Laboratory, Adelphi, USA

³Edgewood Chemical Biological Center, Gunpowder, USA

Our goal here was to explore the neuropathology of blunt TBI when high-velocity impactors strike the head. Such impactors include baseball bats or bullets when the head is helmeted. In the latter case, the helmet slows down or stops the bullet while its material elastically deforms, pushing down on the cranium at high velocity. This type of injury is called "Behind Helmet Blunt Trauma", and is the model of blunt injury used here. Yucatan miniature swine, anesthetized with Propofol, were subjected to ballistic (gunshot) trauma to the head with rifle while wearing a combat helmet. Bullet impact velocities averaged 1800 ft/s. Vital signs were monitored throughout the injury and the post-injury survival. Subjects with stable vitals were euthanized at 4 hours with transcardial perfusion with 4% neutral-buffered paraformaldehyde. If vitals decompensated to critical levels, subjects were euthanized without further delay using the same procedures. Cranial CT was performed before and after the gunshot. Crania were carefully inspected for evidence of fracture. Brain tissues were embedded in paraffin and then sectioned (10 μ m) or processed as floating sections (40 μ m). Serial sections were processed for: general anatomy using Nissl stain (cresyl violet); vascular anatomy/pathology (Masson's

trichrome); and axonal pathology (amyloid precursor protein immunohistochemistry). The initial characterization of lesions in 7 subjects is summarized as follows: In general, there appeared to be a relationship between bullet velocity, severity of cranial fractures, and vital sign outcome. In turn, there was an association between fracture and lobe "bursting", i.e. severe contusion underneath. Coup sites were associated with subarachnoid blood, and multiple superficial linear or round hemorrhagic foci more so than large contusions. There were some contrecoup contusions in olfactory sites. Subarachnoid hemorrhage was ubiquitous. In some cases, there was a more diffuse pattern of small hemorrhages over several gyri, at the white-gray matter border. Multiple fronts of classical APP (+) axonal abnormalities were seen in prefrontal white matter, medial parietal, occipital white matter, next to the roof of frontal horn, splenium, and brain stem, were remarkably similar in distributions to the ones observed with inertia acceleration. There was no obvious association between hemorrhagic foci and axonal abnormalities. These observations suggest that high-velocity blunt trauma is associated with subarachnoid bleeding and diffuse lesions, both vascular and axonal, and axonal pathologies resemble the ones seen with acceleration injuries.

Keywords: Ballistic Injury, Axonal Injury, Hemorrhage, Behind Helmet Blunt Trauma, 3D Brain Mapping

A05-03

EFFECT OF HUMAN NEURAL STEM CELL GRAFTS ON PENETRATING BALLISTIC-LIKE BRAIN INJURY (PBBI) INDUCED BEHAVIORAL DEFICITS

Zhen Hu^{1,4}, Markus Spurlock¹, Shyam Gajavelli¹, Karla Rivera¹, Anil Mahavadi¹, Thomas Hazel³, Deborah Shear², Ross Bullock¹

¹University of Miami, The Miami Project to Cure Paralysis, Miami, USA

²Brain Trauma Neuroprotection and Neurorestoration Center, WRAIR, Silver Spring, USA

³Neuralstem Inc, Neuralstem Inc, Germantown, USA

⁴Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Neurosurgery, Guangzhou, CHINA

We previously reported amelioration of PBBI-induced cognitive deficits following engraftment of NSI-566 human neural stem cells (hNSC). However, the mechanism of such amelioration is unknown. To gain insights we hypothesized that transplant induced behavior modification will depend on transplant location, extent of tissue spared, numbers of cells engrafted derived processes. To test the hypothesis, one million green fluorescent protein (GFP) expressing hNSCs transplanted one week following injury either in the core of injured tissue (intralesional) or in undamaged tissue surrounding the core (perilesional). Same dose of cells were transplanted into an uninjured sham group to uncover hNSCs effects. The animals were tested after 12 weeks (by this time hNSC have been shown to undergo neuronal differentiation) in a standard Morris Water Maze for acquisition (latency on day 5 after 4 days of training) and performance following spatial reversal (day 12). Linear regression to explore relationship between transplant location, lesion volume, cell numbers and latencies was assessed. Surprisingly there was no relationship between transplant location, extent of engraftment and latency to find platform. Similar analysis on additional parameters such as extent of GFP processes in corpus callosum and internal capsule will be presented to gain insights to the mechanism. These results are similar to those with bone marrow stem cell therapies in stroke patients.

Support: Funded by The Department of Defense W81XWH-16-2-0008, BA150111 CDMRP JPC-6.

Keywords: human neural stem cell, PBBI

DIFFERENTIAL EFFECTS OF RESUSCITATION STRATEGIES ON COAGULOPATHY FOLLOWING TRAUMATIC BRAIN INJURY AND HEMORRHAGIC SHOCK

Lai Yee Leung, Katherine Cardiff, Xiaofang Yang, Deborah Shear
Walter Reed Army Institute of Research, Brain Trauma Neuroprotection Branch, Silver Spring, USA

This study examined the changes in blood coagulation profiles in rats exposed to penetrating ballistic-like brain injury (PBBi) combined with hemorrhagic shock (HS), and compared the effect of resuscitation with Hextend, Lactated Ringer's solution (LR) or whole blood (WB) on post-injury coagulation. Rats were assigned into three groups: Hextend, LR, and WB. All animals received a frontal PBBi followed by a 35-minute of HS (mean arterial pressure <45 mmHg). In the Hextend and LR groups, resuscitation fluid was infused followed by shed blood re-infusion. In WB group, shed blood was re-infused 15 minutes after the hypotensive period. Blood samples were analyzed using thromboelastography (TEG) and blood gas analyzer at four time points: Pre-injury baseline, post-PBBi, post-HS, and after the infusion of Hextend, LR or WB. TEG parameters included Reaction Time (R), Kinetics Time (K), Angle (α) and Maximum Amplitude (MA). R was significantly reduced vs. baseline by both PBBi and HS. K was significantly shorter and α was significantly greater immediately following PBBi and HS vs. baseline. MA was significantly greater following PBBi vs. either baseline or post-HS. Hextend, LR and WB produced different coagulation profiles after PBBi and HS insults. Hextend resulted in a significantly smaller α ($p < .05$ vs. WB) and MA ($p < .05$ vs. Baseline and WB), indicating that Hextend infusion leads to a slower coagulation rate and reduced clot strength. In contrast, the parameters in LR and WB groups were comparable to the baseline values. Blood gas analysis revealed a significant reduction in hemoglobin and hematocrit following HS and PBBi, which were restored to baseline levels by WB resuscitation. On the contrary, a significant decrease ($p < .05$ vs. baseline or WB) in ctHb and Hct as well as an increased lactate level were detected following LR or Hextend resuscitation. The current results show that PBBi altered the coagulation parameters, which returned to the baseline levels after LR or WB resuscitation whereas Hextend resuscitation after PBBi/HS resulted in hypocoagulation.

Keywords: Coagulation, Resuscitation, Hemorrhagic Shock, Thromboelastography

A05-05

PENETRATING GUNSHOT HEAD INJURIES WITH PROLONGED SURVIVAL: A POTENTIAL RISK FACTOR FOR CHRONIC TRAUMATIC ENCEPHALOPATHY

Declan McGuone, Jacqueline Nunez, Rebecca D. Folkerth
Office of the Chief Medical Examiner of the City of New York, New York, USA

Post-traumatic neurodegeneration in the form of chronic traumatic encephalopathy (CTE) is associated with a history of traumatic brain injury in susceptible individuals, although the types and number of insults required to initiate neurodegeneration remain incompletely defined. Penetrating gunshot head trauma has not previously been considered a risk factor for neurodegeneration despite being a major cause of traumatic brain injury in many urban centers in the United States. Penetrating gunshot head wounds do not perforate the head and kinetic energy possessed by the projectile is entirely absorbed by the head (including brain). We hypothesized that in penetrating gunshot head

wounds with a survival interval, the crushing and stretch injury of brain tissue by the bullet and the contusion of cortical surfaces from rapid, brief expansion of skull contents, might act as substrates for aggregation of hyperphosphorylated tau. In New York City, gunshot lethality is investigated by the Office of the Chief Medical Examiner (OCME). Over a 6-month period we prospectively examined brains of all gunshot head wounds referred to the forensic neuropathology consultation service at the NYC OCME. Of these, 4 had retained bullet(s) or fragments, and a survival period after injury; these underwent full neurodegenerative disease workup including Bielschowsky silver preparation and immunohistochemistry for tau, beta-amyloid, alpha-synuclein and ubiquitin. All 4 cases were male. Age range at injury was 14 to 29 years and at death was 19 to 50 years. Survival periods ranged from 1 month to 30 years. All victims had neurologic deficits following injury and two had post-traumatic seizure disorder. Neuropathologic evaluation revealed tau deposition in 2: one with rare threads in the bank of the middle frontal gyrus (survival, 1 month), the other with rare threads around cortical vessels in the wound cavity and rare tangles in the locus ceruleus and thalamus (survival, 30 years). No other abnormal protein expression was detected. Detection of tau at autopsy suggests penetrating gunshot trauma represents an independent risk factor for CTE among injured survivors.

Keywords: Gunshot trauma, forensic pathology

A05-06

PREDICTING SURVIVAL AFTER ACUTE CIVILIAN PENETRATING BRAIN INJURIES: THE SPIN SCORE

Susanne Muehlschlegel^{1,2}, Didem Ayturk², Aditi Ahlawat¹, Saef Izzy¹, Thomas M. Scalea³, Deborah M. Stein³, Timothy Emhoff², Kevin N. Sheth⁴

¹University of Massachusetts Medical School (UMMS), Neurology (Neurocritical Care), Worcester, MA, USA

²UMMS, Surgery, Worcester, MA, USA

³R Adams Cowley Shock Trauma Center, University of Maryland, Surgery, Baltimore, MD, USA

⁴Yale University, Neurology (Neurocritical Care and Emergency Neurology) & Neurosurgery, New Haven, CT, USA

Introduction: The predominant experience of penetrating traumatic brain injury (pTBI) derives from battlefield settings, but the civilian experience in Western settings in patients treated after 2005 is limited to only small and single-center studies. As a result, outcome predictors of civilian pTBI in modern trauma and neurocritical care settings are poorly defined. The aim of this study was to identify predictors associated with survival in a contemporary, large, diverse two-center pTBI cohort, and to develop a parsimonious survival prediction score for civilian pTBI.

Methods: Our cohort comprised 415 pTBI patients retrospectively identified from the local trauma registries at two U.S. level-1 trauma centers, of which one was predominantly urban and the other predominantly rural. Predictors of in-hospital and 6-month survival identified in univariate and multivariable logistic regression were used to develop the simple Surviving Penetrating Injury to the Brain (SPIN) Score.

Results: The mean age was 33 ± 16 years, and patients were predominantly male (87%) and black (58%). Survival at hospital discharge and 6-months post pTBI was 42.4%. Motor Glasgow Coma sub-score, pupillary reactivity, self-inflicted injury, transfer from other hospital, female sex, Injury Severity Score and INR were independently associated with survival (all $p < 0.001$; area-under-the-curve 0.962). Important radiological factors associated with survival were also identified but their addition to the full multivariable would have resulted in model overfitting without much gain in the area-under-the-curve.

Conclusions: We developed the SPIN Score, a logistic regression-based risk stratification scale estimating survival after pTBI. While external validation is warranted, this clinical survival prediction tool may provide important information to guide families and physicians during intervention- and goals-of-care decision-making after civilian pTBI.

Keywords: Penetrating Brain Injury, Gun Shot Wound, Prognostication, Outcome Prediction

A05-07

TEMPORAL AND REGIONAL CHANGES IN BRAIN MITOCHONDRIAL BIOENERGETICS FOLLOWING PENETRATING BALLISTIC-LIKE BRAIN INJURY IN RATS

Jignesh Pandya, Ying Deng-Bryant, Hye Hwang, Xiaofang Yang, Lai Yee Leung, Deborah Shear

Walter Reed Army Institute of Research, BTNN, Silver Spring, USA

Mitochondria play a pivotal role in the secondary pathophysiological sequelae following traumatic brain injury (TBI) and thus are leading targets for therapeutic interventions. The current study is designed to characterize the temporal and regional post-injury responses of mitochondrial energy crisis up to 14 days following penetrating ballistic-like brain injury (PBBI) in rats. Adult male rats were subjected to either 10% unilateral PBBI or Sham craniotomy ($n=6/\text{group}$). At 30 min, 3 h, 6 h, 24 h, 3 d, 7 d and 14 d post-PBBI, mitochondria were isolated from the ipsilateral hemisphere of two brain regions: the injury core, i.e. frontal cortex + striatum (FC+ST) and a region distant from the injury core, i.e. hippocampus (HIP). Mitochondrial bioenergetics parameters were measured using a high-throughput procedure of the Seahorse Flux Analyzer. The time-course of FC+ST mitochondria showed a biphasic energy dysfunction response, indicated by a decline in ATP synthesis and maximum respiratory capacity. The first phase of energy crisis started immediately at 30 min (-42% ; $p<0.05$ vs. Sham) and attained baseline levels between 3 h to 6 h (nonsignificant vs. Sham), followed by a second phase of more robust energy crisis observed between 24 h to 14 d post-injury (-55% to -90% ; $p<0.05$ vs. Sham). In contrast, the HIP mitochondria showed a significantly delayed decline in mitochondrial bioenergetics parameters at 7 d (-74% ; $p<0.05$ vs. Sham) and 14 d (-51% ; $p<0.05$ vs. Sham) post-PBBI. Collectively, PBBI produced temporal and region-specific alterations in mitochondrial bioenergetics that are unique to the penetrating, temporary cavity mechanism. More importantly, the results underscore an extended therapeutic window (between 3 h to 24 h post-injury) for mitochondria targeted intervention following PBBI.

Support: US_Army_CCCRP_H_026_2014_WRAIR.

Keywords: Traumatic Brain Injury, Penetrating Ballistic like Brain Injury, Brain Mitochondrial Energy Metabolism, Time Course of Secondary Injury, Seahorse Flux Analyzer, Mitochondria Targeted Therapeutics

A05-08

A MOUSE MODEL OF FOCAL VASCULAR INJURY INDUCES ASTROCYTE REACTIVITY, TAU OLIGOMERS, AND ABERRANT BEHAVIOUR

Brandon Lucke-Wold, Aric Logsdon, Paul Lockman, Charles Rosen, **Rabia Kaiser**

West Virginia University, Neurosurgery, Morgantown, USA

Introduction: Neuropsychiatric symptom development has become more prevalent with 270,000 blast exposures occurring in the past 10 years in the United States. Mechanism of blast injury leading to neuropsychiatric symptomology is currently unknown. Preclinical models of blast-induced traumatic brain injury have been used to demonstrate blood-brain barrier disruption, degenerative pathophysiology and behavioural deficits. Vascular injury is one of the primary effects of neurotrauma that can trigger secondary injury cascades and neurodegeneration. Here we present data from a novel scaled and clinically relevant mouse blast model that was specifically developed to assess the vascular injury occurring after a blast and behavioural outcomes as a result of it.

Methods: We look at the biochemical effects and behavioural changes associated with blast injury in 30 young-adult male BALB/c mice. We report that blast exposure causes focal vascular injury in the Somatosensory Barrel Field cortex measured by increased Texas Red permeability across a damaged blood brain barrier at 72 hours post-blast ($n=15$ for sham and 15 for blast).

Results: This blood brain barrier disruption led to increased perivascular astrocyte reactivity ($F(2,12)=5.73$, $p<0.05$), as well as acute impulsive-like exploratory behaviour on the elevated plus maze ($t=2.61$, $p<0.05$) at 72 hours post-blast. Biochemical analysis revealed that mild blast exposure also invokes tauopathy measured by T22 oligomeric tau ($t=5.59$, $p<0.001$), neuroinflammation measured by TNF α ($F(2,12)=4.53$, $p<0.05$) and iNOS ($F(2,12)=5.60$, $p<0.05$), and oxidative stress measured by NOX4 ($t=4.07$, $p<0.001$).

Conclusion: In conclusion, we propose our model to be useful in evaluating focal blood-brain barrier disruption and its consequences after blast injury and also, to assess human neuropsychiatric symptoms.

Keywords: Ballistic injury, focal vascular injury, Astrocyte reactivity, Behavioral outcome

A05-09

DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC CAFFEINE PRE-EXPOSURE IN THE WRAIR PENETRATING BALLISTIC-LIKE BRAIN INJURY MODEL

Sarah Sanjakdar, William Flerlage, Hyunsik Kang, Alondra Flores, Jacqueline Dougherty, Douglas Napier, Deborah Shear, Andrea Mountney
Walter Reed Army Institute of Research, Brain Trauma Neuroprotection, Silver Spring, USA

Sleep disturbance is a commonly reported behavioral health diagnosis in the military, and Soldiers increasingly rely on pharmacological intervention, such as caffeine, to counteract the effects of sleep-loss. This study assessed the potential therapeutic effect of caffeine on neurobehavioral recovery in the WRAIR PBBI model. Unilateral frontal PBBI was produced in the right hemisphere of anesthetized rats at moderate (7%-PBBI) or severe (10%-PBBI) injury levels. Animals were randomly assigned to pretreatment groups: (1) acute caffeine (25mg/kg CAF) or vehicle (gavage, 1h prior to PBBI), (2) chronic caffeine (0.25g/L CAF) or water (30 days prior to PBBI). Motor function was evaluated on the rotarod (7 and 10 days post). Cognitive performance was evaluated on the Morris water maze (MWM). A single bolus dose of 25 mg/kg caffeine (p.o.) one hour prior to PBBI had no effect on motor outcome. Cognitive deficits were observed in all injury groups with average latency to find hidden platform increased by 130% (PBBI) and 140% (25mg/kg CAF) vs. sham ($p<0.05$). Rats exposed acute caffeine prior to sustaining 10% PBBI displayed a significantly higher thigmotaxic response compared to injured rats not exposed to caffeine, which

may indicate that pre-injury exposure to caffeine exacerbates post-injury anxiety/attention decrements. Results of the chronic caffeine study revealed a significant improvement in motor outcome at 7 and 10 days post-injury in the 7%-PBBI group that had received chronic, pre-injury dosing with caffeine ($p < 0.05$). However, chronic caffeine exposure prior to PBBI significantly increased the latency to locate the platform in the MWM task compared to vehicle at both 7% and 10% injury levels ($p < 0.05$), indicating that chronic pre-injury caffeine exposure may worsen cognitive outcome. Overall, the results of this study indicate that chronic caffeine consumption prior to injury may provide moderate beneficial effects to motor recovery, but may worsen the neurocognitive outcome following TBI.

Keywords: traumatic brain injury, caffeine

A05-10

PERILESIONAL NEURAL STEM CELL TRANSPLANTATION MITIGATES LESION VOLUME FOLLOWING PENETRATING BALLISTIC-LIKE BRAIN INJURY (PBBI)

Markus Spurlack¹, Shyam Gajavelli¹, Zhen Hu¹, Karla Rivera¹, Stephanie Lee¹, Thomas Hazel³, Deborah Shear², Ross Bullock¹

¹University of Miami, Neurosurgery, Miami, USA

²WRAIR, Brain Trauma Neuroprotection and Neurorestoration Center, Silver Spring, USA

³Neuralstem Inc, Neuralstem Inc, Germantown, USA

We previously confirmed engraftment of NSI566 human neural stem cells (hNSC) in PBBI. In this study, we aimed to determine the optimal transplant location relative to PBBI lesion. To do so, we transplanted one million hNSC into each animal one week following injury either in the core of injured tissue (intralesional) or in undamaged tissue immediately surrounding the core (perilesional). We also transplanted the same dose of cells into an uninjured sham group. In a final vehicle group, we transplanted only media with no cells one week following injury. After 12 weeks, we assessed the survival of the transplanted cells and the overall volume of the lesion. Although the survivability of the cells was not significantly different between intra and perilesional groups, there was greater cell survival in the injured groups compared to non injured control. This suggests that engraftment of cells was not adversely by the PBBI lesion milieu. The injury environment after one week may be conducive to transplanted stem cells in terms of survivability and/or proliferation. PBBI-injured animals that received perilesional hNSC engraftments showed a significant reduction in lesion volume compared to animals that received intralesional hNSC transplants [Uninjured + hNSC = $2.64 \pm 0.68 \text{ mm}^3$; PBBI + Vehicle = $64.35 \pm 4.48 \text{ mm}^3$; PBBI + Perilesional hNSC = $27.57 \pm 3.78 \text{ mm}^3$; PBBI + Intralesional hNSC = $55.74 \pm 4.77 \text{ mm}^3$]. The lesion volume was significantly less in the perilesional transplant group when compared to the vehicle group ($p < .05$ vs. PBBI + Vehicle). Both transplant groups had reduced lesion volume compared to vehicle group.

Keywords: human neural stem cell, PBBI

A05-11

ADULT FIREARM-RELATED TRAUMATIC BRAIN INJURY IN UNITED STATES TRAUMA CENTERS

John Yue, Hansen Deng, Ethan Winkler, Sanjay Dhall, Geoffrey Manley, Phiroz Tarapore

University of California, San Francisco, Neurological Surgery, San Francisco, USA

Firearm injuries cause an estimated 33,000 deaths annually in the United States. Management algorithms following gunshot wounds to the head (GSWH) have been static for two decades and outcomes remain in need of systematic characterization. Adult GSWH patients in the National Sample Program (NSP) of the National Trauma Data Bank (NTDB), years 2003–2012, were extracted. Multivariable regression identified predictors for hospital length of stay (HLOS), intensive care unit length of stay (ICU LOS), medical complications, mortality, and discharge disposition. Odds ratios (OR) and 95% confidence intervals (CI) were reported. Statistical significance was assessed at $p < 0.001$. In total, 8,148 patients were included extrapolating to 32,439 national incidents. Age was 36.6 ± 16.4 years. Mean HLOS was 13.9 ± 17.9 days and ICU LOS was 8.6 ± 17.4 days. The mortality was 54.6%. Age, injury intent, Glasgow Coma Scale (GCS) score, Injury Severity Score (ISS), and hypotension were significant predictors as previously reported ($p < 0.001$). Compared to privately insured patients, Medicare/Medicaid patients had longer HLOS (mean increase 4.4 days, 95% CI [2.6–6.3 days], $p < 0.001$). Compared to the Midwest, the South had longer HLOS (mean increase 3.7 days [2.0–5.4 days], $p < 0.001$) and more complications (OR 1.7 [1.4–2.0], $p < 0.001$); the West had less complications (OR 0.6 [0.5–0.7], $p < 0.001$). Compared to handgun, injury from shotgun and hunting rifle had lower odds of death (OR 0.3 [0.2–0.4], $p < 0.001$; OR 0.5 [0.4–0.8], $p < 0.001$). Suicide had lower odds of discharge to home compared to accident (OR 0.5 [0.3–0.7], $p < 0.001$). Relative to Level I trauma centers, Level II trauma centers had lower odds of discharge to home (OR 0.7 [0.5–0.8], $p < 0.001$). In conclusion, firearm type, injury intent, insurance type, and geographic region are important prognosticators for acute recovery and outcome following GSWH, in addition to older age, hypotension, and injury severity. Improved understanding of civilian GSWH is critical to increasing awareness and reducing injury burden from firearm injuries.

Keywords: firearm injury, mortality, penetrating brain injury, traumatic brain injury, epidemiology

A06 BIOMARKER

A06-01

A QUANTITATIVE ASSESSMENT OF MICRORNA BIOMARKERS FOR ACUTE AND SUB-ACUTE TRAUMATIC BRAIN INJURY USING DROPLET DIGITAL PCR

Manish Bhomia¹, Manoshi Gayen¹, Zhao Zhang Li¹, Kevin Wang², Barbara Knollmann-Ritschel¹

¹Uniformed Services University of the Health Sciences, Pathology, Bethesda, USA

²University of Florida, Psychiatry, Gainesville, USA

Traumatic brain injury (TBI) is considered as one of the major causes of disability and death. Currently, there is no FDA approved diagnostic test for diagnosis of TBI. The usefulness of Glasgow coma scale (GCS) and imaging (CT and MRI scans) is limited in the diagnosis of mild TBI. MicroRNAs (miRNAs) are small non-coding RNA molecules which regulate gene expression and have been reported as biomarkers of several diseases due to their stability and ease of detection. We have previously reported a panel of 10 miRNAs as potential acute TBI biomarkers using real-time PCR. In this study, we have used a droplet digital PCR (ddPCR) platform to quantitate the absolute miRNA concentration in a larger cohort of acute and sub-acute TBI cases. Human severe TBI serum and cerebrospinal fluid samples were collected from the patients at 48 hr

and 192 hr post injury. Total miRNA from injury and healthy controls groups was isolated and reverse transcription was performed using primers for the following: miR-195, miR-20a, miR-30d, miR-328, miR-451, miR-362-3p, miR-486, miR-505* and miR-92a. MiRNAs were quantitated (copies / μ l) in serum from severe TBI cases (n=30) at 48 hr post injury and 192 hr post injury and compared to the healthy controls. Data from ddPCR showed significantly higher miRNA concentration of all miRNAs in the 48 hr serum samples in comparison to the healthy controls and their concentrations remained elevated until 192 hr post injury. The results of this study showed that this panel of miRNA biomarkers is promising in detecting acute and sub-acute TBI. Further, ddPCR platform enabled a precise absolute miRNA expression in comparison to real-time PCR.

The opinions expressed herein are those of authors and are not necessarily representative of those of the Uniformed Services University, Department of Defense or, the United States Army, Navy, or Air Force and DMRDP.

Keywords: Biomarker, traumatic brain injury, microRNAs, diagnostics

A06-02

SERUM MIRNA SIGNATURES OF FOCAL OR DIFFUSE TBI

Debbie Boone¹, Harris Weisz¹, Harvey Levin², Claudia Robertson³, Heidi Spratt¹, Douglas DeWitt¹, Donald Prough¹, Helen Hellmich¹

¹University of Texas Medical Branch, Anesthesiology, Galveston, USA

²Baylor College of Medicine, Physical Medicine and Rehabilitation, Houston, USA

³Baylor College of Medicine, Neurosurgery, Houston, USA

Sensitive and accurate assessment of genomic changes plays a major role in personalized medicine. We hypothesized that two broad types of TBI, focal or diffuse injury, can be defined and distinguished using unique signatures of circulating miRNAs. Distinct combinations of circulating miRNAs have been shown to serve as diagnostic and prognostic biomarkers in other neurodegenerative disorders. As a first step in correlating neuroimaging data with serum miRNA levels, CT scan data were used to distinguish two groups of TBI patients (focal or diffuse injury). The RNA isolated from TBI patient serum was assessed for integrity, reverse transcribed, pre-amplified, and run on the Human Serum and Plasma miScript PCR array panels; these panels consist of 84 well characterized miRNAs expressed in biofluids. Global mean analysis was performed in Global Gene software to determine the average Δ CT value for each miRNA, and fold changes were calculated by comparing each TBI sample to controls. One-way ANOVA was used to compare control, focal, and diffuse injury groups, and was followed by the Benjamin-Hochberg test and Tukey's test for multiple comparisons. PCR array data were validated using Droplet Digital PCR analysis of differentially expressed miRNAs. Preliminary analysis shows several miRNAs are differently expressed in human serum samples between control and injury groups, and one differently expressed miRNA distinguishes between focal and diffuse injury. Ingenuity pathway analysis suggests these miRNAs regulate multiple pro-death and pro-survival cell signaling pathways affected by TBI. The development of serum miRNA signatures as prediction tools will help us determine if changes in a small, discrete panel of miRNAs could serve as a surrogate of changes in miRNA expression in different brain areas of TBI patients diagnosed with focal or diffuse brain injury.

Keywords: Brain injury, microRNA expression, Biomarker, Human serum

A06-03

RAT BRAIN CATHEPSIN B PROTEIN LEVELS AND CYSTEINE PROTEASE ACTIVITY AFTER TRAUMATIC BRAIN INJURY

Angela Boutte¹, Brittany Abbatiello¹, Barbie-Ann Stephen¹, Shonnette Grant¹, Gregory Hook², Vivian Hook^{2,3}, Steve Jacobsen², Janice Gilsdorf¹, Deborah Shear¹

¹Walter Reed Army Institute of Research, Brain Trauma Neuroprotection Branch, Silver Spring, USA

²American Life Science Pharmaceuticals, Inc., La Jolla, USA

³University of California, San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences, Dept. of Neurosciences, La Jolla, USA

Cathepsin B is a lysosomal cysteine protease that may be an important therapeutic target and biomarker for traumatic brain injury (TBI). The current study was designed to evaluate the regional and temporal profiles of mature cathepsin B (mCatB) protein levels and cysteine protease activity in the penetrating ballistic-like brain injury (PBBI) model. Rat ipsilateral frontal cortex (FC), hippocampus (HC), and cerebellum (CB) were collected 1–7 d after PBBI or craniotomy alone, compared to unoperated sham controls (baseline for fold change comparison). mCatB increased after 7 d after craniotomy (2.3-fold) and PBBI (11.9-fold) in the FC. HC levels of mCatB were elevated at 3 d (1.6-fold) and 7 d (2.5-fold) after PBBI. CB mCatB was suppressed 3 d after craniotomy (0.37-fold) and 1–3 d after PBBI (range: 0.43–0.55-fold) vs. sham. However, at 7 d post-injury CB mCatB levels in PBBI animals exceeded that of sham (1.2-fold). Craniotomy did not have a significant effect on cysteine protease activity in the FC. PBBI resulted in increased FC cysteine protease activity at 3 d (3.4-fold) and 7 d (2.13-fold) post-injury. In the HC, activity increased 7 d after craniotomy (1.8-fold). Non-linear dysregulation of mCatB protein levels infer that proteolytic processes within proximal and distal brain regions vary with injury severity and time over the course of TBI progression. Differential increases in cysteine protease activity suggest that TBI elicits a complex proteolytic response, inclusive of cathepsin B, thereby incentivizing the need for spatio-temporally defined therapeutic strategies.

Keywords: Cathepsin B, Cysteine Proteases, Craniotomy, Penetrating Ballistic-like Brain Injury, Spatiotemporal Resolution

A06-04

HYPERPHOSPHORYLATED TAU AS A BIOMARKER OF TRAUMATIC AXONAL INJURY IN THE SPINAL CORD

Mitchell Caprelli^{1,2}, Andrea Mothe², Charles Tator^{1,2,3}

¹University of Toronto, Institute of Medical Science, Toronto, Canada

²Toronto Western Hospital, Genetics and Development, Toronto, Canada

³Toronto Western Hospital, Division of Neurosurgery, Toronto, Canada

Clinical trials within the field of traumatic spinal cord injury (SCI) have been largely unsuccessful due to the lack of reliable and consistently accurate measures of patient injury severity. Specifically, the field of SCI lacks biofluid-based biomarkers capable of objectively assessing the severity of a patient's injury. Tau, a microtubule-associated protein, is primarily located within the axonal compartment of central nervous system (CNS) axons. Following traumatic brain injury, tau becomes hyperphosphorylated, and research in this field has proven the utility of hyperphosphorylated tau (p-tau) as a histological and serological biomarker of axonal injury; however, no study

to date has examined the utility of p-tau as a biomarker post-SCI. Therefore, for the current study, we utilized the clip-compression SCI rat model as well as a full spinal cord transection to assess the histological profile of tau hyperphosphorylation; sham-controls consisted of a laminectomy-only procedure. Tau hyperphosphorylation was assessed using antibodies specific for tau when phosphorylated at the serine 396 and threonine 205 amino acid positions; antibody specificity was assessed by using a P301L transgenic Alzheimer's disease mouse and tau knockout mouse. Current results demonstrate that tau becomes hyperphosphorylated within injured white matter axons as early as 4 hours post-clip-compression SCI and persists as late as 7 days post-injury, to a much lesser extent, indicated by significantly reduced p-tau white matter axonal immunoreactivity. Additionally, p-tau white matter axonal immunoreactivity was demonstrated in animals subjected to a full spinal cord transection. Sham-controls displayed no p-tau axonal immunoreactivity. Therefore, similarly to traumatic brain injury, the tau protein becomes hyperphosphorylated within injured spinal cord axons, and antibodies specific for phosphorylated tau amino acid residues are capable of histologically assessing the extent of axonal injury post-SCI. Further experiments are currently being conducted to assess the serological profile of p-tau in animals subjected to both a clip-compression and full transection SCI.

Keywords: Tau, Spinal Cord Injury, Phosphorylation, Biomarker

A06-05

IDENTIFICATION OF AMINO ACID PANEL AS A BIOMARKER FOR TRAUMATIC BRAIN INJURY

Marzieh Hajiaghamemar¹, Todd Kilbaugh², Susan Margulies¹

¹University of Pennsylvania, Bioengineering, Philadelphia, USA

²The Children's Hospital of Philadelphia, Philadelphia, USA

We investigated the plasma concentration of amino acids (AA) as the potential biomarkers of focal and diffuse traumatic brain injury (TBI). Twenty-five female, 4-week-old piglets received a diffuse TBI via rapid non-impact head rotation (RNR, n=13) or a focal TBI via controlled cortical impact (CCI, n=12), and blood samples were obtained pre-injury, and either at 24-hours or 4-days post-TBI. The concentrations of seventeen AAs were determined via HPLC (Agilent 1260 Infinity). Only Glycine, Serine, Taurine and Alanine concentrations were significantly decreased at 24-hours post-injury compared with pre-injury (Mann-Whitney test, $P < 0.05$). Glycine levels continued to remain depressed significantly at 4-days post-TBI while Serine, Taurine and Alanine returned to pre-injury levels at 4-days. In addition, we observed significant increases in Arginine and Lysine levels and significant decrease in Isoleucine at 4-days post-TBI compared with pre-injury. To find a robust TBI biomarker while taking into consideration possible interactions between AA levels, we combined RNR and CCI injuries and used multivariate logistic regression analysis, coupled with the best-subset selection technique and k-fold cross-validation method, to perform a thorough search of all possible subsets of AAs, and evaluated the ability of the subset to correctly identify serum from injured or uninjured animals. Our goal was to determine the optimal multivariate AA biomarker model with the highest TBI prediction capability, assessed using the receiver operating characteristics curve analysis. Selection criteria, such as Akaike's and Bayesian information criteria, adjusted R-squared, and maximum log-likelihood, were used to determine the best subset of AAs for groups of 1-to-17 AAs. Instead of any single AA, the logistic regression combining Glycine, Taurine, and Ornithine was optimal for TBI diagnosis, with 80% sensitivity and 86% overall prediction rate. This optimal model was validated on a separate dataset (4 uninjured

and 5 RNR piglets), yielding excellent TBI diagnostic performance, with 100% sensitivity and 78% overall prediction rate. We propose a new multivariate AA serum biomarker to detect mild-to-moderate. Funded by NINDS U01NS069545.

Keywords: TBI Biomarker, Amino Acids, Multivariate Analysis, TBI Diagnosis, Diffuse and Focal TBI

A06-06

HOW WELL CAN A BLOOD TEST PREDICT A POSITIVE HEAD CT IN TRAUMA PATIENTS?

Margaret Mahan², **Molly Hubbard**^{1,2}, Michelle Chrastek², Samuel Daly², Vikalpa Dammavalam², Drake Burri², Maxwell Thorpe², Kristina Techar², Anne Kleeberger², Erik Warren², David Balser², Abdullah Bin Zahid², Chad Richardson², Walter Galicich^{1,2}, Thomas Bergman^{1,2}, David Tupper², Saul Datwyler³, Justin Rogers³, Jaime Marino³, Beth McQuiston³, Uzma Samadani^{1,2}

¹University of Minnesota, Neurosurgery, Minneapolis, USA

²Hennepin County Medical Center, Minneapolis, USA

³Abbott Laboratories, Abbott Park, USA

The objective of this study was to evaluate blood levels of biomarkers such as GFAP (glial fibrillary acidic protein) and UCH-L1 (ubiquitin carboxyl-terminal hydrolase L1 enzyme) in predicting computed tomography (CT)-positive TBI. As part of preliminary analyses from early enrollees in the CLASSIFY TBI trial, blood samples were collected from 78 trauma subjects within 6 hours of injury who underwent a head CT scan. Biomarker concentrations of subjects with positive (Marshall classification ≥ 2 ; n=34) or negative (Marshall classification=1; n=44) CT scans were compared. In addition, biomarker concentrations of subjects in the trauma group were compared with 35 nontrauma control subjects. Median levels of UCH-L1 for control versus trauma groups were 0.074 and 0.780 ng/mL. Corresponding median levels of GFAP were 0.009 and 0.109 ng/mL. Blood concentrations of both biomarkers were significantly different between the groups (both $P < 0.001$). In trauma subjects, GFAP ($P < 0.001$) and UCH-L1 ($P = 0.008$) were higher in subjects with positive CT scans compared to those with negative CT scans. These findings indicate that concentrations of UCH-L1 and GFAP are increased in patients with TBI. Furthermore, these preliminary data suggest higher levels of GFAP and UCH-L1 predict a positive CT scan with high sensitivity, suggesting that these biomarkers may have clinical utility in the management of brain injury.

Keywords: Positive Head CT, Biomarker, Traumatic brain injury

A06-07

NEUROPHYSIOLOGICAL BIOMARKERS OF TRAUMATIC BRAIN INJURY ENTAILING TO POST-TRAUMATIC EPILEPSY - UPCOMING TWO YEARS FOLLOW-UP STUDY

Samuli Kemppainen, Jarkko Iivarinen

Kainuu Social and Health Care Joint Authority, Department of radiology, Kajaani, Finland

Background: Epilepsy is a life-long neurologic disorder with adverse effects on every-day life and working life. Only symptomatic treatments to control epilepsy are available while curative treatments to prevent the development of epilepsy (epileptogenesis) are lacking. Traumatic brain injury (TBI) is one of the main causes inducing symptomatic epilepsy *i.e.* post-traumatic epilepsy (PTE; 2 to 53 % of cases) forming a group large

enough with high epilepsy incidence for clinical treatment trials. However, sensitive and specific tools for diagnosis and prognosis of TBI and epileptogenesis are lacking forming a major obstacle for overall efficiency and cost effectiveness to enroll study patients.

Hypothesis: We hypothesize that PTE is preceded by changes of electroencephalogram (EEG) since epilepsy is, by definition, seizure disorder driven by uncontrolled synchronous electrical activity of the brain.

Study subjects and methods: Newly diagnosed adult TBI patients are recruited for 24 hour ambulatory EEG recording subacutely (3-4 months) and chronically (2 years) after TBI. The study population consists of severe, moderate and mild TBI subpopulations in order to recognize the whole spectrum of TBI parameters. Healthy subjects and newly diagnosed hemispheric ischaemic stroke patients serve as control groups. Clinical trials featuring full-band high density EEG with a 2 years follow-up period will be conducted. Short- and long-term EEG signals will be analyzed in order to find potential TBI and/or PTE determinants and biomarkers. Furthermore, computational frequency and coherence analysis methods are utilized to recognize atypical EEG patterns indicating TBI or epileptogenesis.

Expected results: Potentially, the study provides EEG determinants leading to validated and clinically relevant biomarkers to recognize patients with at high risk of epileptogenesis. Furthermore, the study may initiate procedures to develop diagnostic biomarker platforms to recognize as well as to predict and to follow therapy response in TBI patients.

Preliminary results: computational methods utilized have been able to recognize EEG changes induced by different external stimuli such as flash light stimulation.

Keywords: electroencephalogram, neurophysiology, frequency, coherence

A06-08

CIRCULATING GFAP LEVELS TO MONITOR THERAPEUTIC RESPONSE TO GLIBENCLAMIDE IN CONTROLLED CORTICAL IMPACT: FINDINGS FROM OBTT

Stefania Mondello¹, Deborah Shear², Helen Bramlett³, C. Edward Dixon⁴, W. Dalton Dietrich³, Kevin Wang⁶, Ronald Hayes⁷, Audrey Lafrenaye⁸, John T. Povlishock⁸, Frank Tortella², Patrick M. Kochanek^{4,5}

¹Messina University, Messina, Italy

²WRAIR, Silver Spring, US

³Miami University, Miami, US

⁴Pittsburgh University, Pittsburgh, US

⁵Safar Center for Resuscitation Research, Pittsburgh, US

⁶Florida University, Gainesville, US

⁷Banyan Biomarkers, Alachua, US

⁸VCU, Richmond, US

Glibenclamide (GLI) is a sulfonylurea receptor antagonist that has shown promise as a therapy to prevent secondary injury following traumatic brain injury (TBI). The present work, part of the Operation Brain Trauma Therapy (OBTT) multi-center pre-clinical drug screening consortium, investigated the effects of GLI treatment on the levels of circulating glial fibrillary acidic protein (GFAP) and the relationships with histopathological and behavioral outcomes after controlled cortical impact (CCI). OBTT demonstrated that GLI treatment reduced contusion volume in CCI; thus, we sought to determine whether circulating GFAP in the initial 24h could inform theranostically on lesion volume at 21 d post-injury. Adult male rats subjected to CCI received a bolus (10µg/kg IP) 15 min after-injury, followed by a continuous SQ infusion (0.2µg/h) via osmotic pumps

throughout 7 d of GLI or vehicle. GFAP levels in blood were measured at 1, 4 and 24h after CCI. Vehicle-treated rats displayed distinct temporal profiles for GFAP vs. GLI-treated rats. GFAP in vehicle-treated animals demonstrated a sustained increase after-injury vs. shams ($p < 0.0001$) peaking at 4h. Conversely, rats treated with GLI initially had high GFAP levels similar to vehicle. However, by 24h GFAP in GLI-treated rats did not differ from sham and were lower ($p < 0.05$) than vehicle. Overall, GLI-treated rats had significantly lower GFAP release (area under the curve) throughout the study vs. vehicle. GFAP at 24h also strongly correlated with contusion volume at 21d ($r = 0.88$, $p < 0.0001$), hemispheric tissue loss ($r = 0.86$, $p < 0.0001$) and MWM latency ($r = 0.68$, $p = 0.0001$). Our findings support a role for GFAP in predicting tissue damage and as a marker of therapeutic response corroborating advantageous effects of GLI in CCI. Circulating GFAP may be useful for high-throughput screening of drugs in pre-clinical investigations. Support: USArmyW81XWH-10-1-0623.

Keywords: Biomarkers, GFAP, TBI, controlled cortical impact, rat, neuroprotection

A06-09

INCREASED MIR-124 CARGO IN CIRCULATING EXTRACELLULAR VESICLES AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Asla Pitkanen¹, Vicente Navarro¹, Mette Heiskanen¹, Niina Vuokila¹, Noora Puhakka¹, David Poulsen², Jenni Karttunen¹

¹Univ. of Eastern Finland, Neurobiology, Kuopio Fin, Finland

²Univ. of Buffalo, the State University of New York, School of Medicine and Biomedical Sciences, Buffalo, USA

Introduction: Post-TBI secondary brain pathologies progress for weeks to months. Extracellular vesicles (EVs) have recently been recognized as mediators of intercellular communication. However, little is known about their contribution to the evolution of post-TBI secondary damage of recovery. We assessed the characteristics of plasma EVs and their contents of brain-enriched miR-124-p3 during the 1st week post-TBI. We also tested whether EV miR-124-3p levels would serve as biomarkers for TBI diagnosis.

Methods: Adult male rats were subjected to lateral fluid-percussion injury. Trunk plasma was collected at 2 or 7 d post-TBI. Naïve and sham-operated animals served as controls. EVs were isolated from plasma using commercial kit based on membrane particle precipitation. The purification method was evaluated using nanoparticle tracking analysis (NTA), scanning electron microscopy, and western blot. The number and size distribution of plasma EVs after TBI were measured with NTA. miR-124-3p concentration was measured from isolated EV-RNA with quantitative PCR. Gene set enrichment analysis (GSEA) was conducted for three EV related gene sets using available mRNA-seq (3 month post-TBI) and microarray (32h post-TBI) data from brain tissue as rank lists.

Results: NTA showed a decrease in the number of plasma EVs at 2 d and 7 d post-TBI. GSEA revealed transcriptomic-level enrichment of gene sets related to EVs, especially in the perilesional cortex. The level of plasma EV miR-124-3p concentration was increased 2 d post-TBI as compared to controls or 7 d post-TBI samples. Receiver operating characteristics analysis indicated that plasma EV miR-124 level differentiated TBI animals from controls (AUC 0.922, $p < 0.05$).

Conclusions: Our data demonstrate dynamic changes in the number of plasma EVs, regulation of genes related to EV production in the brain, and regulation of plasma EV contents of brain-enriched miR-124-3p during the 1st week post-TBI.

Keywords: Exosome, microRNA, Plasma

CSF BIOMARKER LEVELS OF A β 40 AND TAU/A β 42 CORRESPOND TO NEUROPSYCHOLOGICAL OUTCOME IN CHRONIC TBI PARTICIPANTS

Ava Puccio, Milos Ikonovic, Sue Beers, Kathryn Edelman, Steven Benso, Yuefang Chang, Walter Schneider, James Mountz, David Okonkwo

University of Pittsburgh, Neurosurgery, Pittsburgh, USA

Objectives: Traumatic brain injury (TBI) involves axonal injury and accumulation of pathological protein aggregates including amyloid- β (A β) and hyperphosphorylated tau (p-tau). Biomarker analysis of tau and A β concentrations in cerebrospinal fluid (CSF) may be an objective marker of cognitive status after TBI. The goal of the current study was to analyze tau and A β 40–42 in a cohort of military and civilian participants with chronic deficits secondary to TBI, and correlate neuropsychological outcome data with concentrations of tau and A β 42 measured in CSF from the same subjects.

Methods: 19 chronic TBI participants (> 6 months from injury; 16 males, mean age 41yrs, 8 military veterans and 11 civilians) underwent lumbar puncture as well as neuropsychological testing. CSF was analyzed for concentrations of total tau, A β 1–42 (A β 42) and A β 1–40 (A β 40) by ELISA, and tau/A β 42 ratio was calculated. The neuropsychological test battery included measures of memory, processing speed and executive function: California Verbal Learning Test-II (CVLT) Short and Long Delay Free Recall (SDFR, LDFR), Wechsler Adult Intelligence Scale Working Memory Index (WAIS IV) and Trail Making Test Part A/B. Nonparametric correlation (Spearman rho, ρ) was used to relate CSF levels to neuropsychological data, controlling for age.

Results: CSF tau/A β 42 ratio was inversely associated with Trails B (Spearman $p > -0.49$, $p < 0.047$). CSF A β 40 concentration was inversely correlated with CVLT SDFR and LDFR (Spearman $p > -0.51$, $p < 0.032$; $p > -0.50$, $p < 0.034$, respectively). There were no significant correlations between CSF biomarker levels and WAIS neuropsychological measures.

Conclusions: In chronic TBI, neuropsychological outcome on measures of memory and executive function (CVLT and Trails B) corresponded to CSF biomarkers of tau and A β concentrations. Additional studies with a larger cohort of TBI participants are needed to draw meaningful conclusions. The use of CSF biomarkers in ongoing studies will allow us to test more specific hypotheses regarding the link between TBI and chronic neurodegenerative conditions such as chronic traumatic encephalopathy.

Keywords: chronic TBI, amyloid B, tau, neuropsychological outcome

A06-11

SGP130 MODERATES THE RELATIONSHIP BETWEEN CHRONIC IL-6/SIL-6R COMPLEX IN DIFFERENTIATING OUTCOME AFTER SEVERE TBI

Prerna Ranganathan¹, Raj Kumar¹, Amy Wagner^{1,2}

¹*University of Pittsburgh, Department of Physical Medicine and Rehabilitation, Pittsburgh, USA*

²*University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA*

The secondary injury response after traumatic brain injury (TBI) is characterized by an acute innate immune response. While research has focused on acute neuroinflammatory markers, our previous work suggests sub-acute and chronic peripheral inflammatory markers influences long-term global outcomes. Specifically, pro-inflammatory mediators,

including interleukin 6 (IL-6), 2 weeks to 3 months post-injury, were associated with worse global outcomes at 6 and 12 months. Further, the relationship between IL-6 and its soluble receptor, sIL-6R, facilitates a signaling cascade predisposing individuals to a chronic inflammatory state; in contrast soluble gp130 (sgp130), a potent IL-6 inhibitory transmembrane protein, moderates this relationship. To date, no clinical or experimental TBI study has examined the association between IL-6/sIL-6R and sgp130. The objective of this study was to evaluate relationships between serum IL-6, sIL-6R, and sgp130 in the subacute period post-injury for N=100 individuals with severe TBI. Monthly ratios were produced for IL-6: sIL-6R and sgp130:sIL-6R, and IL-6 levels were quartiled using levels from samples collected up to 3 months post-injury. Six-month GOS scores were dichotomized to reflect poor (GOS=2/3) vs. good (GOS=4/5) outcome. Bivariate analysis showed significant differences in sIL-6R by GOS group ($p < 0.0001$), where higher sIL-6R levels were associated with poor outcome. sgp130:sIL-6R ratios also significantly differed by GOS group ($p = 0.0034$), where lower ratios were associated with poor outcome. A multivariate logistic regression model including age, IL-6, sgp130:sIL-6R, and a sgp130:sIL-6R*IL-6 resulted in a significant interaction (OR=5.527, $p = 0.0047$) in predicting 6-month outcome. The interaction suggests sgp130:sIL-6R ratios influence global outcome by attenuating the IL-6/sIL-6R complex, resulting in higher IL-6 levels. These results suggest the preferential binding of sgp130 to sIL-6R selectively blocks progression of inflammation through the inhibition of IL-6 signaling by the sIL-6R after severe TBI. This work has novel implications for understanding how sgp130 potentially serves as a modifiable target for prevention and/or resolution of chronic inflammation post-TBI. Support: DoD-W81XWH-071-0701; NIDILRR-90DP0041; R49-CCR323155.

Keywords: chronic inflammation, traumatic brain injury, innate immunity, interleukin 6, sgp130

A06-12

PHARMACOLOGICAL MRI TO PROBE NMDA-MEDIATED CIRCUIT CHANGES IN THE IMMATURE RAT AFTER FPI

Saman Sargolzaei¹, Yan Cai¹, Naomi Santa Maria², Christopher C. Giza¹, Neil G Harris¹

¹*UCLA, Neurosurgery, Los Angeles, USA*

²*Caltech, Pasadena, USA*

Hippocampal lateral fluid percussion injury (LFPI) has been previously correlated with memory and recognition deficits in developing rats and dysfunction of glutamatergic neurotransmission via the N-methyl-D-aspartate receptor (NMDAR), is a plausible factor. Protein expression studies have confirmed a downregulation of NMDAR protein after experimental traumatic brain injury (TBI). To study the dynamics of NMDARs in vivo, and their role in memory-related network dysfunction after developmental TBI, we acquired pilot pharmacological magnetic resonance imaging (phMRI) data at day 4 following injury (n=6) or sham (n=4) of post-natal day-19 rats. This included a 5 minute baseline Cerebral Blood Volume (CBV) enhanced imaging (7T Bruker spectrometer using a single-shot, gradient-echo sequence, echo/repetition time: 20/1000ms, 300 repetitions, 128×128 matrix, 30×30mm field-of-view and 1mm slice-thickness) followed by systemic injection of 30 mg/kg DCS/ Saline in a 1×4 experimental design. Image acquisition continued 15 minutes post-injection. After typical preprocessing of time-series data and standard space registration, Region of Interest (ROI) based rCBV response analysis (pre vs. post drug challenge) was then performed for five brain regions identified in a prior study as the memory and recognition circuit, Prefrontal Cortex (PFC); Hippocampus (Hip); Thalamus (Tha); Perirhinal (PRh) and Entorhinal (Ent) cortex. No significant changes in regional CBV signal were observed

in control animals following the saline injection. Regional CBV percent signal changes showed DCS-modulated network activation (PFC: +2.29; Ent: +2.64; Hip: +3.46; Prh: +3.05; Tha: +2.99) in sham control rats. Injured brain responses to the DCS challenge were in agreement with our hypothesis of injury induced hippocampal neural network irregularities (PFC: +0.85; Ent: +0.20; Hip: 0.90; Prh: +1.12; Tha: +1.34). Perceived NMDAR-modulated responses were then fed into a multivariate Granger causality analysis which also confirmed the existence of significant ($p < 0.01$) changes in the DCS-mediated hippocampal neural network between injury and sham.

Funding: R01NS27544, R01NS091222, UCLA Easton Labs for Brain Injury, UCLA Steve Tisch BrainSPORT program, UCLA BIRC.

Keywords: Pharmacological MRI, Developmental TBI, NMDA receptor, D-Cycloserine, Bain Connectivity Network

A06-13

ASSOCIATION OF RELEASED TISSUE FACTOR WITH ELEVATED D-DIMER AS A SERUM BIOMARKER OF TRAUMATIC BRAIN INJURY

Eiichi Suehiro¹, Yuichi Fujiyama¹, Miwa Kiyohira¹, Yukari Motoki², Junzo Nojima², Michiyasu Suzuki¹

¹*Yamaguchi University Graduate School of Medicine, Department of Neurosurgery, Ube, Japan*

²*Yamaguchi University Graduate School of Medicine, Faculty of Health Science, Department of Laboratory Science, Ube, Japan*

Objective: Recently, D-dimer blood levels were reported as a biomarker for the outcome of traumatic brain injury (TBI) patients. However, the mechanisms that trigger elevated D-dimer blood levels in TBI remain unclear. The purpose of this study was to evaluate the reliability of D-dimer as a biomarker of TBI and to determine the mechanisms involved in regulating its blood levels.

Methods: Nine patients with moderate to severe (Glasgow Coma Scale score 3-13) isolated TBI were admitted and evaluated at our hospital between May 2013 and June 2014. We collected blood samples from systemic arteries on arrival in the emergency room and at 1, 3, 5, 7, and 14 days after injury. The plasma levels of neuron specific enolase (NSE), D-dimer, and soluble tissue factor were measured.

Results: The plasma levels of NSE (33.4 ng/ml: normal value less than 12.0 ng/ml) and D-dimer (56.1 μ g/ml: normal value less than 1.0 μ g/ml) were elevated on admittance and declined but were still elevated on Day 1 after injury. A significant correlation between NSE and D-dimer was seen on admittance ($R = 0.727$, $p = 0.026$) and on the following days ($R = 0.694$, $p < 0.001$). Furthermore, a significant correlation between soluble tissue factor and D-dimer was seen on admittance ($R = 0.803$, $p = 0.009$).

Conclusion: The level of blood D-dimer accurately reflected the degree of brain damage indicated by NSE levels. Our data suggest that the release of tissue factor induced by brain damage may activate the coagulation cascade leading to elevation in D-dimer levels.

Keywords: traumatic brain injury, coagulopathy, biomarker

A06-14

ASTROGLIAL CELL WOUNDING BIOMARKER ALDOLASE C IS ROBUSTLY ELEVATED IN MILD TBI PATIENTS

Timothy Van Meter¹, Julia Halford², NaZanin Mirshahi¹, Albert Chong², Gerry Shaw³, Ronel Veksler⁴, Stefania Mondello⁵, Tim Meier⁶, Andrew Mayer⁷, Alon Friedman⁴, Frederick Korley⁸, Ina Wanner²

¹*ImmunArray, Program of Neurological Diseases, Richmond, VA, USA*

²*UCLA, Semel Institute, Los Angeles, CA, USA*

³*Encor Biotechnology, Gainesville, FL, USA*

⁴*Dalhousie University, Dept Medical Neuroscience, Halifax, Nova Scotia, Canada*

⁵*Messina University, Dept Biomedical Science, Messina, Italy*

⁶*Medical College of Wisconsin, Dept Neurosurgery, Milwaukee, WI, USA*

⁷*Mind Research Network, LRRRI, Albuquerque, NM, USA*

⁸*University of Michigan Medical School, Dept Emergency Medicine, Ann Arbor, MI, USA*

Diagnostic monitoring of mild traumatic brain injury (mTBI) requires robust biomarkers for precise, sensitive, objective assessment. We recently identified Aldolase C (ALDOC) as a protein released from acutely traumatized astrocytes *in vitro* (Levine et al., 2016). GFAP release and proteolysis was associated with delayed cell death, dependent on injury severity (Halford et al., accepted). The current study examined ALDOC and GFAP levels in clinical samples after TBI. Healthy controls, mTBI, severe TBI and athletic cohorts were studied in replicate electrochemiluminescence-based ELISAs and compared with detectable protein bands in standardized immunoblotting assays. Rank Sum, permutation and paired T-tests were used to measure significant differences between cohorts. Studies demonstrated the C-isoform specificity of the antibodies and showed CNS-specific expression. Both assays confirmed ALDOC elevation after TBI compared to healthy controls (all reported comparisons were significant at $\alpha = 0.05$). A cohort of Italian mTBI patients showed serum ALDOC elevation irrespective of CT-status, while GFAP levels rose only in CT+ mTBI. In HeadSMART mTBI patients, ALDOC was significantly elevated versus healthy controls. ALDOC levels rose significantly in concussed NCAA athletes after injury, versus controls and non-concussed players. In Israeli football players, ALDOC elevation, but not GFAP, was detected in concussed players versus controls. ELISA testing and immunoblotting for GFAP breakdown-products found no GFAP elevation in either athlete study despite elevation in severe TBI and some mTBI patients. Overall, multiple mTBI cohorts of different age, athletic groups, and geographical location support ALDOC as a robust biomarker for mTBI.

Keywords: Biomarker, GFAP, ALDOC, Athlete, mild TBI

A06-15

EXAMINING ADAPTIVE IMMUNE RESPONSE AND RELATIONSHIPS BETWEEN IL-7 AND ANTI-PITUITARY/HYPOTHALAMIC AUTOANTIBODIES AFTER SEVERE-TBI

Sushupta Vijapur¹, Prerna Ranganathan¹, Raj Kumar¹, Zhihui Yang⁴, Penelope Morel³, Kevin Wang⁴, Amy Wagner^{1,2}

¹*University of Pittsburgh, Department of Physical Medicine and Rehabilitation, Pittsburgh, USA*

²*University of Pittsburgh, Safar Center for Resuscitation, Pittsburgh, USA*

³*University of Pittsburgh, Department of Immunology, Pittsburgh, USA*

⁴*University of Florida, Department of Psychiatry, Gainesville, USA*

Traumatic brain injury (TBI) is associated with long-term complications, including persistent hypogonadotropic hypogonadism (PHH), which our studies suggest a link to autoimmunity. Autoantibodies (AAb) to the pituitary (APA) and hypothalamus (AHA) are present up to one-year following TBI and reduced IgM AAb increases PHH-risk among men with TBI. Adaptive immunity, including interleukin 7 (IL-7) production, may promote brain tissue-specific AAb

production, which we hypothesize is neuro-reparative and reduces neuroendocrine dysfunction. Serum IL-7, IgM/IgG APA and AHA levels were evaluated 2-weeks to 12-months post-injury for N=129 individuals with severe TBI. Mean IL-7 levels for 2 wk-3 mos, 4-6 mos, and 7-12 mos were assessed. Age was dichotomized (median=32). Group-based trajectory analyses for APA/AHA IgM and IgG levels identified high, medium, and low trajectory (TRAJ) AAb profiles. Ordinal logistic regression modeled IL-7, dichotomized age, and age*IL-7 interaction effects on AAb TRAJ. At 2wk-3mo, IL-7 was associated with APA IgM TRAJ ($\beta = -0.023$, $p < 0.001$); though age was not a moderator. At 4-6 mos, older age moderated the IL-7 relationship to IgM APA levels, such that at a given age, increases in IL-7 were associated with lower APA IgM TRAJ ($\beta = -0.022$, $p = 0.04$). A similar trend was noted at 7-12 mos for the age*IL-7 interaction ($\beta = -0.026$, $p = 0.08$). IL-7 did not influence IgM AHA production as strongly, with only trends ($p < 0.08$) noted 4-12 mos post-injury. IL-7 was associated with IgG APA across the entire sampling period ($p < 0.04$ all comparisons), while IL-7 was associated with IgG AHA 7-12 mos post-injury ($p \leq 0.015$). These data implicate IL-7 as relevant to the autoimmunity to pituitary proteins and as relevant to ongoing neuroendocrine dysfunction after TBI. Further work should replicate findings and explore how aging and immunomodulation might affect adaptive immunity post-TBI. Support: UPP-Foundation, NIDILRR 90DP0041, DODW81XWH-071-0701, R21-NS085455-01-(KKW).

Keywords: Adaptive Immunity, Autoantibodies, Interleukin 7, Aging

A06-16

MULTIMODAL BIOMARKERS ACCURATELY PREDICT RECOVERY AND ASSESS TRANSPORT AFTER SWINE SPINAL CORD INJURY

Ina Wanner¹, Julia Halford¹, Andrew Mayer², Sean Shen³, Joseph Loo³, Brian Kwon⁴, Peter Crompton⁴, Rachel Kinsler⁵

¹UCLA, Semel Institute for Neuroscience and Human Behavior, Los Angeles, USA

²LRRI, Mind Research Network, Albuquerque, USA

³UCLA, Dept. Chemistry/Biochemistry, Los Angeles, USA

⁴University of British Columbia, ICORD, Vancouver, USA

⁵U.S. Army Aeromedical Research Laboratory, Fort Rucker, USA

Spinal cord injury (SCI) is heterogeneous, hampering treatment optimization and clinical trials. SCI-patient management lacks non-invasive tools evaluating severity and predicting outcome. This project established multimodal tools using novel astroglial-injury-defined (AID) biomarkers (Halford et al., accepted) and MRI assessing transportation effects and outcome after SCI. Moderate thoraco-lumbar contusion in the Yucatan swine with the Vancouver impactor was followed by transportation introducing vehicle shock and vibrations. Quantitative histopathology documented heterogeneous lesions, blood extravasation and lateral white matter sparing that correlated strongly with walking recovery scored by the porcine thoracic injury behavioral scale (PTIBS). T2*-weighted hyperintensity and reduced diffusivity correlated with histopathology and PTIBS. Standardized immunoblot-densitometry and parallel-reaction-monitoring mass spectrometry measured cerebrospinal fluid biomarker levels providing independently validated datasets. Hyperacute panel levels (20min) were higher in non-recovered; elevation was delayed post-SCI in recovered swine. Aldolase C (ALDOC) and brain lipid binding protein (BLBP) remained elevated over one week while GFAP and S100 β decreased after 2 days (d) post-SCI. New

small GFAP-breakdown-products (BDPs) peaked by 2 d post-SCI selectively in non-recovered swine. ALDOC, S100 β and small GFAP-BDPs had each unique kinetic slopes that differed between recovered and non-recovered SCI swine. Total GFAP slopes were similar on recovery but different between transported and non-transported swine. Multivariate logistic regression determined ALDOC kinetic scores as strongest in accurately predicting outcome. Multimodal fluid and imaging markers captured different injury processes and accurately predicted recovery after SCI. Multivariate regression combining GFAP, S100 β and ALDOC kinetics distinguished transported from non-transported SCI-swine. Our multivariable analyses predicted outcome and revealed transport-related prolonged biomarker elevation. The work is significant because SCI outcome critically depends on early post-injury diagnostic monitoring and prevention of injury exacerbation.

Support: USAMRMC:W81XWH-13-2-0047

Keywords: functional recovery, swine, mass spectrometry, kinetics, multimodal analyses, transport

A06-17

TISSUE AND SERUM MICRORNA EXPRESSION IN FOCAL OR DIFFUSE TBI

Harris Weisz¹, Deborah Boone¹, Leela Cherian², Claudia Robertson², Douglas DeWitt¹, Donald Prough¹, Helen Hellmich¹

¹University of Texas Medical Branch, Department of Anesthesiology, Galveston, USA

²Baylor College of Medicine, Department of Neurosurgery, Houston, USA

Minimally invasive biomarkers can distinguish focal or diffuse TBI injury types and may prove clinically useful. Circulating microRNA (miRNA) expression was examined after fluid percussion injury (FPI) and controlled cortical impact (CCI), which produce diffuse and focal injuries, respectively. We hypothesized that there would be unique differential regulation of miRNA in various brain regions dependent on injury type and that these changes would be reflected in serum. Brain regions examined in this study have previously been shown to be functionally affected after TBI. Our long-term goal is to define a panel of miRNAs that can be used to supplement conventional imaging techniques, which currently lack specificity for injury classification. In this study, rats were anesthetized and subjected to either severe FPI or CCI or remained uninjured. Serum was isolated from whole blood in all animals and rats were sacrificed 4 h after TBI. Brains were prepared for laser capture microdissection (LCM) of TBI-affected brain regions. Serum and tissue miRNA were isolated, cDNA was synthesized, and pre-amplification was performed. RT-qPCR was performed using arrays populated with 84 common disease associated miRNAs. Data was analyzed using Geneglobe Data Analysis and Ingenuity Pathway Analysis software (Qiagen). Our analysis shows there are distinct miRNAs which are differentially regulated, in a brain region dependent manner, between either focal or diffuse injuries. Pathway analysis of these tissue-expressed miRNA suggests their involvement in the pathophysiologic response after TBI. Serum miRNA expression revealed significant differences among FPI and CCI comparisons and to naïve controls. In a long term perspective, understanding regulation of tissue miRNAs in TBI injury subtypes and measuring respective changes in serum will improve clinical diagnosis by supplementing imaging modalities. These studies were supported in part by the Moody Project for Translational TBI Research.

Keywords: microRNA, serum, diffuse, focal

A07 AXONAL INJURY

A07-01

IMPROVING REPEATABILITY OF VERTEBRAL KINEMATICS IN A RAT DISLOCATION SPINAL CORD INJURY MODEL

Stephen Mattucci^{1,2}, Jie Liu², Paul Fijal¹, Wolfram Tetzlaff², **Thomas Oxlund**^{1,2}

¹University of British Columbia, Orthopaedics, Vancouver, Canada

²University of British Columbia, ICORD, Vancouver, Canada

Dislocation is the most common clinically observed spinal cord injury (SCI) mechanism, occurring in roughly 45% of SCI cases, however, there are few preclinical models. Despite demonstrating different injury outcomes compared to more common contusion models, the dislocation models exhibit greater variability. Since dislocation injury models involve clamps which grip and displace the spine, a potential cause of variability may be slipping at this interface. The objectives of this study were to: i) design new injury clamps, ii) measure intervertebral kinematics during a high-speed dislocation injury in an in vivo rat model; and iii) quantify relative motion at the vertebrae-clamp interface to determine which clamps provide the most rigid connection.

New clamps were designed to pivot, and self-align when tightened, ensuring each clamp holds both intended vertebrae. The dislocation injury was performed using both the existing and redesigned clamps, where 400 μ m radiopaque tantalum beads were fixed to C3-C6 and the clamps to track relative motion. The injury was performed at C4/C5 on anesthetized rats (n=17 per group), to a displacement of 2.3 mm at a velocity of 700 mm/s. A high-speed x-ray system recorded the tests at 8000 frames per second, and motion of the vertebrae with respect to the clamps was tracked.

The relative motion of C5 with respect to the caudal clamp was significantly less for both translation and rotation for the redesigned clamps, and the maximum observed relative motion was four times less for the redesigned clamps.

This is the first time measurement of clamp-vertebral motion in a high-speed dislocation model. This study demonstrated that relative motion was occasionally present between the existing dislocation clamps and the vertebrae. By redesigning the clamps, relative motion was reduced, and produced more repeatable kinematics during injury. These improvements address a potential source of variability and progress toward a more repeatable rat dislocation model, better suited for investigating SCI interventions, and further shed light on the importance of injury mechanism.

Keywords: biomechanics, spinal cord injury, dislocation, animal model

A07-02

DETERMINING THE RELATIVE MECHANICAL PROPERTIES OF GREY AND WHITE MATTER IN THE RAT SPINAL CORD

Liam Sharkey^{1,2}, C. Antonio Sanchez³, Tim Bhatnagar², Sid Fels³, **Thomas Oxlund**^{1,2}

¹University of British Columbia, Orthopaedics, Vancouver, Canada

²University of British Columbia, ICORD, Vancouver, Canada

³University of British Columbia, Electrical Engineering, Vancouver, Canada

Finite Element (FE) models have been used to determine the relationship between strain and tissue damage in traumatic spinal cord injury. Previous FE studies have used mechanical properties determined from ex vivo loading of spinal cord tissue, despite the variation in results from such tests, and evidence that suggests spinal cord tissue degrades

rapidly after death. The current study aims to develop a method with which to determine the relative mechanical properties of the two primary constituent tissues in the spinal cord; the grey and white matter.

The current study used a quasi-static, 3D FE model to recreate mild contusion-type spinal cord injuries imposed on Sprague-Dawley rats (n=6) by Bhatnagar et al previously. MR images of the undeformed and deformed spinal cords were used to guide the initial mesh generation and the gross cord deformation, respectively. The resulting predicted grey matter deformations were compared with experimental results. The ratio of elastic moduli between the grey and white matter (W/G) was varied and the resulting morphologies of the spinal cords were observed. The morphological similarity was quantified using Dice Similarity Coefficient (DSC). The grey and white matter in each spinal cord were modeled as homogeneous, isotropic, linear elastic solids. W/G values of 0.5, 1.0 and 2.0 were tested with Poisson's ratio held at 0.42.

For all cords, the DSC values ranged between 72% and 87%. The best qualitative agreement was seen for contusions that were central on the spinal cord, for all values of W/G. For the asymmetric contusions, the model accurately predicts deformation at the lateral edges of the grey matter, but predicts greater grey matter deformation at the mediolateral center of the cord than seen in the experimental deformations for all W/G values. These results suggest possible heterogeneity of the spinal cord white matter.

Keywords: biomechanics, spinal cord injury, finite element modelling, material properties

A07-03

BRAIN STRAIN PATTERNS ASSOCIATED WITH FOOTBALL IMPACT RECONSTRUCTIONS

Erin Sanchez¹, Lee Gabler¹, Bethany Rowson², Steven Rowson², Stefan Duma², **Matthew Panzer**¹

¹University of Virginia, Center for Applied Biomechanics, Charlottesville, USA

²Virginia Tech, Center for Injury Biomechanics, Blacksburg, USA

Current football concussion risk assessments were developed based on head kinematics measured using sensors that were worn by players to determine accelerations associated with injury. While head motion is associated with concussion risk, tissue-level strains are the primary mechanism of brain injury. Finite element (FE) head models are used to obtain brain strains for potentially developing more reliable concussion tolerances through simulated head impact. In this study, six-degree-of-freedom head kinematics obtained from laboratory reconstructions of 55 on-field football head impacts (11 concussed cases) were simulated using a FE human brain model. Strain-based metrics including maximum principal (MPS) and cumulative brain strain (CSDM) were calculated in various brain regions for each impact. Estimates of maximum axonal-tract strain (MAS) in the corpus callosum were also considered. Strain-based predictors were evaluated relative to injury outcomes from the players, and used to identify potential tissue-level tolerances for concussion. For all cases, maximum brain strains were highest in cortical grey matter tissue, and cerebral white matter and cerebellum tended to have the lowest strains. Brain strains were higher in concussed players ($p < 0.01$); median MPS was 22% for concussed cases and 14% for non-concussed, CSDM values were overall low (< 0.05) but slightly larger in the concussed group. MAS in the corpus callosum were also higher in concussed cases (5% vs 8% median value, $p < 0.05$). The MPS metric had the highest ROC AUC value (0.822), followed by CSDM (0.815) and MAS (0.711). Additionally, rotational head motion response had the highest correlation with

brain strains in all regions. Although FE brain models are not validated for regional strain prediction, these results highlight the efficacy of developing concussion risk models using tissue-level strains. These methods can be used to improve helmet design to ultimately reduce the incidence of concussion in football. The authors acknowledge the UVa-VTC Neuroscience Seed Fund Program for their support of this research.

Keywords: Brain strain, Concussion, Finite element modeling, Football, Head kinematics

A07-04

SHEAR SHOCK WAVES ARE DIRECTLY OBSERVED IN THE BRAIN WITH HIGH FRAME-RATE ULTRASOUND

David Espindola, **Gianmarco Pinton**

University of North Carolina at Chapel Hill and North Carolina State University, Biomedical Engineering, Chapel Hill, USA

Here we present a high frame-rate (6200 images/second) ultrasound imaging method that can accurately ($<1\ \mu\text{m}$) measure the internal brain motion during the rapid transient events associated with a mild impact in an ex vivo porcine brain. Attempts to measure the in situ nonlinear brain mechanics with imaging methods (MRI, CT) have lacked the penetration, frame rate, or motion detection accuracy to capture the rapid and nonlinear shear wave motion during traumatic injury. The substantial increases in frame-rate and motion detection accuracy of our ultrasound method allow us to investigate previously unexplored biomechanical regimes. We present the first experimental observation of shear shock wave generation in the brain. The highly localized increases in acceleration at the shock front suggests that shear shocks are a previously unappreciated primary mechanism for traumatic injuries.

Our method relies on two main advancements 1) A high frame-rate ultrasound sequence which increases the penetration and improves the image quality compared to conventional high frame-rate methods 2) An adaptive tracking algorithm that can accurately detect the fine shock structure within the brain. To the best of our knowledge no other imaging method has been able to achieve this combination of speed, accuracy, and penetration in the brain.

By imaging brain motion directly we discovered that shear shock waves form within the brain. The measured shock waves have a specific odd harmonic signature predicted by theory describing a cubically nonlinear elastic soft solid. Measurements of the frequency dependent attenuation and dispersion were used to fit this nonlinear theoretical model to our data. This yielded the first estimates of the cubic nonlinear parameter for brain tissue. This previously unobserved shear shock wave phenomenology dramatically amplifies the acceleration at the shock front, deep in the brain, compared with the acceleration imposed at the brain surface (up to a factor 8.5). A 30 g acceleration at the brain surface therefore develops into a 255 g shock wave deep inside the brain. Strain and strain rate are also amplified at the shock front.

NIHR0106052014

Keywords: Shear shock wave, Ultrasound imaging

A07-05

BIOMECHANICS OF A WEIGHT-DROP MODEL OF SPORTS CONCUSSION

Bharat Sanders^{1,2}, Erin Buckley^{1,2}

¹*Emory University, Biomedical Engineering, Atlanta, USA*

²*Georgia Institute of Technology, Biomedical Engineering, Atlanta, USA*

There is a critical need to understand the biomechanical factors influencing mTBI severity in order to guide preventative measures and novel therapies. Numerous existing pre-clinical models of concussion may help address this need, but it is essential that the animal models yield realistic impacts that are comparable to those seen in humans. The “Harvard weight-drop model” claims clinical relevance to sports concussion in that (1) it features blunt impact followed by rotational acceleration and (2) repetitive insults lead to significant cognitive deficits without overt structural brain injury. However, the biomechanical forces of this model have yet to be quantified. Herein, we assess the linear and rotational accelerations incurred in this murine model, and we compare the scaled values to literature reports of human sports concussion. This model involves impact of the intact skull/scalp using a 55 g bolt dropped through a guide tube from 48” followed by rotation in the anterior-posterior plane. To quantify, N = 12 slow motion videos (480 fps) of impact were taken on adult mice. Particle tracking software was used to quantify the position of center of mass of the bolt across 3 frames before/after impact. Using the momentum principle, we related the change in velocity of the bolt pre/post-impact to the linear and the rotational accelerations of the mouse head. Mean (SD) linear acceleration was 83 (27) g and rotational acceleration was 253,000 (75,600) rad/s. Assuming that equal stress is required to reproduce similar injury mechanism in both mice and humans, we scaled our values using the ratio of mice/human brain masses. These accelerations translate to roughly 5.4 (1.8) g and 1,100 (324) rad/s in humans. The scaled *linear* accelerations are significantly lower than reported thresholds for human mTBI; however, the scaled *rotational* accelerations obtained with this model are on the same order of magnitude as human mTBI thresholds. In conclusion, the Harvard weight-drop mouse model yields rotational accelerations that realistically scale to corresponding human sports concussion biomechanics.

Keywords: animal model, rotational acceleration, linear acceleration, scaling

A07-06

DESIGN OF A DISLOCATION SPINAL CORD INJURY MODEL FOR EVALUATING THE EFFECT OF VELOCITY AND TIMING OF RESIDUAL COMPRESSION

Jason Speidel^{1,2}, Stephen Mattucci^{1,2}, Jie Liu², Wolfram Tetzlaff², Thomas Oxlund^{1,2}

¹*University of British Columbia, Orthopaedics and Mechanical Engineering, Vancouver, Canada*

²*International Collaboration On Repair Discoveries, Vancouver, Canada*

The effect of timing of decompression following traumatic SCI remains unclear, as clinical and preclinical studies have demonstrated varying results. Some studies suggest that certain sub-groups of SCI could see greater benefits than others from early decompression. Dislocation is the most commonly seen injury mechanism clinically, but it has never been investigated with respect to residual compression in an animal model. The objective of this study was to develop a model for evaluating the effect of velocity and timing of residual compression in a rodent dislocation SCI model. Based on a previous dislocation model using custom vertebral injury clamps, a novel method for holding residual compression immediately following SCI was developed. This method involves use of an electromagnetic actuator to perform the injuries at both high and low velocity and a

custom stereotaxic frame which rigidly holds the clamps during injury and in residual compression after insult. After residual compression, while still held securely within the stereotaxic frame, the clamps are realigned via linear translation stage where they are rigidly fixed together. The clamps were modified to also function as surgical fixation implants, ensuring spinal stability during all phases of the study. This model was used for a study involving 4 groups of SD rats (n=12) plus shams: 2 timings of decompression (24 minutes, 240 minutes) and 2 velocities (5 mm/s, 500 mm/s). All injuries involve dislocation between the C5/C6 vertebrae in an anterior-posterior direction to 1.45 mm and residual compression of 0.8mm. Animals were evaluated for motor function using the Martinez open field, grip strength, and grooming tests for 6 weeks post-injury. This design is able to precisely create dislocation injuries with immediate residual compression afterwards and represents a novel model of SCI, relevant to the question of timing of decompression.

Keywords: Residual Compression, Dislocation, Animal Model, Decompression, Design, Acute

A07-07

PUBLIC REVIEW OF BIOMECHANICAL DEVICES IN TRAUMATIC BRAIN INJURY (TBI) COMMON DATA ELEMENTS (CDES): NINDS AND DOD VERSION 1.0

Katelyn Gay¹, Adam Bartsch⁴, David B. Camarillo³, **Carol Taylor-Burds²**, Sherita Ala'i¹, Joy Esterlitz¹, Kristen Joseph¹, Patrick Bellgowan²

¹The Emmes Corporation, Bethesda, USA

²National Institute of Neurological Disorders and Stroke, Bethesda, USA

³Stanford University, Stanford, USA

⁴Cleveland Clinic, Cleveland, USA

Objective: The National Institute of Neurological Disorders and Stroke (NINDS) and the Department of Defense (DOD) are collaborating to develop version 1.0 CDE recommendations for Biomechanical Devices in TBI. The goals of this project are to facilitate research data collection by providing standard definitions for commonly used research variables for data sharing and data-mining.

Background: In January 2017, a working group (WG) of experts in blast, blunt and inertial loading related TBI formed to develop Biomechanical Devices in TBI specific CDE recommendations. These CDEs will complement existing NINDS TBI CDE recommendations.

Design/Methods: The WG is reviewing types of raw unprocessed data, how data are acquired, and how data are most commonly analyzed in research involving Biomechanical Devices in the study of TBI.

Results: The WG end products will include CDEs, template case report forms, data dictionaries and guidelines documents. The WG will complete an internal review of recommendations and public feedback will be elicited before version 1.0 recommendations are posted to the NINDS CDE website in September 2017.

Conclusion: The new Biomechanical Devices in TBI CDE recommendations will be valuable for researchers to standardize data collection across studies. By standardizing data collection, more robust metadata and data sharing across studies will be possible in the future. The NINDS CDEs are an evolving resource, as research in the biomechanical device field progresses, updates will be made to these CDE recommendations.

Support: This material is based upon work supported by the U.S Army Medical Research and Materiel Command's Combat Casualty Care Research Program and by HHSN271201200034C.

Keywords: Common Data Elements, Devices, Sensors

A07-08

INVESTIGATION OF CSF CAVITATION AS AN INJURY MECHANISM OF TRAUMATIC BRAIN INJURY

Allen Yu, Joost Op 't Eynde, Cameron Bass

Duke University, Biomedical Engineering, Durham, USA

There is an urgent need to elucidate the injury mechanisms of traumatic brain injury. Blunt impact to the head is hypothesized to generate negative intracranial pressure transients sufficient to cause CSF cavitation. Upon bubble collapse, jet streaming and immense localized pressures may occur, potentially damaging surrounding tissue. The objective for this study was to investigate cavitation bubble formation and collapse as an injury mechanism using a biofidelic head model under impact. A simplified head surrogate with brain phantom was created consisting of a skull (a rigid Lucite container), cerebrospinal fluid (degassed distilled water of 2 mm thickness), and different brain tissue simulants (Perma-Gel, ballistic gelatin composite, Sylgard gel). Brain phantoms were varied in order to investigate cavitation limits from the range of bulk modulus material properties. The head was subject to linear blunt impact loading from a weighted pendulum, with resulting peak linear accelerations ranging from 500–1500 m/s² as measured by a rigidly mounted accelerometer. Hydrophones were externally adhered to the vessel and placed at the coup and contrecoup sites to measure sonoacoustic emissions from bubble formation and collapse. A high speed camera recording at 40,000 fps directly captured fluid behavior. Cavitation was directly observed in the CSF layer and confirmed by acoustic data. The occurrence of cavitation was dependent on the peak acceleration of the system as generated by the impact. Cavitation occurred in all tests above Head Impact Criterion values of 1000, a value for which the loading scenario would create a high risk for injury. Changing brain phantom material and their associated bulk modulus properties altered cavitation thresholds. Bubble formation and collapse elapsed approximately 0.15 to 0.4 msec. Power spectrum analysis of sonoacoustic data showed dominant frequencies ranging from 10–15 kHz during cavitation bubble collapse. This data supports the hypothesis that fluid cavitation is a potential injury mechanism for TBI. Furthermore, the methodology detailed here provides the framework to investigate cavitation in live animal TBI models, as currently there are no empirical studies demonstrating cavitation *in vivo*.

Keywords: Mechanisms of Injury, Cavitation

A07-09

DEVELOPMENT OF CRASH INDUCED INJURY CRITERIA FOR PREDICTING BRAIN INJURIES USING A HUMAN HEAD COMPUTER MODEL

Liying Zhang, Runzhou Zhou, Tushar Arora, Varun Pathak

Wayne State Univ, Biomedical Engineering, Detroit, USA

Finite element (FE) modelling can serve as a powerful tool to study biomechanical process of head and brain injuries that is difficult to investigate experimentally on living human subjects. Recently, a detailed human head model, GHBM (Global Human Body Modelling Consortium) M50, representing a 50th percentile male adult head has been developed, validated and used to develop tissue level injury criteria for TBI. The objective of this study was to validate a detailed GHBM 5th percentile female (F05) head model which accounts for gender related size, geometrical and anatomical differences in order to properly predict injury risk sustained by this population. A number of Crash Induced Injury (CII) criteria for injuries to the skull, face, and brain of various regions were developed to enable the prediction of the injury risk by the

model. Thirty-one sets of published cadaveric head impact experimental data were simulated to validate the biomechanical response of the head model in terms of force-deflection for various facial and cranial bones, intracranial pressure and brain/skull relative displacement for brain of various regions. Then, Forty-four sets of head impact experiments with injurious and non-injurious conditions were simulated to develop CII values for skull fracture, facial fracture, acute subdural hematoma (ASDH), cerebral contusion, and diffuse axonal injury (DAI) at various white matter structures/regions and their associated severities. Model predicted biomechanical responses correlated well to the experimentally measured results with objective ratings greater than 0.7 (1 being perfect). The current GHBM F05 head model has been rigorously validated against all exiting data head impact experiments and responses. The current model is capable of predicting six different head injury types affecting nine regions/locations with reasonable predictive capability. With further improvement and exercises, the human head model can enable assessment of possible real-world injury scenarios to understand the injury mechanisms and allow for engineering improvements to help prevent potential head/brain injury from traumatic events.

Acknowledgment: Global Human Body Modelling Consortium, LLC.

Keywords: Biomechanical Response, Finite Element Human Head Model, Crash Induced Injury Criteria for Head, Diffuse Axonal Injury, Bridging Vein Rupture, Coup and Contrecoup Contusion

A08 CEREBRAL BLOOD FLOW

A08-01

CBF ENHANCES ACCURACY OF IMPACT PROGNOSTIC CALCULATOR

Martina Stippler, Roger Davis, Malia Mcavoy
Harvard, Neurosurgery, Boston, USA

Background: Being able to predict prognosis in severe TBI patients accurately has proven to be difficult. An unbiased prognosis is important for an informed discussion with family members about lifesaving procedures. The IMPACT Prognostic calculator (IPC) is one of the first attempts to predict 6-month mortality and poor outcome. The AUC has been deemed insufficient by some providers. The purpose of this study is to improve the accuracy of the IPC with adding the physiological value of average cerebral blood flow (CBF) during the first 12 hours after the injury.

Methods: All patients with severe TBI and CBF data within the 12 hours after injury included. Their change of 6-month mortality and 6-month poor outcome were calculated and compared with the actual outcome. The average CBF was added to the IPC and the accuracy of the enhanced model was tested. A likelihood ratio test was performed comparing the models.

Results: 147 patients were enrolled. 13.6% were female. Most common mechanism of injury was motor vehicle collision (64%), fall or jump and assault accounted for 16% each. The median GCS score at presentation was 5. The mean age was 35 years.

The C statistics for risk for death of the CORE model was 0.687 and for the CORE+CBF was 0.754 which was statistically significant different ($p=0.0007$). The C statistics for death of the CORE+CT was 0.735 and of CORE+CT+CBF was 0.787 which was statistically significant different ($p=0.0015$).

The C statistics for risk for poor outcome of the CORE model was 0.752 and of the CORE+CBF was 0.787. The C statistics for risk for poor outcome of the CORE+CT was 0.772 and for CORE+CT+CBF was 0.801. Both were statistically significant different $p=0.0116$ and $p=0.0038$ respectively.

Conclusions: We concluded that adding a brain specific physiological measure like CBF to the IMPACT Prognostic calculator helps

to improve accuracy of 6 month outcome prediction in patients with severe TBI. The next direction should be if more readily available brain specific physiological parameter could be identified.

Keywords: Prognosis Prediction, Severe Traumatic Brain injury, Impact Calculator, TBI outcome

A08-02

CHRONIC CEREBROVASCULAR ABNORMALITIES IN A MOUSE MODEL OF REPETITIVE MILD TRAUMATIC BRAIN INJURY

Cillian Lynch^{1,2,3}, Gogce Crynen^{1,2,3}, Scott Ferguson^{1,2,3}, Benoit Mouzon^{1,2,3}, Daniel Paris^{1,2,3}, Joseph Ojo^{1,2,3}, Paige Leary¹, Fiona Crawford^{1,2,3}, Corbin Bachmeier^{1,2,3}

¹The Roskamp Institute, Neuroscience, Sarasota, USA

²The Open University, Life Sciences, Milton Keynes, UK

³James A. Haley Veteran's Administration Center, -, Tampa, USA

Primary Objective: To investigate the status of the cerebrovasculature following repetitive mild traumatic brain injury (r-mTBI).

Research Design: TBI is a risk factor for development of various neurodegenerative disorders. A common feature of neurodegenerative disease is cerebrovascular dysfunction which includes alterations in cerebral blood flow (CBF). TBI can result in transient reductions in CBF, with severe injuries often accompanied by varying degrees of vascular pathology post-mortem. However, at this stage, few studies have investigated the cerebrovasculature at chronic time points following repetitive mild brain trauma.

Methods and Procedures: r-mTBI was delivered to wild-type mice (12 months old) two times per week for 3 months and tested for spatial memory deficits (Barnes Maze task) at 1 and 6 months post-injury. At 7 months post-injury CBF was assessed via Laser Doppler Imaging and, following euthanasia, the brain was probed for markers of cerebrovascular dysfunction and inflammation.

Main Outcomes and Results: Memory impairment was identified at 1 month post-injury and persisted as late as 6 months post-injury. Furthermore, we observed significant immunopathological insult, reductions in global CBF, and downregulation of cerebrovascular-associated markers.

Conclusions: These results demonstrate impaired cognitive behavior alongside chronic cerebrovascular dysfunction in a mouse model of repetitive mild brain trauma.

Keywords: Repetitive Traumatic Brain Injury, Cerebrovascular, Cerebral Blood Flow, Animal Model

A09 CEREBROSPINAL FLUID

A09-01

SUBDURAL EFFUSION/HYGROMA AS THE PATHO-ANATOMIC BASIS FOR TRAUMATIC MENINGEAL ENHANCEMENT

John Butman^{1,3}, Dzung Pham^{3,4}, Martin Cota^{3,4}, Lawrence Latour^{2,3}

¹Clinical Center, NIH, Radiology and Imaging Sciences, Bethesda, USA

²NINDS, NIH, Stroke Branch, Bethesda, USA

³Center for Neurosciences and Regenerative Medicine, Bethesda, USA

⁴Henry Jackson Foundation, Bethesda, USA

Objective: Recently, traumatic meningeal enhancement (TME) seen only on post contrast FLAIR MRI- has been identified as a surprisingly

common manifestation of head trauma. Because of its similarity to subdural enhancement seen in cases of CSF hypovolemia/hypotension, we hypothesized that TME represents the acute development of a post traumatic subdural effusion or hygroma.

Methods: Patients presenting with acute head trauma (n=42) were enrolled in an IRB approved study requiring MRI within two days of injury, including pre- and post-contrast FLAIR and balanced fast field echo (BFFE) sequences.

Pre- and post-contrast FLAIR was used to assess for TME, identified as high signal along the dura seen on post contrast FLAIR without extension into the subarachnoid space. High signal on pre-contrast FLAIR in similar distribution along the dura was considered evidence of subdural hematoma (SDH).

BFFE was used to assess for subdural effusion/hygroma (SDEH) manifest as a separation of the subarachnoid membranes from the inner table of the skull. Such visualization is possible using BFFE due to the combination of high spatial resolution (~500 μ m isotropic scans in 3–4 minutes) and high image contrast between CSF (high signal) and arachnoid membranes (low signal).

Findings: Interpretable and complete data was obtained in 36 cases. TMI was present in 12/36 cases. SDEH was present in 10 of these 12 cases. Of the remaining two cases, one was complicated by SDH, possibly obscuring the SDEH, and one was relatively small and confined to the falx.

Conclusion: Using high resolution BFFE sequences to directly visualize SDEH, we have shown the correspondence of TME and SDEH. Such injuries may represent the milder end of a spectrum of meningeal injury, with subdural hematoma representing a more severe injury or simply the coincidence of a subdural vascular injury with the meningeal injury.

Keywords: mTBI, MRI, acute

A09-02

DISCREPANCIES BETWEEN CONTINUOUS AND INTERMITTENT INTRAVENTRICULAR PRESSURE MONITORING

David Darrow¹, Uzma Samadani³, Theoden Netoff²

¹University of Minnesota, Neurosurgery, Minneapolis, USA

²University of Minnesota, Biomedical Engineering, Minneapolis, USA

³Hennepin County Medical Center, Surgery, Minneapolis, USA

Significant discrepancies between continuous and intermittent pressure monitoring of intraventricular catheters have been found at our institution using the Integra® Camino® Flex Ventricular Catheter. These discrepancies generated clinical uncertainty about the status of cerebral autoregulation of patients suffering severe traumatic brain injury in accordance with Segal's law. Our efforts endeavored to fully characterize the two signals during simultaneous recordings to understand the source of the discrepancy. After IRB approval, simultaneous recordings were obtained from four patients when the external ventricular drain was clamped resulting in more than 40 million data points. Data was sampled at 124 Hz, resulting in 90 hours for comparison. Power spectra were calculated, and mean squared cross coherence was compared. Significant error was found in the low frequency component. Low frequency data less than 0.3 Hz was found to have a correlation coefficient of 0.3 while data above 0.3 Hz was found to be 0.84. False positive readings, when intermittent ICP read above 20 mmHg while continuous ICP remained below, constituted more than 10% of the data, while false negative data constituted less than 0.8%. By examining the low frequency components of the relationship of the two sources of pressure, it was found that the most significant source of discrepancy remained changes in the zero level for the intermittent external ventricular drain readings, corresponding to head position changes or nursing leveling. Continuous ICP monitoring from an intraventricular

pressure sensor consistently reads lower values than an external ventricular pressure sensor while maintaining consistency.

Keywords: Sensor, continuous ICP, flex catheter, Intraventricular

A10 CERVICAL

A10-01

SURGICAL DECOMPRESSION IN PATIENTS WITH COMPRESSIVE MYELOPATHY WHO HAD PAST MEDICAL HISTORY OF CERVICAL SPINAL CORD INJURY

Takeo Furuya¹, Masao Koda^{1,2}, Yasushi Iijima¹, Jyunya Saito¹, Mitsuhiro Kitamura¹, Takuya Miyamoto¹, Kazuhide Inage¹, Sumihisa Orita¹, Masashi Yamazaki^{1,2}, Seiji Ohtori¹

¹Chiba University Graduate School of Medicine, Dept. of Orthopaedic Surgery, Chiba, Japan

²University of Tsukuba, Dept. of Orthopaedic Surgery, Tsukuba, Japan

Objectives: Spontaneous recovery by conservative treatment is often expected in an incomplete spinal cord injury (SCI) case. Multiple factors including the severity of previous stenosis, the neurological symptom before injury are associated with recovery. In some cases, symptom may aggravate again by progression of spondylosis or ossification of the posterior longitudinal ligament in sub-acute or chronic phase of SCI. The purpose of this study is to evaluate the efficacy of surgical decompression in patients with cervical compressive myelopathy who had past medical history of SCI.

Methods: Six patients (four male, two female) who had myelopathy and had past medical history of cervical SCI were treated surgically after 2012 in our institute. Their mean age at surgery was 60 year old. Four patients had ossification of the posterior longitudinal ligament, one had ossified disc herniation and the other had degenerative spondylosis. Decompression surgery was performed an average of 147 days after initial injury. Mean follow-up period was 21 months. ASIA impairment scale (AIS) and JOA score (Japanese Orthopedic Association score) were evaluated.

Results: Two patients showed AIS B, two showed C and rest showed D at the first examination after injury. Spontaneous recovery to D was obtained in all patients in acute phase of SCI. However, patients in this series showed sequential deterioration of the symptom in sub-acute or chronic phase. The average JOA score was 7.5 points (17.0 points in full score) just before surgery and recovered to 12.0 points at final follow-up. The average recovery rate with surgery was 48%.

Conclusion: Relatively good results of surgical decompression for the myelopathy can be expected even if the patient have a past medical history of SCI.

Keywords: surgical decompression, sub-acute phase, chronic phase, clinical outcome, cervical myelopathy, spinal cord injury

A10-02

DUAL TIME COURSE RNA-SEQ REVEALS LEVEL-SPECIFIC NEUROVASCULAR RESPONSE AFTER CERVICAL AND THORACIC SPINAL CORD INJURY

James Hong^{1,2}, Mahmood Chamankhah², Anna Badner^{1,2}, Dario Righelli³, Priscilla Chan¹, Claudia Angelini³, Michael Fehlings^{1,2}

¹University of Toronto, Institute of Medical Science, Toronto, Canada

²Toronto Western Hospital, Genetics and Development, Toronto, Canada

³Istituto Per le Applicazioni del Calcolo, BioinfoLab, Naples, Italy

Traumatic spinal cord injury (SCI) is a devastating neurological condition that occurs primarily at the cervical (cSCI, >65%) and thoracic

(tSCI) levels. Despite the marked neurovascular distinctions of the two levels and strikingly positive response of cSCI to trial drugs such as cethrin compared to tSCI, the mechanisms driving level-specific heterogeneity between their respective milieu remains elusive. We posit that the increased vascularity and grey-white ratio of the cervical cord—relative to the thoracic—results in greater susceptibility to neurovascular disruption, ultimately manifesting a secondary injury of earlier onset, severity, and chronicity. A rat model of moderate clip compression injury was used to induce SCI at the C6-7 and T6-7 levels, with laminectomy-only animals serving as surgical controls. Following sacrifices at 3, 7, 14, and 56 days, samples were subject to RNA-seq, protein work, imaging, and immunohistochemistry. Results of RNA-sequencing revealed striking differences in the onset and temporal profile of astrocytic and pericytic neurovascular processes with canonic *stat3*-dependent gliotic markers—*lcn2*, *gfap* and *serpina3n*—being upregulated in the cervical cord across time. Further, 3D ultrasound and immunostaining revealed rapid tissue loss and hemorrhage starting as early as 3 days post-cSCI with increased *gfap* and *cspg4* staining in the cord. Finally, Western blotting confirmed an increase in *stat3*-dependent gliotic markers accompanied by a loss of key blood-brain-barrier proteins *tpj1* and *ocln* in cSCI across time. Taken together, this data demonstrates—for the first time—the level-specific heterogeneity of SCI, with cSCI having a quicker onset and chronicity compared to tSCI. Further, these results reconcile the potential reasons behind why preliminary tSCI-derived trial paradigms may not be suited—in both strategy and timing—to cSCI, and hopes to engage clinicians and scientists in the design and study of level-specific therapeutics.

Keywords: RNASeq, Neurovascular unit, Secondary injury, Pericyte

A10-03

EXPERIMENTAL RAT MODEL FOR CERVICAL COMPRESSIVE MYELOPATHY

Yasushi Ijima¹, Takeo Furuya¹, Junya Saito¹, Mitsuhiro Kitamura¹, Kazuhide Inage¹, Sumihisa Orita¹, Seiji Ohtori¹, Masashi Yamazaki², Masao Koda¹

¹Chiba University Graduate School of Medicine, Orthopedics, Chiba, Japan

²Tsukuba University, Orthopedics, Tsukuba, Japan

Introduction: Animal models using ‘chronic’ or ‘slow-progressive’ compressive myelopathy are more scarce. Previously a rat model of chronic compressive myelopathy that uses a water-absorbing polymer inserted in the sub-lamina was reported. However, the best size and expansion rate of the sheet have not yet been established. The purpose of the present study was to validate these properties in an ideal rat model of cervical compressive myelopathy.

Methods: We used 30 8-week-old female rats. A sheet of water-absorbing polymer was inserted in the C4–C5 sub-lamina. Rats were randomly divided into 5 experimental groups (n=6 per group). Sheets that expand their volume by 200% were placed in groups 1 (3mm×5mm×0.5mm) and 2 (3mm×5mm×0.7mm). Sheets that expands their volume by 350% were placed in groups 3 (3mm×5mm×0.5mm) and 4 (3mm×5mm×0.7mm). A sheet was placed in the C4–C5 sub-lamina space momentarily in the control group. After the surgery, we evaluated severity of paralysis using a Forelimb Locomotion Scale score, and BBB score for 12 weeks. At 12 weeks after the surgery, the motor neurons in the anterior horn in the C4–C5 segment were counted after staining with cresyl violet, and demyelination in the corticospinal tract at C7 was assessed after staining with Luxol fast blue.

Results: ‘Slow-progressive’ paralysis appeared at 6 weeks post-operatively in group 1 and at 4 weeks in rats in group 2. By contrast,

only temporary paralysis was observed from groups 3 and 4. A loss of motor neurons was observed in all groups except for the control. Demyelination in the corticospinal tract was seen from groups 1 and 2, but not groups 3 or 4.

Conclusions: A polymer sheet that expands its volume by 200% is an ideal material for rat models of cervical compressive myelopathy.

Keywords: cervical compressive myelopathy, rat model

A10-04

DECREASED TIME TO SURGERY AND ICU LENGTH OF STAY IN PATIENTS WITH SPINE TRAUMA FOLLOWING REGIONALIZATION OF A TRAUMA SYSTEM

Michael Kelly¹, Jack He¹, Mary Jo Roach¹, Timothy Moore¹, Michael Steinmetz², Jeffrey Claridge¹

¹MetroHealth Medical Center, Neurosurgery and Surgery, Cleveland, USA

²Cleveland Clinic, Neurosurgery, Cleveland, USA

The effect of regionalized trauma care (RT) on traumatic spine injury (TSI) outcomes in the United States is unknown. The Northern Ohio Trauma System was organized in 2010 as a multi-institution trauma system across northeastern Ohio. We hypothesized that RT would be associated with earlier time to spine surgery and decreased length of stay (LOS). All patients >14 years with a TSI were identified from the RT database using ICD-9-CM diagnostic codes. Data from 2008 through 2012 were analyzed before and after RT in 2010. A total of 4,072 patients were identified; 1904 (47%) pre-RT and 2168 (53%) post-RT. TSI admissions to the regional level 1 center post-RT increased from 67% to 74% (p<0.0001). TSIs in the post-RT group were older (median age: 52 vs. 50, p=0.02). Injury severity scores, Spine Abbreviated Injury Scale scores, and the percentage of TSIs with spinal cord injury were similar between time periods. Post-RT TSIs demonstrated a lower median ICU LOS (0 day vs. 1 days; p<0.0001), underwent spine surgery more frequently (13% vs. 11%; p=0.01), and had a higher rate of spine surgery performed within 24 hours of admission (65% vs. 55%; p=0.02). In patients with tSCI post-RT, ICU length of stay was decreased (1 day vs. 2 days; p<0.0001) and ventilator days were reduced (average days: 2 vs. 3; p=0.006). The post-RT time period was an independent predictor for spine surgery performed in less than 24 hours for all TSIs (OR 1.51, 95% CI: 1.03–2.22, C-stat=0.66). Multivariate linear regression analysis demonstrated an independent effect on reduced ICU LOS post-RT for both TSIs (OR –0.6; 95% CI: –1.04–1.18; R²=0.11) and tSCIs (OR –1.01, 95% CI: –1.6–0.40; R²=0.11). Regionalization of trauma is associated with increased surgical rates, earlier time to surgery, and decreased ICU LOS for patients with TSI.

Keywords: Spine trauma, Spine surgery, Trauma systems, Clinical outcomes

A10-05

HALO VEST IMMOBILIZATION - AN INSTITUTIONAL REVIEW OF SAFETY AND EFFECTIVENESS IN ACUTE CERVICAL SPINE INJURY FROM 2013 TO 2016

Gazanfar Rahmathulla, Laura McLaughlin, Dunbar Alcindor, Carlos Arce, Daryoush Tavanaiepour

University of Florida COM Jacksonville, Neurosurgery, Jacksonville, USA

Objectives: to review the safety and effectiveness of HALO vest immobilization for cervical spine immobilization in acute cervical spine instability, traumatic and non-traumatic.

Method: retrospective review of patients admitted to level 1 trauma center between 2013 to 2016 with acute traumatic spine instability, a majority of cases resulting from trauma. Seventy three patients underwent HALO vest immobilization in order to treat acute cervical instability secondary to trauma at the Occipito-cervical junction, subaxial cervical spine injury and other rare causes. The HALO was used in these patients to achieve spinal fusion either with medical management or as an adjunct to surgical interventions prior to and after till cervical spine fusion was evident.

Results: Majority of HALO orthoses were for traumatic instability with Occipito-cervical injuries in 45 patients, subaxial spinal injury from C5 to C7/T1 region with facet subluxations &/or 3 column injuries with 21 patients and other etiology in 7 cases. The average duration of use was 2.6 months. Adjunct to surgery in 30/72 cases, primary medical management in 36/72 cases and in failure of medical management in 6/72 cases. The most common complication were pinsite infections in 11/73 cases and a pneumonia in 1/73 cases.

Conclusion: HALO orthoses provides a high degree of safe and restricted cervical immobilization for critical patients with acute cervical spine injury. They are effective in primary medical management of appropriately selected patients and, useful as adjuncts in patients with an unstable cervical spine

Keywords: HALO vest immobilization, cervical trauma, cervical instability

A11 CONCUSSION / MTBI

A11-01

CHANGES AFTER SINGLE AND REPETITIVE MILD TRAUMATIC BRAIN INJURY IN A CLOSED-SKULL WEIGHT DROP MODEL

Javier Allende Labastida¹, Shariq Ali², Nasreen Noor³, Tiffany J Dunn¹, Junling Gao¹, Douglas S DeWitt⁴, Donald S Prough⁴, Ping Wu¹

¹University of Texas Medical Branch, Neuroscience and Cell Biology, Galveston, USA

²UTMB, Pharmacology and Toxicology, Galveston, USA

³UTMB, School of Medicine, Galveston, USA

⁴UTMB, Anesthesiology, Galveston, USA

Mild traumatic brain injury (mTBI) represents 75–87% of the reported 1.4–3.8 million cases of TBI per year. Furthermore, athletes often face the risk of repetitive mTBI and its consequences. However, the model of mTBI for research remains less optimal. In order to obtain a better understanding of mTBI-related disorders, we modified the weight drop model developed by Kane et al. in 2012 by adding a velocity sensor to calculate the force of the injury and monitoring impact variability. Fifteen female C57BL/6 mice (about 2.5 months of age) were randomly assigned into 5 groups: Sham, single mTBI with 95- or 120-gram weight (95×1 and 120×1), or repetitive mTBI with 95- or 120-gram weights (95×5 and 120×5). Weight was dropped from one meter height. For repetitive injuries, animals were hit once daily for 5 consecutive days. Kinetic energy (joules) were calculated: Sham=0, 95×1=0.83±0.007J, 95×5=0.82±0.31J, 120×0=1.06±0J, and 120×5=1.05±0.31J. Elevated plus maze (EPM) test was conducted 3 days post injury and brains were collected on day 4 for immunohistochemical analyses. We observed a significant increases in righting reflex following repetitive mTBI. There was a trend towards increased time spent in the open arm of the EPM that also appeared to be dose-dependent. Finally, we observed alterations in immunoreactivity of GFAP, APP and αSMA in multiple areas of the mouse brains. In conclusion, the 120-gram weight at one meter height is the most consistent and optimal injury for modeling mTBI in our hands.

Support: Studies were completed as part of a team funded by The Moody Project for Translational Traumatic Brain Injury Research, Steve Dunn Foundation, Coalition for Brain Injury Research, CONACYT-COPOCYT and Fundación Marrón Cajiga.

Keywords: Sensor, Kinetic Energy

A11-02

DEFINING THE BIOMECHANICAL AND BIOLOGICAL THRESHOLD OF MURINE MILD TRAUMATIC BRAIN INJURY USING CHIMERA

Asma Bashir¹, Dhananjay Namjoshi¹, Wai Hang Cheng¹, Anna Wilkinson¹, Sophie Stukas¹, Kris Martens¹, Tom Whyte², Zelalem Abebe², Kurt McInnes², Peter Crompton², Cheryl Wellington¹

¹University of British Columbia, Pathology and Laboratory Medicine, Vancouver, Canada

²University of British Columbia, Department of Mechanical Engineering, Vancouver, Canada

CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration) is a recently described animal model of traumatic brain injury (TBI) that primarily produces diffuse axonal injury (DAI) characterized by white matter inflammation and axonal damage. CHIMERA was specifically designed to reliably generate a variety of TBI severities using precise and quantifiable biomechanical inputs in a nonsurgical user-friendly platform. The objective of this study was to define the lower limit of single impact mild TBI (mTBI) using CHIMERA by characterizing the dose-response relationship between biomechanical input and neurological, behavioral, neuropathological and biochemical outcomes. Wild-type male mice aged 4–5 months were subjected to a single CHIMERA TBI using six impact energies ranging from 0.1 to 0.7 J, and post-TBI neurological, behavioral, neuropathological and biochemical outcomes were assessed at 6 h, 1 d, 2 d, 7 d, and 14 d time-points. We report that single TBI using CHIMERA induces injury dose- and time-dependent changes in behavioral and neurological deficits, axonal damage, white matter tract microgliosis and astrocytosis. Impact energies of 0.4 J or below produced no significant phenotype (sub-threshold), 0.5 J led to significant changes for one or more phenotypes (threshold), and 0.6 and 0.7 J resulted in significant changes in all outcomes assessed (mTBI). We further show that linear head kinematics are the most robust predictors of duration of unconsciousness, severity of neurological deficits, white matter injury, and microgliosis following single TBI. Our data extend the validation of CHIMERA as a biofidelic animal model of DAI and establish working parameters to guide future investigations of the mechanisms underlying axonal pathology and inflammation induced by mechanical trauma.

Keywords: CHIMERA, Traumatic brain injury, Animal model of TBI, Behavior, Biomechanics, Neuropathology

A11-03

AGE HAS A GREATER INFLUENCE THAN AMYLOID BURDEN ON THE ACUTE RESPONSE TO MILD TRAUMATIC BRAIN INJURY IN MICE

Wai Hang Cheng¹, Sophie Stukas¹, Kris Martens¹, Dhananjay Namjoshi¹, Emily Button¹, Anna Wilkinson¹, Jerome Robert¹, Peter Crompton², Cheryl Wellington¹

¹University of British Columbia, Department of Pathology and Laboratory Medicine, Vancouver, Canada

²University of British Columbia, Department of Mechanical Engineering, Vancouver, Canada

Incidence of traumatic brain injury (TBI) peaks in young and old individuals, and older age is associated with worse outcome and poorer recovery. TBI is also an established risk factor for neurodegenerative conditions such as dementia and chronic traumatic encephalopathy (CTE), and much remains to be learned about how age at injury affects susceptibility to neurodegeneration. We aimed to delineate how TBI, age at injury, and genetic predisposition to amyloid deposition interact. Repetitive mild TBI was induced in APP/PS1 and wildtype (WT) mice using the CHIMERA model at 6 or 13 months of age, and acute behavioural, histological and biochemical changes were assessed up to 14 days post-injury. We observed impaired post-TBI spatial learning in old but not young mice regardless of genotype, and age-dependent post-TBI changes in Ab deposition in APP/PS1 mice. Age at injury and genotype showed a complex interaction with respect to cytokine and microglial responses, where the neuroinflammatory response is exacerbated in young APP/PS1 but blunted in old APP/PS1 mice, whereas in WT mice the response is sensitized by older age. In conclusion, both age at injury and APP/PS1 genotype modify TBI outcomes, with chronological age at injury being the more robust modifier.

Keywords: Alzheimer Disease, CHIMERA, Aging, Traumatic Brain Injury, Neurodegeneration

A11-04

CONVERGENCE OF UNIQUE PLASMA BIOMARKERS IDENTIFIED IN REPETITIVE MTBI AND AD MOUSE MODELS

Fiona Crawford^{1,3}, Joseph Ojo^{1,3}, Gogce Crynen^{1,3}, Prashanti Vallabhaneni¹, Rosa Ajoy¹, Benoit Mouzon^{1,3}, Moustafa Algamal^{1,3}, Paige Leary¹, Jon M Reed¹, Michael Mullan^{1,3}

¹The Roskamp Institute, –, Sarasota, USA

²James A. Haley Veterans' Hospital, –, Tampa, USA

³The Open University, –, Milton Keynes, United Kingdom

Background: Traumatic brain injury (TBI) is a major cause of disability in the military and civilian population, and for many years has been known to be a major risk factor for Alzheimer's disease (AD). Although the existence of this relationship is well recognized, and the overlap and distinction between pathological features of AD and repetitive mTBI, have long been the subject of reporting and discussion, the precise nature of how TBI leads to or precipitates AD pathogenesis is currently not understood. To address this problem we are generating time-dependent molecular profiles of response to repetitive mTBI and AD pathogenesis in mouse models, using proteomic analyses.

Methods: Herein we use the well-validated hTau and PSAPP (APP/PS1) mouse models that develop age-related tau and amyloid pathological features respectively, and our well-established model of repetitive-mTBI in C57BL/6 mice. Plasma samples from these animals were collected at different ages (3–15 months-old for hTau and PSAPP mice), or at different timepoints after repetitive mTBI (24 hrs–12 months post-injury, encompassing pre-, peri- and post-“onset” of the cognitive and neuropathological phenotypes. Liquid chromatography/mass spectrometry (LCMS) approach coupled with Tandem Mass Tag labeling technology were applied to develop molecular profiles of proteins species that are significantly differentially expressed as a consequence of AD or mTBI.

Results: Mixed model ANOVA after Benjamin Hochberg correction identified 31 proteins changing in mTBI groups over time and 13

of these were specifically unique to the injury groups alone when compared with changes overtime in sham mice. LXR/RXR activation, production of nitric oxide and reactive oxygen species and complement systems were the top canonical systems altered in injury groups compared to sham mice. We identified 18 proteins significantly changing in PSAPP mice and 19 proteins in hTau mice compared to their relative wildtype littermates respectively. There was a convergence and coincidental change in 6 unique proteins identified in all three models i.e. repetitive mTBI, hTau and PSAPP mice compared to their controls.

Conclusion: We believe that this work could help inform future translational studies in humans and to confirm the role of these putative biomarkers of repetitive mTBI and prognostic biomarkers for AD.

Keywords: Traumatic Brain Injury, Biomarkers, Tau, Mouse Models

A11-05

CONCUSSION CLASSIFICATION VIA DEEP LEARNING AND A TWO-STEP CROSS-VALIDATION

Yunliang Cai¹, Wei Zhao¹, **Songbai Ji**^{1,2}

¹Worcester Polytechnic Institute, Biomedical Engineering, Worcester, USA

²Dartmouth College, Thayer School of Engineering, Hanover, USA

Robust and objective prediction of traumatic brain injury (TBI) is important for its clinical management. Previous efforts have largely relied on univariate logistic regression and a single “training” injury dataset to evaluate performance. Recently, we developed a deep learning approach to formulate TBI prediction as a supervised binary classification. In this study, we evaluate the injury classifier performance using a two-step cross-validation procedure and compare with four popular injury metrics: Brain Injury Criterion, BrIC, cumulative strain damage measure for the whole-brain (CSDM-WB) and corpus callosum (CSDM-CC), and peak fiber strain in the corpus callosum (Peak-CC). Reconstructed NFL head impacts (25 concussions and 33 non-injuries) were simulated. Peak white matter fiber strains were generated on diffusion tensor imaging voxels to create a 128×128×65 strain-encoded image. The injury dataset was repeatedly (N=20) and randomly split into non-overlapping training (N=39) and cross-validation (N=19) datasets. For each random trial, a fully-connected, five-layer deep learning network was trained in a supervised manner. The same training dataset was used to fit a logistic regression for other four competing metrics. The trained network and regression models were used to predict TBI using the cross-validation dataset, as well as a separate, independent injury dataset (7 injuries and 1 non-injury; reconstructed from automobile crashes and football) for further cross-validation. Using the first validation dataset, the five injury classifier/metrics had largely comparable accuracy, sensitivity and specificity. However, deep learning had a significantly better average accuracy (0.7829 vs. 0.6062, 0.7375, 0.6125, and 0.675 for BrIC, CSDM-WB, CSDM-CC and Peak-CC, respectively) and sensitivity (0.7519 vs. 0.55, 0.7, 0.5571, and 0.6929 for the other four metrics, respectively), statistically, when using the second independent cross-validation dataset. Deep learning has not been used in TBI biomechanics studies before. Our results suggest that this technique may have an improved injury prediction performance than other state-of-the-art injury metrics. They also highlight the need for independent cross-validation

using fresh/unmet injury datasets for more rigorous comparison of injury prediction performances.

Keywords: concussion, injury prediction, traumatic brain injury, deep learning, cross-validation, fiber strain

A11-06

RECLASSIFYING THE CONCUSSION DIAGNOSIS BY DEGREE OF CERTAINTY

Alan Grusky², **Joshua Kamins**^{1,2}, David McArthur², Christopher Giza²

¹UCLA, Neurology, Los Angeles, USA

²UCLA, Neurosurgery, Los Angeles, USA

The lack of an objective, physiological biomarker to detect concussion has resulted in the injury remaining a clinical diagnosis that relies on subjective symptom-based assessments. Historically, concussion grading scales have classified injury without acknowledgement of diagnostic uncertainty and have not resulted in prognostic value. Clinical certainty based scales for diagnosis are currently used in other neurological disorders such as multiple sclerosis and Alzheimer's disease but no such scale is currently used for concussion. A novel classification system was introduced to the literature in 2014 by Kutcher and Giza, which incorporated degree of clinical certainty into the concussion diagnosis. The objectives of the current study are to (1) adapt and refine this concussion classification system and (2) apply it retrospectively to a cohort of mild TBI patients at the UCLA BrainSPORT concussion clinic to determine if it can predict outcome. The classification system categorizes concussions as definite, probable, possible, or not a concussion based on the following clinical criteria: (a) witnessed mechanism of injury, (b) typical symptoms, (c) time of symptom onset, (d) improving time course, and (e) presence/absence of alternative diagnosis. This classification system was used to retrospectively diagnose 160 patients at the UCLA BrainSPORT concussion clinic from June 2015 to October 2016. Post injury symptom scores were assessed with the Sport Concussion Assessment Tool 3 (SCAT-3) graded symptom checklist scores as well as an "Overall rating score" from 0–100% at the initial clinic appointment. Patients were then contacted at a second time point between 6–12 months post injury at which time the patient's SCAT-3 symptom score and overall rating score were reassessed. Concussions classified as "Definite" had a higher likelihood of symptom resolution (<9 symptom score, or >95% overall rating) at 6–12 months post-injury when compared to probable or possible concussions. These findings suggest that a concussion classification system based on degree of certainty can provide value for clinical management by increasing our diagnostic specificity and assisting in prognostication.

Keywords: Concussion, Diagnosis, Prognosis

A11-07

OBJECTIVE ASSESSMENT OF PSYCHOLOGICAL HEALTH IN CHRONIC TRAUMATIC BRAIN INJURY USING MAGNETOENCEPHALOGRAPHY: A TEAM-TBI STUDY

Donald Krieger, **Benjamin Zusman**, Sue Beers, Ava Puccio, Allison Borrasso, Kathryn Edelman, Jane Sharpless, Michael Collins, Anthony Kontos, Walt Schneider, David O Okonkwo

University of Pittsburgh, Neurological Surgery, Pittsburgh, USA

Background: Magnetoencephalography (MEG) enables objective non-invasive examination of human brain function including corre-

lation of changes in global and regional neuroelectric activation with manifestations of neurological disorders including traumatic brain injury. Through the Targeted Evaluation, Action and Monitoring of Traumatic Brain Injury (TEAM-TBI) study, subjects with chronic TBI were assessed with MEG to identify patterns of tonic brain activation which underlie psychological health symptoms. Recordings from the Cambridge(UK) Centre for Ageing and Neuroscience (CamCAN) lifespan normative cohort served as controls.

Methods: MEG was recorded during rest from a subset of the TEAM-TBI cohort ($n=60$, mean age=34.9, $sd=7.5$, 12 female) and from a subset of the CamCAN cohort ($n=75$, mean age=34.3, $sd=7.5$, 19 female). 25 TEAM-TBI subjects have had a 6-month follow up assessment. Normative patterns of neuroelectric activation were identified in the CamCAN cohort using independent components analysis (ICA). The pattern of each member of both cohorts was assigned scores which quantify the similarities of the individual pattern to the ten most dominant normative patterns. The means of those scores differed between cohorts ($p<0.0001$) for ICA's 1, 3, and 6 and not the other ICA's. These 3 ICA's were therefore used to classify each individual, CamCAN vs TEAM-TBI. They were also tested for correlation against the TEAM-TBI participants' Brief Symptom Inventory (BSI) Depression, Somatization, and Anxiety subscales.

Results: The TEAM-TBI cohort differed from the controls ($p<0.0001$) for ICA's 1,3,6. The best classification was achieved using the scores on ICA#1 and 3: CamCAN, 71/75 correct, (94.6%, $p<0.000001$), TEAM-TBI, 58/60 correct, (96.6%, $p<0.000001$). Correlations were significant ($p<0.01$) for ICA#1/depression and ICA#6 weights/somatization. On 6-month follow up with interventions, 7/25 TEAM-TBI subjects had normalization greater than 0.5 sd .

Conclusions: MEG-derived regional brain activation was associated with elevated depression and somatization in chronic TBI. This supports the hypothesis that TBI can induce organic brain changes which lead to psychological health disorders. In this pilot study, the 6-month interventions were associated with improved MEG metrics in over one fourth of chronic TBI subjects.

Keywords: TBI, MEG, somatization, depression, anxiety, Post Traumatic Stress

A11-08

CORRELATION BETWEEN SYMPTOMS AND MEG-DERIVED BRAIN PATTERNS IN CHRONIC TBI

Donald Krieger, **Benjamin Zusman**, James T Becker, Sue Beers, Ava Puccio, Allison Borrasso, Kathryn Edelman, Jane Sharpless, Michael Collins, Anthony Kontos, Walt Schneider, David O Okonkwo

University of Pittsburgh, Neurological Surgery, Pittsburgh, USA

Background: Magnetoencephalography (MEG) enables objective non-invasive examination of human brain function and correlation of changes in global and regional neuromagnetic activity with manifestations of neurological disorders including traumatic brain injury. Through the Targeted Evaluation, Action and Monitoring of Traumatic Brain Injury (TEAM-TBI) study, subjects with chronic TBI were assessed with MEG to identify patterns of tonic brain activity which are associated with psychological health symptoms. Recordings from the Cambridge(UK) Centre for Ageing and Neuroscience (CamCAN) lifespan normative cohort served as controls. A sample of individuals at risk for HIV disease was included to provide additional statistical power and to assess whether the results may be generalized to other clinical populations.

Methods: MEG was recorded during a task free condition from the TEAM-TBI cohort ($n=62$, mean age=34.8, $sd=7.7$, 12 female), the CamCAN cohort ($n=186$, mean age=49.4, $sd=19.0$, 66 female), and

from an HIV sero± cohort ($n=54$, mean age=51.0, $sd=5.9$, 19 female). The Brief Symptom Inventory (BSI) was administered to the TEAM-TBI and HIV± cohorts+. This provides symptom scores for somatization, depression, and anxiety. For each individual, neuro-electric events were identified from the MEG and localized to one of 155 brain regions. Normative patterns of neuromagnetic activity were identified in the CamCAN cohort using independent components analysis (ICA). The pattern of each member of the TEAM-TBI and HIV± cohorts was assigned scores that quantify the similarities of their individual pattern to the ten most dominant normative patterns. Those scores were tested for correlation with each individual's BSI symptom scores.

Results: Significant correlations were found between the normative pattern scores and somatization and depression symptoms scores for both cohorts and for all three scores for the combined cohorts.

TEAM-TBI, $n=62$: somatization, $r=0.38$; $p=0.009$; depression, $r=0.46$; $p=0.007$

HIV-risk, $n=54$: somatization, $r=0.33$; $p=0.014$; depression, $r=0.49$; $p=0.018$

Combined, $n=116$: somatization, $r=0.2$; $p=0.029$, depression, $r=0.33$; $p=0.002$, anxiety, $r=0.29$; $p=0.006$

Conclusions: Patterns of regional brain activity measured with MEG imaging were associated with elevated depression and somatization in chronic TBI and in an HIV-risk cohort. This supports the hypothesis that symptoms of psychological health are due to departure from normal patterns of global brain activation. Quantified comparison with normative patterns may be useful in measuring the biological severity of these symptom clusters.

Keywords: somatization, anxiety, Post traumatic stress, TBI, MEG, depression

A11-09

EXPRESSION OF TRPM4 INDUCES ASTROCYTE SWELLING BUT NOT CELL DEATH FOLLOWING DIFFUSE MODERATE TRAUMATIC BRAIN INJURY IN THE RAT

Karen Gorse¹, Mary Kate Lantzy², **Audrey Lafrenaye¹**

¹Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

²Glen Allen High School, Glen Allen, USA

Traumatic brain injury (TBI) is a highly prevalent disease that has significant costs. Although progress has been made in understanding the complex pathobiological consequences of contusive TBI, questions still remain regarding the diffuse responses to injury. Recently expression of transient receptor potential melastatin 4 (Trpm4), as a component of the sulfonylurea receptor 1- transient receptor potential melastatin 4 (Sur1-Trpm4) channel, has been linked to cell death during hemorrhagic contusion expansion. However, very little is known about the expression patterns of the Sur1-Trpm4 channel following diffuse TBI, in which hemorrhagic contusions are absent. To explore the expression pattern of the Sur1-Trpm4 channel in diffuse TBI adult male Sprague Dawley rats were subjected to a moderate (2.05 ± 0.05 atm) central fluid percussion injury and survived for 6h-8w. Immunolabeling of the Trpm4 component of the Sur1-Trpm4 channel was assessed over time following injury. Hemotoxylin and eosin labeling was undergone to evaluate cell damage/death correlating to Trpm4 expression following injury. To investigate the cell type expressing Trpm4 following diffuse TBI, immunohistochemistry against the common astrocyte marker, GFAP, and the common microglial marker, Iba-1, was done. Finally, ultrastructural assessments were also performed to evaluate the integrity of Trpm4+ cells and

potential for cellular swelling over time following diffuse TBI. Acutely following TBI Trpm4 expression was restricted to GFAP+ astrocytes within the hippocampus. By 1w following diffuse TBI Trpm4+ astrocytes encompassed the hippocampus, fimbria and aspects of the subcortical white mater. This expression pattern, was observed as chronically as 8w post-injury. Correlative assessments of cell damage/death demonstrated little evidence of hippocampal or subcortical white matter damage (<0.0004 damaged cells/ $100\mu m^2$ of hippocampus at all time points), suggesting that expression of Trpm4 by astrocytes does not precipitate cell death in this model of TBI. Additionally, ultrastructural assessments showed Trpm4+ astrocytes have double the area of somatic cytoplasm ($21.46 \pm 9.70\mu m^2$) as compared to Trpm4- astrocytes ($10.26 \pm 4.60\mu m^2$) at 4weeks post-TBI, suggesting cellular swelling with Trpm4 expression. This work was supported in part by NINDS grants 1R01NS096143 and 5P30NS047463 and the Commonwealth fund.

Keywords: TRPM4, central fluid percussion injury, hippocampus, Confocal microscopy, Electron microscopy

A11-10

CONCUSSION MITIGATION STRATEGIES LOWER IMPACT MAGNITUDES IN HIGH SCHOOL FOOTBALL

Michelle LaPlaca^{1,2}, Kristopher Hendershot², Dipro Chakraborty¹, Kelsey Evans², Lindsay Lee², Alessio Medda⁴, Brian Liu⁴, Russell Gore³, David Wright^{1,2}, Tamara Espinoza²

¹Georgia Tech, Atlanta, USA

²Emory University, Atlanta, USA

³Shepherd Center, Atlanta, USA

⁴GTRI, Atlanta, USA

The potential long-term consequences of repetitive head impacts in football and other collision sports highlight a need for improved concussion mitigation strategies. The purpose of this study was to analyze biomechanical impact variables across two high school football seasons to assess whether the use of a helmet add-on contributes to the reduction of head impact magnitudes. We collected helmet impact data using the Riddell Head Impact Telemetry (HIT) system during games and practices on 72 players, $n=42$ in season 1 and $n=30$ in season 2. During season 2 practices, players wore Guardian helmet pads. A total of 33,023 impacts were recorded; with 16,106 (383.5/player) impacts during practices and 6,178 (147.1/player) impacts during games in season 1, and with 3,884 (129.5/player) impacts during practices and 6,855 (228.5/player) impacts during games in the 2nd season. The total number of impacts decreased significantly for practice sessions from season 1 to season 2, reflecting national recommendations for reduced contact practice time. In season 2 practices there was a 13% reduction in linear acceleration ($p<0.0001$) and a 3.3% reduction in rotational acceleration ($p<0.01$). For games, there was an 8.5% reduction in linear acceleration ($p<0.0001$). Interestingly, between skilled players from two different schools in season 2 (i.e. same rules changes) there was no difference in linear or rotational acceleration with or without Guardian cap. However, in linemen, the Guardian-wearing school had significant reductions in both linear and rotational acceleration. Concussion incidence did not significantly change from season to season. A combination of concussion mitigating strategies (rule changes and use of a helmet add-on) can reduce helmet impact frequency and acceleration, yet more research is needed to assess the clinical significance of lower impact magnitudes. Supported by NFL/GE/Under Armour (Head Health Challenge II) and USMRMC W81XWH12C0203.

Keywords: Concussion, Linear acceleration, Rotational acceleration

THE ASSESSMENT OF VISUAL TASK-RELATED BRAIN ACTIVITY AFTER CONCUSSION USING FUNCTIONAL NEAR INFRARED SPECTROSCOPY (FNIRS)

Eileen Storey¹, Lei Wang³, Hasan Ayaz³, Olivia Podolak¹, Matthew Grady², **Christina Master²**

¹The Children's Hospital of Philadelphia, Surgery, Philadelphia, USA

²University of Pennsylvania Perelman School of Medicine, Pediatrics, Philadelphia, USA

³Drexel University, School of Biomedical Engineering, Philadelphia, USA

Functional near-infrared spectroscopy (fNIRS) is a noninvasive and portable neuroimaging modality that detects changes in blood oxygenation related to human brain function. It is a promising objective biomarker for identifying pathophysiologic changes associated with concussion. The objective of this study was to determine the utility of fNIRS to detect and differentiate cortical brain activity between concussed and healthy subjects when performing oculomotor and visual tasks which has not been previously described. A prospective study of 38 concussed subjects and 9 healthy controls was undertaken where all participants completed standard oculomotor and visual tasks, including the King-Devick test (K-D), while wearing an fNIRS headband that recorded anterior prefrontal cortex oxygenation changes with 4 optodes and 12 channels at 4Hz sampling rate. Linear mixed model analysis was performed to compare oxygenation changes in the two cohorts. Among this pilot cohort, healthy controls showed significantly greater oxygenation changes upon initiation of the King-Devick test compared to concussed subjects, but had decreased oxygenation changes over each successive test card ($F_{1,172}=4.06$, $p<0.05$) compared to injured subjects, indicating a habituation to the task over time in healthy controls that did not occur in concussed subjects. There were also significant differences in spatial patterns of oxygenation changes between the left and right prefrontal cortex between concussion subjects and healthy controls with monocular accommodation assessment ($F_{1,66}=12.05$, $p<0.001$) indicating recruitment of different cortical areas for the task in concussed subjects versus healthy controls. Our preliminary experimental results suggest that fNIRS has utility in detecting differences in cerebral blood oxygenation change between concussed and healthy subjects with excellent temporal and spatial resolution based on the given oculomotor or visual task. Further investigation into this neuroimaging modality for quantifying changes in cognitive workload after injury and over the course of recovery is warranted. fNIRS may be useful as an objective measure of injury and recovery, supplementing the clinical assessment and management of concussion.

Keywords: functional near infrared spectroscopy, vision, oculomotor

A11-12

ABNORMAL ACCOMMODATIVE AMPLITUDE IS ASSOCIATED WITH PROLONGED CONCUSSION RECOVERY IN CHILDREN

Christina Master^{1,2}, Stephen Master³, Douglas Wiebe⁴, Eileen Storey¹, Julia Lockyer¹, Olivia Podolak¹, Matthew Grady¹

¹The Children's Hospital of Philadelphia, Surgery, Philadelphia, USA

²Perelman School of Medicine, Pediatrics, Philadelphia, USA

³Weill Cornell Medical School, Pathology, New York, USA

⁴Perelman School of Medicine, Biostatistics and Epidemiology, Philadelphia, USA

Vision and vestibular deficits are common following concussion. It is unknown, however, which of these findings, may be associated with prolonged recovery. The objective of this study was to determine which vision or vestibular deficits are associated with prolonged recovery in children and to identify interrelationships between such deficits. A retrospective cohort study of pediatric patients with concussion was conducted in a subspecialty concussion program within a large pediatric care network. 432 random patient records were abstracted for data. The presence of vision or vestibular deficits upon initial presentation for clinical care at a subspecialty concussion program was determined and the main outcome measure of interest was time to functional clinical recovery. Study subjects ranged from 5–18 years (median = 14). 378 of 432 (88%) patients presented with one of the following vision or vestibular deficits: saccades (82%), balance (68%), smooth pursuits (66%), vestibulo-oculomotor reflex (VOR) (66%), near point of convergence (35%) or accommodative amplitude (22%). A prior history of motion sickness was associated with vision and vestibular dysfunction. Physical examination deficits associated within distinct vision and vestibular clusters. Abnormal accommodative amplitude predicted prolonged recovery time, as did abnormal balance, VOR, and smooth pursuits. Abnormal accommodative amplitude is associated with prolonged concussion recovery in children, as are deficits in balance, VOR and smooth pursuit function. Vision and vestibular deficits associate within specific clusters. A prior history of motion sickness was associated with vision and vestibular deficits following concussion. Vision assessments in concussion must move beyond visual acuity.

Keywords: vision, oculomotor, vestibular, balance

A11-13

PERFORMANCE OF ICD-10 AND DSM-4 CRITERIA FOR DIAGNOSING POSTCONCUSSION SYNDROME: QUALITY OF LIFE OUTCOMES

Stephen McCauley, Elisabeth Wilde, Emmy Miller, Claudia Robertson, James McCarthy, Harvey Levin

Baylor College of Medicine, Neurology, Houston, USA

This study's objective was to investigate the performance of three standard criteria sets for diagnosing postconcussion syndrome/disorder (PCS/PCD).

Comparability of studies of patients with postconcussional syndrome (PCS) are frequently hampered by nonuniformity of symptoms used to diagnose the disorder. Although the International Classification of Diseases, 10th Edition (ICD-10) has clinical and research criteria for PCS, and the Diagnostic and Statistical Manual, 4th Edition (DSM-4) included provisional criteria for postconcussion disorder (PCD), few if any studies strictly observe these criteria. Consequently, little is known about how these criteria sets perform and compare. To explore this issue, 76 participants with mild traumatic brain injury (mTBI) were recruited from consecutive admissions to two Level 1 trauma centers in Houston, TX. Participants ranged in age from 18–50 years (mean age = 29.8 ± 9.1). Measures included the SF-12 and the Rivermead Postconcussion Symptoms Questionnaire (RPSQ) in addition to specific items addressing all ICD-10 and DSM-4 criteria. Because PCD can only be diagnosed at three months postinjury, data from this end point was analyzed for comparability between all criteria sets (mean = 94.5 ± 10.6 days). Incidence rates varied by criteria set used (ICD-10 clinical = 27.6%; ICD-10 research = 21.1%, and DSM-4 = 11.8%). Analyses between those meeting criteria versus not meeting criteria were conducted and significant differences were found using all three criteria sets for SF-12 Mental Health ($p<.002$ to

<.0001) and Physical Health ($p < .02$ to $<.0007$) Summary scores. Significant differences were found for the total RPSQ score ($p < .0001$) for all three criteria sets.

Although the incidence rates varied widely, all three criteria sets appear to identify a subgroup of patients with mTBI who demonstrate significant symptoms at three months postinjury. There was no evidence supporting the use of one criteria set over the others. This may be elucidated employing different outcome measures.

This work was supported by the Congressionally Directed Medical Research Program by the Department of Defense (W81XWH-08-2-0133, PT074693P2).

Keywords: Post concussion Syndrome, Traumatic Brain Injury, Diagnostic criteria, Quality of Life

A11-14

SYSTEMIC AND CEREBRAL HEMODYNAMICS IN MILD AND MODERATE TRAUMATIC BRAIN INJURY PATIENTS WITH PERSISTENT SYMPTOMS

Max McColloster¹, Takashi Tarumi^{1,2}, Marisara Dieppa¹, Justin Repshas², Marcel Turner², Tammy Lewis², Nasreen Sayed¹, Surendra Barshikar¹, Yuemeng Dai¹, Kathleen Bell¹, Rong Zhang^{1,2}, Kan Ding¹

¹UT Southwestern Medical Center at Dallas, Dallas, USA

²Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas, Dallas, USA

Background: Of the 2.5 million traumatic brain injuries (TBIs) in the US each year, 10–15% of those with mild TBIs suffer from persistent symptoms such as headache and cognitive impairment. These symptoms may be associated with impaired cerebral blood flow (CBF). Cardiac baroreflex sensitivity (BRS) and dynamic cerebral autoregulation (dCA) play an important role in maintaining constant CBF during systemic changes in blood pressure (BP).

Objective: To determine whether cardiac BRS and dCA are impaired in patients with mild-to-moderate TBIs with persistent symptoms.

Methods: Nineteen subjects with blunt head TBI ≥ 6 months prior to the study and persistent symptoms at enrollment (Rivermead score 36.1 ± 12.1) were compared to 19 age/sex-matched healthy control subjects. Subjects performed a repeated sit-stand maneuver while beat-to-beat changes in heart rate, BP, and CBF velocity using transcranial Doppler were measured simultaneously. Hemodynamic variability, dCA, and cardiac BRS were assessed with spectral and transfer function analyses.

Results: Compared to healthy control subjects, TBI patients showed attenuated CBF velocity variability under resting conditions whereas during the sit-stand maneuvers, systolic, diastolic, and mean BP variabilities were augmented (all $P < 0.05$). The dCA gain calculated from the low frequency range (~ 0.1 Hz) was increased in TBI patients at rest (2.37 ± 1.03 vs. 1.67 ± 0.628 %/mmHg, $P = 0.04$) whereas during the sit-stand maneuvers, gain was decreased (1.37 ± 0.42 vs. 1.75 ± 0.582 %/mmHg, $P = 0.083$) compared with control subjects. BRS showed a decreasing trend in TBI patients during sit-stand maneuvers (5.52 ± 3.74 vs. 7.33 ± 3.84 ms/mmHg, $P = 0.20$), but no difference at rest (7.67 ± 5.05 vs. 8.12 ± 3.84 ms/mmHg, $P = 0.77$).

Conclusions: TBI patients have augmented BP variability during sit-stand maneuvers with altered dCA gains compared with age/sex-matched healthy subjects. Our results suggest that TBI patients have a diminished capacity to adjust to hemodynamic variability during postural changes.

Keywords: cardiac baroreceptor reflex, dynamic cerebral autoregulation, blood pressure, hemodynamics, heart rate, cerebrovascular circulation

A11-15

FOAM SELECTION IN CLOSED HEAD ANIMAL TBI MODELS MAY AFFECT INJURY OUTCOMES

Kyle Milligan, Eric Gaupp, Michelle LaPlaca
Georgia Institute of Technology, Atlanta, USA

Traumatic brain injury (TBI) is a leading cause of death and disability that is often studied using animal surrogates. Many closed head injury animal models of TBI place the animal on a foam bed during injury; however, the characteristics of these foams are rarely reported and may contribute to variation in TBI severity between laboratories. In this study, adult male rats were anesthetized, placed on one of two types of foam ($n = 7$ each foam; sham, $n = 6$) without head fixation, and given a mild TBI using a controlled cortical impact (CCI) device (3 m/s, 5 mm displacement, 16 mm diameter padded impactor). The two foams were chosen for their divergent properties: one was soft open-pore foam and the other was firmer and closed-pore. Toe pinch response and righting reflex time were recorded immediately following injury, and brains were sectioned 24 hours post-injury and immunostained for glial fibrillary acidic protein (GFAP) expression. Results indicate that GFAP was more highly elevated in brains injured on the firm foam than in those injured on the soft foam, suggesting a difference in TBI severity based on foam stiffness. There was no GFAP expression observed in sham animals. There was no significant difference in toe pinch or righting reflex time between groups. These results demonstrate that the type of foam used in closed head impact models can affect degree of injury for the same impact conditions; there is therefore a need to report and more fully characterize the foams used. We are currently investigating the injury response using other types of foam and additional outcome measures. Supported by R21 NS091832.

Keywords: animal model, foam, GFAP, TBI, concussion, cortical impact

A11-16

DIAGNOSING THE GOSE: STRUCTURAL AND PSYCHOMETRIC PROPERTIES USING ITEM RESPONSE THEORY

Michael Montie¹, Jana Ranson², Lindsay Nelson², Geoffrey Manley³
¹Medical College of Wisconsin, Psychiatry, Milwaukee, WI, USA

²Medical College of Wisconsin, Neurosurgery and Neurology, Milwaukee, WI, USA

³UCSF, Neurosurgery, San Francisco, CA, USA

The Glasgow Outcome Scale – Extended (GOSE) was designed to assess global functional outcome following traumatic brain injury (TBI). Since its introduction, several empirically founded criticisms of the GOSE have been raised, including poor reliability; an insensitivity to small but potentially meaningful changes; a tendency to produce ceiling effects; inconsistent associations with neurocognitive, psychological, and quality of life measures; and an inability to assess the multidimensional nature of TBI outcome. To distill the structural elements and psychometric properties of the GOSE that may contribute to reported shortcomings, the current study used an Item Response Theory (IRT) approach including one-parameter (1PL, or Rasch)

logistic modeling. Data were from the TRACK-TBI Pilot Study, a large ($N=586$), prospective (3M, 6M, 12M), multisite project that included recommended core common data elements (CDEs) and TBI cases of all injury severity levels. To assess the level of latent functional “impairment” captured by facets of the GOSE independent of its conventional 1–8 rating system, GOSE item responses were dichotomized (1=impairment, 0=no or non-debilitating amount of impairment) and combined into composites representing the seven domains (Consciousness, Independence at Home, Independence Outside of the Home, Return to Work, Social and Leisure Activities, Family and Friendships, and Return to Normal Life). CDEs were then used to predict each of the domains at each time point. Results of IPL analyses indicated that, overall, the GOSE’s items captured impairment across a broad disability spectrum at 3, 6, and 12 months. However, there was evidence of item redundancy (multiple items capturing similar levels of impairment), and a deficiency of items that detect lower levels of impairment to possibly explain ceiling effects. Findings also indicated that different CDEs predict different GOSE domains within and across time. The findings illustrate the value of IRT to illuminate the strengths and weaknesses of clinical outcome assessment measures and provides a framework for future measure refinement.

Keywords: Glasgow Outcome Scale Extended, traumatic brain injury, item response theory, Rasch modeling, common data elements, functional outcome

A11-17

CHANGE IN PSYCHOLOGICAL HEALTH FOLLOWING TARGETED INTERVENTION FOR CHRONIC TRAUMATIC BRAIN INJURY: A TEAM-TBI STUDY

David Okonkwo¹, Allison Borrasso¹, Sue Beers¹, Yuefang Chang¹, Kathryn Edelman¹, Maureen McNiff¹, Walter Schneider¹, Anthony Kontos², Michael Collins², Ava Puccio¹

¹UPMC Presbyterian, Dept of Neurosurgery, Pittsburgh, USA

²UPMC, Sports Medicine, Pittsburgh, USA

Objectives: Traumatic Brain Injury (TBI) is a heterogeneous disorder whose consequences can negatively affect individuals’ physical, cognitive, social, and emotional wellbeing. US military Veterans with TBI have high rates of psychological health sequelae associated with combat exposure that further complicate adjustment and reintegration to civilian life. Civilian and military veterans with chronic TBI were enrolled into Targeted Evaluation, Action, and Monitoring of Traumatic Brain Injury (TEAM TBI) study consisting of a 4-day baseline evaluation to prioritize individualized intervention trajectories during a 6-month intervention phase.

Methods: Thirty-nine participants (veterans $n=26$, male $n=29$, mean age 36), completed self-report psychological health measures at baseline and follow up. Psychological health variables included depression, anxiety, somatization and global severity on the Brief Symptom Inventory (BSI-18), Posttraumatic Stress Checklist (PCL), Satisfaction with Life Scale (SWLS), Alcohol Use Disorders Identification Test (AUDIT), Rivermead Post-concussive Symptom Questionnaire (RPQ) and suicidality. Goal setting was conducted to identify participants’ primary concerns.

Results: Based on general estimating equations (GEE) that controlled for age and compliance, there was a significant effect of group by time interaction for depression ($p=.03$), PCL ($p=.02$), and alcohol consumption ($p=.04$) such that veterans showed greater improvement over time. Both groups showed significant improvement BSI anxiety ($p<.0001$), BSI Somatic ($p=.0002$), BSI Global Se-

verity ($p<.001$), and RPQ ($p<.001$). Suicidal ideation occurred in 26.92% of veterans ($n=7$) and 7.69% in civilians ($n=1$) at baseline and decreased to 11.54% within veterans only ($n=3$) at follow-up. Most common identified goals for veterans were to improve employment status and mood (26.92% equally); improved employment was the most common goal for civilians (28.21%).

Conclusion: While both civilian and military subjects in the TEAM-TBI study showed improvement in psychological health, more significant improvements were found among veterans for depression, PCL, and alcohol consumption. Improvements in psychological health may facilitate increased global functioning following TBI, adjustment post-military discharge, attainment of personal goals, and reduce suicide rates.

Keywords: chronic TBI, brain injury, psychological health

A11-18

TEAM-TBI: A MONITORED MULTIPLE INTERVENTIONAL TBI RESEARCH TRIAL

David Okonkwo¹, Anthony Kontos¹, Kathryn Edelman¹, Allison Borrasso¹, Jane Sharpless¹, Dana Williams¹, Dan Pultz¹, Ava Puccio¹, Steven Benso¹, Matthew Butoryak¹, Ryan Soose¹, Ron Poropatich¹, Sue Beers¹, Nora Presson¹, Anne Mucha¹, Thomas Johnson², Walter Schneider¹, Michael Collins¹

¹UPMC Presbyterian, Dept of Neurosurgery, Pittsburgh, USA

²Intrepid Spirit, Concussion Recovery Center, Camp Lejeune, USA

Objectives: Traumatic brain injury (TBI) is a heterogeneous disorder with numerous clinical challenges for military and civilian populations. TEAM-TBI (Targeted Evaluation, Action, and Monitoring of Traumatic Brain Injury) is a monitored, multiple interventional research trial for chronic TBI that identifies specific clinical profiles and then assigns a sequenced, individualized, evidence-based treatment program, with the primary outcome objective being a decrease in symptom severity.

Methods: Inclusion criteria are: age 18-60, history of TBI with refractory clinical sequelae (Post-Concussion Symptom Scale (PCSS) score >30 more than 6 months post-injury). Participants undergo a 4-day comprehensive evaluation of sleep, mood, vestibular, oculomotor, cognitive, neurologic, and neuropsychological function, as well as advanced neuroimaging studies. A multi-disciplinary case review then identifies clinical trajectories and assigns individualized treatment recommendations. Clinical coaches provide telemedicine support and work with treatment providers for coordination of care and undergo a final in-person re-evaluation after the 6-month intervention phase.

Results: Ninety subjects have been enrolled, of whom 52 have completed the study. Primary trajectories identified have been: Psychological Health ($N=38$), Sleep disorder ($N=24$), Vestibular/Oculomotor disorder ($N=22$), Post-traumatic headaches ($N=3$), and Cognition ($N=1$). Subjects were prescribed targeted interventions only for the clinical consequences of TBI each was experiencing. Mean PCSS score across these subjects decreased from 69.9 at screening to 34.9 (50.1% decline) at 6-month follow-up ($p<0.0001$).

Conclusion: In the first cohort to complete the TEAM-TBI study, a Precision Medicine approach to chronic TBI with iterative, targeted treatments produced significant clinical improvement. Symptom burden decreased, on average, by more than half during the 6-month treatment phase. TEAM-TBI is a paradigm-shifting approach to TBI clinical trials to overcome the limitations of past efforts and achieve long-awaited breakthroughs for TBI survivors. TBI clinical trials must move towards identified clinical subgroups with targeted study interventions.

Keywords: chronic TBI, brain injury, symptoms, neurological outcome

BEHAVIOR, PATHOLOGY, AND GLYMPHATIC SYSTEM FUNCTION IN A MOUSE MODEL OF SINGLE AND REPETITIVE MILD CLOSED-HEAD TBI

Zachary Papadopoulos, Aviva Symes

Uniformed Services University of the Health Sciences, Pharmacology, Bethesda, USA

Generally, closed head injury models of TBI result in fewer measurable behavioral and other functional deficits. Repetitive injuries, in addition to potential modeling of injuries in military personnel or athletes, often result in a more significant phenotype. Here, we describe a novel model utilizing repeated impacts on a closed head in male mice that produces substantial behavioral deficits and pathology. In the repeat-injury group, four moderate impacts to the head were performed, with a 24 hour interval between each impact. The single injury group only received one such injury. Repeat-injured mice exhibited hyperactivity and disinhibition in the open field test, social anhedonia in the social interaction maze, and abnormal nesting behavior. No major motor deficits were noted in either model. Behavioral changes were measurable for more than three weeks after injury. Different cohorts were sacrificed at 7, 28, or 56 days post-injury (dpi). Glymphatic function was measured by the distribution of fluorescent tracer injected into the cisterna magna 30 minutes before sacrifice. The distribution of astrocytic aquaporin-4 in the perivascular endfeet was also examined. We found that in injured animals, the tracer had restricted distribution within the brain parenchyma in comparison to that in sham injured animals. Immunohistochemistry revealed primary foci of injury directly under the impact as well as a few secondary discreet injury sites in the single impact model and more widespread pathology in the repeat injury model. Glial pathology from the single injury was largely resolved by 56dpi however astrocytes and microglia were still activated in the repeat injured animals at 56dpi. Fluoro-Jade C staining showed significant neuronal degeneration at 7dpi. Our data show that the repeat closed head injury results in significant behavioral deficits that are present several weeks after injury, together with reduced glymphatic movement of tracer, differential aquaporin 4 expression, increased neuronal degeneration and enhanced glial reactivity. Measurement of glymphatic system function, and nesting behavior are somewhat novel functional indicators after TBI that show deficits in this injury model. This model may therefore be useful for understanding interventions to treat closed head injury.

Keywords: glymphatic, nesting, repeat, repetitive, closed-head, CHI

A11-20

EVALUATION OF ENVIRONMENTAL SENSORS IN MILITARY TRAINING ENVIRONMENTS: PRELIMINARY RELATIONSHIPS BETWEEN EXPOSURE AND OUTCOME

Tyler Rooks, Amanda Kelley, Brad Erickson, Colby Matthews, Carol Chancey

USAARL, Fort Rucker, USA

Given the prevalence of and attention to TBI in both military combat and training environments, the DoD has prioritized identifying and/or developing sensory technologies to detect and quantify head impact exposures. Subsequently, the Environmental Sensors in Training (ESiT) program was developed to evaluate an environmental sensor's (ES) utility to identify potentially concussive events occurring in military-training environments. A portion of the program evaluated

DoD and commercially developed ES in the Basic Airborne Course (BAC). The primary objective is to examine the relationships between sensor measurements and changes in performance on neurocognitive, non-postural dynamic balance/sensory integration, reaction time, and vestibular/oculomotor tasks immediately and at delayed time points following head impacts. Participants were instrumented with multiple ES to record head impact events exceeding specified thresholds. Participants were videotaped during the drills to visually identify head impact events. Participants completed a series of tasks administered on a handheld device comprised of an enhanced virtual reality display, noise-reducing headphones, and a handheld controller. The device effectively provides an immersive environment for multimodal neurologic assessment in remote, noisy, or distracting environments. Additionally, participants completed the MACE, the PCL-M (military version), and a concussion history questionnaire. Testing occurred at in-processing to obtain a baseline and the end of each training week (progressively greater risk for head impact) for a total of four testing sessions. Complete datasets were available for 15 of 20 volunteers (1 female and 14 male). The majority of the participants indicated no prior history of concussion (90 percent), and all participants scored below the accepted cut-off criterion for the PCL-M, suggesting a healthy sample relative to PTSD and concussion. Results of the MACE tests suggest no changes in performance over the course of the training despite the occurrence of head impacts during drills. If results of the functional performance tests show sensitivity to head impacts, given the different time points for assessment, the tests may prove more advantageous for use in the training environment.

Keywords: concussion, wearable sensor, sensor, biomechanics

A11-21

EVALUATING THE ABILITY OF HEAD INJURY CRITERIA TO PREDICT CONCUSSION THROUGH RECONSTRUCTIONS OF COLLEGIATE FOOTBALL HEAD IMPACTS

Bethany Rowson, Steven Rowson, Stefan Duma

Virginia Tech, Biomedical Engineering and Mechanics, Blacksburg, USA

Head injury criteria are used in automotive and sports protective equipment safety standards. The standards currently in use are based on linear head acceleration during impact, and have greatly reduced the number of fatal head injuries in automobile accidents and contact sports like football. However, a large body of research suggests that both linear and rotational acceleration contribute to brain injury mechanisms, and a combination of kinematic parameters may better predict injury risk than a single parameter alone. In order to compare the predictive capabilities of different head injury criteria, we reconstructed head impacts in collegiate football players in a laboratory setting. A total of 11 impacts resulting in concussion and 44 not resulting in concussion were reconstructed. The field impacts were collected with helmet-mounted accelerometers, which compute impact location, peak linear acceleration, and peak rotational acceleration. The laboratory reconstructions were performed to determine the 6-degree-of-freedom linear and rotational kinematics needed to calculate all head injury criteria of interest. The impacts were reconstructed with a pneumatic ram striking an instrumented head and neck assembly fitted with a matched helmet for the field impact. Head kinematics from the reconstructions were used to calculate 10 different head injury criteria. These criteria used either linear kinematics, rotational kinematics, or a combination of both. Receiver operating characteristic (ROC) curves were used to evaluate the predictive capability of all criteria. For all criteria evaluated, the area under the

curve (AUC) was significantly better than random guessing ($p < 0.0007$). Additionally, criteria that included both linear and rotational kinematics performed better than those that only included one or the other. The results of this study have applications toward evaluation of protective equipment to better predict concussion risk and further reduce those injuries.

Acknowledgments: The authors would like to acknowledge the UVa-VTC Neuroscience Seed Fund Program for their support of this research.

Keywords: Acceleration, Linear, Rotational, Risk

A11-22

INTERNAL JUGULAR VEIN COMPRESSION: THINKING OUTSIDE OF THE BOX FOR CONCUSSION, BLAST TBI, AND BLAST INDUCED HEARING INJURY

Brian Sindelar

University of Florida, Department of Neurosurgery, Gainesville, USA

A traumatic brain injury, TBI, is due to acceleration of the head leading to relative motion of the brain parenchyma within the skull causing both macroscopic (hemorrhage) and microscopic injury (diffuse axonal injury). A novel approach to reducing this movement has been proposed through internal jugular vein compression (IJVC). The focus of this presentation will be to present the mechanism and theory behind IJVC and its resultant positive benefits in blunt, blast, and sports related TBI. A historical perspective reviewing the previously performed preclinical rodent models where IJVC demonstrated a reduction in APP, microscopic injury, post blunt injury in a rodent drop weight model (Smith et al., 2012; Turner et al. 2012) and parenchymal hemorrhage, macroscopic injury, in a swine cortical impact model (Sindelar et al. 2016). We will then discuss the two clinical studies demonstrating a reduction in axonal injury seen on DTI in subconcussed high school hockey and football athletes (Myer et al 2016).

Beyond the use in blunt TBI, IJVC has been suggested for use also in blast induced TBI. Due to the compensatory properties of the intracranial space, IJVC results in mild increases in intracranial pressure, but its resultant influence on other structures that communicate with this space, like the cochlea, has only begun to be researched. The discussion will then conclude with the recently completed study demonstrating the significant effects of IJVC specifically in audiological injury following exposure to a blast wind. The author exposed 20 Sprague Dawley rats to a 16.8 ± 0.3 PSI (195.3 dB SPL) right sided shock wave in which 10 had application of a custom IJVC collar in place at the time of injury. IJVC was shown to reduce the incidence of tympanic membrane rupture, initial temporary and permanent threshold shifts on otoacoustic emissions (OAE), and also a significant reduction in hair cell loss at the base of the cochlea secondary to mechanical trauma from the blast wind.

Keywords: internal jugular vein compression

A11-23

AFFECTIVE PROFILING FOR ANXIETY-LIKE BEHAVIOR

Jonathan Statz^{1,3}, Richard McCarron^{1,2}, Jessica Goodrich^{1,3}, Stephanie Ciarlone^{1,3}, Melissa Mehalick¹, Stephen Ahlers¹, Peter Walker¹, Anna Tschiffely¹

¹Naval Medical Research Center, Silver Spring, USA

²Uniformed Services University of Health Sciences, Bethesda, USA

³Henry M. Jackson Foundation, Bethesda, USA

Traumatic brain injury (TBI) is a frequent injury in military conflicts, often caused by exposure to overpressure (shock) waves from devices such as improvised explosive devices. Studies indicate high rates of co-morbid mTBI and posttraumatic stress disorder (PTSD). Specifically, Ahlers et al. demonstrated blast-exposed rats display PTSD-like behavior, indicating possible relationships between blast and PTSD development. However, not every blast-exposed subject will display this behavior and may display phenotypes similar to controls. Therefore, including animals from experimental groups that do not appear anxious may obscure results that would demonstrate differences due to blast. To account for this, normal and abnormal behaviors can be determined from the control group, and the experimental group can be split into those displaying normal behaviors ("unaffected") and anxiety-like behaviors ("affected"). These subgroups can then be compared separately to controls for a better understanding of changes that blast exposure may cause in behavior and molecular variables.

Data sets consist of molecular variables (e.g., blood or protein biomarkers) and behavioral data that identify the "affected" group. Using an affective profiling approach introduced by Richter-Levin and his colleagues, control animal behavioral data was analyzed to determine boundary values for normal behavioral results. These boundary values were then applied to the experimental group's behavioral data, and animals whose behavior fell outside this range were separated into an "affected" experimental group; remaining animals were labeled "blast-unaffected". Using these procedures, data for molecular variables were then compared.

When this method was used on test data containing behavioral data from an elevated zero maze and stathmin and corticosterone levels, clear differences in the average values between "affected" and "unaffected" groups were present. This method shows promise as another method to study effects of blast, while accounting for animals whose data may obscure results due to similarities to control animals.

Keywords: behavior, animal model, PTSD, biomarkers

A11-24

SERUM BIOMARKER CHANGES ARE NOT ASSOCIATED WITH HEAD IMPACT EXPOSURE IN HIGH-SCHOOL FOOTBALL ATHLETES

Jacob R. Joseph², **Jennylee Swallow**¹, Andrew P. Lapointe¹, Kylene Willsey³, Paul Park², Nicholas Szerlip², Steven P. Broglio¹

¹University of Michigan, Kinesiology, NeuroTrauma Research Laboratory, Ann Arbor, USA

²University of Michigan, Neurosurgery, Ann Arbor, USA

³University of Michigan, Pediatrics, Ann Arbor, USA

Introduction: Previous studies indicate a subset of athletes without clinically diagnosed concussion have evidence of neurophysiological deficit on imaging studies. In this study, we aim to determine if there are changes in serum biomarkers associated with neuronal or axonal damage in nonconcussed athletes with repeated head impact exposure. We further seek to determine if these changes are correlated with helmet accelerometry metrics.

Methods: Varsity high school football players (N=16, mean age 16.6y, mean height 183cm, mean weight 94kg) underwent preseason and postseason serum biomarker testing for tau, neurofilament light(NF-L), glial fibrillary acidic protein(GFAP), ubiquitin carboxy-terminal hydrolase L1(UCH-L1), and spectrin breakdown products (SBDP). Athletes were monitored using the Helmet Impact Telemetry System (HITS) at all practices (n=36) and games (n=9) Cumulative hit count, cumulative linear acceleration (cLA), and cumulative rotational acceleration (cRA) were calculated. Three athletes were excluded due to diagnosed concussion. Paired sampled t-tests evaluated differences

between groups. Bivariate Pearson correlations were performed on absolute changes in biomarkers and accelerometry metrics.

Results: Twelve nonconcussed athletes met criteria for inclusion. Mean(\pm SEM) preseason values in pg/mL were: tau= 0.88 ± 0.09 ; NF-L= 5.05 ± 0.76 ; GFAP= 39.85 ± 10.05 ; UCH-L1= 230.3 ± 56.3 SBDP= 23.3 ± 4.3 . A 64.7% increase in serum levels of tau (1.45 ± 0.19 , $p=.003$) and a 62.6% increase in UCH-L1 (374.5 ± 66.5 , $p=.026$) were seen at the end of the season. There were no significant changes in NF-L, GFAP, or SBDP. Mean number of head impacts was 440.8 ± 20.3 , mean cLA was 1.17×10^4 g, and mean cRA was 5.28×10^5 rad/s². No significant correlations between accelerometry metrics and changes in serum biomarkers were seen ($p's > 0.05$).

Conclusion: Even in absence of a diagnosed concussion, high-school football athletes have evidence of neuronal and axonal injury over the course of a season as determined by serum biomarkers. These changes are not related to the cumulative impact or acceleration burden accrued over the course of the season.

Keywords: Concussion/ mTBI, Biomarker, Accelerometry, Pediatric

A11-25

REDUCTION OF LARGE CORTICOSPINAL MOTOR NEURONS PRECEDES THE INFLAMMATORY RESPONSE AND UPREGULATION OF P-TAU AFTER REPEAT MILD TBI

Gretchen Thomsen, Noell Cho, Mor Alkaslasi, Navpreet Dhillon, Patricia Haro, Galinos Barmparas, Eric Ley
Cedars-Sinai Medical Center, Regenerative Medicine Institute, Los Angeles, USA

Few experimental models attempt to recapitulate the progressive brain atrophy and tau pathology that defines human chronic traumatic encephalopathy (CTE). The absence of stringent diagnostic tools together with variable disease progression limit the understanding of this disease. Here, we use a recently described novel rat model of CTE to examine temporal changes in cortical neuron loss, inflammation, and hyper-phosphorylated tau (P-tau) following repeat concussion.

Methods: Mild, bilateral TBI was administered to rats at post-natal day (p) 60 once weekly for either 2 or 5 weeks. Sham and TBI rats were euthanized at acute (p90), short (p144) or long (p235) time points. TBI rats were classified into "mild" or "severe" injury groups based on rotarod performance. Brain tissue was collected for stereological analysis to quantify large layer V corticospinal motor neurons (CTIP2+ cells >300 μ m), inflammatory cells (Iba1), and P-tau (AT8) over time.

Results: TBI rats with mild rotarod deficits did not demonstrate a significant reduction in large corticospinal motor neurons at any time following injury. However, those with severe deficits showed a significant loss in large corticospinal motor neurons at the acute time point, which was sustained over time. This loss of motor neurons preceded both the significant cortical inflammatory response and up-regulation of cortical P-tau that was evident in both severity groups at both short and long time points, but not at the acute time point.

Conclusions: Following repeat concussion, animals that displayed severe functional deficits exhibited a significant reduction in large corticospinal motor neurons that preceded the microglia response and the upregulation of P-tau. This suggests that early targeting of neuronal cell damage could be beneficial to altering the progression of CTE caused by repetitive head injury. By continuing to elucidate the underlying mechanisms of this disease, appropriate treatment strategies can be developed and applied.

Keywords: rat model, inflammation, neuronal loss, corticospinal motor neurons

A11-26

PRE-INJURY SOMATIC COMPLAINTS AND NEGATIVE EMOTIONALITY PREDICT SYMPTOM RECOVERY AFTER SPORT-RELATED CONCUSSION

Alexa Wild, Jana Ranson, Michael A. McCrea, Lindsay D. Nelson
Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

It is increasingly being recognized that athletes vary in their recoveries from sport-related concussion (SRC) and that pre-morbid individual patient factors play a role in their risk for persistent post-concussive symptoms. We previously published evidence that higher pre-injury somatic symptoms prolong symptom recovery via their influence on acute post-concussive symptom burden. This study aimed to replicate this previous finding and additionally examine the degree to which broad personality traits, shown to predict other health outcomes, contribute to symptom presentation and recovery after SRC. High school and collegiate football athletes (N=917) completed preseason evaluations. Athletes who sustained a concussion during the season (n=62) completed serial post-injury assessments (<6 and 24–48 hours; 8, 15 and 45 days). Cox proportional hazard modeling was conducted to predict symptom duration (in days) from various pre-injury and acute (<48-hour) post-injury demographic, injury, and clinical assessment variables. The best overall predictors of symptom duration were neurologic complaints (Minnesota Multiphasic Personality Inventory-2-Restructured Form, MMPI-2-RF, NUC scale score), somatic complaints (MMPI-2-RF RC1 scale score), and post-concussive symptom burden (Sport Concussion Assessment Tool-3 symptom severity) assessed within 48 hours of injury ($p's < .009$). Additionally, path analyses indicated that both pre-injury somatic complaints and trait negative emotionality (Multidimensional Personality Questionnaire, MPQ) contribute to prediction of symptom duration by exacerbating acute post-injury head pain and neurologic symptoms. The findings replicate previous reports that pre-injury somatic symptoms affect clinical recovery from SRC and point to negative emotionality as an additional individual difference construct with relevance to concussion recovery. These data align with a broad literature pointing to the predictive value of negative emotionality for diverse health outcomes. Clinicians and athletic staff could use these findings to identify and focus treatment efforts on concussed athletes who are at higher risk of prolonged recoveries. Future work should investigate associations between these pre-injury characteristics and neurobiological outcomes and develop early interventions to maximize the recovery of athletes at high risk of persistent symptoms.

Keywords: High School and Collegiate Football, Sport related concussion, Personality, Somatic Complaints, Recovery

A11-27

PHASES OF HEMODYNAMIC RECOVERY AFTER MILD TRAUMATIC BRAIN INJURY

Corey Thibeault¹, **Seth Wilk**¹, Samuel Thorpe¹, Michael O'Brien¹, Nicolas Canac¹, Mina Ranjbaran¹, Ilyas Patanam¹, Mateo Scheidt¹, Danielle Seth-Hunter¹, Leo Martinez¹, Ramon Diaz², Robert Hamilton¹
¹Neural Analytics, Los Angeles, USA
²University of Pennsylvania Perelman School of Medicine, Department of Neurology, Philadelphia, USA

The lack of biomarkers which measure specific pathophysiologic mechanisms has hindered the development of improved therapeutic

and diagnostic tools for managing traumatic brain injuries (TBI). Recent research has identified abnormalities in cerebral blood flow and it is clear that the microvasculature is prominently affected after TBI, including mild TBI (mTBI). Detailed understanding of hemodynamic alterations following mTBI has the potential for providing an objective diagnostic aid as well informing the design of therapeutic trials aimed at cerebrovascular dysfunction. In this study, we assessed cerebral hemodynamic changes in high school sports participants 14–19 years old after suffering clinically diagnosed mTBI using transcranial Doppler (TCD). Cerebral blood flow velocity of mTBI subjects ($n=109$, age 16.2 years (1.16)) were compared to age matched controls ($n=70$, age 16.1 years (1.56)). We identified two phases of hemodynamic change after concussion not previously reported. During the first 48 hours, Pulsatility Index (PI), Resistivity Index, and peak ratio (P2R), showed a significant difference from the controls ($U=-3.10$; $P<0.01$, $U=-2.86$; $P<0.01$, and $U=2.62$; $P<0.01$, respectively). In addition, PI and P2R were not correlated ($r_p=-0.36$; $P=0.23$). After 48 hours post-injury, differences in pulsatile features were no longer observable. However, breath holding index (BHI), a measure of cerebral vascular reactivity, was significantly increased after concussion. BHI was measured 2–3, 4–5, and 6–7 days post-injury with $U=2.72$; $P<0.01$, $U=2.46$; $P=0.014$, and $U=2.38$; $P=0.018$, respectively. Measurements were repeated for 30 days following injury and longitudinal results will be presented. Understanding of the multiple phases of hemodynamic recovery after mTBI may provide further insight into the initial mechanical injury and subsequent recovery.

Keywords: transcranial Doppler

A11-28

A BIOFIDELIC MOUSE MODEL OF IMPACT CONCUSSION THAT RECAPITULATES ACUTE-SUBACUTE BRAIN PATHOLOGIES ASSOCIATED WITH MILD TBI AND EAR

Mark Wojnarowicz^{1,2}, Olga Minaeva^{1,2}, Andrew Fisher², Erich Franz², Ning Hua¹, Juliet Moncaster¹, Bertrand Huber^{1,3}, Bruce Lamb⁴, Alon Friedman^{5,6}, Patric Stanton⁷, Ann McKee^{1,3}, Lee Goldstein^{1,2}

¹Boston university school of medicine, Boston, USA

²Boston University College of Engineering, Boston, USA

³VA Boston Healthcare System, Boston, USA

⁴Cleveland Clinic, Cleveland, USA

⁵Dalhousie University, Halifax, Canada

⁶Ben-Gurion University at the Negev, Beersheba, Israel

⁷New York Medical College, Valhalla, USA

The mechanisms underpinning impact concussion, traumatic brain injury (TBI), and chronic traumatic encephalopathy (CTE), and the relationships between these disorders, are poorly understood. We examined postmortem brains from teenage athletes in the acute-subacute period after mild closed-head impact injury and found astrogliosis, myelinated axonopathy, microvascular injury, perivascular neuroinflammation and phosphorylated tau pathology. We developed a mouse model of impact concussion that uses momentum transfer to induce traumatic head acceleration in non-anesthetized C57BL/6 and Ccr2RFP/Cx3cr1GFP mice. Experimental head injury recapitulated brain pathologies associated with mild TBI and early CTE in humans. Unilateral closed-head injury resulted in ipsilateral neuronal dropout, neuroinflammation, and traumatic microvascular injury with serum albumin extravasation that was detectable in living mice by dynamic contrast-enhanced MRI. Delayed microgliosis and infiltrating myeloid cells were detected by flow cytometry

and confirmed by fluorescence microscopy in Ccr2RFP/Cx3cr1GFP mice. Phosphorylated tauopathy was detected in ipsilateral cortex by 24 hours post-injury and progressed to both hemispheres where tau pathology persisted for at least 5.5 months post-injury. These pathologies were accompanied by persistent bilateral impairments in hippocampal axonal conduction and defective long-term potentiation (LTP) in the prefrontal cortex. TBI and CTE brain pathologies and neurophysiological defects did not correlate with neurobehavioral measures of acute concussion. Closed-head impact injury, independent of concussion signs, can induce mild TBI and trigger early brain pathologies and functional sequelae associated with CTE.

Acknowledgments: NIH, VA, DoD, Private Foundation, Concussion Legacy Foundation, WWE.

Keywords: Animal Modeling, Tau Protein, Microvascular Injury, Neuroinflammation

A11-29

PROSPECTIVE ASSESSMENT OF CONCUSSION SCREENING IN THE EMERGENCY DEPARTMENT

David Wright¹, Rachel Koval Patzer¹, Timothy Moran¹, Jasmine Moore³, Daniel Wu², Jonathan Ratcliff¹

¹Emory University, Emergency Neurosciences, Atlanta, USA

²Emory University, Emergency Medicine, Atlanta, USA

³Grady Memorial Hospital, Trauma Program, Atlanta, USA

The goal of this study was to describe current concussion screening, diagnosis, and management patterns in the emergency department (ED) of a large urban county hospital. This pilot was part of a larger initiative, *Screen-Inform-Prevent*, which was developed by a national multidisciplinary workgroup to improve concussion identification in the ED setting.

We implemented a prospective screening tool embedded within the electronic medical record and then examined the number of patients who received a concussion diagnosis, corresponding ICD-10 code, and discharge instructions. Clinicians were not alerted to the results of the initial screen. Chart review was used to document specific elements in the physician note and the ICD-10 codes assigned. Injury mechanism and complex (i.e. isolated head injury vs. multiple injuries) predicted whether or not a patient received a diagnosis of concussion. Over half of the population that screened positive for concussion did not have any elements of a concussion exam documented.

Of the 98 patients who screened positive, concussion was included in the differential diagnosis for 45 patients and in the final diagnosis for 36 patients; 15 patients received discharge instructions, and 6 patients received a concussion ICD-10 code. Patients with and without a concussion diagnosis were primarily distinguished by their injury complex (i.e. isolated head injury vs. multiple injuries), whether a head CT was ordered, and whether concussion was included in the differential diagnosis. Similarly, patients who received discharge instructions were distinguished by their injury complex (i.e. isolated head injury vs. multiple injuries).

Our study provides additional support that ED patients with likely concussion do not receive an examination necessary to identify a concussion, and of those diagnosed, half did not receive concussion-specific discharge instructions. Improved recognition and patient education has the potential to prevent and reduce concussion morbidity. More work is needed to improve concussion screening and evaluation in the ED setting.

Keywords: Screen, Inform, Prevent, Concussion

MULTI-DIRECTIONAL DYNAMIC INJURY METRIC FOR MILD BRAIN TRAUMA DETECTION

Kaveh Laksari¹, **Lyndia Wu**¹, Taylor Nguyen¹, Mehmet Kurt², Michael Fanton³, Jesse Ruan⁴, Saeed Barbat⁴, David Camarillo^{1,3}

¹Stanford University, Bioengineering, Stanford, USA

²Stevens Institute of Technology, Mechanical Engineering, Hoboken, USA

³Stanford University, Mechanical Engineering, Stanford, USA

⁴Ford Motor Company, Dearborn, USA

Background: Mild traumatic brain injury (mTBI), or concussion, has received heightened awareness due to its devastating effects on professional athletes, military personnel and more broadly general public. Mounting evidence suggests heightened risk of chronic neurodegeneration with repeated mTBI. Return to play guidelines and legislations try to protect athletes from repeat trauma, which is the implicated cause of long term brain damage. Despite increasing awareness of mTBI, timely diagnosis and prevention of repeat injury are difficult due to a lack of understanding of injury mechanisms.

Methods: We included human injury (49) and non-injury (1140) datasets from multiple loading regimes, including football head impacts, soccer headers, voluntary head motions and car accidents. We investigated multiple established kinematics injury metrics, and also used a novel under-damped lumped-parameter model in 3 dimensions to represent the brain-skull interface, which was validated finite element simulation results.

Results and Discussion: We fitted a multi-regression logistic model to classify the injured and non-injured kinematics, and we compared the performances using deviance, area under the receiver-operating curve. Various metrics fared differently in different loading conditions of loading magnitude and duration, therefore a model accounting both head kinematics and brain-skull dynamics showed superior classifying performance.

Conclusion: In this study, we used a combination of datasets from contact sports, voluntary experiments and car accidents to develop a lumped-parameter multi-directional metric that takes into account both the head kinematics and skull-brain dynamics. We formally compensated for the effect of incidence rate in contact sports in developing the metric and showed that it significantly affects the injury threshold. We showed that current government endorsed metrics such as BrIC perform reasonably well in the high angular velocity regimes (longer durations) but not as well in high acceleration regimes (shorter durations).

Keywords: Concussion Diagnostic, Injury Criteria, Reduced Order Modeling

A11-31

TRAUMATIC OPTIC NEUROPATHY FROM CONCUSSIVE BRAIN INJURY IN THE MOUSE: INSIGHTS INTO MOLECULAR MECHANISMS

Leyan Xu¹, Jiwon Ryu¹, Amit Patel², Santiago Gonzalez¹, Nikolaos Ziogas¹, Don Zack², Derek Welsbie², Vassilis Koliatsos¹

¹Johns Hopkins University, Pathology/Division of Neuropathology, Baltimore, USA

²Johns Hopkins University, Ophthalmology, Baltimore, USA

We have recently established a model of primary multifocal axonopathy in the mouse after diffuse TBI using impact acceleration (IA) (Xu et al, 2016). Among the various CNS tracts involved, the visual system was greatly prominent, with IA dose -dependent retinal ganglion cell (RGC) axonal and perikaryal degeneration and with neuropathy-related neu-

roinflammation. This model can serve not only to replicate the specific conditions of traumatic optic neuropathy but also to more broadly model the problem of traumatic axonal injury because of the well-defined origin, course, and termination of the optic tract and the accessibility of RGCs to experimental manipulations. Here we delve further into molecular mechanisms operating in traumatic optic axonopathy after IA, with focus on the dual leucine zipper kinase (DLK)-jun terminal kinase (JNK) axis. Using relatively mild IA settings, we performed a time course of changes in expression of key members of the DLK-JNK axis and we then began to delete specific members of the axis and explore outcomes. By immunohistochemistry and Western, we found that phosphorylation of c-jun in RGCs increased significantly at day 1 and 3 and started to drop at day 7; similar trends were observed with other members of the DLK-JNK axis. Using gamma-synuclein as marker of RGCs, we found a significant RGC loss and optic nerve degeneration at 14 and 28 days after injury (19% and 37% cell loss, respectively). A CTB pulse in retina was blocked in the distal third of the optic nerve when chased at day 2 but appeared to have recovered after day 14. We are presently exploring the effect of deleting dlk expression with Cre-lox recombination strategies on c-jun phosphorylation on day 3 post-injury. We are also exploring the effect of deleting both dlk and lz on the survival of RGCs 8 weeks post-injury. We propose that traumatic optic neuropathy after IA is a powerful model for traumatic axonopathy and, because of the accessibility of the retina to genetic and pharmacological manipulations, it is especially appealing for exploring molecular mechanisms of traumatic degeneration, including the role of mixed-lineage kinases.

Keywords: concussive injury, traumatic optic neuropathy, apoptosis, axonal injury

A11-32

AUTOMATED DECISION SUPPORT SYSTEM TO DETECT AND EVALUATE HEMATOMA IN PATIENTS WITH ACUTE TRAUMATIC BRAIN INJURY

Heming Yao¹, S.M. Reza Soroushmehr^{2,3}, Craig Williamson^{2,4}, Negar Farzaneh^{1,2}, Kevin Ward^{2,3}, Kayvan Najarian^{1,2,3}

¹University of Michigan, Department of Computational Medicine and Bioinformatics, Ann Arbor, USA

²University of Michigan, Michigan Center for Integrative Research in Critical Care, Ann Arbor, USA

³University of Michigan, Department of Emergency Medicine, Ann Arbor, USA

⁴University of Michigan, Departments of Neurosurgery and Neurology, Ann Arbor, USA

Traumatic brain injury (TBI) is a massive public health problem worldwide. Approximately 1.5 to 2 million Americans sustain a traumatic brain injury each year with the lifetime and total costs associated with these injuries estimated at \$60 billion. Accurate and fast automatic detection of hematoma in brain are important for TBI diagnosis, which can efficiently decrease the medical cost, reduce the error from visual examination and provide guidance for appropriate medical treatment. The evaluation of hematoma regions is also important for epidemiologic studies and agent efficacy estimation. In this study, we developed an automated system to detect and segment hematoma regions in head Computed Tomography (CT) images of patients with acute TBI. Our proposed method has three main stages as follows. 1) Pre-processing, 2) Feature extraction and 3) Training a learning model. We first process head CT images to extract regions of interest and decrease noises and variances among patients. Then, we extract clinically relevant features from CT images as well as statistical, texture, and geometrical features to capture characteristics of

hematoma regions. After that, we combine uncertainty-based active learning strategy to adaptively select the most samples to train a SVM classifier. In this study, we used CT images of 40 patients from Treatment of Traumatic Brain Injury Experimental Clinical Treatment Trial (ProTECT) and our final SVM model achieved the area under curve (AUC), specificity and sensitivity of 0.909, 0.674, and 0.985, respectively. Our results show that the proposed automatic hematoma detection system can overcome the challenge of the limited, highly unbalanced and varied dataset.

Keywords: acute TBI, image processing, segmentation, active learning

A11-33

AUTOMATED DECISION SUPPORT SYSTEM TO QUANTITATIVELY EVALUATE THE VOLUME OF SUBDURAL HEMATOMA IN TRAUMATIC BRAIN INJURED PATIENTS

Negar Farzaneh^{1,2}, S.M. Reza Sorousmehr^{2,3}, Craig Williamson^{2,5}, Cheng Jiang⁴, Ashok Srinivasan⁶, Jayapalli Bapuraj⁶, **Heming Yao**¹, Kevin Ward^{2,3}, Frederick Korley³, Kayvan Najarian^{1,2,3}

¹University of Michigan, Department of Computational Medicine and Bioinformatics, Ann Arbor, USA

²University of Michigan, Michigan Center for Integrative Research in Critical Care University of Michigan, Ann Arbor, USA

³University of Michigan, Department of Emergency Medicine, Ann Arbor, USA

⁴University of Michigan, Department of Computer Science, Ann Arbor, USA

⁵University of Michigan, Departments of Neurosurgery and Neurology, Ann Arbor, USA

⁶University of Michigan, Departments of Radiology, Ann Arbor, USA

Traumatic brain injury (TBI) is a serious public health problem in the U.S. that could result in permanent disability. However, the impact of the injury could be limited/decreased by early management and treatment. TBI can cause subdural hematoma (SDH), diffuse axonal injury, lacerations and other injuries. The focus of our study is on SDH detection as it is one of the most common sequelae of TBI and can be difficult to detect through visual examination. In this study, we propose fully automated image processing and machine learning based approaches for detecting convexity subdural hematomas and measuring the volume of SDH quantitatively. We analyze head Computed Tomography (CT) images and extract textural, statistical and geometrical features of sample points from the intracranial region to train a classifier. We implement a tree bagger classifier to classify each point as hematoma or no-hematoma. We apply our method on 42 CT image sets where 35 sets captured from patients with SDH from a University of Michigan Hospital database. The total number of CT images used in our analysis is 866 and our method could yield sensitivity and specificity values of 85.02% and 73.74%. Specificity coefficient in 6 out of 7 of the control cases are over 97.5%.

Keywords: subdural hematoma, segmentation, image processing, machine learning

A11-34

PREINJURY MEDICAL COMORBIDITIES ARE ASSOCIATED WITH PERSISTENT POSTCONCUSSIONAL SYMPTOMS FOLLOWING MILD TRAUMATIC BRAIN INJURY

John Yue¹, Maryse Cnossen², Ethan Winkler¹, Sourabh Sharma¹, Hansen Deng¹, Caitlin Robinson¹, Hester Lingsma², Alex Valadka³, David Okonkwo⁴, Geoffrey Manley¹

¹UCSF, Neurosurgery, San Francisco, USA

²Erasmus-MC, Public-Health, Rotterdam, Netherlands

³VCU, Neurosurgery, Richmond, USA

⁴UPMC, Neurosurgery, Pittsburgh, USA

Over 70% of traumatic brain injuries are classified as mild (mTBI), which present heterogeneously, leading to challenges in management and prognosis. Improved characterization of baseline medical comorbidities may delineate their status as risk factors for unfavorable recovery. Patients from the TRACK-TBI Pilot study with Glasgow Coma Scale 13–15, completing three- and six-month outcomes (Glasgow Outcome Scale-Extended (GOSE); Acute Concussion Evaluation (ACE): physical/cognitive/sleep/emotional subdomains) were extracted. Presence/absence of 12 preinjury medical comorbidities and intracranial pathology on acute computed tomography (CT) were considered. Statistical significance was assessed at $p < 0.001$ correcting for multiple comparisons. Two-hundred sixty mTBI patients were aged 44.0 ± 18.7 -years, 70.4%-male, 78.6%-Caucasian; 36.2% were CT-positive. Comorbidities of highest incidence were psychiatric-30.0%, cardiac-20.4%, gastrointestinal-15.8%, asthma-13.1%, headache/migraine-11.5%, seizures-8.5%. Overall three- and six-month GOSE were 6.83 ± 1.14 , 6.92 ± 1.13 respectively. ACE symptomatology did not differ significantly between CT-positive/negative groups. In CT-negative patients, psychiatric-history associated with decreased three- and six-month GOSE ($p = 0.001$); seizure-history associated with decreased six-month GOSE ($p = 0.001$). At three-months, increased ACE-physical symptomatology associated marginally with gastrointestinal-history ($p = 0.004$), increased ACE-cognitive symptomatology associated with seizure-history (2.53-vs.-1.12-symptoms; $p < 0.001$), ACE-sleep symptomatology associated with gastrointestinal-history (1.56-vs.-0.77-symptoms; $p = 0.001$) and headache/migraine-history (1.58-vs.-0.76-symptoms; $p < 0.001$), ACE-emotional symptomatology associated with headache/migraine-history (2.04-vs.-0.91-symptoms; $p < 0.001$) and marginally with seizure-history ($p = 0.003$). At six-months, ACE-physical associated with headache/migraine-history (5.08-vs.-2.71-symptoms; $p < 0.001$) and marginally with seizure-history ($p = 0.003$), ACE-sleep associated marginally with hepatic-history ($p = 0.005$), and ACE-emotional associated with seizure-history (2.79-vs.-1.37-symptoms; $p < 0.001$). Psychiatric-history associated with increased symptomatology across all ACE subdomains at three- and six-months (range: 0.71-to-2.36 mean increase in symptom number; $p < 0.001$). In CT-positive patients, thyroid-history associated marginally with ACE-physical ($p = 0.003$), and hepatic-history associated with ACE-emotional (3.67-vs.-0.91-symptoms; $p = 0.001$). The contribution of baseline comorbidities to evolving symptomatology within six-months post-mTBI deserves increased scrutiny. In CT-negative patients, comorbidities associating with postconcussional symptoms across three-and six-month time-points include psychiatric-history, and to a less conserved extent, headache/migraine- and seizure-history. Gastrointestinal-history may increase symptoms burden prior to three-months.

Keywords: medical comorbidities, mild traumatic brain injury, outcome measures, postconcussional symptoms, prognosis

A11-35

FACIAL FRACTURE IN THE UNCOMPLICATED MILD TRAUMATIC BRAIN INJURY PATIENT: EVALUATION OF SIX-MONTH FUNCTIONAL DISABILITY

John Yue¹, Sourabh Sharma¹, Ethan Winkler¹, Maryse Cnossen², Hansen Deng¹, Caitlin Robinson¹, Hester Lingsma², Alex Valadka³, David Okonkwo⁴, Geoffrey Manley¹

¹UCSF, Neurosurgery, San Francisco, USA

²Erasmus-MC, Public-Health, Rotterdam, Netherlands

³VCU, Neurosurgery, Richmond, USA

⁴UPMC, Neurosurgery, Pittsburgh, USA

The status of facial bones as a protective cushion for cranial contents, versus risk factor for cranial injury, during traumatic brain injury (TBI) is unclear. The contribution of acute facial fractures on long-term functional disability following uncomplicated mild TBI (mTBI) remains to be investigated. Adult subjects suffering acute TBI requiring clinically-indicated brain computed tomography (CT) scan <24-hours of injury from the prospective Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot), with Glasgow Coma Scale (GCS) score 13-15, without intracranial CT pathology nor history of stroke, brain tumor, and/or learning/developmental disability, completing six-month disability measures [Glasgow Outcome Scale-Extended (GOSE), Craig Handicap Assessment and Reporting Scale-Short Form (CHARTSF) subscales) were extracted. Outcomes were analyzed by CT evidence of facial fracture. Multivariable regressions controlling for age, education, employment, psychiatric and seizure history, GCS, loss-of-consciousness, and polytrauma were performed for univariate analyses with $p < 0.10$. One-hundred sixty-five uncomplicated mTBI subjects were included, aged 41 ± 16 -years, 67%-male, and 75%-Caucasian. Facial fracture ($n=20$) was associated with hospital admission (70%-vs.-39%; $p=0.018$). Most patients achieved good recovery at six-months (GOSE ≥ 7 ; facial fracture=61%, no fracture=70%). On univariate analysis, facial fracture was associated with lower CHARTSF-Social Integration scores (82.2 ± 31.5 vs. 92.9 ± 16.3 ; $p=0.017$) and marginally lower CHARTSF-Economic Self-Sufficiency scores (64.0 ± 39.4 vs. 77.6 ± 32.9 ; $p=0.092$). On multivariable analysis, the association between facial fracture and decreased CHARTSF-Social Integration persisted (adjusted-mean 75.7 ± 5.0 vs. 84.7 ± 3.4 ; mean difference = -9.1, 95% confidence interval [0.5–17.7]; $p=0.039$). Adjusted Social Integration scores for fracture/non-fracture groups were below/above the 50th percentile CHARTSF normative data for all-severity TBI, respectively. Subjects suffering facial fractures in the setting of acute uncomplicated mTBI may be at risk for impaired return to social participation independent of other injury factors, and may constitute a subpopulation benefitting from heightened surveillance and/or therapeutic intervention. Confirmation in larger datasets is however warranted.

Keywords: CHART-SF, disability, facial fracture, mild traumatic brain injury, social integration

A11-36

SELECTIVE SEROTONIN REUPTAKE INHIBITORS FOR TREATING NEUROCOGNITIVE AND NEUROPSYCHIATRIC DISORDERS AFTER TRAUMATIC BRAIN INJURY

John Yue, John Burke, Pavan Upadhyayula, Ethan Winkler, Hansen Deng, Caitlin Robinson, Catherine Suen, Sourabh Sharma, Adam Ferguson, Laura Ngwenya, Geoffrey Manley, Phiroz Tarapore
UCSF, Neurosurgery, San Francisco, USA

Traumatic brain injury (TBI) is a leading cause of death and permanent disability worldwide, affecting an estimated 10 million people annually. The prevalence of Axis I and/or Axis II disorders following TBI is 20-50%. Although there is a great need for evidence-based treatment for psychiatric impairments after TBI, such research is scant. Disorders of mood and cognition may remain even after recovery of neurologic function is achieved. One mechanism by which

this occurs is disruption of brainstem neurons during TBI, which effectively disrupts pontine and medullary serotonergic projections, resulting in impaired metabolism of serotonin and associated deficits in mood regulation, executive control and homeostasis. Selective serotonin reuptake inhibitors (SSRI) are highly specific antidepressant agents modulating arousal, emotion, and working memory. They constitute first-line treatment for a variety of neurocognitive and neuropsychiatric disorders. This review investigates the utility of SSRIs in treating post-TBI sequelae. Twenty-seven unique reports were consolidated from the Cochrane Central Register for Controlled Trials and National Library of Medicine PubMed databases (seven randomized controlled trials, nine open-label studies, 11 case reports). SSRIs are associated with improvement of depressive symptoms but not cognitive symptoms; pooled results using the Hamilton Depression Scale (HAM-D) demonstrate a significant mean decrease of depression severity following sertraline administration compared to placebo. A number of case reports and small open-label studies cite mood improvement following SSRI administration, while effects on cognitive and functional recovery were largely absent or negative. No studies specifically focused on SSRI treatment effects for post-TBI post-traumatic stress disorder (PTSD). Discernment of SSRI efficacy for TBI-induced PTSD represents an important direction of future research; in addition, placebo-controlled studies with extended follow-up periods and concurrent biomarker, neuroimaging and behavioral data are necessary to accurately delineate the attributable pharmacological effect of SSRIs in the TBI population.

Keywords: cognition, depression, postconcussive disorder, selective serotonin reuptake inhibitor, traumatic brain injury, posttraumatic stress disorder

A12 EPILEPSY / SEIZURE

A12-01

CHARACTERIZATIONS OF ENHANCED SEIZURE SUSCEPTIBILITY FOLLOWING SEVERE PENETRATING AND SEVERE CLOSED-HEAD BRAIN INJURY IN RATS

Jenny Browning, Lai Yee Leung, Krista Caudle, Ying Cao, Zhilin Liao, Weihong Yang, Ashley Whiteman, Deborah Shear, Xi-Chun Lu
Walter Reed, BTNN, Silver Spring, USA

Traumatic brain injuries (TBI) carry a risk for the development of post traumatic epilepsy (PTE). Currently, animal models that produce clinically relevant spontaneous seizures are lacking. The aim of the study was to characterize PTE following severe penetrating TBI (pTBI) and severe closed-head TBI (cTBI) in rats. Severe pTBI was induced by stereotactically inserting a perforated steel probe into the brain of an anesthetized rat and rapidly inflating the probe's elastic tubing into an elliptical shaped balloon. Two trajectories (frontal and lateral) and two severity levels (10% and 12.5%) were used. Severe cTBI was induced by launching a steel ball bearing (10.5 mm in diameter) aimed at the dorsal-lateral skull surface of anesthetized rats. Investigation of spontaneous seizure activity was carried out to 1 or 6 months post-injury. At 1 and 6 months post-injury, seizure susceptibility was tested in all animals by pentylenetetrazole (PTZ) injection (30 mg/kg). Eight pTBI groups (2 trajectories \times 2 severities \times 2 time points) and 2 cTBI groups (2 time points) were included in the study. Among 80 pTBI rats and 20 cTBI rats included in the study, no spontaneous convulsive behavior was detected out to 6 months post-injury. Spontaneous and daily recurrent nonconvulsive seizures were detected in only one cTBI rat via EEG recording at 6

months post-injury. Increased seizure susceptibility in the PTZ test was observed in 50–80% of all injured animals with higher seizure scores and shorter seizure latencies detected in pTBI injured animals. In the cTBI model, seizure susceptibility to the PTZ challenge was moderately increased 1 month post-injury and even to a lesser degree at 6 months post-injury. Overall, while results of the current study showed lowered threshold to the PTZ challenge in both brain injury models, it failed to demonstrate consistent, reproducible evidence of spontaneous PTE in either model, in the absence of the PTZ challenge.

Keywords: Post-traumatic seizures, Post-traumatic epilepsy, model development, Ballistic-like brain injury

A12-02

ASTROGLIAL GLUTAMATE TRANSPORTER GENETIC ASSOCIATIONS WITH POST-TRAUMATIC SEIZURES

Raj Kumar¹, Anne Ritter¹, Amery Treble-Barna¹, Conley Yvette², Amy Wagner^{1,3}

¹University of Pittsburgh, Physical Medicine and Rehabilitation, Pittsburgh, USA

²University of Pittsburgh, Nursing, Pittsburgh, USA

³University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

Individuals with TBI have an estimated 50-times increased relative risk for unprovoked seizure versus healthy age-matched controls. Published studies have identified factors that increase risk for post-traumatic seizure (PTS), like subdural hematoma and craniectomy; however, there remains significant unexplained variance in clinical prognostic models. Variation in genes involved in both secondary injury after TBI and seizure pathology (e.g. glutamate transport/excitotoxicity), may help address this knowledge gap. *SLC1A3*, which encodes the glial glutamate transporter excitatory amino acid transporter-1, clears extracellular glutamate from synaptic clefts across astroglial membranes, impacting local bioenergetics, ammonia homeostasis and GABA-glutamine production, which may each lead to increased PTS risk. The study objective was to assess the association between genetic variation in *SLC1A3* and time until first seizure over the first 3 years post-injury in N=267 self-reported White adults with severe TBI and no pre-injury history of seizure disorder. Eighteen tagging single nucleotide polymorphism (SNPs) were examined for independent associations between minor allele variant carriers and PTS. Adjustment for multiple comparisons was made using the minimum number of effective tests (Meff) method. Rs4869682 (GG vs. T-carriers) was associated with time until first seizure (log-rank $p=0.003$). PTS incidence for GG homozygotes was 29.7% versus 16.9% for T-carriers. After adjustment for subdural hematoma, rs4869682 T-carriers had a 53% reduced PTS risk versus GG-homozygotes (aHR=0.47, 95% CI: 0.25, 0.89, $p=0.021$). No other SNPs were associated with PTS after multiple comparisons adjustment. Our data suggest variation in the glial transporter gene, *SLC1A3*, is associated with epileptogenesis, such that T-minor allele carriers for rs4869682 are protected against PTS. These study results should be validated in independent samples; though, the relevance of this glial transporter gene in PTS pathophysiology makes it a candidate to use for 1) PTS risk stratification and 2) as a potential treatment target. Supported by NIH-R01HD048162; NIH-R01NR013342; DoD-W81XWH-071-0701, CURE Epilepsy Award; NIDILRR-90DP0041.

Keywords: Post-traumatic seizure, Astroglia, Candidate gene study

A12-03

COMBINATION THERAPY OF ANTI-EPILEPTIC DRUGS AGAINST POST-TRAUMATIC NONCONVULSIVE SEIZURES IN RATS

Xi-Chun Lu, Ying Cao, Zhilin Liao, Jenny Browning, Frank Tortella, Deborah Shear

Walter Reed Army Institute of Research, Brain Trauma Neuroprotection & Neurorestoration/Psychiatry and Neuroscience, Silver Spring, USA

We previously demonstrated synergistic effects of combination therapy using phenytoin paired with levetiracetam (PHT+LEV) or ethosuximide (PHT+EXM) for mitigating acute post-traumatic nonconvulsive seizures (NCS) induced by penetrating ballistic like brain injury (PBBI) in rats. We also observed sub-additive effects of LEV paired with gabapentin (LEV+GBP). In this study we evaluated two different pairs of combinations of these drugs: EXM+LEV and PHT+GBP, in the PBBI model. All rats received a frontal PBBI and skull EEG electrodes implantation. EXM+LEV were tested at four dose ratios: EXM/LEV: 5.6/6.3, 11.1/12.7, 22.2/25.4, or 44.5/50.7 mg/kg. PHT+GBP were tested at five dose ratios: PHT/GBP: 1.8/0.6, 3.6/1.3, 7.2/2.5, 14.5/5.0, or 28.9/10.0 mg/kg. Treatments were given intravenously twice/day for three days following the injury. The NCS were detected by continuous EEG recording for 72h. Similar to previous studies, approximately 65% of PBBI-vehicle treated animals showed evidence of post-traumatic NCS within the first 72h following injury. PBBI animals treated with EXM+LEV showed reduced NCS incidence to 33–44% across all dose ratios tested and dose-dependently decreased NCS frequency and duration by 10–84% ($p<0.05$ at 2 highest dose ratios) and 2–88% ($p<0.05$ at 2 highest dose ratios), respectively. However, for PHT+GBP treatments, only the highest dose ratio reduced NCS incidence to 41% and significantly reduced seizure frequency by 78% ($p<0.05$) and seizure duration by 82% ($p<0.05$). Isobolographic analysis further indicated that EXM+LEV combination therapy achieved synergism because the observed effects exceeded the expected additive effects, whereas PHT+GBP had sub-additive effects. These results extended our previous findings on AED combinations involving PHT, EXM, and LEV and indicated their favourable interactions when any two pairs were combined at lower dose constituents than their monotherapy doses. On the other hand, similar to the lack of beneficial effects of LEV+GBP combination therapy, PHT+GBP also failed to improve their anti-seizure activities, suggesting that GBP may not be a good candidate for combination therapy against post-traumatic NCS.

Keywords: post-traumatic nonconvulsive seizures, penetrating brain injury, anti-epileptic drugs, combination therapy, EEG, rats

A12-04

LOWER DOES OF PROPHYLACTIC LEVETIRACETAM IN THE ELDERLY FOR SUBACUTE AND CHRONIC SUBDURAL HEMATOMAS AFTER SURGICAL EVACUATION

Jacqueline O'Niell, B.S., B.A., Laila Mohammad, MD, **Christine Meadows**, Ross S. Green, MD, Howard Yonas, MD

University of New Mexico School of Medicine, Neurosurgery, 87131, USA

Objective: Chronic subdural hematomas (cSDH) are considered a disease of the elderly, with an incidence increased from 1.72/100,000/year to 7.35/100,000/year in patients aged 70–79. The literature

reports an incidence of post-operative seizures in cSDH evacuations between 1.0%–23.4%. Seizures as a post-operative complication of cSDH evacuation are associated with higher morbidity and mortality. Use of prophylactic anticonvulsants has well-documented adverse side effects, particularly concerning in the elderly. Due to limited research in this area, there are no definitive conclusions regarding the utilization of anticonvulsants as prophylaxis in subdural evacuations. The aim of this study is to determine if a decreased dosage of prophylactic levetiracetam is equally effective as standard dosing in the elderly population.

Methods: We conducted a retrospective review of 24 patients, 70 years and older, with a diagnosis of subacute SDH (saSDH) or cSDH, admitted to the University of New Mexico Hospital July 2014–January 2016, who underwent surgical intervention. Patients were excluded for incomplete medical records or a history of epilepsy or traumatic brain injury. The cohort was divided by prophylactic levetiracetam dosage, 500 mg versus 1,000 mg twice a day. We measured the incidence of witnessed seizures, seizures on electroencephalogram (EEG), and epileptiform activity on EEG between groups. Statistical analysis was conducted utilizing Fisher's Exact comparisons.

Results: Of the 24 patients, 20/24 (83%) received 500 mg, while 4/24 (17%) received 1,000mg. In the 500mg group, 16/20 (80%) had no post-operative seizures, 3/20 (15%) had witnessed seizures, 1/20 (5%) had EEG seizures, and 2/20 (10%) had epileptiform activity on EEG. This is compared to the 1,000mg group, who had 3/4 (75%), 1/4 (25%), 0/4 (0%), and 0/4 (0%), respectively. There was no statistically significant difference between witnessed seizures, EEG seizures, or epileptiform activity on EEG between the groups.

Conclusions: Our limited data suggests that 500 mg of levetiracetam has the same prophylactic benefit as 1,000 mg in saSDH and cSDH patients, 70 years-of-age and older, who undergo surgical evacuation. Administering a lower dosage of prophylactic levetiracetam may reduce the incidence and degree of adverse medication effects in this patient population.

Keywords: Subdural hematoma, Prophylactic, Optimal dosage, Seizures

A12-05

ABNORMAL Ca^{2+} SIGNALING IN REACTIVE ASTROCYTES CONTRIBUTES TO SPONTANEOUS SEIZURES IN A NEW MOUSE MODEL OF POST-TRAUMATIC EPILEPSY

Oleksii Shandra, Stefanie Robel

Virginia Tech Carilion Research Institute, Roanoke, USA

Traumatic brain injury (TBI) is the most common cause of acquired epilepsy. Currently available anti-epileptic therapy is ineffective for post-traumatic seizures prevention pointing to differences in the pathogenesis. To date nearly all research has focused on neuronal dysfunction. Yet, the field has identified other potential underlying causes that individually lead to generation of epileptiform activities, including reactive astrogliosis and calcium $[Ca^{2+}]$ -dependent gliotransmission. These mechanisms are actively induced by TBI. The role of astrocytes and their Ca^{2+} transients in epilepsy remains largely unknown due to uncertainty of how they affect seizures. Even less is known how these phenomena contribute to post-traumatic epilepsy (PTE). Clinically, even after mild TBI, the risk for PTE is increased. Today, spontaneous seizures were only confirmed in two PTE models, where TBI is induced by moderate-to-severe penetrating trauma, PTE incidence is low and profound tissue loss following brain injury interferes with the emergence of a concise picture detailing disease

mechanisms and biomarkers. We developed a new model of PTE induced by repetitive mild non-penetrating TBI. The TBI was induced (100 g weight, 50cm height, 3 impacts, 45min inter-injury interval) in a small cohort of mice. After the final righting time was assessed, mice were fitted with electrodes for electroencephalographic (EEG) recordings. EEG monitoring was started 4 days post-injury. All recorded animals had at least two seizures within 6 weeks post-injury. The first seizure was captured on day 21' post-injury. Seizures occurred in a subset of mice and were accompanied by behavioral abnormalities including freezing, facial automatisms, tail extension, rearing, and falling. Using 2-photon imaging we determined abnormal astrocyte Ca^{2+} signaling acutely and within first 4 days post-injury. We provide proof-of-concept evidence that our model of PTE with recurrent unprovoked spontaneous electrographic and behavioral seizures in the settings of repetitive diffuse mild TBI is a promising animal model for studying the pathogenesis of PTE and will help in finding new potential therapeutic targets. Early post-traumatic abnormalities in astrocyte Ca^{2+} and downstream mechanisms are promising targets to explore for their role in post-traumatic epileptogenesis.

Keywords: Calcium Oscillations, Reactive Astrocytes, Spontaneous Seizures, Post-Traumatic Epilepsy

A12-06

OPTICAL AND ELECTROGRAPHIC CHANGES IN THE BRAIN AFTER TRAUMA

Jenny Szu¹, Danielle Ornelas², Som Chaturvedi¹, Junze Liu², B. Hyle Park², Devin Binder¹

¹*University of California, Riverside, of Biomedical Sciences, Division Riverside, USA*

²*University of California, Riverside, Department of Bioengineering, Riverside, USA*

Traumatic brain injury (TBI) is a major cause of morbidity and mortality in persons under the age of 45 and is a primary public health concern in both the civilian and military populations. Post-traumatic epilepsy (PTE) is a long-term negative consequence of TBI in which one or more unprovoked seizures occur at least one week after the initial trauma and accounts for up to 20% of all symptomatic epilepsy. Severity of injury and early posttraumatic seizures are risk factors associated with PTE, however, predictors of PTE still remain highly variable. Current studies of PTE have focused on pharmacologically testing seizure threshold using pentylenetetrazole and imaging studies, while limited, have not identified a correlation between trauma and seizure susceptibility. In our study, we utilized optical coherence tomography (OCT) to detect functional and structural changes in the brain in a controlled cortical impact (CCI) injury mouse model of TBI. Additionally, animals underwent long-term video-electroencephalography (vEEG) recording to identify spontaneous seizures after TBI and intrahippocampal electrical stimulation to quantitatively define electrographic seizure threshold (EST) and duration (ESD). OCT imaging detected alterations in optical attenuation, a measure of both scattering and absorption of the biological sample, with and without vascular contribution at different time points after TBI. Additionally, injured animals exhibited altered EST and ESD compared to naïve and sham controls. Altogether, our data suggest that seizure threshold can be quantitatively measured and OCT imaging can potentially offer useful information to identify predictors of PTE in our model.

Keywords: Optical coherence tomography, Seizure threshold, EEG

A13 IMAGING

A13-01

THE ROLE OF NETWORK SIZE AND ARCHITECTURE IN RECOVERY AFTER TARGETED INJURY IN NEURONAL ISLANDS

Modupe Adegoke, Olivia Teter, David Meaney
University of Pennsylvania, Bioengineering, Philadelphia, USA

Experimental models of dissociated neuronal cultures have offered insight into the single-cell pathophysiology of neuronal injury, but fail to incorporate the modular architecture inherent to the brain. Without this modular architecture, it is difficult to directly evaluate the role of architecture in driving neural activity, particularly across different scales of brain organization. In this study, we explored how network size affected neuronal activity during recovery after injury *in vitro*. Primary rat cortical cultures were patterned as multi-neuron isolated clusters of neurons (islands) ranging in size from 750 microns (μm) to 1.4 mm. Selective injury of 20–30% randomly chosen neurons in islands was performed using a microinjector device, at a level of injury that does not cause cell death. A genetically-encoded calcium indicator (GCaMP6f) was used to estimate neural activity at baseline, immediately after, and 6–20 hours following injury at 13–15 days *in vitro* (DIV). We computed single-cell Ca^{2+} transient rates, and estimated functional connectivity among neurons using previously published methods. Neuronal islands progressed from non-synchronous patterns of activity to full network bursts as they matured. Ten days following plating, we observed slow-paced, full-system bursts (0.8 ± 0.33 events/minute) in smaller islands, with faster rates of non-synchronous activity appearing in larger islands (11.3 ± 7 events/minute, $p < 0.01$ relative to smaller islands). At the single cell level, two characteristic neuronal populations appeared: (1) fast-pacing neurons characterized by periodic transients with a peak at 0.33 Hz in the frequency domain, and (2) regular pacing neurons with activity in the 0.03–0.1 Hz range. Following injury, activity is perturbed in islands with non-synchronous activity, but maintained in cultures with bursting pre-injury patterns. Additionally, there is a transient increase in the proportion of fast-pacing neurons 6 hours after injury, suggesting that they are more resilient to injury or potentially serve as re-initiators of activity. Taken together, these data suggest that activity state, as determined by network size, plays a role in the response to and recovery after targeted injury.

Keywords: *in vitro* model, controlled neuronal networks, targeted injury, Calcium imaging, functional connectivity

A13-02

FACTOR ANALYSIS TO DETERMINE WHITE MATTER INJURY PATTERNS UNDERLYING COGNITIVE CHANGES FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY

Brenda Bartnik Olson¹, Nirmalya Ghosh², Udochukwu Oyoyo¹, Barbara Holshouser¹, Joy Nichols², Jamie Pivonka-Jones², Karen Tong¹, Samuel Barnes¹, Stephen Ashwal²

¹Loma Linda University, Radiology, Loma Linda, USA

²Loma Linda University, Pediatrics, Loma Linda, USA

Traumatic brain injury (TBI) is associated with widespread disruption in white matter (WM) integrity. Diffusion tensor imaging (DTI) shows promise as a tool to detect changes underlying neurocognitive outcome. Factor analysis can condense a large number of variables into relatively few new variables that best characterize the data. These factors can be used to predict outcomes of interest. The purpose of our study was to use

factor analysis to identify patterns of WM injury that predict neurocognitive outcome in pediatric TBI patients. 30 children (ages 4–18) with moderate or severe TBI were enrolled. DTI was acquired at 3T using 30 directions and $b=0$ and 1000 s/mm^2 . Patients were imaged 6–17 days post injury. Cognitive testing was done 1 year after injury and included the Wechsler Abbreviated Scale of Intelligence (FSIQ), Performance Intelligence Quotient (PIQ), and the Test of Everyday Attention-Children (TEA-CH G). TBSS was used to create FA, ADC, AD and RD maps. Individual cortical, combined regions and whole brain DTI measures were subjected to factor analysis. Principal components of individual variables were used in a logistic regression to determine the factors ability to predict neurocognitive outcome. Three factors were identified, accounting for 69.8% of the variance in the original data set. Factor 1 and 2 included ADC, AD, and RD measurements from individual cortical, combined, and total WM regions. Factor 3 included the mean FA of individual cortical, combined, and total WM regions. All factors predicted impairments in FSIQ and TEA-CH G with 82.5% and 87.5% accuracy. Factor 3 was the best predictor of PIQ at 86.2%. Our findings show that factor analysis of DTI in pediatric patients after a TBI can successfully identify patterns of WM injury that predict impairments in cognitive function. Supported by NIH/NINDS R01-NS054001

Keywords: diffusion tensor imaging, pediatric, factor analysis, cognition

A13-03

MAGNETIC RESONANCE SPECTROSCOPY ALONG TRACT IN PEDIATRIC TRAUMATIC BRAIN INJURY

Emily Dennis¹, Talin Babikian², Jeffery Alger³, Faisal Rashid¹, Julio Villalon-Reina¹, Yan Jin¹, Alexander Olsen⁴, Richard Mink⁵, Christopher Babbitt⁶, Jeffrey Johnson⁷, Christopher Giza⁸, Paul Thompson¹, Robert Asarnow²

¹USC Keck School of Medicine, IGC, SNII, Los Angeles, USA

²UCLA, Dept. Psychiatry Biobehav Sciences, Los Angeles, USA

³NeuroSpectroScopics LLC, Sherman Oaks, USA

⁴NTNU, Psychology, Trondheim, Norway

⁵Harbor-UCLA Medical Center, Torrance, USA

⁶Miller Children's Hospital, Long Beach, USA

⁷LAC+USC Medical Center, Pediatrics, Los Angeles, USA

⁸Mattel Children's Hospital, UCLA BIRC, Los Angeles, USA

Many studies over the last decade have used dMRI (diffusion magnetic resonance imaging) to investigate TBI, due to its sensitivity to white matter (WM) disruptions post-injury. However, while dMRI can identify areas of WM disruption post-injury, it is limited in its abilities to interpret those regions of disruption. Lower FA can indicate demyelination, but it can also indicate axonal loss, inflammation, and changes to axonal packing. We collected whole brain magnetic resonance spectroscopy (MRS) to combine with dMRI and give more detail about areas of WM disruption. We scanned 29 TBI patients 2–5 months post-injury and again 13–19 months post-injury, along with 38 well-matched controls. Earlier analyses identified 2 subgroups based on interhemispheric transfer time (IHTT) collected using ERP (event-related potentials), with differences in cognitive function and WM structural organization. To combine dMRI and MRS, we co-registered the modalities and extracted summaries of 6 measures (FA – fractional anisotropy, MD – mean diffusivity, RD – radial diffusivity, AD – axial diffusivity, NAA – *N*-acetylaspartate, Cho – choline) within 19 WM bundles. Group differences in NAA corresponded to group differences in dMRI measures, while there was no correspondence between dMRI measures and differences in Cho. Using a partial-*F* test, we found that a model including MRS measures explained

significantly more variance in cognitive outcome than a reduced model including only demographic, dMRI, and IHTT variables. Our results suggest demyelination plays an important role in TBI-related WM disruption, and that multi-modal data improves outcome prediction.

Keywords: MRS, dMRI, tractography, longitudinal

A13-04

VALIDATION OF A NEW APPROACH TO READING POST-TRAUMATIC CRANIAL IMAGING

Daniel Evans¹, Vijay Ravindra MD, MSPH¹, Brenden Ronna¹, Andres Rubiano MD, PhD², Gregory Hawryluk MD, PhD¹

¹University of Utah, School of Medicine, Salt Lake City, USA

²El Bosque University, Neurosurgery, Bogota, Colombia

Traumatic Brain Injury (TBI) is an important public health concern. Computerized Tomography (CT) of the head is the gold standard for initially evaluating moderate to severe TBI patients. Adequate and timely evaluation of head CT in TBI is important for immediate management. Current ATLS guidelines for CT evaluation in TBI do not incorporate Brain Trauma Foundation (BTF) guidelines. We aim to investigate a novel method of evaluating trauma head CT. The objective of this study is to compare a new standardized method (ABCDE) of initially assessing head CT with the Advanced Trauma Life Support (ATLS) recommendations in patients with TBI. This is a cross-sectional survey study to assess the performance of a novel imaging interpretation method. Eight CT scans of patients were sent to 18 participants, randomized and blinded to 2 reading methods: the novel method (ABCDE) and ATLS guidelines (ATLS 9th edition). Participants included residents in surgery, emergency medicine, neurology, neurosurgery and medical students. 144 total readings were collected and compared to a gold standard (attending neurosurgeon). Both ABCDE and ATLS methods showed equivalence or near equivalence in identifying abnormal CT (ABCDE sensitivity 85%, ATLS 83%), blood on CT imaging (ABCDE sensitivity 81%, ATLS 83%), presence of basal cistern compression (ABCDE sensitivity 77%, ATLS 76%), correctly identifying the underlying CNS pathology (ABCDE 74%, ATLS 79%) and identifying the need for surgical decompression (ABCDE sensitivity 60%, ATLS 73%). ABCDE was superior in correctly identifying the grade of basal cistern compression (ABCDE 50%, ATLS 37.5%). Limitations include the small sample size and heterogeneity and level of expertise between the two groups. Both methods showed similarity in identifying the need for surgical decompression. Method ABCDE was superior in identifying the grade of basal cistern compression. Being equal, utilization of ABCDE, which incorporates BTF guidelines in CT evaluation, is recommended. Increasing compliance with BTF guidelines is an important determinant of quality measures.

Keywords: Computed Tomography

A13-05

INITIAL ROTTERDAM SCORE PREDICTS DISCHARGE GOSE BUT NOT NEUROLOGIC DETERIORATION

Paul McMahon¹, Jeffrey Pollock², **Holly Hinson**³

¹Oregon Health and Science University, Neurosurgery, Portland, USA

²Oregon Health and Science University, Radiology, Portland, USA

³Oregon Health and Science University, Neurology, Portland, USA

Objective: In order to minimize secondary injury after severe traumatic brain injury (TBI), clinicians need a method of determining those at highest risk of deterioration during the first 48 hours of hospitalization. The Rotterdam Score for computed tomography scans (CT scans) is a well validated tool for predicting outcome at 6 months (Glasgow outcome scale-extended or GOSE), but has not been evaluated to predict acute outcomes.

Methods: We retrospectively assessed the utility of the Rotterdam score calculated from the admission Head CT to predict neurologic deterioration and GOSE at discharge in a subset of patients with TBI admitted to the ICU at a single institution from a prospective database of adult trauma patients. Neurologic deterioration during the ICU course was defined as a decrease of 2 points in the GCS between assessments not attributable to medication. Logistic and linear regression models were constructed to evaluate the relationship between Rotterdam score and outcomes.

Results: Fifty patients with severe TBI (GCS 3-8 on admission) were identified from the larger cohort (n=268). Within the group of TBI patients, the median Rotterdam score was 2 [IQR 1-2], and 43% (n=21) of the group exhibited neurologic deterioration. The median GOSE at discharge was 3 [3-5]. There was no clear relationship between Rotterdam scores and neurologic deterioration (P=0.80, OR=0.94, 95% CI: 0.56 to 1.55). In contrast, higher Rotterdam scores were associated with worse GOSE scores at hospital discharge: for each 1-point increase in Rotterdam, the GOSE will decrease by 0.53 (P=0.046, 95% CI: -1.06 to -0.01).

Conclusion: Admission Head CT Rotterdam score was not predictive of neurologic deterioration during the first 48 hours after admission, but was associated with GOSE at discharge.

Keywords: Rotterdam Score, Neurologic Deterioration

A13-06

QUANTIFYING THE EFFECTS OF CEREBRAL MICROHEMORRHAGES DUE TO MILD TRAUMATIC BRAIN INJURY UPON WHITE MATTER PROPERTIES DURING AGING

Andrei Irimia¹, Paul Vespa²

¹University of Southern California, Leonard Davis School of Gerontology, Los Angeles, USA

²University of California, Los Angeles, Departments of Neurosurgery and Neurology, Los Angeles, USA

Cerebral micro-bleeds (CMBs) are frequent acute neuroimaging findings in patients with mild traumatic brain injury (mTBI), particularly in older ones. Nevertheless, the clinical significance and potential interpretation of TBI-related CMBs remains controversial partly because their long-term effects are poorly understood. Here we report the development of a neuroimaging-based framework for the longitudinal quantification of CMB effects upon the white matter (WM) of aging mTBI victims. The multimodal neuroimaging approach involves T_1 - and T_2 -weighted structural magnetic resonance imaging (MRI), fluid-attenuated inversion recovery (FLAIR), diffusion weighted and diffusion tensor imaging (DWI and DTI, respectively) and susceptibility weighted imaging (SWI). Co-registration of neuroimaging volumes is first accomplished four-dimensionally (across space and time) and across modalities. CMBs are then identified at each acquisition time point based on SWI, which is particularly amenable to CMB detection. WM fiber bundle reconstruction is performed via DTI-based streamline interpolation using a novel algorithm for topologically-consistent spatiotemporal mapping of WM connections. WM bundles passing through the vicinity of CMBs (i.e.

through umbral and/or penumbral regions) are identified automatically and their properties [fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), etc.] are calculated. The framework is (A) amenable to an arbitrary number of time points at which neuroimaging is performed, and (B) scalable to include an arbitrary number of imaging modalities. The workflow was validated based on a neuroimaging dataset acquired from (A) 10 healthy volunteers aged 22 to 69 who were scanned repeatedly over a 6-week period and (B) 10 mTBI patients aged 21 to 67 scanned acutely and 6 months post-injury. Validation results indicate minimal differences across scans pertaining to WM fiber bundle properties [mean differences across subjects and scans: $1.2 \pm 0.3\%$ (FA); $0.8 \pm 0.5\%$ (MD); $2.1 \pm 0.6\%$ (AD)]. Thus, our analytic approach is sufficiently robust for enabling the study of CMB effects upon the aging mTBI brain.

Keywords: blood-brain barrier, magnetic resonance imaging, micro-hemorrhage, penumbra

A13-07

ASSOCIATION BETWEEN ACUTE MICRO-HEMORRHAGES DUE TO MILD TRAUMATIC BRAIN INJURY AND CHRONIC CORTICAL ATROPHY IN OLDER ADULTS

Andrei Irimia¹, Paul Vespa²

¹University of Southern California, Leonard Davis School of Gerontology, Los Angeles, USA

²University of California, Los Angeles, Departments of Neurosurgery and Neurology, Los Angeles, USA

It has long been known that older adults exhibit increased permeability of the blood-brain barrier, defective neuroinflammatory responses to traumatic brain injury (TBI) and abnormal microglial activation subsequent to the onset of TBI-related cerebral microbleeds (CMBs). Nevertheless, the nature of the association between CMBs and the long-term trajectories of grey matter (GM) and white matter (WM) structure and volumetrics remains poorly understood, particularly throughout senescence. Here we report the results of a longitudinal analysis of this association based on neuroimaging scans acquired from 36 aging mTBI victims (age range: 51–78 years; $\mu = 66.4$ years; $\sigma = 5.2$ years). Volunteers were scanned with IRB approval both acutely and 6 months post-injury using a protocol involving T_1 - and T_2 -weighted magnetic resonance imaging (MRI), fluid attenuated inversion recovery (FLAIR), diffusion tensor imaging (DTI) and susceptibility weighted imaging (SWI). In all volunteers, the only acute neuroimaging findings included the presence of CMBs (as obviated by SWI) and CMB-related penumbral edema (as obviated by FLAIR). At follow-up, all volunteers were found to have suffered brain atrophy (mean brain volume change: $-2.4 \pm 1.2\%$) and GM loss (mean GM volume loss: $-1.6 \pm 0.9\%$). Additionally, cortical thickness was found to have decreased in various regions, including parahippocampal gyrus (mean cortical thickness change: $-12.8 \pm 4.2\%$), prefrontal sulcus ($-3.3 \pm 2.4\%$), anterior cingulate gyrus ($-4.2 \pm 2.5\%$), middle temporal gyrus ($-8.3 \pm 2.8\%$) and orbital gyrus ($-4.5 \pm 1.7\%$). When interpreted together, our preliminary results suggest that, in older adults, the presence of acute CMBs is correlated with detectable neuroanatomical changes, including decreases in total brain volume, total GM volume as well as cortical thinning across a variety of brain regions. Future research in a larger sample should aim to determine the relationship between age at injury and the significance of the association between acute CMB occurrence and GM loss, particularly in older adults.

Keywords: blood-brain barrier, cerebral micro-bleed, grey matter, susceptibility weighted imaging

A13-08

DIFFUSION TENSOR IMAGING CAN PREDICT SURGICAL OUTCOMES OF PATIENTS WITH CERVICAL COMPRESSSION MYELOPATHY

Mitsuhiro Kitamura¹, Satoshi Maki¹, Masao Koda², Takeo Furuya¹, Yasushi Iijima¹, Jyunya Saito¹, Takuya Miyamoto¹, Kazuhide Inage¹, Sumihisa Orita¹, Masashi Yamazaki², Seiji Ohtori¹

¹Chiba University Graduate School of Medicine, Orthopedic Surgery, Chiba, Japan

²Faculty of Medicine, University of Tsukuba, Orthopedic Surgery, Tsukuba, Japan

Introduction: Diffusion tensor imaging (DTI) can be used to evaluate patients with CCM quantitatively because it can provide microstructural information regarding the spinal cord with quantitative diffusion parameters. The objective of this study was to assess whether preoperative DTI parameters can predict surgical outcomes of patients with CCM.

Methods: We enrolled 20 patients with CCM who had undergone surgery and were followed up for more than 6 months. Japanese Orthopaedic Association (JOA) score for cervical myelopathy was evaluated before and 6 months after surgery. Surgical outcomes were measured by both change and recovery rate of JOA score, and were regarded as good if change in JOA score was 3 points or higher or the recovery rate of JOA score was 50% or higher. The patients were examined using a 3.0 T magnetic resonance system before surgery. Regions-of-interest were determined based on the geometry of the cord and measured DTI parameters were fractional anisotropy (FA). The correlations between FA value and surgical outcomes were analyzed. The predictive performance of FA value for good surgical outcomes was evaluated by the area under the receiver operator characteristic (ROC) curve.

Results: JOA score was 8.9 preoperatively and 11.6 at 6 months after surgery and improved significantly ($p < 0.001$). Change of JOA score moderately correlated with FA ($r = 0.51$, $p = 0.02$). Moreover, the recovery rate of JOA score correlated moderately with FA ($r = 0.49$, $p = 0.03$). The area under the ROC curve for prognostic precision for surgical outcomes evaluated by change and recovery rate of JOA score were 0.76 and 0.89, respectively, indicating good model prediction by FA.

Conclusion: It is feasible to predict surgical outcomes of patient with CCM using DTI. DTI can be used as an imaging biomarker for surgical prognosis of CCM patients.

Keywords: Diffusion Tensor Imaging, prediction, cervical myelopathy

A13-09

DIFFUSION TENSOR IMAGING CHARACTERIZES LESION HETEROGENEITY AND IS ASSOCIATED WITH MOTOR FUNCTION IN CHRONIC CANINE PARALYSIS

Melissa Lewis¹, Pew-Than Yap², Natasha Olby¹

¹North Carolina State University, College of Veterinary Medicine/ Department of Clinical Sciences, Raleigh, USA

²University of Chapel Hill, School of Medicine, Department of Radiology, Chapel Hill, USA

Lesion heterogeneity amongst chronically paralyzed dogs after acute, complete thoracolumbar spinal cord injury (TLSCI) is poorly described. We hypothesized that Diffusion Tensor Imaging (DTI) would define and quantify the continuum of lesion severity in this population. Our objective was to use fractional anisotropy (FA), mean diffusivity (MD) and tractography to quantify lesion severity and investigate associations with hind limb motor function. Dogs were recruited that had suffered

clinically complete TLSCI and failed to recover hind limb sensation. They underwent thoracolumbar MRI with DTI and gait analysis. FA and MD were calculated on regions of interest at the lesion epicenter and cranial and caudal to the visible extent of the lesion. Tractography was performed to detect trans-lesional fibers. Gait was quantified using an ordinal scale (OFS) ranging from 0-12 with scores ≥ 4 indicating weight bearing stepping. Relationships between FA, MD, presence of trans-lesional fibers and OFS were investigated using logistic regression or Wilcoxon rank sum. Twenty-two dogs were enrolled with median OFS of 2 (0-6); 5 had an OFS ≥ 4 . Median values were FA: 0.188 (0.107-0.320), MD: 2.06×10^{-3} ($1.33-2.96 \times 10^{-3}$) at the lesion epicenter, FA: 0.420 (0.391-0.516), MD: 1.52×10^{-3} ($1.03-1.87 \times 10^{-3}$) cranial, and FA: 0.369 (0.265-0.513), MD: 1.31×10^{-3} ($0.82-2.08 \times 10^{-3}$) caudal to the lesion. Four dogs had no trans-lesional fibers. Injury duration (median: 18 months, 3-64) was associated with MD ($p < 0.05$) but not FA values. FA at the lesion epicenter and presence of trans-lesional fibers were associated with OFS ($p \leq 0.03$). These findings suggest DTI quantifies injury severity amongst chronically paralyzed dogs after severe TLSCI.

Keywords: diffusion tensor imaging, canine, chronic paralysis

A13-10

MICROSTRUCTURAL TISSUE AND VASCULAR INJURY WITHIN REGIONS OF ENCEPHALOMALACIA IN CHRONIC TRAUMATIC BRAIN INJURY

Margalit Haber¹, **Tawny Meredith-Duliba**¹, Franck Amyot^{2,3}, Kimbra Kenney^{2,3,4}, Carol Moore³, Erika Silverman¹, Yi-Yu Chou³, Dzung L. Pham⁴, Eric Wasserman⁴, Ramon Diaz-Arrastia¹, Danielle Sandmark¹

¹University of Pennsylvania, Neurology, Philadelphia, USA

²USUHS, Neurology, Bethesda, USA

³CNRM, HJF, Neurology, Rockville, USA

⁴NINDS, NIH, Neurology, Bethesda, USA

Magnetic resonance imaging (MRI) is a powerful tool for visualizing traumatic brain injury-related pathology. Trauma-induced encephalomalacia is frequently identified by Fluid-attenuated inversion recovery (FLAIR) MRI. Often thought to represent gliosis, the underlying microstructural tissue and vascular injury within FLAIR hyperintensities are not well understood. The current study utilized a multimodal MRI protocol to assess mean diffusivity (MD), fractional anisotropy (FA), and vascular alterations within FLAIR normal and abnormal regions in chronic TBI subjects. MRI was performed on TBI subjects (subjects with FLAIR abnormalities, $n=13$; subjects without FLAIR abnormalities, $n=8$) and healthy controls ($n=15$). Subjects received MPRAGE-T1, FLAIR, Diffusion Tensor Imaging (DTI), Arterial Spin Labeling (ASL), and Blood-oxygen level dependent imaging (BOLD) with hypercapnia challenge to assess cerebral vascular reactivity (CVR). MD, FA, cerebral blood flow (CBF), and CVR within regions of FLAIR hyperintensities were compared to normal appearing tissue. All values were converted to z-scores using the pool of healthy controls from the study. There was a significant reduction in FA, CBF, and CVR with a complementary increase in MD within regions of FLAIR abnormalities ($p < 0.05$ for all comparisons). Grey matter regions without FLAIR hyperintensities did not show a reduction in CBF ($p < 0.05$). However, normal appearing grey matter in TBI patients with and without FLAIR hyperintensities exhibited significantly reduced CVR relative to controls ($p < 0.05$). A standardized symptoms questionnaire (RPQ-13) did not reveal differences between TBI subjects with FLAIR hyperintensities versus TBI subjects without FLAIR abnormalities ($p > 0.05$), consistent with prior studies indicating that FLAIR abnormalities are poorly associated with clinical outcome. These findings lend insight into the neurobiology underlying FLAIR

abnormal regions in chronic TBI, and indicate that CVR is a more sensitive measure of microstructural integrity.

Keywords: MRI/FLAIR, Cerebral Vascular Reactivity, Chronic TBI, Diffusion Tensor Imaging

A13-11

IN VIVO RETINAL IMAGING OF NEUROINFLAMMATION IN A MOUSE MODEL OF IMPACT CONCUSSION

Olga Minaeva^{1,2,3}, James D. Akula^{4,5}, Mark W. Wojnarowicz^{1,6}, R. Daniel Ferguson⁷, Mircea Mujat⁷, Erich S. Franz^{1,2}, Andrew M. Fisher^{1,2}, Juliet A. Moncaster^{1,3,6}, Bertrand R. Huber^{6,8}, Lee E. Goldstein^{1,2,3}

¹Boston University School of Medicine, Molecular Aging & Development Laboratory, Boston, USA

²Boston University, College of Engineering, Boston, USA

³Boston University Photonics Center, Boston, USA

⁴Boston Children's Hospital, Dept. of Ophthalmology, Boston, USA

⁵Harvard Medical School, Dept. of Ophthalmology, Boston, USA

⁶Boston University School of Medicine, Boston, USA

⁷Physical Sciences, Inc, Biomedical Optics, Andover, USA

⁸VA Boston Healthcare System, Boston, USA

Despite growing awareness of links between concussion, traumatic brain injury (TBI), and long-term effects of neurotrauma, the underlying mechanisms in the brain and possible changes in the retina are unknown. In this study, we used a new mouse model of concussion and a state-of-the-art retinal eye scanner to investigate post-traumatic inflammatory responses in the brain and retina. We utilized Ccr2RFP/Cx3cr1GFP mice (Jackson Laboratory) to enable immune cell visualization by class (monocyte, microglia), origin (peripheral, brain, retina), morphology, and location. We used a new closed-head impact injury mouse model (Tagge et al., submitted) that recapitulates key features of human concussion. We used a multimodal adaptive optics small-animal imager (MAOSI) (Physical Sciences, Inc.) for *in vivo* retinal studies. MAOSI has several channels: adaptive optics optical coherence tomography (AO-OCT) and adaptive optics scanning laser ophthalmoscopy (AOSLO) with reflectance and fluorescence channels. Before injury, Ccr2RFP/Cx3cr1GFP mice showed normal cellular distribution and morphological phenotype of microglia in retina and brain. We observed significant increase in microglia in retina and brain post-injury. Moreover, microgliosis in both retina and brain was notable for reactive cellular phenotype transformation with overlapping ramification fields. Closed-head impact injury is associated with similar reactive inflammatory responses and sequelae in both retina and brain. The retina can be used as a "brain proxy" for noninvasive diagnosis, prognosis, and monitoring of neuroinflammation after closed-head injuries.

Acknowledgment: DoD W81XH-14-1-0592.

Keywords: Impact Concussion, Mouse Model, Retinal Imaging, Neuroinflammation

A13-12

THE CONTRIBUTION OF AQUAPORIN 4 IN DIFFUSION TENSOR ANISOTROPY IN THE JUVENILE BRAIN

Andre Obenaus^{1,2}, Jacqueline Coats², Andrew Fukuda², Wei Sun², Jerome Badaut^{2,3}

¹University of California, Irvine, Pediatrics, Irvine, USA

²Loma Linda University, Basic Sciences, Loma Linda, USA

³University of Bordeaux, CNRS UMR5287, Bordeaux, France

Objectives: Diffusion tensor imaging (DTI) is frequently used to study brain microstructure non-invasively and then to provide prognostic information about the TBI patient's white matter (WM). So far, it has been proposed that either/or a decrease/increase in the relative anisotropy (RA) from DTI reflects the loss of axons and myelin in the WM. However, little empirical data exists about the cellular mechanisms that modulate anisotropy measures. One such cellular regulator could be the expression levels of water channels, such as aquaporin 4 (AQP4). AQP4 modulates brain water movement on DWI and hypothesize that AQP4 can regulate DTI measures including RA.

Methods: Rats underwent two stereotactic deliveries of silencing RNA targeting AQP4 (siAQP4) 48hrs apart. Three days after siRNA (or siGlo, non-targeted siRNA – controls) animals were sacrificed for DTI MRI and immunohistochemical analysis. DTI was undertaken at 11.7 T using a 7-direction paradigm with $b=0$ and 2013.2 s/mm^2 and parametric DTI maps were generated. Confirmatory staining for glial (GFAP), microglia (IBA1) and axons (NF200) was under taken.

Results: We demonstrate in the siRNA targeting AQP4 in the uninjured cortex, resulted in a significant decrease in radial diffusivity and an increase in relative anisotropy (RA) associated with a decrease in AQP4 staining, without modification of GFAP, IBA1 and NF200.

Conclusions: The current work demonstrates that a decrease in the cortical expression of AQP4 is associated with increased RA but without significant alterations in cellular structure. Therefore, elucidating the molecular mechanisms of anisotropy alterations in white and gray matter can impact the clinical interpretation of DTI findings often reported in pathological conditions, such as traumatic brain injury.

Keywords: gray matter, diffusion tensor imaging, juvenile, water mobility

A13-13

MICROHEMORRHAGES LOCALLY DECREASE FRACTIONAL ANISOTROPY ON DIFFUSION TENSOR IMAGING

Dzung Pham, Snehashis Roy, Yi-Yu Chou, Leighton Chan, John Butman

Henry Jackson Foundation, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

Objective: Microhemorrhages are considered a marker of traumatic axonal injury. Reductions in global measures of white matter (WM) integrity based on diffusion tensor imaging (DTI) correlate with increasing microhemorrhages in TBI patients. The goal of this work is to evaluate the local changes in fractional anisotropy that occur at microhemorrhage sites in WM.

Methods: Twenty patients with microhemorrhages were selected from a cohort of mild to moderate TBI patients enrolled in a natural history study. Microhemorrhages were identified on susceptibility weighted imaging (SWI) by placing a 2 mm radius digital sphere at its location. Fractional anisotropy (FA) was computed from a 60 direction DTI acquisition. Both FA and SWI were rigidly co-registered to the subject's T1-weighted image, which had its interhemispheric fissure aligned to a central plane within the image volume. This alignment simplified identification of a contralateral "control" location for each microhemorrhage. Because FA estimates are noisy in gray matter regions, only microhemorrhages within WM were considered. The WM mask was defined by segmentation of the T1-weighted image, followed by a morphological erosion of 1mm.

Results: A total of 478 microhemorrhages were identified. After masking to ensure that both microhemorrhages and their contralateral

location were within WM, 100 microhemorrhages remained. The average FA computed at the site of microhemorrhage was 0.465 ± 0.142 , lower than 0.511 ± 0.158 that was computed in the contralateral location ($p < 0.01$, paired t-test).

Conclusions: FA values at the sites of WM microhemorrhages are reduced as compared to contralateral WM regions without microhemorrhage. One interpretation is that this reduction represents a real change in axonal integrity. However, DTI is based on an EPI sequence with a long echo time and is hence subject to susceptibility induced signal loss from presence of hemorrhages. It is possible that at least some of the FA reduction is an artifact of this phenomenon. If the latter is true, interpretations of DTI measurements in TBI patients with microhemorrhages should be made with caution. Future work is needed to verify the actual cause of these changes.

Keywords: susceptibility weighted imaging, diffusion tensor imaging, microhemorrhage, fractional anisotropy

A13-14

ULTRA HIGH RESOLUTION SUSCEPTIBILITY WEIGHTED IMAGING AT 3 TESLA

Harshan Ravi, Wen-Tung Wang, **Dzung Pham**, John Butman
Henry Jackson Foundation, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

Introduction: Susceptibility weighted imaging (SWI) is a technique to evaluate veins and hemorrhages. Typical SWI uses image resolution on the order of 0.5–1.0 mm in-plane resolution and 1–2.0 mm slice thickness. To visualize microstructures at ultra-high (UH) resolution, sufficient SNR is required motivating the use of UH field MRI (7 tesla), as SNR scales with field strength. However, the availability of human UH field MRI units is still quite limited. A similar SNR advantage could, in principle, be achieved by signal averaging at 3 tesla. In this study we show results of the multi average ultra-high resolution SWI acquired at 3 tesla MRI.

Methods: Subjects were scanned on a Philips 3 tesla Achieva system using a 32-channel head coil. A 3D segmented EPI acquisition technique in combination with high acceleration ($3 \times$ acceleration in both phase and slice encoding direction) was used. Parameters were TR/TE = 60/29 ms, resolution = $380 \mu \times 380 \mu \times 400 \mu$, echo train length 15, flip angle 18° for a scan duration of 120 s. Each scan was repeated 20 times (total 40 minutes). Magnitude, real and imaginary data were saved. The magnitude image of each repetition was coregistered to the first (using FLIRT) and the resulting transformation was applied to the real and imaginary data to generate a complex data set. The 20 repetitions were averaged to generate complex data from which magnitude and phase images were generated.

Results: Maximum subject motion averaged 4.45 ± 3.33 mm over the 40 minute acquisition. Without registration – small veins were blurred and transcortical vessels could not be seen. With registration we were reliably able to delineate microvasculature structures (e.g. transcortical vessels). Microhemorrhages and contusions were clearly depicted in the TBI patient.

Discussion: Here we demonstrate the feasibility of generating UH resolution of $400 \mu\text{m}$ at 3 tesla using multiple averages and image registration. Although the imaging times are not reasonable for routine clinical application, the method opens a window for the investigation of the association of traumatic microhemorrhage and microvasculature in a clinical research environment.

Keywords: susceptibility weighted imaging, high resolution, microhemorrhage, motion correction

CRANIOTOMY ALONE RESULTS IN DEFAULT MODE NETWORK DYSFUNCTION IN THE IMMATURE RAT

Saman Sargolzaei¹, Yan Cai¹, Naomi Santa Mari², Afshin Paydar¹, Christopher C. Giza¹, Neil G. Harris¹

¹University of California Los Angeles, Neurosurgery, Los Angeles, USA

²California Institute of Technology, Pasadena, USA

It remains controversial whether rodents with a craniotomy-only are required, or even appropriate to serve as a sham group to control for the effect of surgery after experimental TBI. Published data show significant molecular and behavioral changes that occur due to craniotomy compared to naïve controls, indicating that craniotomy alone likely constitutes a brain insult. We hypothesized that these confounding effects of craniotomy are also accompanied with alterations in neural circuit dysfunction. We tested this by acquiring resting state functional-MRI data from male, 23 day-old Sprague Dawley rat pups at day 4 post-craniotomy (3mm diameter, -3mm, +4mm left-lateral; intact dura) as well as from age-matched, naïve controls with no craniotomy but with time-matched exposure to isoflurane anesthesia (n=5/group). Imaging data were acquired on a 7 T Bruker spectrometer using a single-shot, gradient-echo sequence, echo/repetition time: 20/1000ms, 300 repetitions, 128×128 matrix, 30×30mm field-of-view and 1mm slice-thickness). After typical preprocessing of the time-series data, voxel-wise functional connectivity analysis was then performed by calculating Pearson correlation coefficients between all brain voxels. The Root Mean Square of the correlation values for each voxel were calculated as an index of global functional connectivity (fc), clusterized for the presence of 30 voxels or more. Large scale, significant (p<0.01) differences in fc were found between the two groups following group ANOVA. Center of mass for the peaks of the clusters that survived statistical correction for multi voxel comparison were located predominantly in regions previously assigned to the rodent default mode network: bilaterally in auditory, temporal association, and primary visual cortex, and in right retrosplenial cortex and hippocampus. These network alterations provide additional evidence to support the idea that craniotomy-alone constitutes a brain injury, and that it might not always serve as an appropriate control. Funding: R01NS27544, R01NS091222, UCLA Easton Labs for Brain Injury, UCLA Steve Tisch BrainSPORT program, UCLA BIRC.

Keywords: Craniotomy, Default mode network, Developing Brain, MRI, Experimental model of TBI

A13-16

RELATIONSHIP BETWEEN MICROVASCULAR FUNCTION AND REGIONAL BRAIN VOLUMES AFTER CHRONIC TBI

Margalit Haber¹, **Erika Silverman**¹, Franck Amyot^{2,3}, Kelly Flesher², Kimbra Kenney^{2,3,4}, Carol Moore³, Yi-Yu Chou³, Dzong Pham³, Eric Wasserman⁴, Ramon Diaz-Arrastia¹

¹University of Pennsylvania, Neurology, Philadelphia, PA

²USUHS, Neurology, Bethesda, MD

³CNRM/HFJ, Neurology, Rockville, MD

⁴NINDS/NIH, Neurology, Bethesda, MD

Traumatic Cerebral Vascular Injury (TCVI) is a universal feature of traumatic brain injury (TBI) and likely contributes to TBI-related disability. Prior studies have shown a significant decrease in whole brain, gray and white matter cerebrovascular reactivity (CVR) in TBI subjects. The relationship between regional CVR measures and brain parenchyma integrity is unknown. To investigate whether TCVI correlates with brain

parenchymal volume loss, analysis was performed on chronic TBI subjects and healthy controls. TCVI was measured via Blood Oxygen Level Dependent magnetic resonance imaging (MRI-BOLD) with hypercapnia challenge to generate CVR maps, as well as arterial spin labeling (ASL) to generate cerebral blood flow (CBF) maps. Cerebral volumes were calculated with the Freesurfer image analysis suite. The segmentation maps were imported into MATLAB and superimposed onto CBF and CVR maps to calculate mean values for each region of interest (ROI). Volume measurements were compared to CBF and CVR values within each ROI. CVR was significantly decreased in multiple subcortical ROIs in the chronic TBI subjects relative to healthy controls (p<0.001), while CBF was not. TBI subjects exhibited significantly more ROIs with reduced volume and cortical thickness (z-score<-2.5, p<0.05). Group level analysis did not reveal significant volume changes in any particular ROI between the TBI and healthy control groups. Regional volumetric changes, CBF, and CVR were not significantly correlated over multiple ROIs. Cortical gyri with a substantial reduction in thickness (z-score<-2.5) in TBI subjects did not show a concurrent abnormal CBF or CVR reduction (z-score>-2.5). This suggests that regional deficits in CBF and CVR in chronic TBI reflect direct vascular injury and dysfunction, and are not a consequence of neural injury. Vascular and neuronal injuries represent distinct TBI endophenotypes, which warrant independent study to further understand their contributions to TBI.

Keywords: MRI, Cerebral Vascular Reactivity, Chronic TBI, MRI-BOLD, Arterial Spin Labeling

A14 INTRACRANIAL PRESSURE

A14-01

MINNESOTA EXTERNAL VENTRICULAR DRAIN GRADING SYSTEM

Kim Uy, David Darrow, Coridon Quinn, Stephen Haines

University of Minnesota Medical School, Neurosurgery, St. Paul, USA

Background: The safety and accuracy of EVD placements remain a challenge in daily practice and publications. A need to develop a rapid and standardized EVD grading system in neurosurgical practice is desired. We developed a three-dimensional Minnesota EVD grading system that account for the number of times the catheter crosses the ependyma, the trajectory and the depth of the catheter tip with respect to Foramen of Monro (FOM). Our study aims to compare the strengths and weaknesses of Minnesota EVD grading system against previously proposed EVD grading systems.

Methods: CT scans of 104 patients who underwent a ventriculostomy were reviewed retrospectively. Three resident physicians were chosen to be the raters. Pre-procedure CT scans were distributed to the graders to estimate the level of difficulty of a catheter placement (e.g. Easy, Intermediate, Hard). Then post-procedural CT scans were given to the raters to grade using the Minnesota EVD scale, O'Leary's, Huyette's, Karkala's and Janson's grading systems. All the scans were distributed to the raters in a random order. Intraclass coefficients were calculated to determine the inter- and intra-rater reliabilities of each scale. The efficiency and practicality of the grading scales were compared by measuring the time required to grade each scan and the frequency of specialized software use during the grading session, respectively.

Results: Inter-rater reliability of Karkala's, Janson's and Minnesota EVD grading system were similar and higher than O'Leary's and Huyette's scales. Minnesota EVD system has the highest intra-rater reliability compared to the rest of the grading scales. Furthermore, Minnesota EVD grading system predicted 76.7% of the difficulty score which is higher than all the 4 grading scales combined.

Conclusion: The Minnesota EVD grading system is an efficient and practical method to use to grade the accuracy of EVD placement. It also has improved inter- and intra-rater reliabilities compared to other existing scales.

Keywords: External ventricular drain, grading system, ventriculostomy

A14-02

ANALYSIS OF OUTCOMES OF BARBITURATE THERAPY FOR REFRACTORY INTRACRANIAL HYPERTENSION AFTER TBI

Aditya Vedantam, Claudia Robertson, Shankar Gopinath
Baylor College of Medicine, Neurosurgery, Houston, USA

Recent clinical trials have shown inconsistent clinical benefit for decompressive craniectomy or hypothermia to treat intracranial hypertension in TBI. Barbiturate therapy continues to be used as a third-tier option for refractory intracranial hypertension. The aim of this study was to determine if ICP control with barbiturate therapy is associated with improved clinical outcomes in a series of TBI patients treated with barbiturate therapy for refractory intracranial hypertension. Patients with closed head injury who received pentobarbital for control of refractory intracranial hypertension from 1986–2012 were reviewed. Average ICP prior to and during barbiturate coma was analyzed. Outcomes were recorded at 6 months after injury using the Glasgow Outcome Scale. Primary outcomes of this study included ICP control during barbiturate coma and mortality at 6 months after injury. Univariate and multivariate regression analysis for primary outcomes were performed for clinical and imaging parameters. A total of 176 patients were included in this study. ICP was adequately controlled during barbiturate therapy in 47 of 146 patients (32.2%), who received pentobarbital for a minimum of 24 hours. Diffuse injury on initial CT scan ($p=0.04$) and higher motor GCS score ($p=0.046$) was associated with higher likelihood of ICP control. ICP control was associated with lower rates of mortality (86.5% vs 13.5%, $p<0.001$) and higher rates of severe disability in patients with ICP control (67.9% vs 32.1%, $p<0.001$). There was no significant difference for the rates of good recovery ($p=0.12$), moderate disability ($p=0.21$) or persistent vegetative state ($p=0.65$) based on ICP control. Uncontrolled ICP during barbiturate coma was significantly associated with 6-month mortality (OR 9.7 95% CI 3.8–24.9, $p<0.001$). ICP control with barbiturate therapy was superior in patients with diffuse injury on initial CT scan and higher admission motor GCS scores. Although ICP control with barbiturate therapy in TBI was associated with significant survival advantage, higher rates of severe disability were observed among survivors.

Keywords: barbiturate therapy, pentobarbital coma, glasgow outcome scale

A15 MONITORING

A15-01

INNOVATIONS IN DATA ACQUISITION FOR MULTIMODAL BRAIN MONITORING: A KEY COMPONENT OF A NEUROTRAUMA PROGRAM

Jeff Chen, Evan Tobias, Patrick Chen, Ronald Sahyouni, Diem KT Tran
University of California, Irvine, Neurotrauma/Neurological Surgery/Medical Device Informatics, Orange, USA

Objectives: Our hospital, an American College of Surgeons Level I trauma center has 60 ICU beds spread over 3 floors that are available to neurotrauma patients. There is a premium on ICU beds, and ICU nurses are cross-trained to care for neurotrauma patients in all the units. Our goal was to establish usage of multimodal brain monitoring across the ICUs.

Methods: Each device required development of computer interfaces to allow direct transfer of information to our EMR. For example, the NeuroOptics NPi 200 pupilometer interfaces with our EMR via a small form-factor PC with an HID (Human Interface Device) reader attached, which gathers information from a microchip located on the device headpiece. The data is collected with the iSirona DeviceConX program and imported to the EMR immediately. Camino(ICP), Licox(oxygenation), and Hemedex(Cerebral Blood Flow) monitors integrate with our EMR via NantHealth Device ConX. Cerebral Microdialysis (CMD) requires chemical analysis using the ISCUS Flex analyzer. This analyzer is housed in the chemistry lab and maintained by the analytical pathology department. Hourly samples are collected by the ICU nurses and sent via the pneumatic tube system to the lab. The analyzer reports the results directly to the EMR via the Sunquest Download protocol. Training of the ICU nursing staff was accomplished via podcasts and in-person in-services.

Results: The pupilometer download was instituted in May 2015, ICP in 2012, Licox in September 2015, Hemedex in November 2016, and CMD in March 2017. Data acquisition/storage from multimodal brain monitoring has become seamless and routine. The granularity of the data stored is minute to minute.

Conclusion: Direct data download to our EMR from the pupilometer, ICP, Licox, Hemedex, and CMD has become an integral component of our multispecialty ICUs. The information is stored and archived in the patient's EMR, readily accessible to the treating physicians. Reduced manual data entry achieves improved accuracy and allows nursing staff to focus more on patient care.

Keywords: multimodal brain monitoring, Cerebral Microdialysis, informatics, Pupilometer

A15-02

MULTIMODALITY MONITORING IN SEVERE TRAUMATIC BRAIN INJURY: CLINICAL FEASIBILITY AND RELIABILITY

Laura Ngwenya, Erica Stoddard, Norberto Andaluz, Jed Hartings, Brandon Foreman
University of Cincinnati, Cincinnati, USA

The cornerstone of management of severe traumatic brain injury (sTBI) patients involves the identification and treatment of increased intracranial pressure (ICP). However, the detection and prevention of secondary brain injury requires a multimodality monitoring (MMM) approach that provides data capture of not only ICP, but also brain tissue oxygen (PbtO₂), cerebral blood flow (rCBF), and electrocorticography (dEEG). We present our institutional experience with MMM using a four-lumen bolt to facilitate simultaneous measurement of multiple intracranial parameters through a single bur hole. Data was retrospectively collected from consecutive adult sTBI patients admitted to our Neuroscience Intensive Care Unit (NSICU) between April 2015 and March 2017 who underwent MMM. Demographics, injury characteristics, duration of monitoring for each probe, device related complications, and length of stay (LOS) were recorded. Forty patients were included (mean age=43.0±17.3 SD). Bolt and probe placement occurred a median of 17.7 hours (interquartile range [IQR]: 8.3–16.9 hours) from time of injury. Insertion related minor complications (e.g. small tract hemorrhage, intracranial bone chips, or

pneumocephalus) occurred in 37.5% of cases, but none were clinically significant or required intervention. Median total monitoring time was 103.5 hours (IQR 48.8–133.6 hours). Probe reliability analysis showed that ICP data was the most reliable, with data available a mean of 94.8% of the total monitoring time with 1.2% error time. PbtO₂ and dEEG data were reliable for >90% of total monitoring time with less than 2% error time. The least reliable measure was rCBF, with 77% of total monitoring time and 17.9% error time. Average NSICU and hospital LOS was 12.2 and 15.2 days, respectively. We conclude that MMM in sTBI patients can be done through a single bur hole without clinically significant complications. Our experience with this alternative to solitary ICP monitoring yields reliable patient data that can support individualized patient care.

Keywords: multimodality monitoring, severe traumatic brain injury, brain tissue oxygen, electrocorticography

A16 NEUROCRITICAL CARE

A16-01

EPIDEMIOLOGY, MANAGEMENT, AND OUTCOMES OF TRAUMATIC BRAIN INJURY IN SUB-SAHARAN AFRICA

Jessica Eaton^{1,4}, Asma Bilal-Hanif², Joanna Grudziak³, Anthony Charles^{2,3,4}

¹University of Louisville School of Medicine, Louisville, USA

²Kamuzu Central Hospital, Lilongwe, Malawi

³University of North Carolina, Chapel Hill, USA

⁴UNC Project Malawi, Lilongwe, Malawi

Trauma accounts for 4.7 million deaths each year, with an estimated 90% of these occurring in low- and middle-income countries (LMICs). Approximately half of trauma-related deaths are due to central nervous system injury, most often in the form of traumatic brain injury (TBI). Odds of mortality following TBI are twice as high in LMICs as in high-income countries. An understanding of TBI in LMICs is essential to mitigate mortality; however, there is a paucity of data on TBI in these settings. This retrospective review of prospectively collected data from Kamuzu Central Hospital, a tertiary care center serving 6 million people in central Malawi, includes all patients admitted from October 2016 through February 2017 with a history of head trauma plus altered consciousness and/or radiographic evidence of TBI. 202 patients met inclusion criteria. 159 (70.1%) were male, with mean age 28.0±16.2. Mean GCS was 11.1±3.8. Road traffic crashes constituted the most common injury mechanism (125, 62.9%). 105, or 52.0%, of patients received a CT scan, with the most common findings being contusions (49, 24.4%), skull fractures (44, 21.9%), and subdural hematomas (15, 7.5%). 61 (30.1%) patients had severe TBI, defined as a GCS of ≤8, of whom 10 (16.4%) were intubated, while 5 (8.2%) received tracheostomies. Overall mortality was 26.6%. Of patients who survived, 81.5% made a good recovery, 10.3% were moderately disabled, 6.2% were severely disabled, and 1.4% were in a vegetative state. An adjusted survival time analysis, with failure defined as mortality or poor functional outcome, was done. Female sex was found to be protective. On Cox analysis, deterioration of GCS in the first 24 hours of hospitalization was significantly predictive (HR = 1.25, 95% CI: 1.15, 1.37). Implementation of proven in-hospital interventions for these patients, aimed at preventing secondary brain damage and detecting neurological deterioration in a timely manner, is critical to attenuate TBI-related morbidity and mortality in resource-poor settings.

Keywords: global neurosurgery, low-resource settings, sub-Saharan Africa, epidemiology, low- and middle-income countries, global health

A16-02

EVIDENCE FOR A NEURAL-RESPIRATORY-INFLAMMASOME AXIS IN TRAUMATIC BRAIN INJURY-INDUCED ACUTE LUNG INJURY

Nadine Kerr¹, Juan Pablo de Rivero Vaccari¹, Ronald Zambrano¹, Sam Abbassi¹, Shu Wu¹, Dalton Dietrich¹, Robert Keane¹

¹University of Miami, Neurological Surgery, Miami, USA

²University of Miami, Department of Pediatrics, Miami, USA

³University of Miami, Department of Physiology and Biophysics, Miami, USA

Acute Lung Injury (ALI) is a common systematic complication of severe Traumatic Brain Injury (TBI). However, the pathophysiologic mechanisms of TBI-induced ALI are not well known. We have previously shown that the inflammasome plays a critical role in TBI-induced secondary pathophysiology and that inflammasome proteins are released in Extracellular Vesicles (EV) after TBI. Here we investigate whether EV-mediated inflammasome signaling contributes to the etiology of TBI-induced ALI. In this study, C57/BL6 mice were subjected to Controlled Cortical Impact Injury (CCI) and brains and lungs were examined for inflammasome activation, pyroptosome formation, and ALI. Also, an adoptive transfer experiment was performed by injecting serum-derived EV from sham and TBI injured mice into healthy mice and lungs were analyzed for inflammasome protein expression and ALI. Our findings indicate that brain and lungs of CCI-injured mice showed a significant increase in the expression of AIM2, IL-1b, caspase-1, IL-18 and HMGB1 acutely after TBI. Moreover, injured lungs also showed evidence of pyroptosis. Lungs of CCI-injured animals demonstrated evidence of ALI as determined by thickening of the alveolar septum, alveolar edema and inflammation, thus resulting in a higher ALI score. Adoptive transfer of serum-derived EV from TBI mice, but not sham mice, into healthy mice induced higher expression of inflammasome proteins in lungs and higher ALI scores. Interruption of this axis by administration of Enoxaparin or an anti-ASC significantly inhibited inflammasome activation and improved ALI scores. In conclusion, our data show that inflammasome activation plays a major role in TBI-induced ALI. These data provide strong evidence for activation of a Neural-Respiratory Inflammasome Axis, and demonstrate that targeting this axis with Enoxaparin or anti-ASC antibody may provide a novel therapeutic approach for neurotrauma-induced ALI.

Funding: Miami Project to Cure Paralysis, NIH grants: R42NS086274, F31 HL132425-01A1

Keywords: Traumatic Brain Injury, Acute Lung Injury

A16-03

THE DESIGN OF THE HYPERBARIC OXYGEN BRAIN INJURY TREATMENT (HOBIT) TRIAL

Frederick Korley, Gaylan Rockswold, Byron Gajewski, Renee Martin, Robert Silbergleit, William Barsan

University of Michigan Medical School, Emergency Medicine, Ann Arbor, USA

Objective: To select the combination of treatment parameters of hyperbaric oxygen therapy (hyperoxia) that are most likely to demonstrate improvement in the rate of good functional outcome following severe traumatic brain injury (TBI).

Methods: This is a multicenter, prospective, randomized, adaptive phase II clinical trial. Individuals aged 16 to 65 who present to one of

14 enrolling sites with severe TBI (GCS 3 to 8) will be eligible for inclusion. Individuals with GCS of 7 or 8 and a Marshall CT score of 1 or 2, and those with a GCS of 3 and bilaterally mid-position and nonreactive pupils will be excluded. Participants will be randomized to one of 8 interventions and treated for up to 5 days. There will be an initial burn-in period of 53 participants during which participants will be enrolled in a fixed randomization ratio of 6:6:6:6:6:6:1:1. A constant proportion of 20% of participants will be randomized to the control arm throughout the study. After the initial burn-in period, response adaptive randomization (RAR) will be performed. During this period, interim analysis of outcome data will be performed quarterly and the results will be used to adjust randomization probabilities to favor the better performing arms. Hyperoxia treatment will be initiated within 6 hours of hospital presentation for those not requiring craniotomy and within 12 hours of presentation for those requiring craniotomy/other major surgery. The primary outcome is functional recovery measured by a sliding dichotomized Glasgow Outcome Scale Extended (GOS-E) at 6-months post-injury. We will calculate the probability that a treatment arm is both superior to control and has a >50% chance of phase III success. A maximum of 200 subjects will be enrolled during a planned enrollment period of 3 years.

Conclusion: The HOBIT trial is designed to determine the most effective treatment parameters for hyperoxia and yield data that will support a clear go/no-go decision regarding whether the hyperoxia should proceed to a phase III trial.

Keywords: hyperbaric oxygen, Severe traumatic brain injury, clinical trial, adaptive design

A16-04

LOCAL BRAIN TISSUE WATER CONTENT IN TRAUMATIC BRAIN INJURY PATIENTS

Bornali Kundu, James Gee, Al-Wala Awad, Min Park, Gregory Hawryluk

University of Utah, Neurosurgery, Salt Lake City, USA

Brain edema predicts poor neurological outcomes in traumatic brain injury (TBI) patients, however, it is indirectly assessed by intracranial pressure (ICP) monitoring. Intraparenchymal thermal conductivity-based probes measure local cerebral blood flow but more recently have been used to derive a measure of tissue water (TW) content in the brain (i.e. local cerebral edema). Hypertonic saline boluses lower the TW content measure in neurosurgical patients. However, how this measure responds over time through a patient's ICU course has yet to be established. We investigated whether TW content responds to hyperosmolar and hypothermic therapy administered over time in neurosurgical patients. TBI patients with ICP monitoring placed due to poor neurological status were reviewed. A Camino Bolt (Integra Life Sciences Inc) monitoring system or an extraventricular drain was used. Patients were chemically paralyzed and cooled to a goal temperature of 34–35 degrees Celsius. A QFlow500 probe (Hemedex Inc.) was placed in the hemisphere containing more intact brain tissue according to current guidelines. Patient were monitored for several days. Correlation between TW and ICP, TW and serum sodium, and TW and temperature were calculated. N = 15 patients were included in this study. Results showed that there is a positive correlation between TW and ICP over time (mean $r=0.17$, $p<0.001$). An ICP of 17–20 mmHg is related to 75–76% TW content. Overall, there is a negative relationship between TW and serum sodium, though this seems to depend on the prior state of the brain, i.e. time from injury. Bolusing with hypertonic saline was related to lower TW content. Fi-

nally, there is a positive correlation between TW and body temperature over time for the patients whom were cooled ($n=3$). Local TW content is a novel measure that can be used as part of current multimodal neuromonitoring systems to allow for both global and local, targeted treatment of brain injury.

Keywords: Hemedex

A16-05

WHAT PROGNOSTIC INFORMATION DO TBI FAMILIES NEED AND PHYSICIANS DELIVER? RESULTS FROM A MULTI-CENTER QUALITATIVE STUDY

Thomas Quinn¹, Jesse Moskowitz¹, Muhammad W. Khan¹, Lori Shutter², Robert Goldberg³, Nananda Col⁴, Kathleen M. Mazor⁵, **Susanne Muehlschlegel**¹

¹*University of Massachusetts Medical School (UMMS), Neurology, Worcester, USA*

²*University of Pittsburgh Medical School, CritCare&Neurol, Pittsburgh, USA*

³*UMMS, Quant Health Sci, Worcester, USA*

⁴*Shared Decision Making Resources, Georgetown, USA*

⁵*UMMS, Meyers Prim. Care Inst. & Int Med, Worcester, USA*

Objective: Shared decision-making (SDM) is a collaborative family-centered process aimed at standardizing communication, enhancing family members' understanding of prognosis, actively weighing treatment risks/benefits, and matching them to patient values and preferences. As part of the ongoing research to create a SDM tool for goals-of-care decisions in critically-ill traumatic brain injury (ciTBI) patients, we sought to understand key communication preferences and practices by decision stakeholders (surrogates and physicians).

Methods: We conducted a qualitative study employing semi-structured interviews with 16 ciTBI surrogate decision-makers from two level-1 trauma centers, and 20 attending physicians representing geographic (Northeast, Mid-Atlantic, South, West, Midwest) and subspecialty diversity (neurocritical care, neurosurgery, trauma, palliative care). Two independent reviewers analyzed transcribed interviews using deductive and inductive approaches (NVIVO-software) to identify major themes. Theme saturation determined the final sample size.

Results: The majority of surrogates (82%) preferred numeric estimates describing the patient's prognosis, feeling it would limit prognostic uncertainty, which in turn surrogates perceived as frustrating. Conversely, 75% of physicians reported intentionally omitting numeric estimates during prognostication meetings due to low confidence in surrogates' abilities to appropriately interpret probabilities, worry about creating false hope, and distrust in the accuracy and data quality of existing TBI-outcome models. Physicians felt that TBI-outcome models are for research only and should not be applied to individual patients. Surrogates valued compassion during prognostication discussions, and acceptance of their goals-of-care decision by clinicians. Physicians and surrogates agreed on avoiding false hope.

Conclusions: We identified fundamental differences in the communication preferences of prognostic information between ciTBI patient surrogates and physicians. These findings inform the content of a SDM tool for goals-of-care discussions in ciTBI patients, but may also have important implications for improving communication practices in the neuroICU independent of a formal SDM tool.

Funding: NIH/NICHD 5K23HD080971

Keywords: Shared decision-making, Prognostication, Family-centered care, critical care

ACUTE AND HYPER-ACUTE MINI-OPEN LATERAL CORPECTOMY FOR THORACOLUMBAR TRAUMATIC BURST FRACTURES

William Smith^{1,2}, Nick Ghazarian³, Ginger Christian¹

¹Western Regional Center for Brain and Spine Surgery, Las Vegas, USA

²University Medical Center of Southern Nevada, Neurosurgery, Las Vegas, USA

³Valley Hospital Medical Center, Las Vegas, USA

Objective: Historically, spinal burst fracture treatment within 24 hours post-injury was considered an “acute” treatment when using conventional surgical approaches. Minimally disruptive lateral approaches for thoracolumbar corpectomy may minimize resource requirements. This study examined the feasibility of acute (<24 hours) and hyper-acute (<8 hours) treatment of thoracolumbar burst fractures to maintain or improve spinal injury scores.

Methods: Sixteen patients treated within 24 hours with a mini-open lateral corpectomy for traumatic spinal pathology were reviewed for preoperative, perioperative, and postoperative data. Neurologic status was assessed using ASIA scores. Fractures occurred primarily from L1 to L3. Wide-footprint expandable titanium devices were used in 75% of patients. All patients received supplemental fixation.

Results: Average time from injury to hospital (ER) was 1.8 hours, average time from the ER to operating room (OR) of 8.2 hours and an average OR time of 2.7 hours. Eight patients required ≤8 hours from injury event to surgical initiation while 7 were completed between 8 and 24 hours (one patient with incomplete surgical timing record). Blood loss averaged 646 mL without intraoperative complication. Length of hospital stay averaged 6 days. Average follow-up of 8.6 months with 15 of 16 patients available for neurologic status assessment. Eleven of the 15 patients (73%) experienced at least one ASIA grade improvement and 2 patients treated ≤8 hours showed improvements in 2 or 3 grades. No neurologic deteriorations were observed. One postoperative pleural effusion was treated with a chest tube and resolved without sequelae. One patient developed an asymptomatic inferior vertebral body compression fracture following cylindrical VBR subsidence.

Conclusion: These results suggest that mini-open lateral approaches, using direct visualization, allow safe and immediate decompression through hyper-acute (<8 hours) treatment of spinal burst fractures in eligible patients. Additionally, low perioperative and postoperative morbidity allow for hastened recovery.

Keywords: Thoracolumbar spine burst fractures, acute treatment, minimally invasive lateral corpectomy, ASIA scores, outcomes, complications

A16-07

ASSOCIATION BETWEEN HYPERNATREMIA AND MORTALITY IN PATIENTS WITH SEVERE TBI

Aditya Vedantam, Claudia Robertson, Shankar Gopinath

Baylor College of Medicine, Neurosurgery, Houston, USA

Hypernatremia is independently associated with increased mortality in critically ill patients; however, there is limited data on the effect of hypernatremia on TBI patients. The aim of the present study was to evaluate the impact of hypernatremia in patients with severe TBI treated in a neurocritical care unit. Patients with severe TBI treated in

a single neurocritical care unit from 1986-2012 were reviewed. Patients with a minimum of three recorded serum sodium values were selected. Patients with a diagnosis of diabetes insipidus or hypernatremia on admission were excluded. Hypernatremia was classified based on the highest serum sodium, and was classified as no hyponatremia (≤150 mEq/L), mild (151–155 mEq/L), moderate (156–160 mEq/L) and severe (>160 mEq/L). The independent effect of hypernatremia on mortality was assessed using a multivariate cox regression analysis. A total of 588 severe TBI patients were studied. Initial GCS was 3/15 in 220 patients (37.4%). The median number of serum sodium measurements for patients in this study was 17 (range 3–190). Mannitol was used in 109 patients (18.5%) and no patient received hypertonic saline. No hypernatremia was seen in 371 patients (63.1%), mild hypernatremia in 77 patients (13.1%), moderate hypernatremia in 50 patients (8.5%) and severe hypernatremia in 90 patients (15.3%). At discharge, 148 patients (25.2%) died. Mild (hazard ratio 3.4, 95% CI 1.94–5.97, $p<0.001$), moderate (hazard ratio 4.4, 95% CI 2.47–8.02, $p<0.001$), and severe (hazard ratio 8.4, 95% CI 5.13–13.86, $p<0.001$) hypernatremia were significant independent predictors of mortality. Significantly lower survival rates were observed for TBI patients with greater degrees of hypernatremia (log rank test, $p<0.001$). In this study, hypernatremia after admission was observed in 36.9% of severe TBI patients treated in a neurocritical care unit. In addition to severe hypernatremia, mild and moderate hypernatremia was associated with early mortality. These results highlight *additional* mortality risk associated with hypernatremia after severe TBI.

Keywords: hypernatremia, severe traumatic brain injury

A16-08

PHYSICIAN COMMUNICATION STRATEGIES IN EMERGENT BRAIN TRAUMA

Theresa Williamson, Peter Ubel

Duke University, Neurosurgery, Durham, USA

Background: 1.7 million people suffer traumatic brain injuries annually in the US, leading to 275,000 hospitalizations, 52,000 deaths, and an estimated 60 billion dollars in direct and indirect medical costs (Finkelstein et al 2006). The enormity of the problem lies in stark contrast to the paucity of research addressing how health care decisions are made in cases of severe traumatic brain injury. Health behavioral economics is defined as a method of analysis that applies psychological insights into human behavior to explain decision-making.

Specific Aims: This project aims to characterize the variability in physician description of prognosis, hospital course, and recommendations in patients with severe traumatic brain injury who meet criteria for neurosurgical intervention.

Methods: Using a case description, a qualitative study was used to determine what neurosurgery residents and faculty would communicate to a patient's family and questions pertinent to content, communication style, patient and physician factors were analyzed to characterize variability.

Results: Results include a qualitative description of physician preference and communication style in severe traumatic brain injury.

Conclusion: Overall, results will initiate a discussion of the way that human nature influences physicians and patients as they make crucial decisions in a disease process that affects millions.

Keywords: traumatic brain injury, physician communication, patient decision making

CONTINUOUS, NONINVASIVE OPTOACOUSTIC MONITORING OF CEREBRAL VENOUS BLOOD OXYGENATION IN PATIENTS WITH TRAUMATIC BRAIN INJURY

Satoshi Yamamoto¹, Claudia Robertson², Yuriy Petrov³, Irene Petrov³, Rinat Esenaliev³, Donald Prough¹

¹University of Texas Medical Branch, Anesthesiology, Galveston, USA

²Baylor College of Medicine, Neurosurgery, Houston, USA

³University of Texas Medical Branch, Center for Biomedical Engineering, Galveston, USA

Introduction: Clinical traumatic brain injury (TBI), the leading cause of death in North America for young persons, lacks specific treatments. In some centers, jugular bulb oximetry has been used to assess cerebral venous oxygen saturation (O₂sat) and to assist treatment, with a threshold of intervention <50%. We used a prototype, noninvasive optoacoustic system to assess real-time O₂sat in the superior sagittal sinus (SSS) in severely injured TBI patients and, in a subset of patients, compared optoacoustic SSS O₂sat with invasive jugular bulb O₂sat.

Methods: We studied TBI patients admitted to the Neuro ICU at Ben Taub Hospital, Houston. Patients were enrolled between May, 2009 and May, 2014. For short-term monitoring, we manually positioned an optoacoustic probe over the SSS and continuously measured during several-minute intervals of stable blood pressure. We compared mean optoacoustic SSS O₂sat with simultaneous jugular bulb O₂sat, while acknowledging that those two variables would be expected to be similar but not identical.

Result: 29 patients were enrolled. Measurements of SSS O₂sat were attempted in 25 patients. Satisfactory data were obtained in 16. Bland-Altman analysis of agreement between SSS and jugular bulb O₂sat in six patients in whom jugular bulb catheters were placed calculated bias and standard deviation of -1.8% and 4.7%, respectively.

Discussion: The optoacoustic system provides real-time noninvasive SSS O₂sat data that resemble simultaneous data from invasive jugular bulb oximetry. In previous experiments in anesthetized sheep comparing optoacoustic SSS O₂sat and SSS hemoximetry in blood from the same site, the measurements were highly correlated. Therefore, we speculate that optoacoustic SSS O₂sat may be an accurate, noninvasive assessment of matching between cerebral oxygen supply and demand.

Keywords: optoacoustic O₂ saturation, superior sagittal sinus (SSS), jugular bulb O₂ saturation, cerebral venous blood oxygenation

A16-10

AIR-EVACUATION-RELEVANT HYPOBARIA FOLLOWING TRAUMATIC BRAIN INJURY PLUS HEMORRHAGIC SHOCK WORSENS LUNG INJURY AND MORTALITY

Pratistha Tamrakar, Juliana Medina, Ashley Moore, Parisa Rangghran, Julie Proctor, Turhan Coksaygan, Gary Fiskum

University of Maryland Baltimore, Baltimore, USA

Background: Rats exposed to aeromedical evacuation (AE) relevant hypobarica within 72 hr after isolated traumatic brain injury (TBI) exhibit greater neurologic injury than those maintained under normobarica. This study tested the hypothesis that exposure to hypobarica worsens neurologic outcomes or mortality following polytrauma (PT)

consisting of controlled cortical impact (CCI) TBI followed by hemorrhagic shock (HS).

Methods: Following CCI, rats were subjected to HS, (MAP 35–40 mm Hg), for 30 min. Resuscitation utilized Hextend followed by blood re-infusion. At 24 hr post-surgery, rats were exposed to either normobarica (sea level) or hypobarica (=8000 ft altitude) for 6 hrs under normoxic (21 or 28%) or hyperoxic (100% O₂) conditions. Behavior was tested weekly for 4 weeks. Cortical lesion volumes were determined stereologically.

Results: Polytrauma injured rats experience a mortality rate of 30–35% which increases to 45% when exposed to AE relevant normoxic hypobarica and to 60% when hypobarica is combined with hyperoxia. Histologic evidence indicates that the differences in mortality were due to differences in lung injury. There was no difference in cortical neuropathology between the normobaric and hypobaric groups or between the normoxic and hyperoxic subgroups. While rats in all PT groups exhibited worse neuroscores and greater balance-beam foot-faults at 7 days, there were no effects of hypobarica or hyperoxia.

Conclusions: Exposure of rats after PT to 6 hr of hypobarica or hyperoxia or both results in increased mortality. Based on lung histopathology at 2 days following PT, the cause for this increase in mortality is exacerbation of lung injury. These findings are consistent with clinical studies indicating that critical care complications during AE can be reduced by increasing aircraft cabin pressures to the equivalent of 4000 ft altitude, compared to the commonly employed level of 8000 ft. Supported by US Air Force FA8650-15-2-6D21.

Keywords: Polytrauma, hypobarica, oxygen, lung injury, Aero-medical evacuation

A17 NEUROPATHOLOGY

A17-01

TRAUMATIC BRAIN INJURY SURVIVAL IS ASSOCIATED WITH WIDESPREAD CEREBRAL AMYLOID ANGIOPATHY

Josie Fullerton¹, Victoria E. Johnson², Sonam Ansel¹, Douglas H. Smith², William Stewart¹

¹University of Glasgow, Queen Elizabeth University Hospital, Glasgow, United Kingdom

²University of Pennsylvania, Penn Centre for Brain Injury and Repair and Department of Neurosurgery, Pennsylvania, USA

Introduction: There is growing recognition of increased risk of late neurodegenerative disease, including chronic traumatic encephalopathy, in survivors from traumatic brain injury (TBI). Neuropathological observations in late survivors of TBI describe a complex pathology including abnormalities in tau, amyloid-beta (A-beta) and blood-brain barrier disruption. To date, while increased A-beta plaque deposition has been described as a frequent observation in late survivors of TBI, other aspects of A-beta pathology remain less well documented. Given the widespread vascular pathology documented in TBI, of particular interest is its influence on cerebral amyloid angiopathy (CAA).

Methods: From the Glasgow TBI Archive cases with a history of long-term survival (>6 months) following a single moderate or severe TBI (n=44; median age 53.5 y) and uninjured, age-matched controls (n=47; median age 55 y) were selected. Using A-beta immunohistochemistry, multiple cortical regions were examined for presence, distribution and extent of CAA, with sections scored using standardised semi-quantitative techniques. Material was also prepared for APOE genotyping.

Results: Incidence of CAA was lower in long-term survivors of TBI (6/44) than in uninjured, age-matched controls (14/47; $p=0.029$). However, while CAA was localized to a single brain region in the majority (83%) of controls, where present, in late TBI survivors all cases with CAA showed more widespread disease with two or more regions involved ($p=0.001$). Further, CAA in late survivors of TBI was considerably more extensive than in controls (CAA score TBI 6.3 ± 3.4 v 2.1 ± 1.1 controls; $p<0.0005$).

Conclusion: In this cohort of patients surviving 6 months or more from single moderate or severe TBI, where present, CAA is considerably more widespread and extensive than in age matched controls. These data add to our knowledge of the complex pathologies in TBI survivors, shedding light on further significant A-beta pathology after TBI. Supported by grants DOD PT110785, NIH NS056202/NS038104.

Keywords: traumatic brain injury, amyloid, cerebral amyloid angiopathy, chronic traumatic encephalopathy

A18 NEUROPROTECTION

A18-01

EVALUATION OF MINOCYCLINE IN THE MIAMI FLUID PERCUSSION MODEL OF TRAUMATIC BRAIN INJURY: AN OBTT CONSORTIUM STUDY

Helen Bramlett, Ofelia Furones-Alonso, Juliana Sanchez-Molano, Jessie Truettner, William Moreno, Ryan Treu, W. Dalton Dietrich
University of Miami Miller School of Medicine, Neurological Surgery, Miami, USA

Minocycline (MIN) has been studied in various TBI models which have been positive. This may be due to the drugs pleiotropic effects targeting microglial activation, reduced cellular apoptosis and inflammation. Minocycline was chosen as the tenth drug for testing by the multicenter consortium Operation Brain Trauma Therapy. The University of Miami site tested Minocycline in our model of fluid percussion TBI. Male Sprague-Dawley rats were anesthetized and underwent moderate fluid percussion (FP; 1.8-2.1atm) TBI or sham surgery. Rats were randomized and administered a bolus (30 mg/kg, IV) of Minocycline followed by 72h infusion (2 mg/kg/h) or vehicle beginning 15 min after TBI. This protocol produced steady state blood levels mimicking those seen in the successful phase II clinical trial in human SCI. Animal groups were TBI-MIN ($n=15$), TBI-Veh ($n=15$) or Sham ($n=15$). Rats were tested on day 7 post-injury for sensorimotor function. On days 13–21, rats were assessed for cognition utilizing the simple place task, probe trial and working memory task. On day 21, brain tissue was processed for histology. One-way ANOVA was not significant for the sensorimotor tasks. For the hidden platform task, two-way repeated measures ANOVA for latency was significant for group ($p<0.05$) but not for group \times day. MIN appears to provide some improvement on latency with a trend towards TBI-MIN better than TBI-Veh ($p=0.062$). There was no significant difference between groups for the probe trial. Repeated measures ANOVA for working memory latency was not significant for group ($p<0.05$). We conclude that treatment with Minocycline after FP did not improve sensorimotor but did slightly improve cognitive function. At this time, behavioral findings of Minocycline treatment in the FP model in rats do not support its further testing across models in OBTT, but may merit additional testing in combination therapy, particularly in mild TBI models. DoD W81XWH-10-1-0623 W81XWH-14-2-0018.

Keywords: traumatic brain injury, OBTT, fluid percussion

A18-02

EVALUATION OF MINOCYCLINE IN THE CONTROLLED CORTICAL IMPACT MODEL OF TRAUMATIC BRAIN INJURY: AN OBTT CONSORTIUM STUDY

C. Edward Dixon¹, Hong Yan¹, Xiecheng Ma¹, Philip Empey^{1,2}, Samuel Poloyac^{1,2}, Keri Feldman¹, Patrick M. Kochanek¹

¹University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

²University of Pittsburgh, Pharmacy, Pittsburgh, USA

Several studies have reported positive effects with minocycline therapy in experimental traumatic brain injury via multiple mechanisms. Minocycline was chosen as the tenth drug tested by the multicenter consortium Operation Brain Trauma Therapy. The University of Pittsburgh site tested minocycline in the controlled cortical impact (CCI) model. Male Sprague-Dawley rats were anesthetized and underwent CCI (4m/sec, 2.8 mm deformation) or sham surgery. Rats were randomized into three groups ($n=10$ /group) and administered a minocycline regimen that mimics steady state blood levels seen in a successful phase II clinical trial in spinal cord injury (single IV bolus 30 mg/kg 15 min after CCI followed by infusion [2 mg/kg/h] for 72hr), CCI followed by vehicle treatments, or sham surgery. Functional outcomes were tested via beam balance and beam walking tests (days 3–5), Morris water maze (MWM) acquisition (days 14–18) and probe trial (day 18). Rats were sacrificed on day 21 for histology. A repeated-measures ANOVA revealed no significant group effects for beam balance and a significant group effect for beam walk latencies ($p<0.016$). None of the injured groups differed from each other. In the beam walking test, only the CCI+Mino group differed from the sham group. A significant group effect ($p=0.0001$) was found for the MWM test. While swim latencies for the injured groups differed significantly from shams, they did not differ from each other. There were no group differences in the probe trial. There were no treatment effects on lesion volume or hemispheric tissue loss. We conclude that treatment with minocycline after CCI did not improve function or histopathology. Although biomarker data are pending, these findings do not support further testing of minocycline monotherapy in the CCI model in OBTT. Support: US DoD W81XWH-10-1-0623 W81XWH-14-2-0018.

Keywords: OBTT, Neurobehavioral Function, Controlled cortical impact, Traumatic brain injury

A18-03

COMBINATION THERAPY OF LEVETIRACETAM AND SIMVASTATIN IN A RAT MODEL OF PENETRATING BALLISTIC-LIKE BRAIN INJURY

Caitlin Groeber Travis, Starlyn Okada-Rising, Rebecca Pedersen, Justin Sun, Krista Caudle, Kristen DeDominicis, Jenny Browning, Sindhu Kizhakke Madathil, Xi-Chun May Lu, Janice Gilsdorf, Jignesh Pandya, Deborah Shear

Walter Reed Army Institute of Research, Brain Trauma Neuroprotection & Neurorestoration, Silver Spring, USA

Levetiracetam (LEV) is an antiepileptic drug and Simvastatin (SIM) is a drug used to reduce serum cholesterol. Both drugs have shown neuroprotective effects when tested individually in several pre-clinical models of traumatic brain injury (TBI). Our previous monotherapy studies of these drugs confirmed their neuroprotective efficacy in a model of penetrating ballistic-like brain injury (PBBI) in rats. Based on the monotherapy dose-response profile of each drug,

isobolographic analysis was used to construct fixed-dose ratios for a combination therapy of LEV and SIM to identify potential additive or synergistic effects in the rat model of PBBI. Unilateral frontal PBBI (10%) was induced in the right hemisphere of isoflurane anesthetized rats. LEV and SIM were tested with the following dose ratios (LEV/SIM, mg/kg): 6.25/0.01, 12.5/0.02, 25/0.04, 50/0.06, and 100/0.08. Both drugs were administered intravenously (LEV by bolus injection; SIM by infusion) at 30 min and 6 hours post-PBBI. Additional treatments were given daily (LEV 2x/day; SIM 1x/day) for 10 days post-PBBI. The order of administration was counter-balanced. The rotarod task was used to evaluate motor function 7 and 10 days post-PBBI. The Morris water maze was used to evaluate cognitive performance 13–17 days post-PBBI. Brains were collected 22 days post-PBBI for histopathological analysis. There were significant motor and cognitive deficits in all injury groups compared to sham. Isobolog analysis of the results identified sub-additive effects between Levetiracetam and Simvastatin in the PBBI model indicating this drug combination may not be useful for neuroprotective therapy.

Keywords: Levetiracetam, Simvastatin, TBI, Penetrating Ballistic-Like Brain Injury, Rat, Behavior

A18-04

NEUROPROTECTIVE EFFECTS OF NANO-PULSED LASER THERAPY (NPLT) IN THE RAT FLUID PERCUSSION INJURY MODEL

Jutatip Guptarak¹, Margaret A Parsley¹, Kathia M Johnson¹, Ian J Bolding¹, Irene Petrov², Yuriy Y Petrov², Rinat O Esenaliev^{2,3}, Donald S Prough¹

¹University of Texas Medical Branch, Anesthesiology, Galveston, USA

²University of Texas Medical Branch, Center for Biomedical Engineering, Galveston, USA

³University of Texas Medical Branch, Department of Neuroscience and Cell Biology, Galveston, USA

Background: Effective therapeutic interventions after traumatic brain injury (TBI) are aimed at prevention of further brain damage after the primary impact. We have previously shown that transcranial delivery of Nano-Pulsed Laser Therapy (NPLT) is neuroprotective in rats with blast-induced neurotrauma. NPLT combines near-infrared laser light (808nm) and laser-generated, low-energy optoacoustic waves for therapeutic effects. Due to the success of NPLT in prior studies, we investigated its neuroprotective effects when administered shortly after injury in the rat fluid percussion injury (FPI) model of TBI.

Methods: Adult male Sprague-Dawley rats were randomly assigned to receive FPI or SHAM surgery. NPLT was delivered transcranially for five minutes at one hour post-injury. One day after treatment, brains were removed and processed for Fluoro Jade C (FJC; indicative of cell injury) and for activated caspase 3 immunofluorescence (indicative of apoptosis). FJC⁺ cells were counted by two investigators in a blinded fashion.

Results: FJC⁺ cells were not present in the brains of SHAM and SHAM+NPLT rats. In FPI rats, FJC⁺ cells were present in the cortex, hippocampus and thalamus. Active caspase-3 immunoreactivity colocalized with FJC suggesting that these cells were undergoing apoptosis. The number of FJC⁺ cells was reduced in the brain of FPI+NPLT rats as compared to FPI rats.

Conclusions: Early NPLT treatment after FPI reduces neuronal cell death in the hippocampus, cortex and thalamus. Further investigation on the effects of NPLT beyond one day and the mechanisms mediating them will help elucidate therapeutic potentials of this non-invasive, novel therapeutic strategy to treat TBI.

Funding: These studies were completed as part of an interdisciplinary research team funded by The Moody Project for Translational Traumatic Brain Injury.

Keywords: Fluid percussion injury, Nano-Pulsed Laser Therapy, Fluoro Jade C, Active caspase-3, Sprague-Dawley rats

A18-05

EVALUATION OF TAURINE NEUROPROTECTION IN THE CONTROLLED CORTICAL IMPACT MODEL OF BRAIN INJURY IN AGED RATS

Raeesa Gupte, Sarah Christian, Paul Keselman, Janna Harris
University of Kansas Medical Center, HoUniversity glund Brain Imaging Center, Kansas City, USA

Despite higher rates of hospitalization and mortality following traumatic brain injury (TBI) in patients over 65 years old, this age group is highly underrepresented in drug development and treatment studies. Worse outcomes in elderly individuals compared to younger adults could be attributed to exacerbated injury mechanisms including oxidative stress, inflammation, blood-brain barrier disruption, and bioenergetic dysfunction. Taurine, an endogenous amino acid and nutritional supplement that functions as an antioxidant, anti-inflammatory, cellular osmolyte, and neuromodulator is neuroprotective in adult rats with TBI. However, its effects on the aged brain have not been explored. Therefore, the objective of our study was to determine whether taurine is neuroprotective in an aged rat model of TBI. Aged male F344 rats (21 months old) were anesthetized and subjected to unilateral controlled cortical impact injury to the sensorimotor cortex (5 m/s, 2.5 mm depth). Rats were then randomized into four groups (n=9–10 per group) and administered taurine (200 mg/kg, 50 mg/kg, or 25 mg/kg i.p.) or saline 20 minutes post-injury and then daily for 7 days. Sensorimotor functional outcomes were assessed using beam walk and bilateral adhesive removal test at 1, 3, 7, 10 and 14 days post-TBI. Magnetic resonance images were obtained at day 14 post-TBI and analyzed using ImageJ to estimate lesion volumes. Experimenters were blinded to the treatment group for the duration of the study. MRI analysis showed a dose-dependent decrease in TBI lesion volume in taurine-treated groups compared to saline. However, taurine did not significantly improve sensorimotor outcomes. This suggests that while taurine promotes tissue sparing in aged rats following TBI, this may not be sufficient to rescue gross function of the remaining tissue.

Supported by: Funds from NIH (R21 NS091920) and a University of Kansas Lied Basic Science grant to Dr. Harris. The Hoglund Brain Imaging Center is supported by a generous gift from Forrest and Sally Hoglund.

Keywords: Taurine, Neuroimaging, Sensorimotor function

A18-06

PHENELZINE ADMINISTRATION FOLLOWING TRAUMATIC BRAIN INJURY IMPROVES SYNAPTIC AND NON-SYNAPTIC MITOCHONDRIAL RESPIRATORY FUNCTION

Rachel Hill, Indrapal Singh, Juan Wang, Jacqueline Kulbe, Edward Hall
University of Kentucky, SCoBIRC/Neuroscience, Lexington, USA

Introduction: Traumatic brain injury (TBI) results in the production of peroxynitrite (PN), leading to oxidative damage of lipids and

protein. PN-mediated lipid peroxidation (LP) results in production of 4-hydroxy-2-trans-nonenal (4-HNE). We had previously demonstrated that pharmacological inhibition of LP by phenelzine (PZ), a 4-HNE scavenger, preserved total cortical mitochondrial bioenergetics. The goal of these studies was to evaluate differences in PZ-mediated protection between the non-synaptic or synaptic cortical mitochondrial populations following TBI.

Methods: Male Sprague-Dawley rats received a severe (2.2 mm) controlled cortical impact (CCI)-TBI. PZ was administered subcutaneous at 15 min (3, 10 & 30 mg/kg (24 hr group) and 10 mg/kg (48 hr group)) with a maintenance dose at 24 hpi (5 mg/kg; 48 hr group only). Non-synaptic and synaptic cortical mitochondria were isolated at either 24 or 48 hpi and respiratory rates were measured using a Clarke-type electrode.

Results: At 24 hrs post-TBI vehicle-treated animals had significantly lower non-synaptic and synaptic mitochondrial respiration rates compared to Sham. Administration of PZ at the 10 mg/kg dosing paradigm significantly improved non-synaptic mitochondrial respiration rates for all States compared to vehicle-treated animals. Synaptic mitochondrial rates were all improved by the 10 mg/kg dose, and significantly for States III, V (I) and V (II). At 48 hpi vehicle-treated animals had significantly lower non-synaptic and synaptic mitochondrial respiration rates compared to Sham. Administration of PZ significantly improved non-synaptic mitochondrial respiration rates for all States compared to vehicle-treated animals. Synaptic mitochondrial rates were all improved by PZ, however, only RCR reflected any significant improvement compared to vehicle-treated animals.

Conclusions: These results demonstrate that PZ administration preserves mitochondrial bioenergetics at 24 hrs and that this protection can be maintained out to 48 hrs post-injury. These results extend previous results from studies looking at the effect of PZ treatment on total mitochondrial function following TBI.

Acknowledgments: This work was supported by NIH/NINDS R01 NS084857.

Keywords: Traumatic Brain Injury, Synaptic and Non-Synaptic Mitochondria, LP-Mediated Oxidative Damage, 4-HNE, Phenelzine

A18-07

PHARMACOLOGICAL INHIBITION OF OXIDATIVE DAMAGE BY THE LAZAROID U-74389G IMPROVES CORTICAL MITOCHONDRIAL FUNCTION FOLLOWING TBI

Rachel Hill, Indrapal Singh, Juan Wang, Jennifer Brelsfoard, Edward Hall

University of Kentucky, SCoBIRC/Neuroscience, Lexington, USA

Traumatic brain injury (TBI) results in hemoprotein breakdown leading to cellular accumulation of free iron. Iron (Fe) can react with hydrogen peroxide (H₂O₂) and/or lipids resulting in post-traumatic Fe-mediated oxidative damage. The lazaroid U-74389G (desmethylated Tirilazad; TZ), an inhibitor of Fe-dependent lipid peroxidation (LP), was used to protect mitochondrial function following TBI in young adult male rats. Male SD rats received a severe (2.2 mm) controlled cortical impact-TBI. TZ was administered intravenous (iv) at 15 min and 2 hrs post injury (hpi) followed by an intraperitoneal (ip) dose at 8 hpi at the following doses (mg/kg): 0.3 (iv) +1 (ip), 1+3, 3+10, 10+30. Total cortical mitochondria were isolated at 72 hpi and respiratory rates were measured using a Clarke-type electrode. Mitochondrial 4-HNE and acrolein were evaluated as indicators of LP-mediated oxidative damage by quantitative Western immunoblot. At 72 hrs post-TBI injured animals had

significantly lower mitochondrial respiration rates compared to Sham. Administration of TZ at the 1 mg/kg dosing paradigm significantly improved mitochondrial respiration rates for State II, State III, Complex II driven State V and RCR compared to vehicle-treated animals. At 72 hrs post-TBI injured animals had significantly higher levels of mitochondrial 4-HNE and acrolein compared to Sham and administration of TZ reduced reactive aldehydes levels compared to vehicle-treated animals. The aim of this study was to explore the hypothesis that interrupting secondary oxidative damage via acute pharmacological inhibition of Fe-mediated LP by TZ following a CCI-TBI would provide mitochondrial neuroprotective effects in a dose-dependent manner. We found that acute administration of TZ to injured rats resulted in improved mitochondrial function and lowered the levels of reactive aldehydes in the mitochondria. These results establish not only the most effective dose of TZ treatment to attenuate LP-mediated oxidative damage, but also set the foundation for further studies to explore additional neuroprotective effects following TBI.

This work supported by NIH/NINDS R01 NS083405.

Keywords: Traumatic brain injury, Mitochondria, Iron-mediated lipid peroxidation, 4-HNE, Acrolein

A18-08

EXPRESSION PATTERNS OF HEAT SHOCK PROTEIN 27, 70 AND 90 DEPENDING ON TIME COURSE IN CONTUSIVE SPINAL CORD INJURED RATS

Kyungwon Yang^{1,2}, Youngkyung Kim^{1,2,3}, Koeun Lee⁴, Youngwook Yoon³, **Junesun Kim**^{1,2}

¹*Korea Univ. College of Health Science, Dept. of Health Science, Seoul, Korea South*

²*BK 21 program, Korea Univ. Grad. Sch., Seoul, Korea South*

³*Korea Univ. College of Medicine, Dept. of Physiology, Seoul, Korea South*

⁴*Korea National Research Institute of Health, Cheongju, Korea South*

Traumatic spinal cord injury (SCI) causes sensory and motor dysfunction below the injured level. A poor intervention in the primary damage period may result in permanent handicap. Heat Shock Proteins (Hsp), proteins families of the molecular chaperone, are induced by cell stressful conditions. Recently, Hsp70 has been found to play an important role for neuroprotection relevance to cell death after SCI. However, the underlying mechanisms of the contribution Hsp70 on neuroprotection after SCI remain unclear. Moreover there is a lack of study about the role of Hsp27, and 90 following the damage of spinal cord. Thus, we investigated the changes of Hsp27, 70, and 90 expression patterns in spinal cord after injury. The contusive SCI was made by New York University (NYU) impactor at the T10 level from 12.5 mm height under anesthesia. To assess the changes of Hsp27, 70, and 90 expressions was performed immuno-blotting in rostral, epicenter, caudal and remote site at 4 h, 8 h, 12 h, 24 h, 3 d, and 7 d after injury. Hsp27 expression was slightly increased in all spinal segment from 4 h to 7 d after SCI. A significant change was shown in 7 days after damage. Hsp70 was increased immediately after damage in the epicenter but it was returning back around basal level. In contrast to Hsp27 and 70, the expression of Hsp90 was decreased to less than basal level until 7 days after SCI. Our findings suggest that in studying mechanisms of Hsp, a critical time point for neuroprotection is different depending on target Hsp families.

This study is supported by NRF funded by the Ministry of Science, ICT, and Future planning Grant (2014M3C1B2048632)

Keywords: Heat shock protein, HSP, Spinal cord injury

NEUROPROTECTIVE EVALUATION OF THE COMBINATION PHENELZINE AND CYCLOSPORINE A FOLLOWING SEVERE CONTROLLED CORTICAL IMPACT TRAUMATIC

Jacqueline Kulbe, Indrapal Singh, Juan Wang, Edward Hall
University of Kentucky College of Medicine, Spinal Cord and Brain Injury Research Center, Lexington, USA

There are currently no FDA-approved pharmacotherapies able to prevent the devastating neurological deficits caused by traumatic brain injury (TBI). Due to complex secondary injury mechanisms which occur following TBI, combinational therapies should enhance neuroprotection above single agents alone. Two pivotal secondary injury mechanisms, lipid peroxidation (LP) and mitochondrial dysfunction, peak 72 h following injury. Therefore, continuous infusion of neuroprotective agents over the first 72 h following injury may also be required for optimal neuroprotection. Mitochondria are essential mediators of the TBI secondary injury cascade, including lipid peroxidation. As such, mitochondria are promising therapeutic targets for the prevention of cellular death and dysfunction following TBI. Phenelzine (PZ), an anti-depressant capable of scavenging LP-derived neurotoxic aldehydes, and cyclosporine A (CsA), an immunosuppressant capable of inhibiting the mitochondrial permeability transition pore (mPTP), have previously been shown to be partially neuroprotective, including protection of mitochondria, following TBI. This is the first study to evaluate the effects of a continuous 72 h infusion by subcutaneous osmotic pump of PZ, CSA, or PZ + CsA on mitochondria 72 h following severe controlled cortical impact injury in rats. Although neither PZ, CsA, nor PZ + CsA were able to improve mitochondrial respiratory function in the first dosing paradigm evaluated (PZ: 10mg/kg s.c. 15min post-injury +10mg/kg/day/72 h; CsA: 20mg/kg i.p. 15 min post-injury +10mg/kg/day/72 h), individually PZ and CsA, but not the combination PZ+CSA, were able to attenuate modification of mitochondrial proteins by LP-derived neurotoxic aldehydes. However, it is premature to conclude that combining aldehyde scavenging with inhibition of mPTP is not neuroprotective following TBI. Therefore, kinetics studies, assessment of additional outcome measures, and evaluation of additional PZ mechanisms of action, such as monoamine-oxidase inhibition, will be pursued in order to optimize dosing paradigms.

This research is funded by NIH-NINDS 5R01 NS083405 and 5R01 NS084857. JR Kulbe is currently supported by NINDS F30 NS096876

Keywords: Mitochondria, Combinational Therapy, Neurotoxic Aldehydes, Lipid Peroxidation, Phenelzine, Cyclosporine A

A18-10

EVALUATION OF MINOCYCLINE IN THE WRAIR PBBI MODEL: STUDIES FROM THE OPERATION BRAIN TRAUMA THERAPY (OBT) CONSORTIUM

Starlyn Okada-Rising, Jignesh Pandya, Krista Caudle, Jenny Browning, Rebecca Pedersen, Justin Sun, Deborah Shear
Walter Reed Army Institute of Research, Brain Trauma Neuroprotection, Silver Spring, USA

Minocycline was the 10th drug selected for testing by the OBT consortium. Minocycline is a broad-spectrum tetracycline antibiotic shown to be neuroprotective in neurodegenerative diseases and experimental models of stroke, spinal cord injury (SCI), and brain

trauma. It has been reported to inhibit microglial activation, prevent oligodendrocyte and/or neuronal apoptosis, decrease oxidative damage, and reduce inflammation. A phase II clinical trial for acute SCI found Minocycline safe and tended to improve several motor recovery outcomes. The WRAIR site evaluated the effectiveness of Minocycline in the penetrating ballistic-like brain injury (PBBi) model. PBBi (10%) was performed unilaterally in the right hemisphere of anesthetized rats. A 30 mg/kg Minocycline loading bolus was administered intravenously 15 mins post-PBBi followed by continuous 72-hour infusion (2 mg/kg/hr). This protocol was shown in PK studies by our group to produce steady state blood levels ranging between 5–10 mcg/mL, mimicking those in the successful phase II clinical trial. Groups consisted of TBI-MIN (n = 14), TBI-Veh (n = 19) or Sham (n = 16). Motor and cognitive performance were assessed using rotarod (days 7 and 10 post-PBBi) and Morris water maze (MWM, days 13–17 post-PBBi). On day 21, brain tissue was processed for histology. Motor testing showed significant injury-induced deficits versus sham (p < .05). Overall rotarod latencies were reduced by 51.8 ± 5.5% (TBI-Veh) and 47.4 ± 8% (TBI-MIN) vs. sham. No significant therapeutic effects were detected on the rotarod task. MWM results revealed significant injury-induced deficits with latencies to locate the submerged platform increased by 56.8 ± 14% (TBI-Veh) and 52.9 ± 14.8% (TBI-MIN) versus sham. Positive trends towards improved memory retention (probe trial) and reduced lesion size were observed, but not significant. Current findings do not support further testing of Minocycline using this dosing regimen. However, additional testing may be warranted using higher doses and/or extended treatment durations in order to overcome peak microglial and inflammatory responses in the PBBi model. Supported by U.S. Army Grant W81XWH-10-1-0623.

Keywords: traumatic brain injury, OBT

A18-11

MITOCHONDRIAL TRANSPLANTATION FOLLOWING CONTUSION SPINAL CORD INJURY

Samirkumar Patel¹, Jenna Gollihue¹, Khalid Eldahan¹, David Cox¹, Patrick Sullivan², Alexander Rabchevsky¹

¹University of Kentucky, Spinal Cord & Brain Injury Research Center, Dept of Physiology, Lexington, USA

²University of Kentucky, Spinal Cord & Brain Injury Research Center, Dept of Neuroscience, Lexington, USA

The current study tested whether transplantation of exogenous mitochondria into the contused rat spinal cord results in 1) their incorporation into various host cell types, 2) improving overall bioenergetics of host cells, and 3) increasing long-term functional neuroprotection. For visualization of transplanted mitochondria, we used transgenically modified PC12 cells in which mitochondria were labeled with tGFP, and for clinical relevance, we also used mitochondria isolated from rat soleus muscle. Freshly isolated tGFP (50, 100 or 150 µg/cord) or muscle (50 or 100 µg/cord) mitochondria were microinjected into the penumbra of severely contused spinal cords within 1 hr after spinal cord injury (SCI) at L1/L2 (250 kdyn using IH Impactor) in adult female Sprague-Dawley rats. Depending on outcome measures, they survived 24 hr, 48 hr, 7 days or up to 6 weeks. Results showed that transplantation of either tGFP or muscle mitochondria significantly maintained bioenergetics of injured spinal cord tissues 24 hr after injury, with maximum effects at the 100 µg dosage. Confocal imaging showed prominent rostro-caudal spread of

exogenous tGFP mitochondria from injection sites after 24–48 hr that dissipated by 7 days. At the earlier time points, tGFP mitochondria co-localized conspicuously with microglia/macrophages and endothelial cells, with less incidences in astrocytes and oligodendrocytes, and none in neurons. Assessments of hindlimb functional recovery (BBB-LRS) and paw withdrawal latencies (Von Frey hair) over 6 weeks after transplanting 100ug tGFP or muscle mitochondria showed no significant differences in over-ground locomotion or mechanical hypersensitivity compared to vehicle-injected injured groups. Morphometric analyses further showed no differences in grey or white matter tissue sparing. In summary, intraspinal injections of mitochondria after contusion SCI improved cellular bioenergetics acutely, but that such maintenance of respiration did not translate into improved long-term functional neuroprotection. Supported by NIH/NINDS R21NS096670 (AGR), NRSA F31NS093904 (JLG).

Keywords: Respiration, Enzyme activities, Hindlimb locomotor recovery, Von Frey hair

A18-12

NRF2 AND P53 TRANSCRIPTION FACTOR MODULATORS ENHANCE NEUROPROTECTION IN AN IN VITRO MILD TBI MODEL

Whitney Ratliff^{1,2}, Jessica Chang^{1,2}, David Driscoll¹, Miseanne Tang¹, Sarah Neyra¹, Nigel Greig³, Bruce Citron^{1,2}

¹Bay Pines VA Healthcare System, R&D, Bay Pines, USA

²Morsani College of Medicine, University of South Florida, Molecular Medicine, Tampa, USA

³National Institute on Aging, NIH, Translational Gerontology Branch, Baltimore, USA

In the US, approximately 1.7 million people sustain a traumatic brain injury (TBI) each year. In our military and athletes, TBI remains a major cause of morbidity and mortality. The majority of TBIs are mild, but can result in deleterious cognitive effects for which there is no effective treatment. tBHQ is an activator of the transcription factor, Nrf2, and has been shown to induce production of several neuroprotective factors. Pifithrin- α compounds, which inhibit p53, demonstrated neuroprotective effects in an *in vitro* excitotoxicity model and an *in vivo* head injury model. To evaluate the effects of a repetitive physical injury on a neuronal cell line and characterize the potential protective effects of treatment, we used an *in vitro* “injury in a dish” model. tBHQ plus the sulfur containing inhibitor, PFT- α , produced significantly improved neuronal function over single treatments measured with an MTS assay. We investigated treatment with tBHQ, oxygen containing inhibitor, PFT- α (O), and a combination to determine whether activation of Nrf2 and inhibition of p53 would provide a synergistic neuroprotective effect. Observations indicated that treatment with tBHQ plus PFT- α (O) improved overall neuronal survival and neurite health, when compared to untreated cells or those that received only one treatment. In addition, qRT-PCR array data has identified differentially regulated genes in response to combination treatment. Anti-inflammatory heme oxygenase 1 mRNA was increased, which may account for some neuroprotective effects. This suggests that simultaneous activation of Nrf2 and inhibition of p53 produces enhanced neuroprotective effects in response to neuronal injury and could represent a novel treatment in response to head injury.

Keywords: transcription factors, in vitro injury, Nrf2, p53, synergistic treatment

A18-13

THE NEUROPROTECTIVE EFFECTS OF A NOVEL HYBRID COMPOUND FOR TRAUMATIC BRAIN INJURY

Dong Sun, Omar Protzuk, Andrew Rolfe, Jacob Fulp, Shijun Zhang
Virginia Commonwealth Univ., Anatomy and Neurobiology, Richmond, USA

Following TBI, the brain undergoes serious pathological changes, which compromise neuronal survival and plasticity, and contributes to numerous functional deficits. Thus far, published studies have found that two natural products, curcumin (a dietary product) and melatonin (the major pineal hormone), have beneficial effect for TBI recovery. Recently, we have developed a novel hybrid compound derived from curcumin and melatonin, ZCM-I-1. In a mouse transgenic model of Alzheimer's disease, this novel compound showed significant effect in reduction of oxidative stress and microglial activation, enhancement of mitochondrial function and synaptic plasticity. Based on this observation, we explored the effect of ZCM-I-1 for TBI treatment. In this study, adult male Sprague-Dawley rats were subjected to a moderate cortical impact injury. At 30 minutes, 1 day, and 2 days following injury, 50 mg/kg of ZCM-I-1 was administered i.p. for a total of 3 doses. Sensorimotor functions were tested using beam walking, rotarod, and neurological score testing methods. Cognitive functions were assessed using Morris Water Maze latency and probe trial tests. Animals were sacrificed at 2 or 28 days post-injury in order to assess acute and chronic response to treatment, respectively. Brain tissues were processed for histological examination to assess injury-induced degenerative neurons with FJB staining. Cortical lesion volume and neuronal cell survival were also assessed. We demonstrated that following TBI, ZCM-I-1 administration significantly reduce the number of degenerative neurons in the injured cortex and hippocampal neurons. Injured animals received ZCM-I-1 treatment also had significant improvement in both motor and cognitive functions. Our data suggest that our novel compound has neuroprotective effects for TBI. Further study examining the effect of ZCM-I-1 treatment on post-TBI neuroplasticity is ongoing.

Keywords: traumatic brain injury, neuroprotection, cell death, cognitive function, neuroplasticity

A18-14

NEUROPROTECTIVE AND COGNITIVE BENEFITS OF ACUTE PHOSPHODIESTERASE 4B INHIBITION AFTER TRAUMATIC BRAIN INJURY

Nicole Wilson¹, Mark Gurney², W. Dalton Dietrich¹, Coleen Atkins¹

¹University of Miami Miller School of Medicine, Neurological Surgery, Miami, USA

²Tetra Discovery Partners, Grand Rapids, USA

Finding molecular targets to attenuate traumatic brain injury (TBI)-induced pathologies and cognitive deficits is of critical importance for the estimated 3–5.3 million people currently living with TBI-related disabilities in the United States. Previously, we reported that acute inhibition of the cAMP-hydrolyzing enzyme, phosphodiesterase 4B (PDE4B), reduced inflammation and cortical contusion volume after TBI. However, it was unknown whether the therapeutic benefits of acute PDE4B inhibition led to reduced behavioral deficits, neuronal loss and atrophy after TBI. To determine whether acute PDE4B inhibition attenuated TBI-induced behavioral deficits and pathology, adult male Sprague Dawley rats received sham surgery or moderate parasagittal fluid-percussion brain injury (2 ± 0.2 atm) and were then

treated with a PDE4B-selective inhibitor, A33, or vehicle for up to 3 days post-surgery. Animals were assessed from 1 to 6 weeks post-surgery for forelimb placement asymmetry, contextual fear conditioning, water maze performance and spatial working memory. We found that A33 treatment significantly improved contextual fear conditioning and water maze retention at 24 hrs post-training. However, this treatment did not rescue sensorimotor or working memory deficits. At 2 months after surgery, atrophy and neuronal loss were assessed. A33 treatment significantly reduced neuronal loss in the pericontusional cortex and hippocampal CA3 region. This treatment paradigm also reduced cortical, but not hippocampal, atrophy. These results suggest that acute PDE4B inhibition may be a viable treatment to reduce pathology and memory deficits after TBI. Altogether, the multifactorial and beneficial effects observed with A33 treatment support the use of PDE4B inhibitors as an anti-inflammatory, and possibly neuroprotective, treatment strategy for TBI.

This work was supported by NINDS F31 NS089351 and R01 NS056072, NIMH R44 MH091791 and The Miami Project to Cure Paralysis.

Keywords: PDE4B, cAMP

A18-15

A COMBINATION OF FA-GC AND BPV IMPROVE LOCOMOTOR RECOVERY AND BLADDER FUNCTION AFTER CONTUSIVE SPINAL CORD INJURY IN ADULT RATS

Xiangbing Wu^{1,2,3}, Wei Wu^{1,2,3}, Heqiao Dai^{1,2,3}, Vanessa A. Wilson^{2,3}, Xiao-Ming Xu^{1,2,3}

¹Indiana University School of Medicine, Dept. Neurological Surgery, Indianapolis, USA

²ISCBIRG, Indianapolis, USA

³Stark Neuroscience Research Institute, Indianapolis, USA

Our previous studies showed that ferulic acid-glycol chitosan (FA-GC), a nanoparticle, or bisperoxovanadium (bpV), an inhibitor of phosphatase and tensin homolog (PTEN), promoted neuroprotection and functional recovery after a contusive spinal cord injury (SCI) in adult rats. Here we investigated whether a combination of the two would maximize the effect on neuroprotection. A moderate contusive SCI was performed at the 10th thoracic level using the Louisville Injury System Apparatus (LISA, n=61). FA-GC (20 mg/kg), bpV (400 ug/kg), or their combination was injected through the jugular vein after SCI. The bpV was injected intraperitoneally daily for an additional 6 days in the bpV and combination groups. Rats received Basso-Bresnahan-Beattie (BBB) locomotor rating scale, transcranial magnetic motor evoked potential (tcMMEP), and metabolic cage assessments for 6 weeks post-injury. All rats were sacrificed at 7 weeks post-injury. Our BBB results showed significantly improved locomotor function in the combination group compared with the vehicle group, and moderately better motor function compared with either of the single treatments. The tcMMEP showed the highest response rate in the combination group (95%) compared with the FA-GC (70%), bpV (69%), or vehicle group (60%). The metabolic cage study showed significantly decreased void volume in the combination, FA-GC, and bpV groups, and significantly increased void frequency in the combination and FA-GC groups compared with the vehicle group. Bladder size was significantly reduced by 24%, 21%, and 13% in the combination, FA-GC, and bpV groups, respectively. The combination also significantly reduced the lesion area and increased the white matter sparing. All these data suggest that the combination of FA-GC and bpV promotes greater motor and bladder recoveries than either treatment alone following a moderate contusive SCI in adult rats.

Supported by DOD W81XWH-12-1-0562 and ISDH13679 (XW).

Keywords: Spinal cord injury, contusion, FA-GC, bpV

A18-16

MICRORNA-711 AS A THERAPEUTIC TARGET FOR NEUROPROTECTION AND RECOVERY IN SPINAL CORD INJURY

Junfang Wu, Boris Sabirzhanov, Bogdan Stoica, Jessica Matyas, Marina Coll-Miro, Laina Yu, Alan Faden

University of Maryland School of Medicine, Anesthesiology & Center for Shock, Trauma and Anesthesiology Research (STAR), Baltimore, USA

Despite great research efforts, there is still no established effective treatment to improve recovery following spinal cord injury (SCI). In part, this reflects incomplete understanding of the complex secondary pathobiological mechanisms involved and the historical emphasis on targeting single injury mechanism. There are many processes that contribute to the pathophysiology of SCI, such as neuronal cell death, axonal damage, vascular disruption, neuroinflammation, among others. It is increasingly clear that effective clinical strategies will require multi-functional drugs. MicroRNAs (miRs) are small (20–23 nucleotide) non-protein-coding RNAs that negatively regulate target gene expression at the posttranscriptional level by binding to their mRNAs and inducing its degradation and/or inhibiting translation. As single miRs can simultaneously modulate the expression of various proteins in multiple pathways, miRs are attractive candidates as upstream regulators of the secondary SCI progression. Here we examined miRs expression changes in the injured spinal cord using a moderate contusion SCI model in mice. We observed rapid and persistent up-regulation (approximately 10-fold) of miR-711, in response to specific post-traumatic transcriptional activation. This was associated with down-regulation of the pro-survival protein Akt, a target of miR-711, with sequential activation of GSK3 α/β and pro-apoptotic BH3-only molecules PUMA. Moreover, inhibition of miR-711 attenuated the injury-induced down-regulation of Akt and sequential activation of GSK3 β and pro-apoptotic Bcl2 family members. Furthermore, Angiopoietin-1, a predicted target of miR-711, is decreased in the injured spinal cord and elevated by inhibition of miR711. Importantly, miR711 inhibitors reduced neuronal loss and axonal damage, rescued epicenter blood vessels, and improved recovery of motor function and coordination. Together, our data indicate that miR-711 elevation after SCI serves to several endogenous neuroprotective pathways, leading to neuronal cell death and axonal and blood vessel damage. Thus, miR-711 represents a potential therapeutic target that may lead to novel, effective clinical therapeutic strategies.

Keywords: MicroRNA 711, spinal cord injury, neuroprotection, locomotor function

A18-17

TESTING THE EFFECT OF PROTEIN KINASE INHIBITORS ON TRAUMATIC BRAIN INJURY-RELEVANT CELL-BASED MODEL OF TAU HYPERPHOSPHORYLATION

Hamad Yadikar, Kevin Wang, Richard Yost, Isabel Torres

University of Florida, Department of Psychiatry, Gainesville, USA

Traumatic brain injury (TBI) increases the subsequent risk of chronic neurodegenerative disorders, including Alzheimer disease and chronic traumatic encephalopathy (CTE). Tauopathies are neurodegenerative

disorders that are related to the abnormal aggregation of hyperphosphorylated tau protein into oligomers. It was found in both human and animal model of TBI, Tau is also hyperphosphorylated post-injury. However, the biological mechanism through which tau get hyperphosphorylated need further elucidation. In this study, okadaic acid (OA, a protein phosphatase PP1/PP2A inhibitor) was used to induce Tau hyperphosphorylation and oligomerization as model for TBI. This model was used to set up a screening method for studying various protein kinase inhibitors on tau hyperphosphorylation using rat primary cortical culture (CTX) and mouse neuroblastoma (N2A) cells. The inhibitors used were pan kinase inhibitors (k252a and STS), GSK3b inhibitors (LiCl, AR-A014418 and A-1070722), CDK5 inhibitor (Roscovitine), Calcineurin inhibitor (Cyclosporin), Calmodulin antagonist (Calmidazolium), Casein kinase II inhibitor (tBBB), fyn/src kinase inhibitor (Saracatinib) or calcium chelators (EGTA and EDTA). The phosphorylation and oligomerization of Tau protein was studied by western blotting. Identification of tau oligomers was achieved by antibodies that detects total tau at 102–140 (DA9). Phospho-specific epitopes studied were pSer202/pThr205, pThr181, pSer202, pSer396/pSer404, pThr231. Treatment of OA for 24 hours achieved tau hyperphosphorylation at multiple TBI-related phospho-epitopes. In N2A cells, treatment with OA lead to the formation of tau oligomers observed at ~170 kDa, which were completely reversed when cells were pre-treated with tBBB. The hyperphosphorylation was partially blocked by EDTA, EGTA and k252a and completely inhibited by tBBB and cyclosporine. As for CTX cells, LiCl, AR-A014418 and A-1070722) were the most potent inhibitors of Tau hyperphosphorylation followed by tBBB and roscovitine. Identification of protein kinases that inhibit tau hyperphosphorylation in TBI can form the basis for screening of compounds and could provide potential therapeutic targets for the treatment of TBI-induced tauopathy-related neurodegenerative diseases.

Keywords: Tauopathy, Tau hyperphosphorylation, TBI, Kinase Inhibitors, Cell culture

A18-18

TARGETING KCNQ CHANNELS TO TREAT MOTOR AND SENSORY DYSFUNCTION AFTER SPINAL CORD INJURY

Qing Yang, Zizhen Wu, Tao Hong, Lin Li

University of Texas McGovern Medical School, IBP, Houston, USA

Traumatic injury to the spinal cord results in acute necrosis and delayed secondary expansion of neurological damage, and leads to a lifetime of paralysis, sensory dysfunction, and chronic pain. Excessive excitation is a primary source of neural injury evoked by various insults, causing neuronal cytotoxicity, and slowly expanding lesions. The proposed studies attempt to reduce persistent hyperexcitability of neurons during acute phases of spinal cord injury (SCI), thereby preserving neurological function that would be lost during the chronic stage. KCNQ/Kv7 channels are abundant in spinal neurons and axons, controlling their excitability. Retigabine, an FDA-approved drug for anti-epilepsy that specifically opens KCNQ/Kv7 channels, could be a plausible treatment to reduce SCI-induced pathology. We produced contusive SCI at T10 in adult, male rats, which then received 10 consecutive days' treatment with retigabine or vehicle 3 hours or 3 days after contusion. Two different concentrations and two different delivery methods were used. With pumps (250 mg/kg/hr) delivery 3 hours after contusion, retigabine promoted recovery of locomotor function, not intraperitoneal delivery. Remarkably, retigabine delivery in both method significantly attenuated the development of mechanical stimuli-induced hyperreflexia (hindlimb and torso) although there were no significant difference in thermal threshold. Retigabine delivered by pump 3 days after contusion only significantly attenuated the development of mechanical hypersensitivity,

no effect on locomotor function. Finally, we found early application of retigabine protect gray matter, not the white matter after SCI. Our result indicates that very early opening of KCNQ/Kv7 channels promotes locomotor function recovery and preemptively mitigates development of neuropathic pain following SCI.

Support: Supported by grants from the Mission Connect -TIRR Foundation, and the Department of Defense USAMRAA.

Keywords: KCNQ/Kv7 channels, locomotor function, chronic pain, spinal cord injury, T10 contusion

A18-19

ESTROGEN REDUCES INTRACELLULAR CALCIUM AFTER MILD RAPID STRETCH INJURY IN ADULT RAT PRIMARY CORTICAL NEURONS IN VITRO

Yaping Zeng, Jutapit Guptarat, Margaret O Parsley, Donald S Prough, Douglas S DeWitt

Department of Anesthesiology, University of Texas Medical Branch, Galveston, Texas, Anesthesiology, Galveston, USA

Traumatic brain injury (TBI) initiates numerous processes that can lead to neuronal injury, behavioral/cognitive dysfunction, seizures and neurodegenerative diseases (e.g. Alzheimer's, Parkinson's) Estradiol has neuroprotective effects in models of experimental traumatic and/or ischemic brain injury. In the present study, we examined the effects of 17-beta estradiol (E2) on intracellular calcium (Ca^{2+}) and neuron viability in adult rat brain-derived primary cortical neuron or hippocampal cells subjected to one of three levels of rapid stretch injury (RSI), an *in vitro* model that replicates many features of TBI *in vivo* using a model 94A cell injury controller II. Rat hippocampus and cerebral cortex were sliced at 0.5 mm and digested with papain at 30°C for 30 min. and triturated to release cells. The cells were plated onto poly-L-lysine-coated 2-mm-thick Silastic bottom 25-mm-diameter Flex Plate wells. Rat neuron cells in Neurobasal_A/B27+ bFGF medium were cultured to confluency in 95% air, 5% CO₂ at 37°C and then were subjected to mild RSI (30, 40 psi for 50ms) and treated with vehicle or E2 (80nM) for 30 min immediately post-injury. 1,3,5 and 24 hours post-RSI, Intracellular Ca^{2+} levels were measured with Fura-4/AM and the neuron viability was also assessed with P.I and Hoechst. Mild RSI increased intracellular Ca^{2+} levels and reduced neuron survival. Post-injury E2 treatment significantly reduced stretch-induced increases in Ca^{2+} influx and neuronal death assessed 1 hr after 30 psi RSI. Our results suggest the E2 improved of neuronal cell survival by reducing intracellular Ca^{2+} after mild stretch injury.

Supported by the Moody Project for Translational Traumatic Brain Injury Research

Keywords: Adult rat brain primary cortical neuron, Rapid stretch cell injury, Intracellular calcium, Estrogen, Neuron cell survival

A19 NUTRITION

A19-01

PHARMA NUTRIENT SUPPLEMENTATION TO ENHANCE INTESTINAL BLOOD FLOW FOLLOWING EXPERIMENTAL SPINAL CORD INJURY

Gregory Holmes, Margaret McLean, Gina Deiter

Penn State Univ. College of Medicine, Neural & Behavioral Sciences, Hershey, USA

The benefit of early enteral nutrition in the care of critically injured patients is well established. Further benefits are derived by administering immune-enhancing formulas including glutamine, arginine,

nucleotides, and omega-3-fatty acids. Despite this recognized standard of care following trauma, the application of clinical nutrition for individuals with spinal cord injury (SCI) remains controversial and poorly defined. Adult male Wistar rats received a 300kdyn T3-SCI or surgical control. Three days post-injury, both groups underwent *in vivo* experimentation to quantify mesenteric and duodenal blood flow in response to enteral delivery of low (10 mM) or high (100 mM) doses of arginine or glutamine. In accordance with previous data, mean arterial pressure (MAP) and superior mesenteric artery (SMA) irrigation of the viscera were significantly lower in T3-SCI rats. In T3-SCI rats, 30 or 60 minutes of enteral supplementation with either 10mM or 100mM arginine provoked a reduction in local duodenal blood flow while SMA flow and MAP remained unchanged. This reduction in local duodenal blood flow was significantly lower than control animals. Conversely, enteral delivery of 10 mM glutamine provoked a significant elevation in local duodenal blood flow at 30 min while SMA flow and MAP remained unchanged. Control animals did not demonstrate a significant elevation in local duodenal blood flow until 60 min. The 100mM dose of glutamine provoked a reduction in local duodenal blood flow in T3-SCI rats while remaining unchanged in control rats. The reduction in local duodenal blood flow of T3-SCI rats was significantly lower than controls. Our data suggest that unlike glutamine supplementation, the microvascular benefits derived from enteral arginine supplementation are not realized in the SCI individual. Therefore, accepted standards of care for maintaining GI health such as administering pharmaconutrients, such as arginine, that require active transport but do not provide a local energy substrate for the gut may be detrimental to GI integrity in the SCI population. Support: CH Neilsen #295319

Keywords: Mesenteric blood flow, Gastrointestinal, Postprandial hyperemia, Enteral nutrition

A20 POST-TRAUMATIC STRESS

A20-01

IMPACT OF REPETITIVE MTBI ON FEAR MEMORY, DEPRESSIVE BEHAVIOR, AND MARKERS OF SYNAPTIC PLASTICITY IN A MOUSE MODEL OF CHRONIC PTSD

Moustafa Algamil^{1,2}, Joseph O Ojo^{1,2}, Carlyn Lungmus¹, Philip Muza¹, Joan Owens¹, Benoit Mouzon^{1,3}, David Diamond³, Michael Mullan^{1,2}, Fiona Crawford^{1,2,3}

¹Roskamp Institute, Sarasota, USA

²The Open University, Milton Keynes, United Kingdom

³James A. Haley Veterans' Hospital, Tampa, USA

Background: Comorbid mTBI and PTSD can be clinically challenging to diagnose, primarily because of the heterogeneity and clinical overlap shared by both conditions. Clinical studies exploring comorbid mTBI and PTSD are often complicated by variances in type and severity of injury, time post-trauma, underlying comorbidities, and predisposing risk factors. These inherent limitations emphasize the urgent need to develop an etiologically relevant animal model whereby variables can be efficiently controlled. In this study, we use our refined mouse model of PTSD, and our established model of repetitive mTBI to assess the impact of mTBI on consolidation and/or extinction to a conditioned traumatic memory, including additional behavioral and neurobiological measures post-exposure.

Methods: C57BL/6J male mice were exposed to a 21-day stress paradigm at 3 and 5 month of age followed by a battery of behavioral testing for fear-memory, anxiety, depression. Mice were euthanized 10days and 3month post-exposure, with brain and plasma samples

collected for molecular profiling. The 21-day stress paradigm involved many randomized exposures to a danger-related predator odor (TMT) whilst immobilized, daily unstable social housing, and physical trauma in the form of five (separate) repeated inescapable footshocks. Animals receiving r-mTBI (x5) and stressors were exposed to a closed head injury 1hr after each conditioned footshock.

Results: Stressed mice showed significant weight loss, recall of traumatic memories, anxiety and depressive-like behavior when compared to control mice. Interestingly, repeated mTBI abrogated conditioned fear memory and depressive behavior in the forced swim test. Baseline TNF α plasma levels were elevated in stress only groups compared to controls, and repetitive mTBI mitigated this response in stressed mice. Biochemical analysis of hippocampal and amygdala homogenates revealed overlapping and unique changes to the HPA axis, glutamatergic and serotonergic signaling in stress and mTBI mice.

Conclusion: Our results demonstrates that our mouse model of PTSD develops persisting traits that capture critical aspects of PTSD symptomatology as defined by DSM-V. Unique traits were also observed with the comorbid presentation of mTBI and stress, and this was explained by some of our neurobiological measures. We anticipate that our model will be a useful platform to explore the neurobiology of PTSD and mTBI.

Keywords: PTSD, mTBI, Fear Memory, Depression, Mouse model, Closed Head Injury

A20-02

TRAUMATIC BRAIN INJURY LEADS TO LONG-TERM ENHANCED AUDITORY FEAR LEARNING AND STIMULUS GENERALIZATION

Ann Hoffman^{1,2}, Ellen Hsieh², Nishtha Chavda², Jamie Lam², David Hovda¹, Christopher Giza¹, Michael Fanselow²

¹UCLA, Neurosurgery, Los Angeles, USA

²UCLA, Psychology, Los Angeles, USA

Cognitive impairments and emotional lability are common long-term consequences of Traumatic Brain Injury (TBI). Increasing prevalence of comorbid TBI and Post-Traumatic Stress Disorder (PTSD) emphasizes an urgency for a better understanding of how injury affects interactions between sensory, cognitive, and emotional systems that may underlie maladaptive responding. We have previously shown changes in auditory-emotional network activity and enhanced contextual fear learning following white noise fear conditioning early after TBI, prior to the resolution of typical physical symptoms. In the current study, we asked whether TBI would have chronic effects on auditory fear learning and responses to novel stimuli. Four weeks following either mild-moderate lateral fluid percussion injury (FPI) or sham surgery, adult male rats were fear conditioned to either white noise or unsignaled shocks. All groups were tested for contextual fear memory and context extinction then subsequently tested for fear responses to either pure tones or white noise auditory stimulus trials in a new context. While FPI did not impact freezing across acquisition trials, FPI led to increased shock reactivity if white noise preceded the shock. As expected, unsignaled conditioned groups had greater contextual fear relative to noise-shock groups. However, FPI noise-shock animals froze more to the context than respective shams, consistent with our previous findings. During white noise cue testing, FPI noise-shock rats had increased freezing on the first trial of white noise cue testing compared to respective shams. Interestingly, when presented with novel pure tones FPI noise-shock animals displayed robust fear to the novel, untrained auditory stimulus compared to noise-shock shams. These data indicate an injury-induced increase in auditory

stimulus generalization, and a unique phenotype chronically following diffuse TBI. This novel finding illustrates both a cognitive impairment and increased fear in the chronic phase after TBI, where stimulus generalization may underlie maladaptive fear and hyperarousal common to comorbid TBI-PTSD.

Keywords: amygdala, learning and memory, fear, PTSD, fear generalization

A20-03

MODEL OF COMORBID MILD TBI AND FEAR CONDITIONING/RETENTION IN MICE THAT PRODUCES COGNITIVE, BEHAVIORAL AND INFLAMMATORY IMPACTS

Gary Kaplan¹, Stephen Heinrichs¹, Lei Wang^{2,3}, Talita Glaser^{2,3}, Kimberly Leite-Morris¹, Kendra Rumbika¹, Olivia Nguyen¹, Yang Teng^{2,3}

¹VA Boston Healthcare System/Boston Univ Sch Medicine, Psychiatry and Pharmacology, Boston, USA

²VA Boston Healthcare System, Division of SCI Research, Boston, USA

³Dept. Neurosurgery and PM&R, Harvard Medical School, Boston, USA

The high rates of comorbid TBI and PTSD diagnosed in veterans in recent years have resulted in increased research investigation into these conditions and putative treatments. In this study, mild traumatic brain injury (mTBI) and posttraumatic stress disorder (PTSD) were modeled in male C57BL/6 mice in order to determine the comorbid effects of mTBI and PTSD on cognition, behavior and neuroinflammation within 3 weeks of injury. Mice with mTBI induced by fluid percussion injury (FPI) at one of two intensity levels (0.7 and 1.7 atmospheres), and also sham FPI mice, were subsequently fear conditioned (FC). FC was used to model aspects of PTSD and fear cues and contexts produced near-complete freezing behavior. Mice were then assessed for novel object recognition and fear retention/extinction. Novel object recognition was impaired in FPI groups compared to sham FPI and naive controls. Re-exposure to the fear cue and context several days after fear acquisition showed the retention of freezing behavior in FPI/FC and sham FPI/FC groups. Following behavioral testing, brains were harvested to for immunohistochemical (IHC) studies. IHC staining of activated macrophage/microglia marker CD68, and pro-inflammatory marker TNF- α produced evidence of neuroinflammation in cortical, hippocampal and subcortical brain regions as a consequence of FPI/FC (vs. controls). IHC staining of beta-amyloid precursor protein was used as a measure of diffuse axonal injury and this protein was induced in several regions in FPI/FC groups. This FPI/PTSD mouse model shows translational efficacy as a model for mTBI/PTSD and produces subacute cognitive impairment and fear retention accompanied by neuroinflammation and axonal injury in cortical, subcortical and hippocampal regions. This mouse model can serve as a platform to examine longer term behavioral and neural effects and potential therapeutics. Supported by a VA Merit Award to GBK and Gordon Project/C.S.C./U.W.S.F. fellowships to LW and TG.

Keywords: fluid percussion injury, fear conditioning, neuroinflammation, comorbidity

A20-04

A POTENTIAL PROTECTIVE ROLE FOR AUTOPHAGY REGULATION IN TBI-INDUCED STRESS DAMAGE TO EXTRA-CRANIAL ORGANS

Yinlong Liu¹, Zhongyuan Bao¹, Xiupeng Xu¹, Honglu Chao¹, Chao Lin¹, Zheng Li¹, Yan Liu², Xiaoming Wang³, Yongping You¹, Ning Liu¹, Jing Ji¹

¹the First Affiliated Hospital of Nanjing Medical University, Neurosurgery, Nanjing, china

²Nanjing Medical University, Institute for Stem Cell and Neural Regeneration, School of Pharmacy, Nanjing, china

³Nanjing Medical University, Department of Immunology, Nanjing, china

Extra-cranial organ injury is one of severe complications in patients with traumatic brain injury (TBI) and is a predictor of poor clinical outcome, involving in increased apoptosis, enhanced inflammatory response, and excessive oxidative stress in extracranial organs. Autophagy is known as a primary homeostatic process that can promote cell survival under stress. Thus, we hypothesized that autophagy could be an effective strategy for prevention of cell death in TBI-induced stress damage to extracranial organs. TBI can lead to lung and intestine injury, and induce autophagy in lung tissue and mitophagy in the intestine tissue in our model. Because extracellular signal-regulated kinase 1/2 (ERK1/2) is known to play an important role in regulating autophagy, we investigate the effect of the ERK1/2 signaling pathway on autophagy in extracranial organs after TBI by using PD98059 (ERK inhibitor). Autophagy or mitophagy was assessed by electron microscopy (EM) and biochemical evidences (western blot, immunobiochemical staining). Rats were pre-conditioned with autophagy promoter rapamycin or inhibitor 3-methyladenine before they were challenged with TBI. We found that autophagy can suppress apoptosis, inflammation and oxidative stress, which were activated after TBI. Moreover, autophagy was mediated by the ERK1/2/mTOR/Stat3 signaling pathway in the lung and mitophagy was mediated by the ERK1/2/Nrf2/HO-1 signaling pathway in the intestine after TBI, which may serve to reduce the stress injury and improve intestinal or pulmonary function. It appears that autophagy plays a protective role in TBI-induced stress damage to extracranial organ and this effect may be enhanced by moderately improving autophagy level. Meanwhile, modulating ERK1/2 signaling pathway may be important for the prevention and treatment of of symptoms associated with TBI.

Keywords: TBI, Extra-cranial organ injury, Autophagy, oxidative stress, inflammatory response, apoptosis

A21 REHABILITATION

A21-01

REPETITIVE ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION IMPROVES OUTCOME AT THREE WEEKS BUT NOT ONE WEEK AFTER TBI IN MICE

Olga Bragina, Devon Lara, Yirong Yang, Claude Shuttleworth, Edwin Nemoto, **Denis Bragin**

University of New Mexico School of Medicine, Albuquerque, USA

Objectives: Traumatic brain injury (TBI) causes long-term neurological dysfunction in 70% of survivors for which there is no clinically proven therapies. Transcranial direct current stimulation (tDCS) is being tested clinically after TBI but mechanisms and optimal stimulation parameters have not been determined. We tested the efficacy of repetitive anodal tDCS stimulation applied at one and three weeks after TBI on neurologic recovery and cerebral blood flow (CBF) in mice.

Methods: TBI was induced by controlled cortical impact and subjected to anodal tDCS or false stimulation. Sham control mice had a craniotomy only. Repetitive anodal tDCS (0.1 mA/15min) or false stimulation was done under anesthesia over 4 weeks for 4 consecutive days with 3-day intervals beginning 1 or 3 weeks after TBI (n=10/group). The anode was placed over the craniotomy and the cathode on

the thorax. CBF was obtained pre and post stimulation by MRI; microvascular tone and CBF (mCBF) and tissue oxygenation (NADH) were measured by two-photon microscopy. Rotarod, passive avoidance, and Y-maze were used to evaluate neurological recovery.

Results: TBI produced contusions in the cortex and hippocampus; CBF fell to $35 \pm 8\%$; arteriolar diameters, number of functioning capillaries, and tissue oxygenation decreased in peri-contusional areas ($P < 0.05$). Anodal tDCS immediately increased CBF, mCBF and tissue oxygenation by dilatation of arterioles in both sham and TBI mice ($P < 0.01$). Motor function assessed by Rotarod was better in tDCS mice compared to false stimulation mice. Step-through retention latency of tDCS mice in passive avoidance was longer than in false stimulated mice. In the Y-maze, tDCS mice entered the new-opened arm more frequently compared to false-stimulated mice. Only the group in which stimulation started at 3 weeks after TBI recovered significantly better ($P < 0.05$ for all tests) than when stimulation started one week after TBI.

Conclusions: Repetitive anodal tDCS improves neurologic recovery when started at three weeks but not one week after TBI. These results suggest that anodal tDCS stimulation may be defines the window of brain tissue recovery after TBI also reflected by increased CBF and tissue oxygenation.

Support: NIH-NIGMS P20GM109089.

Keywords: Transcranial Direct Current Stimulation, Metabolism, Vascular Tone, Cerebral Microcirculation

A21-02

A COMBINATION OF ENVIRONMENTAL ENRICHMENT AND NICOTINAMIDE AFTER TBI IN JUVENILE RATS LEADS TO TRANSFER EFFECTS IN COGNITIVE TASKS

Ryan Holden, Aidan Smith, Shawn Roller, James Essepreis, McKensy Hudson, Michael Hylin
Southern Illinois University Carbondale, Carbondale, USA

Few effective therapeutic interventions have demonstrated the ability to mediate behavioral deficits following damage in juvenile populations. Even fewer have shown long-term beneficial effects. Most treatment regimens consist of single treatments, whereas combinational therapies may serve to augment rehabilitation. Utilization of a combined treatment may not only maximize recovery, but also provide a sustained effect in the absence of the therapy. The current study used a combination of continuous environmental enrichment (EE) and a 500mg/kg dose of nicotinamide (NAM) given three times following a bilateral controlled cortical impact (CCI) or sham surgery on post-natal day 28 rats. After surgery, animals were divided into 5 groups: CCI+EE, CCI+NAM, CCI+NAM+EE, CCI+noEE+noNAM, and sham no treatment. Rats were tested on motor (Foot Fault and Beam Walk) and cognitive tasks (Morris Water Maze and Radial Arm Maze). Results showed that the combinational therapy group performed similar to the sham group in both motor tasks. There was also significance improvement in spatial memory performance of the combined group in the water maze, as the latencies were comparable to those of the sham group. After cessation of enrichment, the positive effects of the combination group were transferred to a different spatial memory task (Radial Arm Maze). Specifically, the combinational therapy made significantly fewer reference and working memory errors relative to the other injured groups. Overall, these findings point to the benefits of combining environmental enrichment with pharmacological treatments when compared to individual treatments alone in treating cognitive dysfunction following juvenile brain trauma. When administered together combinational therapy may be more efficacious with respect to long-term functional recovery.

Keywords: polytherapy, nicotinamide, development, transfer

A21-03

OUTCOME AFTER POST-ACUTE REHABILITATION IN TRAUMATIC BRAIN INJURY PATIENTS

Stefanie Howell, Charan Singh, Sarah Finley, Grace Griesbach
Centre for Neuro Skills, Research, Irving, USA

Objective: To investigate the sustainability of post-acute rehabilitation following traumatic brain injury (TBI).

Materials and Methods: TBI patients that participated in a full-time comprehensive post-acute rehabilitation program ($n=38$) were interviewed by phone at least 1 year post-discharge (3.6 ± 2.23). The mean latency from TBI was 4.74 ± 2.69 yrs. All subjects had at least 12 years of education. The primary measures of assessment were as follows: Disability Rating Scale (DRS), Mayo-Portland (MPAI), Neuro-QoL, Supervision Rating Scale (SRS), Brief Test of Adult Cognition by Telephone (BTACT) and an occupational status questionnaire.

Results: At initial follow-up, analysis of employed and unemployed subjects revealed that 68% had an occupational position equal to that of pre-injury. Those who were employed at the time of follow-up had lower levels of disability upon discharge than the unemployed group. Both the employed and unemployed groups showed a decrease in disability during the rehabilitation period ($p < .001$); however, the employed subjects continued to improve post-discharge while the unemployed remained stable across time ($F(1,36)=13.656, p < .001$). DRS at discharge, was correlated with the below listed follow-up measures (all $p < 0.05$): delayed word recall ($r = -.437$), digits backwards ($r = -.363$), category fluency ($r = -.517$), red/green baseline switched ($r = -.460$), and backwards counting ($r = -.573$). Analysis of MPAI scores showed similar findings. NeuroQoL scores indicated that, compared to the unemployed, the employed group had an increased sense of well-being, a greater ability to participate in social roles and activities and were more satisfied with their social roles ($p < .05$ for all measures).

Conclusion: Individuals who experience positive rehabilitative outcomes are more likely to regain their former occupation and quality of life. These preliminary findings support the longitudinal sustainability of the beneficial effects of rehabilitation.

Keywords: TBI, rehabilitation, outcome, follow-up, longitudinal

A21-04

IMPACT OF COMPLETE SPINAL CORD INJURY ON NEO-VASCULARIZATION AND TISSUE GRANULATION IN MOUSE MODEL OF SKIN PRESSURE ULCERS

Suneel Kumar¹, Martin Yarmush^{1,2}, Francois Berthiaume¹

¹*Rutgers, The State University of New Jersey, Biomedical Engineering, Piscataway, USA*

²*Massachusetts General Hospital and Shriners Burns Hospital, Center for Engineering in Medicine, Boston, USA*

Pressure ulcers or sores (PUs) are a very common debilitating secondary complication of traumatic spinal cord injury (SCI) beside sensory and motor functional deficit. It tends to occur in soft tissues located around bony prominences. There is however little known about the impact of SCI on wound healing due to the lack of suitable animal model to perform relevant studies in controlled experimental settings. Herein we describe a reproducible and clinically relevant mouse model of PUs in the context of complete SCI. Adult male (BALB/c) mice were subjected to thoracic (T9-T10) complete SCI. Immediately after, a skin fold on the back of the mice was lifted and sandwiched between two magnet discs held in place for 12 hours, thus

creating an ischemic area that developed into a pressure ulcer over the following days. The wounded areas demonstrated tissue edema and epidermis disappearance by day 3 after magnets removed. The PUs spontaneously healed slowly ($p < 0.001$) in SCI mice compared to Control mice (35 versus 21 days). Histology data showed that there was slower migration of epidermal cells over the granulation tissue in SCI group compared to Control group. The SCI group also showed smaller thickness of epidermis and dermis, lower blood vessel density, decreased numbers of proliferating cells, and decreased expression alpha-smooth muscle actin compared to Control group on their wound closing day. Taken together, these data suggest that SCI significantly slows down the dynamics of healing process in experimental pressure wounds in mice. This work is supported by grant of New Jersey Commissions on Spinal Cord Research.

Keywords: Spinal cord injury, Pressure ulcers, Neovascularization, Granulation, Proliferation, Alpha-smooth muscle actin

A21-05

NOVEL SELF-GUIDED REHABILITATION TASK PRESERVES COGNITIVE PERFORMANCE AFTER DIFFUSE TRAUMATIC BRAIN INJURY IN THE RAT

L. Matthew Law^{1,2}, Daniel Griffiths^{1,2}, Jonathan Lifshitz^{1,2}

¹University of Arizona, Department of Child Health, Phoenix, USA

²Barrow Neurological Institute at Phoenix Children's Hospital, Translational Neurotrauma, Phoenix, USA

Traumatic brain injury (TBI) is not a transient event from which all people recover; the resulting damage can evolve into neurological disease. In rats, TBI disrupts memory circuits, expressed as impaired cognitive performance. Experimental rehabilitation strategies, such as enriched environment and exercise, have limited success in alleviating symptoms. The persistent symptoms of TBI are heterogeneous and therefore require focused therapies to alleviate neurological symptoms. In our hands, diffuse brain injury by midline fluid percussion leads to cognitive impairments at 1 month post-injury. Therefore, we hypothesize that rehabilitation targeting spatial and contextual memory circuit will prevent the onset of injury-induced memory impairments. Rehabilitation occurs in a box with a peg board floor (24" x 24") that allows for 3" plastic pegs to be inserted at 1" intervals in designated layouts; termed Peg Forest Rehabilitation. Uninjured and brain-injured rats were exposed to the rehabilitation task (15 min/day), allowing free navigation through random layouts of the peg-filled arena for 10 days over 2 weeks. Controls were exposed to an open-field arena (15 min/day) or served as caged-controls for 10 days over 2 weeks. One week post-rehabilitation (1 month post-injury), cognitive performance was tested for short-term memory (novel object recognition), long-term memory (novel location recognition), and working memory (temporal object recognition). Brain-injured animals exposed to Peg Forest rehabilitation showed similar levels of object discrimination to uninjured rats on all three object recognition tasks. Whereas, brain-injured open field and caged-control rats performed significantly worse than shams on all three object recognition tasks, demonstrating injury-induced cognitive impairment without benefit of rehabilitation. Sham animals had no cognitive benefit from Peg Forest rehabilitation. Thus, passive, intermittent rehabilitation targeting specific neurological impairments can prevent cognitive symptomatology. The Peg Forest is a viable rehabilitation strategy to explore cellular and molecular mechanisms underlying preservation of neurological function. Supported by the Diane and Bruce Halle Foundation

Keywords: rehabilitation, cognition, diffuse axonal injury, memory preservation

A21-06

THE IMPACT OF LIGHT CYCLE REVERSAL UPON THE THERAPEUTIC EFFECTIVENESS OF ENVIRONMENTAL ENRICHMENT FOLLOWING TRAUMATIC BRAIN INJURY

Paige Niemet¹, Ryan Holden¹, Anthony Moore², Pramod Dash², Michael Hylin¹

¹Southern Illinois University-Carbondale, Psychology, Carbondale, USA

²University of Texas Medical School, Neurobiology and Anatomy, Houston, USA

Environmental enrichment (EE) is a versatile tool that affords animals the opportunity to engage in social interactions, explore novel stimuli, as well as freely explore a larger living space. The effects of EE have been shown to promote neural plasticity after traumatic brain injury and is an acceptable form of rehabilitation. The use of abbreviated EE, which is defined as access to an enriched environment for a set amount of time each day, is comparable to rehabilitation programs performed in the clinic. Furthermore, proteins associated with neural plasticity peak during the day when the rats are less active and are at their lowest during the night, when the rats tend to be in a more active state. These proteins are important for the maintenance and consolidation of long-term memories. The objective of the current study was to investigate how the time at which EE was administered during the light cycle impacts performance in the Morris Water Maze (MWM). Adult male Sprague-Dawley rats either received a bilateral controlled cortical impact (CCI) or sham surgery. Animals received 6 hours of EE per day, starting 1 week post injury and began MWM training 2 weeks post injury. Testing began after the 6 hours of EE during the light phase or dark phase of the daily cycle. We hypothesized that rats given EE during their light phase will experience recovery in the MWM when compared to rats tested in the dark phase possibly due to peak levels of neural plasticity proteins. The results concluded that receiving EE and MWM training during the dark phase negates the beneficial effects of abbreviated EE. Further research is needed to study the cyclical effects of proteins such as glycogen synthase kinase-3 Beta (GSK3 β) following treatment and the role it plays in the modulation of neural plasticity proteins associated with EE treatment.

Keywords: Environmental Enrichment, Chronotherapeutics, Circadian Rhythm, Neural Plasticity Proteins

A21-07

THE IMPACT OF UNILATERAL VERSUS BILATERAL CRANIECTOMY CCI MODELS UPON RECOVERY OF FUNCTION IN JUVENILE ANIMALS

Phaedra Norrell, Aidan Smith, Ryan Holden, Paige Niemet, Michael Hylin

Southern Illinois University Carbondale, Psychology, Carbondale, USA

In juveniles under the age of 15, traumatic brain injury (TBI) is the leading cause of death and disability. While prior research has demonstrated that a bilateral craniectomy increases axonal strain and leads to cell death in the contralesional hemisphere; unilateral craniectomy models are commonly used. However, it remains to be seen what differences exist when it comes to induced recovery between these two models. Through utilizing both bilateral and unilateral models in this study, we aimed to assess mechanisms underlying recovery of function in the juvenile rat model. The animals were given a controlled cortical impact (CCI) or sham surgery on postnatal day 28 (PND 28). CCI subjects were given either a unilateral or a bilateral craniectomy, with

the other opening occurring over the identical region in the opposite hemisphere. In both models, an impact was delivered to the right parietal cortex at a velocity of 5.5 m/s with a 2-mm deformation of the cortex. To facilitate recovery of function post-injury, we utilized a 24-hour environmental enrichment (EE) protocol starting one day post-injury, where EE subjects remained in EE throughout the entirety of the experiment. The recovery of both EE-treated animals was compared to animals that remained in a standard housing environment. While we found no behavioral differences (as assessed with the Morris water maze (MWM)) between the two injury models; we did observe significant differences with respect to EE treatment. EE-treated subjects with a unilateral craniectomy demonstrated significant improvement in the MWM when compared to subjects with bilateral craniectomies. Future research will look to evaluate how EE treatment impacts the histological differences between the two injury models with respect to axonal strain. Through examining differences between these two models we can better understand the mechanisms surrounding functional recovery after treatment and better improve rehabilitative strategies for injury models with varying degrees of injury severity improving recovery of function post-injury.

Keywords: Enriched Environment, Juvenile Injury, Recovery of Function, Neural Plasticity

A21-08

EFFECT OF NEUROREHABILITATION ON BRAIN CIRCUITS AFTER MILD TBI: AN EXPERIMENTAL FUNCTIONAL RSfMRI STUDY

Afshin Paydar¹, Diego Rubio¹, Shanmukha Srinivas¹, Anthony E. Kline², Neil G. Harris¹

¹UCLA, Neurosurgery, Brain Injury Research Center, Los Angeles, USA

²University of Pittsburgh, Physical Medicine & Rehabilitation, Pittsburgh, USA

We present an update of our previous pilot work to determine the effects of environmental enrichment on brain circuitry after controlled cortical impact injury (CCI) in the adult male rat. We tested whether resting state fMRI (rsfMRI) functional connectivity can be used to monitor brain circuitry in response to rehabilitation in expanded cohorts of injured and anesthetic-sham rats that were housed immediately after injury for 4 weeks in either standard (STD) or environmental enriched (EE) conditions (n=9-11/group). We acquired rsfMRI data at 1 and 4 wks post-injury, and then 4 wks later following resumption of STD conditions for all groups (8 wks post-injury). We analyzed data for network-based connectivity differences over 96 brain regions using graph theory at both global and regional levels. There was no effect of time on sham-group network parameters over the 8 wk study indicating good reproducibility. There was no effect of EE on sham-group brain circuitry compared to sham-STD for any global network parameter at any time-point apart from small-worldness (SW), which was reduced immediately following 4 wks of EE ($P < .05$), but this did not endure after resumption of STD conditions. Compared to our published work in moderate/severe TBI, global network parameters after mild CCI in STD conditions were relatively unaffected at both 1 and 4 wks post-injury, apart from temporary reduced modularity and SW ($P < .05$), indicating potential circuit reorganization. By 8 wks however, most network parameters trended towards alterations similar to 4 wks post-injury in moderate TBI. This was highlighted by the regional development of hyperconnectivity (increased nodal $P < .05$ and edge strength, $P < .005$) in multiple brain regions compared to sham-STD rats at 8 wks. There was no effect of EE on CCI for any global network parameter compared to sham+EE at any time-point except for reduced modularity and SW at 4 wks ($P < .05$), possibly indicating EE-induced reorganization. This did

not persist into STD conditions at 8 wks. CCI+EE resulted in only sparse areas of hyperconnectivity compared to sham-EE (bilateral caudate, hippocampus, $P < .05$), so that compared to CCI+STD, EE resulted in reductions in bilateral retrosplenial and sensory cortex ($P < .05$).

Acknowledgments: UCLA Brain Injury Research Center; NIH NINDS R01NS091222

Keywords: Environmental Enrichment, Traumatic Brain Injury, Reorganization, rsfMRI, Functional Connectivity

A21-09

COMBINATION OF EPIDURAL SPINAL CORD STIMULATION AND BODY WEIGHT SUPPORT TREADMILL TRAINING FOR SEVERE SPINAL CORD INJURY

Junya Saito¹, Masao Koda², Takeo Furuya¹, Yasushi Ijima¹, Mitsuhiro Kitamura¹, Takuya Miyamoto¹, Sumihisa Orita¹, Kazuhide Inage¹, Masashi Yamazaki², Seiji Ohtori¹

¹Chiba University Graduate school of Medicine, Department of Orthopedic Surgery, Chiba, Japan

²University of Tsukuba, Department of Orthopedic Surgery, Tsukuba, Japan

The neural control of rhythmical hind-limb locomotion is known to be organized by a central pattern generator (CPG) in the lumbar enlargement. In some previous reports, epidural spinal cord stimulation (SCS) for CPG enabled to elicit full weight-bearing standing and stepping in patients with motor complete spinal cord injury. However, the precise mechanism of improvement by SCS is still unclear. The purpose of this study was to elucidate efficacy and molecular mechanism of SCS with body weight support treadmill training. Ten female Sprague-Dawley rats were used in this study. All rats were performed spinal cord transection at the T8-T9 level under general anesthesia. Two weeks after the spinal cord transection, rats were divided into SCS group and control group. The rats in SCS group were performed laminectomy at L3-4 level followed by implantation of a SCS electrode at L2 level. SCS and body weight support treadmill training were performed for 30 minutes per day, 5 days per week, for 4 weeks. The rats in control group were performed laminectomy without putting an electrode. Rats in control group were set to do treadmill training without stimulation during the same periods of SCS group. We evaluated hind-limb locomotion function by using Basso-Beattie-Bresnahan (BBB) score. We also assessed force for full extension of hind-limb as evaluation of spasticity before treadmill training, two and four weeks after treadmill training. For histological examination, we performed immunohistochemical staining for glutamic acid decarboxylase (GAD)65 of lumbar enlargement after behavioral assessment. There was no significant difference of BBB score between two groups, but spasticity of hind-limb in SCS group was significantly reduced. Histological assessment revealed that expression of GAD65 tended to increase in the SCS group. SCS ameliorated hind-limb spasticity through up-regulation.

Keywords: epidural spinal cord stimulation

A22 THERAPEUTICS / DRUG DISCOVERY

A22-01

ADMINISTRATION OF PERAMPANEL FOLLOWING TRAUMATIC BRAIN INJURY IN RATS REDUCES COGNITIVE DEFICITS

Virginia Aida¹, Omari Turner¹, Tracy Niedzielko¹, Betty Pat¹, JeVana Pickens¹, Jerzy Szaflarski², Candace Floyd¹

¹Univ. of Alabama at Birmingham, Dept of Physical Med & Rehab., Birmingham, USA

²Univ. of Alabama at Birmingham, Department of Neurology, Birmingham, USA

The United States Centers for Disease Control and Prevention indicate that the incidence rate for traumatic brain injury (TBI) has increased over the last decade. Unfortunately, protective treatment options remain limited. We hypothesize that antagonists of AMPA receptors may confer acute protection after TBI. The goal of the current study is to evaluate the effects of post-injury administration of a non-competitive AMPA antagonist, perampanel (FYCOMPA®), on cognitive behavioral outcomes in adult male rats after moderate TBI. Perampanel is clinically approved as an antiepileptic. Following moderate lateral fluid percussion TBI (3.0ATM) or sham uninjured control procedure, rats were randomized to receive 0, 0.2, 1.0, or 2.0 mg/kg of perampanel (i.p.) starting at 1 hour post-TBI and then once per day for 7 days. Anxiety-like behaviors were assessed on post-TBI day 4 via the elevated plus maze. Learning and memory were evaluated using the Morris Water Maze on post-TBI days 7 – 11. Motivation was measured via the female encounter test on post-TBI day 25. We found that TBI induced deficits in learning and memory as compared to uninjured controls and that post-injury administration of perampanel ameliorated these impairments, regardless of dose. Additionally, rats in the TBI group spent less time with the female in the female encounter test than uninjured controls and the 1.0mg/kg dose of perampanel reduced this effect. No significant effects of TBI or perampanel were observed in the elevated plus maze. Taken together, these data suggest that acute administration of perampanel after TBI reduces injury-induced deficits in learning and memory as well as motivation. Thus, acute administration of perampanel following TBI may be an effective protective agent. Supported by a Research Contract from Eisai, Inc. to JPS.

Keywords: Moderate TBI, Perampanel (FYCOMPA®), Lateral Fluid Percussion Model, Cognitive Deficits

A22-02

PHARMACOLOGICAL INHIBITION OF MYOSTATIN IN A MODEL OF SPINAL CORD INJURY IMPROVES KEY CHARACTERISTICS OF MUSCLE PATHOLOGY

Gregory Bigford¹, Dalton Dietrich¹, Micah Webster², Adriana Donovan², Mark Nash¹

¹University of Miami Miller School of Medicine, Miami Project to Cure Paralysis, Miami, USA

²Scholar Rock, Inc., Cambridge, USA

Spinal cord injury (SCI) leads to hypokinesia and skeletal muscle atrophy. The association of muscle deficit with metabolic dysfunction makes preservation of lean mass a clinical priority. Myostatin, a member of the TGF β family of growth factors, is a key negative regulator of skeletal muscle mass and is associated with muscle pathophysiology in many degenerative processes. The objective of this study was to determine the effect of anti-myostatin pharmacotherapy on sub-lesional skeletal muscle following experimental SCI. Myostatin signaling was blocked by systemic delivery of a highly selective monoclonal antibody, muSRK-015P, that blocks release of active growth factor from the latent form of myostatin. muSRK-015P is a parental counterpart of Scholar Rock's lead compound, SRK-015, expressed on a mouse IgG1 framework. SCI was induced by subjecting mice to severe contusion (65 kDyne) at thoracic level T9. Mice were administered test articles, muSRK-015P or controls (either vehicle or IgG), immediately following injury. A sham group was subjected to laminectomy only. Test articles were re-administered 1-week post-SCI. Animals were sacrificed at 2-weeks post-SCI. Treatment con-

trol groups had significant sub-lesional muscle atrophy in soleus and gastrocnemius ($p < 0.05$ compared to sham), while muSRK-015P treatment significantly improved maintenance of mass in both muscles ($p < 0.05$ compared to controls; not significant compared to sham). Oil Red O staining showed significant lipid infiltration into control muscles, which was absent in sham ($p < 0.05$) and significantly reduced in muscles from the muSRK-015P group ($p < 0.05$ compared to controls). These improvements in muscle health were associated with improved grip strength ($p < 0.05$) and BMS functional scores ($p < 0.05$) at 1- and 2-weeks post SCI in the muSRK-015P group compared to controls. These results indicate that pharmacological inhibition of myostatin signaling with muSRK-015P reduces atrophy and fatty infiltration, and improves muscle function following SCI.

Keywords: Musculoskeletal, Metabolism, Treatment, Rehabilitation

A22-03

EFFECTS OF FLUID PERCUSSION TRAUMATIC BRAIN INJURY AND TARGETED IMMUNOTHERAPY ON GENE EXPRESSION USING CUSTOM PCR ARRAY

Ian Bolding¹, Kathia Johnson¹, Margaret Parsley¹, Deborah Boone¹, Clark Andersen², Helen Hellmich¹, Donald Prough¹, Douglas DeWitt¹, Bridget Hawkins¹

¹University of Texas - Medical Branch, Anesthesiology, Galveston, USA

²University of Texas - Medical Branch, Preventative Medicine and Community Health, Galveston, USA

Traumatic brain injury (TBI) can cause brain region-specific changes in mRNA and miRNA expression. We hypothesized that an immunotherapy that targets removal of tau oligomers given one hour post-TBI would result in decreased expression of neurodegenerative genes or increased expression of neuroprotective genes in the hippocampus. Adult male Sprague Dawley rats received either a single fluid percussion or sham injury before assessing the duration of suppression of the righting reflex. An intracerebroventricular injection of anti-tau oligomer-specific monoclonal antibody (TOMA) or nonspecific Immunoglobulin G control was administered one hour post-TBI. Animals underwent cognitive behavior testing and were euthanized fifteen days later for tissue collection. Based on our previous studies and a literature review, we created a custom Qiagen PCR plate array that targets 86 TBI-related genes and five housekeeping genes; the array also includes five wells that control for genomic contamination, RNA, PCR and RT quality. Normalized gene expression was analyzed by ANOVA for each gene, and differences among treatment groups were assessed by Tukey-adjusted contrasts followed by Benjamini-Hochberg control of false discovery rate. Our results show that up-regulation of neuroprotective genes in the TOMA treated animals varied with brain region, suggesting TBI may initiate brain region-specific injury and/or recovery processes. Studies including additional time intervals post-TBI are warranted. These studies were funded by The Moody Project for Translational TBI Research, Darrell K Royal Research Fund for Alzheimer's Disease, Mission Connect (program of the TIRR Foundation), and the UTMB Technology and Commercialization Program.

Keywords: Immunoglobulin G, PCR Array, Monoclonal Antibody, Tau Oligomers, Tau

A22-04

DATA DRIVEN MODELS SUPPORT NOVEL TBI THERAPY

H Alex Choi¹, Jude Savarraj¹, Georgene Hergenroeder¹, Ryan Kitagawa¹, Anthony Moore², Pramod Dash²

¹University of Texas Health Science Center McGovern Medical School, Neurosurgery, Houston, USA

²University of Texas Health Science Center McGovern Medical School, Neurobiology and Anatomy, Houston, USA

Objective: Cytokines are signaling molecules that modulate immune response, regulate cell populations, and cascade to alter a variety of cell functions. Alterations in cytokine levels have been associated with outcomes after traumatic brain injury (TBI). We hypothesized that: (1) systemic cytokine/chemokine (CC) levels and interactions among CC are associated with clinical outcomes; and (2) galantamine – an acetylcholinesterase inhibitor used to treat Alzheimer’s disease – modulates the CC clusters generated from patient data.

Methods: 41 serum cytokines in 76 TBI patients were measured 0–24 hrs (T1) and 24–48 hrs (T2) after injury. Clinical outcomes were assessed with the modified Rankin scale (mRS) at discharge: good (mRS <4) and poor (mRS ≥4). The Mann-Whitney U test compared groups. Pearson’s correlation coefficient (Pcc) was computed for pairs, and hierarchical clustering grouped cytokines with the most interactions across both outcome groups. Median cytokine values for all patients at T2 were used to evoke the most likely molecular pathways using Qiagen’s Ingenuity Pathway Analysis (IPA).

Results: Cluster analysis identified two strongly inter-correlated CC clusters at both time points. Poor outcomes after TBI were associated with (1) elevated levels of IL6 and IL10 and lower levels of PDGF and RANTES, and (2) activated mechanisms involving inflammatory and colony-stimulating factors. Pathway analysis evoked a key network at T2 with a central histone hub and a sub-network focused on Complement C5. Galantamine modulated 14 of 35 molecules in this key network through 7 intermediaries, including nicotinic acetylcholine receptor subunit alpha-7.

Conclusion: TBI is characterized by specific CC clusters associated with clinical outcomes. In addition to pre-clinical data, these human data-driven results suggest that galantamine therapy should be evaluated for treatment of TBI patients.

Keywords: traumatic brain injury, bioinformatics, pathway analysis

A22-05

ROADMAP FOR SUCCESSFUL TRANSLATIONAL PRE-CLINICAL TRAUMATIC BRAIN INJURY THERAPY TESTING

Bridget Hawkins¹, Stefania Mondello², C. Edward Dixon³, Ronald L. Hayes⁴, Douglas H. Smith⁵, W. Dalton Dietrich⁶, Helen M. Bramlett⁶, William M. Armstead⁷, Edward D. Hall⁸, Kathryn E. Saatman⁹, Michelle C. LaPlaca¹⁰, Linda J. Noble-Haeusslein¹¹, Adam R. Ferguson¹², Pramod Dash¹³, Alex B. Valadka¹⁴, David F. Meaney¹⁵, Samuel M. Poloyac¹⁶, Liying Zhang¹⁷, Cameron R. Bass¹⁸, Pamela VandeVord¹⁹, Sandy R. Shultz²⁰, András Büki²¹, Deborah A. Shear²², Joseph B. Long²³, Col Sidney R. Hinds²⁴, Claudia S. Robertson²⁵, Donald S. Prough¹, Patrick M. Kochanek²⁶, Douglas S. DeWitt¹

¹University of Texas Medical Branch (UTMB), Moody Project for Translational Traumatic Brain Injury Research, Department of Anesthesiology, Galveston, USA

²University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italy

³University of Pittsburgh School of Medicine, Department of Neurosurgery, Brain Trauma Research Center, Geriatric Research, Education and Clinical Center in the Veteran’s Affairs Pittsburgh Health Care System, Pittsburgh, USA

⁴Banyan Biomarkers, Inc., Alachua, USA

⁵University of Pennsylvania, Center for Brain Injury and Repair and Department of Neurosurgery, Perelman School of Medicine, Philadelphia, USA

⁶University of Miami Miller School of Medicine, Department of Neurological Surgery, Miami, USA

⁷University of Pennsylvania, Department of Anesthesiology and Critical Care, Perelman School of Medicine, Philadelphia, USA

⁸University of Kentucky, Anatomy & Neurobiology, Neurology, Neurosurgery and Physical Medicine & Rehabilitation, Lexington, USA

⁹University of Kentucky, Spinal Cord and Brain Injury Research Center, Department of Physiology, Lexington, USA

¹⁰Georgia Institute of Technology, Coulter Department of Biomedical Engineering, Atlanta, USA

¹¹University of California at San Francisco, Departments of Neurological Surgery and Physical Therapy and Rehabilitation Science, San Francisco, USA

¹²University of California at San Francisco, Brain and Spinal Injury Center, Department of Neurological Surgery, San Francisco, USA

¹³University of Texas Health Science Center, Department of Neurobiology & Anatomy, McGovern Medical School, Houston, USA

¹⁴Virginia Commonwealth University, Department of Neurosurgery, School of Medicine, Richmond, USA

¹⁵University of Pennsylvania, Department of Engineering, Philadelphia, USA

¹⁶University of Pittsburgh School of Pharmacy, Department of Pharmaceutical Sciences, Pittsburgh, USA

¹⁷Wayne State University, Department of Biomedical Engineering, Detroit, USA

¹⁸Duke University, Department of Biomedical Engineering, Durham, USA

¹⁹Virginia Tech University, Department of Biomedical Engineering and Mechanics, Blacksburg, USA

²⁰The University of Melbourne, Department of Medicine (Royal Melbourne Hospital), Parkville, VIC, Australia

²¹Medical University of Pecs, Department of Neurosurgery, Pécs, Hungary

²²Walter Reed Army Institute of Research, Brain Trauma Neuroprotection and Neurorestoration Branch, Center for Military Psychiatry and Neuroscience, Silver Spring, USA

²³Walter Reed Army Institute of Research, Blast-Induced Neurotrauma Branch, Center for Military Psychiatry and Neuroscience, Silver Spring, USA

²⁴United States Army Medical Research and Materiel Command, Department of Defense Blast Injury Research Program Coordinating Office, Fort Detrick, USA

²⁵Baylor College of Medicine, Department of Neurosurgery, Houston, USA

²⁶University of Pittsburgh School of Medicine, Department of Critical Care Medicine, Anesthesiology, Pediatrics and Clinical and Translational Science, Safar Center for Resuscitation Research, Pittsburgh, USA

Considerable interest exists to translate novel therapies into human clinical trials for the treatment of traumatic brain injury (TBI). We present key findings from the Moody TBI Symposium held in Galveston in which invited experts in clinical and preclinical TBI provided their opinions on preclinical study design. The purpose of this initiative was to seek perspectives on a number of translational issues that included: 1) model and therapy selection, 2) outcomes and data collection procedures, 3) analysis of results and reproducibility of studies, 4) data management and sharing plans, and 5) communications between labs during large scale multi-site projects. It is anticipated that the knowledge generated from this initiative will help to guide future scientific discussions and expert guidelines towards translation of novel therapies to improve TBI outcomes. The Moody TBI Symposium was generously supported by The Moody Project for Translational Traumatic Brain Injury Research and Mission Connect, a program of the TIRR Foundation.

Keywords: consensus, roadmap, preclinical

A NOVEL CICLOSPORIN FORMULATION FOR TRAUMATIC BRAIN INJURY TREATMENT

Michael Karlsson^{1,3,4}, Bryan Pukenas^{1,2}, Ross Plyler², Madeline Stelow², Melissa Byro¹, Johannes Ehinger^{3,4}, Matilda Hugerth³, Eskil Elmér^{3,4}, Magnus Hansson^{3,4}, Susan Margulies², Todd Kilbaugh^{1,2}

¹Children's Hospital of Philadelphia, Philadelphia, USA

²University of Pennsylvania, Philadelphia, USA

³NeuroVive Pharmaceutical AB, Lund, Sweden

⁴Lund University, Lund, Sweden

Alterations in mitochondrial function is a critical component of the secondary injury cascade initiated by traumatic brain injury. We hypothesize that improving mitochondrial function will improve outcomes. Ciclosporin A (CsA) primarily exerts its neuroprotective effect through inhibiting Cyclophilin D-mediated activation of the mitochondrial transition pore. NeuroSTAT[®] is a novel lipid emulsion containing CsA. Our objective was to evaluate the pharmacokinetics and efficacy of NeuroSTAT using a large animal model of focal controlled cortical impact injury (CCI). Four-week-old (7–9 kg) piglets were studied. A mild-to-moderate injury was induced using a spring-loaded CCI device. One hour post-CCI, a loading dose of CsA was administered followed by a continuous infusion for either 24 h or 5 days. In a 24-hour bioequivalence study, piglets were randomized to 20 mg/kg/day (IV) of either NeuroSTAT or Sandimmune formulation of CsA (N=3/group). In a pharmacokinetic dose escalation study: 5, 10, 20 or 40 mg/kg/day of NeuroSTAT (N=3/group) was administered over 24 h, and CsA levels were measured in brain tissue to determine optimal dosing. Finally, in a randomized blinded placebo controlled study, animals were treated for 5 days with continuous infusion (20 mg/kg/d NeuroSTAT[®], N=10) or placebo (N=13), and multiple efficacy outcome metrics were obtained: blood and CSF biomarkers, microdialysis, mitochondrial respiration and reactive oxygen species generation, and advanced neuroimaging. Mean brain tissue concentrations of Sandimmune and NeuroSTAT (436 ± 187 ng/g vs. 566 ± 80 ng/g, P=0.3538), indicated equivalent penetration. NeuroSTAT brain concentrations significantly increased with each escalation from 10 to 40 mg/kg/day. Finally, animals treated with NeuroSTAT (20 mg/kg/day) for 5 days displayed a trend towards higher maximal oxidative phosphorylation (OXPHOS) (P=0.252) and a decrease in reactive oxygen species generation (P=0.122) compared to placebo. Analysis of the additional outcome metrics are ongoing.

Keywords: Mitochondria, Ciclosporin A, Focal controlled cortical impact injury

A22-07

OVERVIEW OF THE FIRST 12 THERAPIES EVALUATED BY OPERATION BRAIN TRAUMA THERAPY, A PRE-CLINICAL MULTI-CENTER CONSORTIUM FOR TBI

Patrick Kochanek¹, Helen Bramlett², C. Edward Dixon¹, Frank Tortella³, W. Dalton Dietrich², Stefania Mondello⁴, Ronald Hayes⁵, Kevin Wang⁶, John Povlishock⁷, Audrey Lafrenaye⁷, Samuel Poloyac¹, Philip Empey¹, Janice Gilsdorf³, Deborah Shear³

¹University of Pittsburgh, Safar Center, Pittsburgh, USA

²University of Miami, Miami, USA

³Walter Reed Army Institute of Research, Silver Spring, USA

⁴University of Messina, Messina, Italy

⁵Banyan Biomarkers, San Diego, USA

⁶University of Florida, Gainesville, USA

⁷Virginia Commonwealth University, Richmond, USA

Operation brain trauma therapy (OBT) is a multi-center pre-clinical drug and biomarker screening consortium supported by the US DoD. OBT screens therapies across three models in rats (parasagittal fluid percussion injury [FPI], controlled cortical impact [CCI] and penetrating ballistic-like brain injury [PBB]) reflecting a range of phenotypes. Our goals include 1) to define therapies efficacious across models with the best chance for clinical translation, and 2) to define model-dependent effects to guide trials in targeted pathologies. The results of the first 5 therapies tested (nicotinamide, erythropoietin [EPO], cyclosporine [CsA], simvastatin, and levetiracetam) were published. OBT has now screened 10 therapies, with ongoing testing of drugs 11–12. Drugs 6–12 include glibenclamide, kolidon-VA64, AER-271, amantadine, minocycline, E-64d and P7C3-A20. Levetiracetam showed benefit in multiple models. The second most successful drug, glibenclamide showed multiple benefits in CCI. Other therapies showed model dependent effects: tissue sparing by nicotinamide in CCI, benefit from CsA in FPI but toxicity in PBB, benefit from amantadine in PBB. Serum glial fibrillary acidic protein (GFAP) and Ubiquitin carboxy-terminal hydrolase-L1 were assessed at 4 and 24 h. GFAP predicted 21 d histology and drug effects. Levetiracetam merits additional testing and is being evaluated in our large animal model. Glibenclamide and Amantadine merit additional testing in contusion and penetrating brain injury, respectively. Our data support theranostic use of serum GFAP. Support: W81XWH-10-1-0623; W81XWH-14-2-0018.

Keywords: consortium, rat, reproducibility, rigor, pre-clinical, therapy

A22-08

KCC2, A NOVEL THERAPEUTIC TARGET IN TRAUMATIC BRAIN INJURY

Pavel Lizhnyak, Andrew Riddler, John Povlishock, Andrew Ottens
Virginia Commonwealth University, Anatomy and Neurobiology,
Richmond, USA

There remains a need for more effective therapeutics in the treatment of traumatic brain injury (TBI). In this study, we tested whether an effective TBI intervention could be developed around a post-translational target occurring within an opportune treatment window as guided by temporal proteomics. Of interest were delayed-onset processes best managed after patient stabilization on the intensive care unit, focusing on events initiated a day or more after insult, out to two weeks following controlled cortical impact injury. We identified a subset of protein changes with the temporal profile of interest utilizing a self-organizing map approach. Enriched in this map were post-translational processes tied with ionic dysregulation, among which was a highly dynamic processing of neuron-specific K⁺-Cl⁻ cotransporter 2 (KCC2), an essential component for maintaining chloride homeostasis that is critical to inhibitory neurotransmission. We identified a potential therapeutic window of opportunity starting on day 1 preceding unique acetylation, phosphorylation and ubiquitination events guiding the functional loss of KCC2. To test this window, we administered the KCC2-targeting compound CLP290 daily (50 mg/kg, p.o.) before, at, and after the identified 1-day point of KCC2 post-translational processing. The therapy was most effective at 1-day, preserving plasmalemmal KCC2 within perilesional somatosensory neocortex needed to maintain chloride homeostasis and effective inhibitory neurotransmission. Furthermore, TBI-impacted sensorimotor integration improved significantly with the 1-day intervention on rotarod and whisker adhesive removal task assessments. Together, our findings demonstrate the therapeutic targeting of post-

translational processes revealed with temporal proteomics to effectively preserve KCC2-mediated chloride homeostasis with improved functional recovery. Furthermore, the approach defines an effective administration window that can be extended for the intervention of other post-translational events. Lastly, KCC2-targeted therapy may be extended to other neurological insults known to involve chloride dysregulation such as epilepsy and stroke.

Supported by NINDS NIH 1F31NS100322-01, Department of Anatomy and Neurobiology, and VCU Neuroscience PhD Program.

Keywords: KCC2, Proteomics, Inhibition, CCI, Chloride, Post-translational

A22-09

USE OF BEHAVIORAL MEASURES TO ASSESS THE THERAPEUTIC VALUE OF NANO-PULSED LASER THERAPY AFTER FLUID-PERCUSSION INJURY

Nicole Hausser¹, Rinat Esenaliev^{2,3}, Irene Petrov³, Yuriy Petrov³, Kathia Johnson¹, Ian Bolding¹, Margaret Parsley¹, Douglas DeWitt¹, Donald Prough¹, Maria Micci¹, **Stacy Sell**¹

¹Univ. Texas Medical Branch, Anesthesiology, Galveston, USA

²Univ. Texas Medical Branch, Neuroscience and Cell Biology, Galveston, USA

³Univ. Texas Medical Branch, Biomedical Engineering, Galveston, USA

Traumatic brain injury (TBI) is considered a chronic condition due to the fact that it can both cause and accelerate disease; thus, the search for non-invasive, non-pharmaceutical therapies continues. Here, we assessed a novel, non-invasive nano-pulsed laser therapy (NPLT) system that combines near-infrared laser light (808 nm) and laser-generated, low-energy optoacoustic waves using fluid-percussion injury (FPI) in rats. Adult, male Sprague-Dawley rats were divided into four groups (SHAM, FPI, SHAM+NPLT, FPI+NPLT). All rats experienced two days of training followed by baseline assessment using the Neuroscore, Beam-Balance, and Beam-Walk tests prior to surgical preparation. Rats were anesthetized, intubated and a craniotomy was trephined lateral-right of the sagittal suture. Half of the rats received a moderate (2.0–2.2 atm) FPI and half received sham surgery. NPLT was applied for five minutes at one hour post-surgery. Neurological and vestibulomotor function were assessed using Neuroscore, Beam-Balance, and Beam-Walk for five consecutive days post-surgery. Cognitive function was assessed using a working memory version of the Morris water maze on post-injury days 11–15. Data were analyzed using a mixed model ANOVA to test for differences between treatments, days, trials, and in the case of the working memory test, the trials delta (Trial 1 – Trial 2). Results indicated that NPLT restored the performance of injured rats to that of sham-injured rats treated with NPLT, providing evidence of the neuroprotective effect of NPLT and supporting the continued investigation of NPLT as a therapeutic intervention after TBI. Funded by The Moody Project for Translational TBI Research

Keywords: nano-pulsed laser therapy, working memory, fluid-percussion injury, rodent behavior

A22-10

TARGETING THE NEURAL INJURY LANDSCAPE

Amanda Witten¹, Jonathan Lifshitz^{2,3,4}, **Sarah Stabenfeldt**^{1,4}

¹Arizona State University, Biomedical Engineering, Tempe, USA

²Barrow Neurological Institute at Phoenix Children's Hospital, Phoenix, USA

³University of Arizona College of Medicine-Phoenix, Department of Child Health, Phoenix, USA

⁴Arizona State University, Neuroscience Program, Tempe, USA

Traumatic brain injury (TBI) is the leading cause of injury related death in America. Current clinical treatment modalities for TBI focus on minimizing the secondary symptoms and complications associated with TBI; however, no clinical treatments currently exist to address the underlying neuropathology for any level of TBI severity ranging from mild to severe. The long-term goal of this project is to develop novel intervention strategies that directly tackle neurodegenerative cues present in the extracellular matrix to shift the balance toward regeneration. The primary objective of this study was to identify unique targeting moieties of the neural injury microenvironment in order to mask neurodegenerative cues in injured neural tissue. We employed the molecular biology screening technique, phage display, to identify nanobodies that bind to markers that are more abundant in injured neural tissue versus healthy neural tissue. Here, we focus on the chondroitin sulfate proteoglycan neurocan. We successfully completed biopanning screens against neurocan, confirmed via high throughput ELISAs. Subsequent concentration dependent ELISAs with four top clones demonstrated a superior nanobody clone displaying preferential binding to neurocan compared to non-specific targets. Recombinant protein expression/production of this nanobody is underway where we are conducting additional binding affinity assays to determine the robustness of its targeting capabilities. Ultimately, this approach will contribute significant advances to improved understanding of how the extracellular microenvironment impacts neural regeneration after brain injury.

Keywords: Glial scar, nanobody, neurocan

A22-11

EFFECTS OF LOW-DOSE CARBON MONOXIDE INHALATION ON INFLAMMATION AND MOTOSENSORY FUNCTION AFTER EXPERIMENTAL SPINAL CORD INJURY

Yang Teng¹, Inbo Han¹, Xiang Zeng¹, Zaid Aljuboory¹, Ross Zafonte¹, Augustine Choi²

¹Harvard Medical School, PM&R and Neurosurgery, Charlestown, USA

²Weill Cornell Medical College, Cornell University, New York, NY, Medicine, New York, USA

Inflammation plays major roles in the pathogenesis and recovery after acute spinal cord injury (SCI). Carbon monoxide (CO) confers anti-inflammatory and pro-healing effects in rodent models and clinical cases of lung lesions when applied in low concentrations. The goal of this project was to investigate the impacts of low-dose CO ventilatory exposure on the inflammatory events and spinal cord neuronal survival following T9-10 injury of 35 g × 5 min compression in adult female S-D rats. Three-four hours after SCI, 4 groups of rats were randomly housed inside whole body exposure chambers that were maintained with room air or 100, 250 or 500 ppm CO (n=7/group), respectively. The rats underwent 1 hour CO exposure/24 hours for 12 consecutive days. In addition to hindlimb locomotion, lesion volume, white matter sparing and neuronal survival were evaluated up to 6 weeks post T9-10 compression. Anti-inflammation and neural protection parameters were assessed by immunohistochemical, histopathological and stereological quantifications. We found that CO treatment demonstrated a dose-dependent therapeutic effect for neural

repair. The 500 ppm CO-treated rats showed the highest level of hindlimb functional improvement, lesion volume reduction, white matter sparing and neuronal preservation, compared to the control group; furthermore, 500 ppm CO inhalation significantly decreased pro-inflammatory cytokine (e.g., IL-1 β and TNF- α) expression, macrophage/microglia activation (e.g., CD68+), reactive gliosis, and neuronal protein nitration. CO ventilation therapy directed and sustained macrophage polarization towards an arginase 1+ M2 phenotype to promote neural recovery. Importantly, the total number of interneurons and the numbers of GAD67+ inhibitory and ChAT+ excitatory interneurons were significantly higher in the 500 ppm CO-treated spinal cords. The results of 500 ppm ventilation were independently replicated. Taken together, the studies demonstrate that low-dose CO inhalation may mitigate inflammatory damage and support functional recovery by enhancing interneuron and neurite survival, suggesting a potential treatment for clinical neurotrauma.

Keywords: Spinal cord injury, Carbon monoxide, Inflammation, Macrophage, Interneuron, Recovery Neurobiology

A22-12

FLUBENDAZOLE ANTIMITOTIC THERAPY TARGETING PROLIFERATION OF B CELLS AND ASTROCYTES FOR SPINAL CORD INJURY

Chen-Guang Yu, Christina K. Oistilli, Kavi Dayaram, Hina Iqbal, Madison Sands, James W Geddes

Univ. of Kentucky, Neuroscience, Spinal Cord and Brain Injury Res. Center (SCBIRC), Lexington, USA

Traumatic SCI significantly causes mitotic cell proliferation of B cells and astrocytes, which leads to inflammation, astroglial scar formation, autoimmunity, locomotor deficits and pain. However, no approved anti-B cell or anti-astroglial products are available for SCI indications. Flubendazole (FluBZ) is a novel tubulin-binding antimitotic agent that has been widely used in the treatment of intestinal and neural parasites in human and can be safely administered long term up to two years in human without side effects. The objective is to evaluate whether FluBZ anti-mitotic therapy reduces proliferating signaling pathways of B cells and astrocytes and demonstrates potent pain relief and improved locomotor function after SCI. Intraperitoneal (IP) injection with 5 and 10 mg/kg/day (n=10/group) of FluBZ to Sprague-Dawley rats for 2 or 4 weeks started at 3 or 5 hrs post-SCI (180 kdyn) at T10. These FluBZ treatments resulted in improved locomotor function (BBB scores and kinematic analysis) 7 weeks after SCI and reduced pain behaviors 4 weeks after excitotoxic SCI compared to vehicle-treated controls (n=9/group). FluBZ IP treatment also improved total tissue sparing, white matter sparing, and gray matter sparing at 7 weeks after SCI. Mechanistic studies revealed that FluBZ reduced splenic population of CD45RA-positive B cells and spinal GFAP-positive astrocytes at 4 or 7 weeks post-injury and suppressed production of antibody IgG at lesion site post-injury. Moreover, we found that FluBZ inhibited mitotic signaling activation of mitogen-activated protein kinase pERK1/2 and mitotic cell cycle protein activator cyclin B1 at lesion site 4 weeks post-injury. In conclusion, our results demonstrate that FluBZ antimitotic therapy inhibiting proliferation of B cells and astrocytes as well as mitotic signaling is a valid therapeutic strategy for treating paralysis and pain following SCI.

Keywords: flubendazole, B cells, astrocytes, spinal cord injury, neuropathic pain, paralysis

A23 VASCULAR

A23-01

VASCULAR INTEGRITY AFTER MODERATE TRAUMATIC BRAIN INJURY IS IMPROVED WITH HUMAN UMBILICAL CORD PERIVASCULAR CELL THERAPY

Tanya Barretto^{1,2}, Eugene Park², Denis Gallagher³, Elaine Liu², Andrew Baker^{1,2}

¹*University of Toronto, Institute of Medical Science, Toronto, Canada*

²*St. Michael's Hospital, Toronto, Canada*

³*Create Fertility Centre, Toronto, Canada*

There are currently no therapeutic treatments for traumatic brain injury (TBI). Primary injury from the direct impact to the skull followed by secondary injury mechanisms consisting of delayed cellular and molecular pathways can contribute to breakdown of the blood brain barrier (BBB) among other events. Following TBI, vascular disruption leads to edema formation that is associated with increased intracranial pressure adversely affecting outcome. Human umbilical cord derived perivascular cells (HUCPVCs) have been shown to express mesenchymal, neurotrophic and vascular factors, making them a potential therapeutic strategy. A TBI was modeled using the rat fluid percussion injury (FPI) device. Rats were systemically infused with 1.5×10^6 cells either 1 hour pre-injury or 1.5 hours post injury, and survived for 24 h, 48 h or 7 days. Brains were assessed for Evan's blue vascular leakage and edema formation. Immunohistochemistry for RECA-1 at 24 h and 48 h was performed to assess microvascular density. Western blot was used to examine the expression of tight junction proteins after trauma. At 24 h and 48 h Evan's blue vascular leakage was 6.4 $\mu\text{g/g}$ and 15.5 $\mu\text{g/g}$, respectively and 1.7 $\mu\text{g/g}$ in sham rats. Pre-injury treatment values were 4.0 μg and 5.6 μg at 24 and 48 hours, respectively. Post-injury leakage values were 5.5 $\mu\text{g/g}$ and 3.3 $\mu\text{g/g}$ at 24 and 48 hours, respectively. At 7 days post-injury, FPI rats demonstrated leakage values similar to sham rats. Edema formation was not significantly different across treatment groups. At 24 h and 48 h, vascular density, expressed as fluorescence/area was 0.056 and 0.064 respectively compared to 0.081 for sham, and 0.076 and 0.082 for HUCPVC treated animals at 24 h and 48 h respectively. Preliminary results indicate some therapeutic capacity of HUCPVCs in mitigating acute BBB pathophysiology after TBI.

Keywords: Mesenchymal stromal cells, Fluid percussion injury, Umbilical cord derived stem cells, Tight junctions

A23-02

GASTROINTESTINAL VASCULAR PERMEABILITY AFTER SPINAL CORD INJURY

Juan Herrera¹, Kurt Bockhorst¹, Deepa Bhattarai², Karen Uray^{2,3}

¹*UTHealth Medical School at Houston, Diagnostic and Interventional Imaging, Houston, USA*

²*UTHealth Medical School at Houston, Department of Pediatric Surgery, Houston, USA*

³*University of Debrecen, Department of Medicinal Chemistry, Debrecen, Hungary*

Gastrointestinal (GI) dysfunction is observed clinically after spinal cord injury (SCI) and contributes to the diminished long-term quality of life after SCI. GI vascular permeability changes can result in undiagnosed abdominal complications after SCI. Our study examined the acute and chronic GI vascular changes that occurs following spinal

cord injury. We demonstrated that the GI vascular tract in mice becomes compromised during the acute phase of injury and persists into the chronic phase of injury. The contusion injury was delivered using the Infinite Horizon Impactor (60 kDynes with a 1 second dwell time) at thoracic level 8. The study examines GI vasculature permeability was measured using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) at 48 hours, 2 and 4 weeks following injury. Our hypothesis was that the observed vascular permeability could be attenuated by an intravenous (IV) administration of angiopoietin-1 (Ang-1). Ang-1 is a vascular stabilizing protein expressed constitutively by endothelial cells, pericytes, astrocytes, smooth muscle cells, and fibroblasts. Our results indicated that a single administration of Ang-1 reduced vascular permeability at 48 hours but the effect was only transient, as there were no significant difference between treated and the vehicle control treated group at 2 and 4 weeks. When the treatment paradigm was changed from a single dose to multiple doses of Ang-1 following contusion injury. Ang-1 (150 μ g, IV for 7 days), our DCE-MRI data indicated a significant decrease in GI vascular permeability 4 weeks after injury compared to vehicle control treated animals. We also demonstrated that Ang-1 reduced the expression of sICAM-1 in the ileum compared to the vehicle treated group. Together this data indicates that the GI vasculature is compromised into the chronic phase of injury and that multiple administrations Ang-1 can attenuate GI vascular permeability.

Keywords: gastrointestinal, vascular permeability, dynamic contrast enhanced imaging, angiopoietin-1

A23-03

TRAUMATIC BRAIN INJURY ALTERS CORTICAL VASCULATURE IN A SEX SPECIFIC MANNER

Amandine Jullienne¹, Arjang Salehi¹, Bethann Affeldt¹, Mohsen Baghchechi¹, Angela Avitua¹, Mark Walsworth¹, Isabelle Enjalric¹, Elizabeth Haddad¹, Saburi Eliamani¹, Mary Hamer¹, Sonali Bhakta¹, Andre Obenaus^{1,2}

¹Loma Linda University, Basic Science, Loma Linda, USA

²University of California Irvine, Pediatrics, Irvine, USA

Traumatic brain injuries (TBI) occur in 1.7 million people each year in the USA. Little is known about how the cerebrovasculature is altered after TBI. We previously reported that TBI elicits acute decrements in cerebral vessels near the injury site in rats followed by revascularization over the subsequent 2 weeks. Sexual dimorphism of the brain is well documented and different hormonal levels in males and females differentially modify the recovery process after brain injury. However, little is known about the effects of biological sex on the temporal evolution of revascularization following moderate TBI. Using a model of controlled cortical impact in male and female mice, we set out to determine if the injury and the repair process are affected by sex. Lesion volume was assessed using MRI T2-weighted imaging at 1 and 7 days post-injury (dpi). To evaluate the vascular network, we used a new "vessel painting" technique that uses a fluorescent dye (DiI) to stain blood vessels. Vascular parameters such as vessel numbers and complexity were analyzed using Angiotool and FracLac, ImageJ plugins. Blood-brain barrier (BBB) alteration, neurodegeneration, inflammation and endothelial activation were assessed through immunohistochemistry. We found no sex differences in lesion volume, BBB alteration or neurodegeneration. However at 1 dpi, females exhibited more astrocytic hypertrophy, whereas males presented with increased endothelial activation and expression of beta-catenin, which has been shown to be involved in angiogenesis. At 7 dpi, we observed an increase in the number of vessels and an enhancement in vessel complexity in injured

cortex of males compared to females. Our results imply that the cerebrovasculature recovers differently after TBI, suggesting sex should be considered when patients are being treated. Further studies are needed to determine if these sex differences are beneficial or deleterious.

Keywords: biological sex, beta-catenin

A23-04

CEREBRAL ARTERY DAMAGE WITH LARGE DEFORMATIONS

Matthew Converse, **Kenneth Monson**

University of Utah, Mechanical Engineering, SALT LAKE CITY, USA

Cerebral vessels commonly tear and bleed as a consequence of traumatic brain injury (TBI). It follows that neighboring vessels experience deformations that are also significant, though not severe enough to produce bleeding. Although these vessels remain intact, little is known about potential changes to their integrity and their prognosis as functioning vessels. Preliminary work from our laboratory shows that both the mechanical properties and contractility of cerebral arteries are altered by deformation. We hypothesized that these observed changes would correlate with damage to elastin and collagen in the extracellular matrix (ECM) and thus aimed to characterize ECM changes following large deformations. Middle cerebral arteries were dissected from euthanized sheep, attached to an isolated vessel testing device, and subjected to overstretch in either the axial or circumferential direction. The tested vessels were incubated in fluorescently-tagged collagen hybridizing peptide (CHP), a newly discovered marker of tropocollagen disruption, and evaluated via confocal microscopy. Elastin was assessed using autofluorescence. Extent of damage was correlated with magnitude and direction of applied overstretch. Damaged collagen was found following subfailure overstretch in both directions, but it was located primarily in the media after circumferential loading and in the adventitia following axial overstretch, consistent with known dominant fiber directions in the arteries. Extent of collagen damage, defined by the intensity of CHP fluorescence, increased linearly with overstretch, but only above thresholds of 1.15 and 1.23 times the in vivo diameter and length for the circumferential and axial directions, respectively. We also commonly observed disruption of the internal elastic lamina prior to complete rupture of the vessel, occurring, on average, at 1.31 times the in vivo length, compared to 1.57 for failure. It was common for multiple disruptions to occur in a single vessel prior to failure. Findings show that vessel ECM is damaged in cerebral arteries experiencing large deformations below the threshold of complete vessel disruption. To our knowledge, such damage has not yet been observed in vivo, but these changes could explain the observed changes in function and structural properties and may be relevant to TBI.

Keywords: extracellular matrix, collagen, internal elastic lamina, overstretch

A23-05

TRAUMATIC BRAIN INJURY RESULTS IN ACUTE RAREFICATION OF THE VASCULAR NETWORK

Andre Obenaus^{1,2}, Michelle Ng², Amanda Orantes², Eli Kinney-Lang², Faisal Rashid², Mary Hamer², Richard DeFazio⁶, Jiping Tang³, John H. Zhang^{3,4}, William J. Pearce⁵

¹University of California, Irvine, Pediatrics, Irvine, USA

²Loma Linda University, Pediatrics, Loma Linda, USA

³Loma Linda University, Physiology Pharmacology, Loma Linda, USA

⁴Loma Linda University, Anesthesiology Neurosurgery, Loma Linda, USA

⁵Loma Linda University, Center for Perinatal Biology, Loma Linda, USA

⁶University of Michigan, Molecular and Integrative Physiology, Ann Arbor, USA

Objectives: The role of the cerebrovascular network and its acute response to TBI is poorly defined and emerging evidence suggests that cerebrovascular reactivity is altered. We explored how cortical vessels are physically altered following TBI using a newly developed technique, vessel painting.

Methods: We tested our hypothesis that a focal moderate TBI results in global decrements to structural aspects of the vasculature. Rats (naïve, sham-operated, TBI) underwent a moderate controlled cortical impact. Animals underwent vessel painting perfusion to label the entire cortex at 1 day post TBI followed by whole brain axial and coronal images using a wide-field fluorescence microscope.

Results: Cortical vessel network characteristics were analyzed for classical angiographic features (junctions, lengths) wherein we observed significant global (both hemispheres) reductions in vessel junctions and vessel lengths of 33% and 22%, respectively. Biological complexity can be quantified using fractal geometric features where we observed that fractal measures were also reduced significantly by 33%, 16% and 13% for kurtosis, peak value frequency and skewness, respectively.

Conclusions: Acutely after TBI there is a reduction in vascular network and vascular complexity that are exacerbated at the lesion site and provide structural evidence for the bilateral hemodynamic alterations that have been reported in rodents and patients after TBI.

Support: NIH NINDS 1P01NS082184, Project 3

Keywords: vessel painting, vascular repair, fractal, network

A23-06

OUTCOMES IN BLUNT CEREBROVASCULAR INJURY FOLLOWING THE USE OF AN EVIDENCE BASED TREATMENT PROTOCOL

Richard Rodgers¹, Freeman Zane², Melillo Anthony², Kovanda Timothy¹

¹Indiana University School of Medicine, Goodman Campbell Brain and Spine, Indianapolis, USA

²Marian College of Osteopathic Medicine, Indianapolis, USA

Objectives: Literature suggests blunt cerebrovascular injury (BCVI) occurs in about 1% of blunt trauma victims. Multiple different criteria have been established for BCVI screening. Treatment for BCVI varies by institution, ranging from observation to the use of antiplatelet agents and/or anticoagulants. Treatment recommendations are generally based on retrospective reviews with poor follow up for outcome measures. Although high-level evidence to support different treatment algorithms is lacking, multiple different treatment options appear to improve outcomes in BCVI. Goodman Campbell Brain and Spine has developed an evidence based protocol for managing BCVI. In this study, the authors critically analyze BCVI outcomes in a patient population treated using this protocol.

Methods: A retrospective review was performed to assess outcomes for BCVI patients presenting to a major trauma center in Indianapolis, IN from 2013-2016. During this time period, BCVI patients were managed using an evidence based treatment protocol.

Results: 70 patients with 80 blunt cerebrovascular injuries were identified. There were 37 carotid artery injuries and 43 vertebral artery injuries. Patient age ranged from 18 to 90. Treatment rates occurred as follows: Aspirin 54.3% (38/70), Aspirin and Plavix 12.9% (9/70), anticoagulation with heparin, enoxaparin and/or Coumadin 11.4% (8/70), other 21.4% (15/70). Overall mortality rate was 17.1% (12/70). 12.9% (9/70) of patients had a stroke attributable to their vascular injuries.

Conclusion: The authors recommend the use of an evidence based protocol for the management of BCVI. Protocol use can help to standardize care within this patient population while potentially providing medicolegal protection. Prospective and long-term follow up data is needed to further analyze the benefit of the proposed treatment protocol.

Keywords: Blunt cerebrovascular injury, treatment protocol, antiplatelet medication, anticoagulation

A23-07

LONGITUDINAL ASSESSMENT OF CEREBROVASCULAR DYSFUNCTION AFTER TRAUMATIC BRAIN INJURY USING FUNCTIONAL NEAR-INFRARED SPECTROSCOPY

Michael Sangobowale¹, Franck Amyot^{2,3}, Hasan Ayaz⁴, Yerram Reddy⁴, Erika Siverman¹, Tawny Meredith-Duliba¹, Megan Moyer¹, Danielle Sandsmark¹, Ramon Diaz-Arrastia¹

¹University of Pennsylvania, Neurology, Philadelphia, USA

²Uniformed Services University of the Health Sciences, Neurology, Rockville, USA

³Center for Neuroscience and Regenerative Medicine, Neurology, Bethesda, USA

⁴Drexel University, Biomedical Engineering, Philadelphia, USA

Traumatic cerebrovascular injury (TCVI) is common after TBI and is responsible for a significant portion of TBI-related disability. Development of therapies targeting TCVI will require reliable and inexpensive biomarkers to measure vascular dysfunction and document target engagement. Cerebrovascular reactivity (CVR) is impaired following TBI and methods which reliably and non-invasively measure CVR are available, making CVR an attractive candidate predictive and pharmacodynamic biomarker for TCVI-directed therapies. This study was designed to assess CVR longitudinally after TBI in humans using functional Near Infrared Spectroscopy (fNIRS) from the acute to the subacute and chronic stages. We also studied the effect of treatment with a phosphodiesterase 5 inhibitor, sildenafil citrate, in order to assess the utility of fNIRS as a pharmacodynamic biomarker in future clinical trials. 7 participants with complicated mild TBI were studied in the acute (within 72 after injury) and subacute (14 days after injury) stages, in addition to 4 age-matched healthy controls (HC), who were studied once. CVR was assessed by measuring the changes in oxygenated hemoglobin (ΔHbO) and deoxygenated hemoglobin (ΔHbR) concentration produced by mild hypercapnia (5% CO_2). The change in CVR before and one hour after the administration of single dose of sildenafil citrate (60 mg orally) was also assessed. Mean (\pm SEM) CVR was lower in TBI patients compared to HC (HC: $0.100 \pm 0.028\%$ /mmHg; and TBI: CVR $0.057 \pm 0.011\%$ /mmHg, $p=0.22$). Sildenafil administration did not result in an increase in CVR in HC ($t=0.1463$ $df=3$, $p=.89$) whereas TBI patients show a clear potentiation of the CVR response ($p<.08$). These findings support the hypothesis that vascular injury represents a distinct endophenotype following TBI and PDE5 inhibition as a potential therapy for TCVI.

Keywords: FNIRS

B01 BEHAVIORAL FUNCTION

B01-01

EXECUTIVE FUNCTION IMPAIRMENTS AFTER FRONTAL LOBE BRAIN TRAUMA IN MALE RATS

Corina Bondi, Ian Marshall, Melissa Nicholas, Lindsay Kutash, Ihuoma Njoku, Megan LaPorte, Hannah Radabaugh, Anthony Kline
University of Pittsburgh, Physical Medicine and Rehabilitation, Pittsburgh, USA

More than 10 million people worldwide sustain a traumatic brain injury (TBI) each year. Many survivors suffer long-lasting cognitive impairments associated with frontal lobe disturbances, as well as psychopathological consequences. Previously, we demonstrated that a controlled cortical impact (CCI) injury over the parietal cortex produced deficits in executive function in the attentional set-shifting test (AST) in rats, a complex cognitive paradigm analogous to the Wisconsin Card Sorting Test, which measures strategy-switching deficits in patients with frontal lobe damage, TBI, and psychiatric disorders. Considering that a large percentage of TBIs occur via direct impact to the frontal part of the skull (e.g., windshield during a car accident), this study aimed to investigate complex cognitive deficits in rats subjected to frontal lobe CCI. Isoflurane-anesthetized adult male rats were subjected to CCI (2.0, 2.2, and 2.4 mm cortical tissue deformation at 4 m/sec) or sham injury over the prefrontal cortex region in the right hemisphere. Rats were tested on the AST at four weeks post-surgery. The test involves a series of increasingly difficult discriminative stages to obtain food reward, including simple and compound discriminations, stimulus reversals, and intra- and extradimensional (ED) shifts. Frontal CCI produced significant deficits in attentional performance on the ED stage and stimulus reversals of AST at four weeks post-injury, seen as increased total trials to reach criterion and significantly higher total errors compared to SHAM rats ($p < 0.05$ for Injury, $n = 7-8/\text{group}$). These effects were particularly robust in the two more severe injury groups, namely 2.2 and 2.4 mm cortical deformation depth ($p < 0.05$). These results suggest that frontal lobe injury negatively impacts complex cognitive functioning. Ongoing and future studies will focus on further disentangling brain constructs and neurotransmitter alterations responsible for such attentional deficits following brain trauma, as well as identifying necessary pharmacotherapies for cognitive performance and advance rehabilitation research.

Supported by NIH grants NS094950, NS099683, UPP/UPMC Academic Foundation and UPMC Rehabilitation Institute (COB); HD069620, HD069620-S1, NS060005, and NS084967 (AEK).

Keywords: Controlled cortical impact, Attentional set-shifting, Frontal injury, Executive function

B01-02

DELAYED ADMINISTRATION OF INTRANASAL INSULIN PROVIDES LIMITED COGNITIVE IMPROVEMENT AFTER MODERATE CCI

Kimberly Byrnes^{1,2}, Nicole Hockenbury^{1,2}, Shalini Jaiswal³, William Frey⁴

¹Uniformed Services University, Anatomy, Physiology and Genetics, Bethesda, USA

²Uniformed Services University, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

³Uniformed Services University, Translational Imaging Core, Bethesda, USA

⁴HealthPartners Neuroscience Research, St. Paul, USA

Following traumatic brain injury (TBI), there is an acute period of elevated glucose demand followed by a period of cerebral hypometabolism, which directly affects long term patient outcome and is associated with cognitive and physical deficits. Our previous research demonstrated that intranasal insulin administration within 4 hours after injury significantly increased glucose uptake in the hippocampus and improved cognitive function after a moderate controlled cortical impact (CCI) in rats. In order to more fully understand the therapeutic potential of intranasal insulin administration for TBI, this study aimed to evaluate the effect of delaying treatment. Male adult Sprague Dawley rats were exposed to a moderate CCI injury followed by intranasal insulin or saline treatment beginning 24 hours post-injury and continuing with daily administration for 14 days. Glucose uptake was assessed by measuring ¹⁸Fluorodeoxyglucose (FDG) uptake in positron emission tomography (PET) scans at 2 and 10 days post-injury. Motor and cognitive testing were performed through 60 days post-injury. Significant differences in FDG uptake were noted in the cortex and hippocampus at 2 and 10 days post-injury, respectively, in control and insulin-treated groups. In addition, analysis of the novel object recognition task showed that insulin treated rats demonstrated significant improvement at 2 days post-injury in comparison to rats that received saline. However, injured animals treated with intranasal insulin demonstrated no significant difference in cognitive performance on the MWM or motor function in the beam walk task, unlike our previous study. These data suggest that more acute treatment with intranasal insulin is optimal for observing functional effects of the therapy, and may suggest that the acute hyperglycolysis and elevated glucose demand are the primary target of insulin administration. However, delaying treatment to 24 hours may still offer some benefit.

Keywords: insulin, glucose, intranasal, PET

B01-03

COMPARABLE IMPEDIMENT OF SPATIAL LEARNING IN FEMALE AND MALE RATS SUBSEQUENT TO DAILY ADMINISTRATION OF HALOPERIDOL AFTER TBI

Jeffrey Cheng, Kristin E. Free, Anna M. Greene, Patricia B. de la Tremblaye, Naima Lajud, Corina O. Bondi, Anthony E. Kline
University of Pittsburgh, Physical Medicine and Rehabilitation, Pittsburgh, USA

Antipsychotic drugs, such as haloperidol (HAL), are prescribed in the clinic to manage traumatic brain injury (TBI)-induced agitation. While preclinical studies have consistently shown that HAL hinders functional recovery after TBI in male rats, its effects in females is unknown. Hence, the objective of this study was to directly compare neurobehavioral and histological outcomes in both sexes to determine whether the reported deleterious effects of HAL extend to females. Anesthetized adult female and male rats received either a controlled cortical impact (CCI) or sham injury and then were randomly assigned to a dosing regimen of HAL (0.5 mg/kg, i.p.) or vehicle (VEH; 1 mL/kg, i.p.) that was initiated 24 hours after injury and continued once per day for 19 consecutive days. Motor function was tested using established beam-balance/walk protocols on post-operative days 1-5 and acquisition of spatial learning was assessed with a well-validated Morris water maze task on days 14-19. Cortical lesion volume was quantified at 19 days. No statistical differences were revealed between the HAL and VEH-treated sham groups and thus they were pooled for each sex. HAL impaired motor recovery in males, but not females. However, HAL significantly diminished spatial learning in both sexes. Taken together, the data show that daily HAL does not prohibit motor recovery in females, but does negatively impact cognition. The

deleterious effects of HAL on cognition in females replicate that observed in males and suggests that other treatments need to be explored to combat agitation after TBI.

Supported, in part, by NIH grants HD069620, HD069620-S1, NS060005, NS084967 (AEK), NS094950, NS099683 (COB), and the University of Pittsburgh Physicians/UPMC Academic Foundation (COB).

Keywords: Controlled cortical impact, Water maze, Antipsychotics

B01-04

LONG-TERM EFFECTS OF ADOLESCENT CHRONIC STRESS ON TBI COGNITIVE AND EMOTIONAL IMPAIRMENTS IN ADULT MALE RATS

Patricia De la Tremblaye, Hannah Radabaugh, Kileigh Nassau, Jody Wellcome, Corina Bondi, Anthony Kline

University of Pittsburgh, Department of Physical Medicine & Rehabilitation, Pittsburgh, USA

Exposure to early life stress has lasting effects on behavior and brain function due to dynamic plasticity occurring in the developing adolescent brain. However, it is yet to be determined how stress exposure in this developmental period influences functional recovery post traumatic brain injury (TBI) later in life. Thus, the goal of this study was to test the hypothesis that stress in adolescence would confer deleterious effects on behavioral impairments post TBI in adulthood. Adolescent male Sprague-Dawley rats (n=40) were exposed to 4 weeks (postnatal day, PND, 30–60) of chronic unpredictable stressors (CUS) or no stress, and after a 1-month resting period (PND 60–90), were anesthetized and received a cortical impact of moderate severity (2.8 mm tissue deformation at 4 m/s) or sham injury. After one week of recovery, anxiety-like behavior in the open field test (OFT) and elevated plus maze (EPM), and cognitive performance in the novel object recognition (NOR) task and, Morris water maze (MWM) were measured, and the brains collected 25 days post TBI for histological analysis. Preliminary results show increased time spent in the anxiogenic zones of the OFT and EPM, and improved NOR memory after a 24 h delay, and reduced time to reach the platform in the MWM for CUS groups compared to no-stress groups, although TBI rats remained significantly more anxious and cognitively impaired compared to sham controls. These results suggest that aversive environmental conditions in adolescence induces adaptive behavioral responses in TBI rats, albeit, without leading to full functional recovery.

Keywords: Early-Life Stress, Anxiety-Like Behavior, Cognitive Behavior, Controlled Cortical Impact (CCI)

B01-05

VISUAL DYSFUNCTION OF THE OPTOMOTOR RESPONSE IN APOE TRANSGENIC MICE AFTER TBI

Scott Ferguson, Benoit Mouzon, Tiffany Smith, Destinee Aponte, Michael Mullan, Fiona Crawford

Roskamp Institute, Sarasota, USA

Introduction: Our mouse model of repetitive mild TBI (r-mTBI) produces chronic optic nerve pathology and retinal degeneration. The APOE4 allele has previously been shown to be associated with poor outcome after head injury. In order to assess the visual function of APOE transgenic mice, we have optimized a mechanical assay to assess the optomotor response.

Methods: The optomotor apparatus consisted of a rotating drum containing black and white stripes at varying angular resolutions at 2 rpm. Mice were acclimated to the apparatus for a period of 5 minutes

in photopic lighting. Optomotor testing at each resolution consisted of pairs of 2 minute trials with 1 trial in a clockwise rotation followed by 1 trial in a counter-clockwise rotation with an inter-trial time of 30 seconds. After the completion of all 4 trials the mouse was returned to the home cage and the next mouse was tested. All trials were recorded with Noldus Ethovision XT using both manual and automatic quantification.

Results: Optomotor testing and optimization revealed a quantifiable optokinetic response of the mice which decreased with decreasing stripe thickness, and was significantly impaired in APOE4 transgenic mice after TBI. Ethovision XT based quantification of the head velocity failed to show a stripe-dependent decrease of the optomotor response, but more advanced pattern recognition algorithms may allow for more accurate automated detection of the optomotor response in the future.

Conclusions: By varying the resolution of the stripes we were able to increase the difficulty of the task and determine the optimal conditions for discriminating subtle vision deficits. APOE4 transgenic mice show a significant vision impairment compared to sham controls, but current commonly used animal tracking software is insufficient for discriminating between the optomotor response and random head movements.

Keywords: APOE, TBI, Vision, Optomotor

B01-06

EXPLORING SPATIAL MAP VERSUS COMPENSATORY STRATEGY FORMULATION FOR PLACE-LEARNING IN THE MORRIS WATER MAZE AFTER EXPERIMENTAL TBI

David Fine¹, Prerna Ranganathan¹, Edda Thiels³, Amy Wagner^{1,2}

¹*University of Pittsburgh, Physical Medicine and Rehabilitation, Pittsburgh, USA*

²*University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA*

³*University of Pittsburgh, Department of Neurobiology, Pittsburgh, USA*

Explicit learning and memory deficits after traumatic brain injury (TBI) have implications for skill learning, reacquisition, retention, and cognitive rehabilitation approaches that leverage implicit-learning networks, which remain relatively intact, as compensatory mechanisms. The Morris water maze (MWM) assesses learning/memory deficits through metrics like latency and target zone time allocation (TZTA). MWM protocols include extra-maze cues to formulate spatial maps (explicit-learning) representing the escape platform's location (place-learning). However, latency improvements can be achieved via alternative strategies without clear spatial map formulation. We hypothesized MWM place-learning post-experimental TBI results primarily from acquiring non-spatial (Implicit-learning) strategies, and post-injury cognitive training (CT) on the MWM's implicit components facilitates further compensatory reductions (via implicit-learning networks) in platform latencies, and supports increased spatial strategy selection. Adult male Sprague-Dawley rats (n=50) underwent controlled cortical impact (CCI) injury or sham surgery. Beginning D8 post-surgery, CCI/Shams completed 6 days of non-spatial CT or no-training (NT). Acquisition trials [AC (D14–D18, D20–D24)], and periodic short/long-term retention probe trials (PT) were performed. Platform latencies, peripheral zone time allocation (PZTA), TZTA, and search strategies were assessed. CCI-CT and CCI-NT groups improved latency and TZTA by D18 (p<0.001). PZTA improved by D24 (p<0.01). Shams improved latency, PZTA, and TZTA by D18 (p<0.05, all comparisons) and further reduced PZTA by D24 (p<0.001). Compared to CCI-NT animals, CCI-CT had lower AC latencies (p<0.001), higher TZTA (p<0.01), and lower PZTA (p<0.01) than CCI-NT on long-term retention PTs, where CT-Shams also had lower PZTA than NT-Shams (p<0.01). Frequency

analysis showed CT groups used more spatial strategies than NT counterparts with long-term retention PTs (D19:CCI-CT=25%, CCI-NT=0%, Sham-CT=66.67%, Sham-NT=44.4%; D25:CCI-CT=40%, CCI-NT=18.18%, Sham-CT=90%, Sham-NT=33.3%). These findings suggest MWM improvements can be achieved via new learning involving implicit networks, and CT on the MWM's implicit components improves place-learning and facilitates spatial map retention post-TBI. Support: NIH-1R21HD071728.

Keywords: Morris Water Maze, cognitive rehabilitation, implicit learning networks, Spatial Map Formation, Experimental TBI

B01-07

ALBEIT NOCTURNAL, RATS SUBJECTED TO TBI DO NOT DIFFER IN NEUROBEHAVIORAL PERFORMANCE WHEN TESTED DURING THE DAY OR NIGHT

Anthony Kline, Peter J. Niesman, Hannah L. Radabaugh, Kileigh L. Nassau, Jeffrey P. Cheng, Naima Lajud, Patricia B. de la Tremblaye, Corina O. Bondi

University of Pittsburgh School of Medicine, Department of Physical Medicine & Rehabilitation, Pittsburgh, USA

The majority of behavioral assessment studies are conducted during the day, which is not when rats are most active. This discrepancy may preclude optimal performance. Hence, the goal of this study was to determine if differences in neurobehavior exist in traumatic brain injured (TBI) rats when assessed during the day vs. night. The hypothesis was that the night group would perform better than the day group in all behavioral tasks. Isoflurane-anesthetized adult male rats received a controlled cortical impact (2.8 mm depth at 4 m/sec) or sham injury and were randomly assigned to either day (1:00 - 3:00 p.m.) or night (07:30 - 09:30 p.m.) testing. Motor function (beam-balance and beam-walk) was conducted on post-operative days 1-5 and cognitive performance (acquisition of spatial learning) was assessed on days 14-18. CORT levels were quantified at 24 hr and 21 days after TBI. No significant differences were revealed between the TBI rats tested during the day vs. night for beam-balance, beam-walk, or water maze (p 's < 0.05). CORT levels were higher in the TBI and sham groups tested at night at 24 hr (p < 0.05), but returned to baseline and were no longer different by day 21 (p > 0.05), suggesting an initial, but transient stress response, that did not affect neurobehavioral outcome. These data suggest that the time rats are tested has no impact on their performance, which does not support the hypothesis. The finding is important because it validates the interpretations from numerous studies conducted when rats were tested during the day vs. their biologically active period.

Supported by NIH grants HD069620, HD069620-S1, NS060005, NS084967 (AEK), NS094950, NS099683 (COB), and the University of Pittsburgh Physicians /UPMC Academic Foundation (COB)

Keywords: Controlled cortical impact, day vs night, spatial learning, Morris water maze, motor

B01-08

EFFECT OF GALANTAMINE ON ATTENTIONAL SET-SHIFTING PERFORMANCE AFTER EXPERIMENTAL BRAIN TRAUMA

Ihuoma Njoku, Lindsay Kutash, Darik O'Neil, Ian Marshall, Hannah Radabaugh, Jeffrey Cheng, Anthony Kline, Corina Bondi

University of Pittsburgh, Physical Medicine & Rehabilitation, Safar Center for Resuscitation Research, Center for Neuroscience, Pittsburgh, USA

Traumatic brain injuries (TBI) impact millions of Americans, with older patients being more likely to have a co-occurring condition, particularly dementia. Galantamine (GAL), a first-line drug used to treat dementia, acts primarily as an acetylcholinesterase inhibitor and has been reported to positively impact cognitive function in older adults. Previously, we demonstrated that a controlled cortical impact (CCI) injury produced significant impairments in executive function in the attentional set-shifting test (AST), a complex cognitive paradigm analogous to the Wisconsin Card Sorting Test, which is used to measure strategy-switching deficits in patients with frontal lobe damage, TBI, and psychiatric disorders. In the current study, we predicted that daily GAL injections would normalize AST performance after a parietal lobe TBI in rats. Isoflurane-anesthetized adult male rats were randomly assigned to either a CCI or sham group. Surgery was administered following a previously established CCI protocol. Following surgery, rats were randomly distributed into three treatment groups: saline or GAL (1 or 2 mg/kg/day), until the test day 4 weeks later. AST results indicated that TBI significantly impairs performance on the first reversal stage, deficits attenuated by both GAL chronic doses (p < 0.05). In particular, GAL (2 mg/kg/day) also significantly reduced TBI-induced cortical lesion volumes (p < 0.05). In summary, chronic GAL administration provides an efficacious treatment for higher-order cognitive recovery following TBI. Further studies will investigate whether these results are maintained when using aged Sprague-Dawley rats in order to mirror the elderly segment of adults typically treated with GAL in the clinic, as well as assess protein expression of brain cholinergic markers involved in the GAL mechanism of action for restoring executive function after TBI.

Keywords: Controlled Cortical Impact, Attentional Set-Shifting, Executive Function, Acetylcholine, Galantamine

B01-09

INTERACTION OF BRAIN TRAUMA AND CHRONIC STRESS ON COGNITION, ANXIETY, AND MARKERS OF NEUROTRANSMISSION AND NEUROINFLAMMATION

Darik O'Neil, Lindsay Kutash, Patricia B. de la Tremblaye, Ian Marshall, Jeffrey Cheng, Hannah Radabaugh, Naima Lajud, Anthony Kline, Corina Bondi

University of Pittsburgh, Safar Center for Resuscitation Research/PMR, Pittsburgh, USA

Traumatic brain injury survivors endure cognitive impairments associated with frontal lobe disturbances, and are vulnerable to neuropsychiatric disorders. Research has highlighted chronic unpredictable stress (CUS) as a major risk factor for many psychopathological conditions. Herein, we began to assess clinically-relevant cognitive- and anxiety-like dimensions sensitive to both TBI and CUS. We hypothesized that moderate TBI produced by controlled cortical impact (CCI) injury, as well as CUS exposure will render cognitive impairments in rats in an attentional set-shifting test (AST), reduced sucrose preference, open field exploration, blunted weight gain, as well as increased inflammatory markers and altered brain neurotransmission markers. Adult male rats were subjected to a CCI (2.8 mm deformation, 4 m/s) or sham injury over the right parietal cortex. Following surgery, rats were assigned to receive CUS (21 days) or handling (CTRL). Upon cessation of stress, rats were tested for anxiety (open field test) and anhedonia (reduced preference of 1% sucrose-water). At 4 weeks post-surgery, rats were tested on the AST, which involves a series of increasingly difficult discriminative tasks. Results demonstrate that CUS leads to a 5-10% weight gain reduction compared to CTRL, yet the combination of TBI and CUS does not negatively

impact exploration in the open field, or sucrose preference (n=8–12/group). TBI and CUS rendered cognitive deficits when given alone, as expected, but not when given in combination, suggesting a resilience or compensatory interaction. Ongoing directions include assessing serum levels of immune markers such as IL-4, IL-6, IL-10, IL-12 and TNF α , as well as protein levels in discrete brain regions for serotonergic, dopaminergic and adrenergic markers relevant to neurotransmitter synthesis, release and reuptake. This project will provide novel outcomes pertaining to cognition, anxiety- and depressive-like symptoms following overlapping chronic stress and the recovery phase of TBI.

Supported by NIH grants NS094950, NS099683, UPP/UPMC Academic Foundation and Rehabilitation Institute (COB), HD069620, HD069620-S1, NS060005, NS084967 (AEK).

Keywords: controlled cortical impact, chronic unpredictable stress, western blot, attentional set-shifting, inflammatory markers, open field test

B01-10

HIPPOCAMPAL NETWORK CHANGES AFTER MILD TRAUMATIC BRAIN INJURY

Rosalia Paterno¹, Brian Johnson², Hannah Metheny², John F. Burke¹, G Xiong², Akiva S. Cohen²

¹UCSF, Neurosurgery, San Francisco, USA

²CHOP, Department of Anesthesiology and Critical Care Medicine, Philadelphia, USA

Traumatic brain injury (TBI) is the primary cause of death and disability in children and young adults. TBI causes substantial cognitive impairments such as learning and memory alteration in humans. However, little is known about the causal mechanisms mediating this pathology. Previous research has demonstrated that TBI preferentially leads to hippocampal damage in both the human condition and rodent models of TBI. However, the precise pathophysiological changes that occur in the hippocampus after TBI are not known. In order to model mild TBI (mTBI) in mice, we employed lateral fluid percussion injury (LFPI) and we investigated electrophysiological alterations in hippocampal activity. Specifically, we recorded hippocampal neural activity using chronic 32-channel silicon probes with recording electrodes distributed across areas CA1, CA3, and DG sub-regions of the hippocampus. We recorded local field potentials across the entire hippocampus while the animals were in the home cage (often resting or sleeping) and while performing hippocampal-dependent memory task interspersed with rest sessions in the home cage. Electrodes were implanted 6–8 days post-injury and we recorded the hippocampal activity 1 week after implantation. We have previously demonstrated that LFPI causes regional hippocampal imbalances in excitatory and inhibitory synaptic transmission. Optimal brain function requires a delicate balance between excitatory and inhibitory neurotransmission (E/I balance). Furthermore, E/I balance is essential for the induction and maintenance of neuronal oscillations, which underlie cognitive function and working memory. In this study, we found a multitude of spectral changes especially in the theta and gamma narrow bands that occur after TBI. Furthermore, we found differential activity in CA1 versus CA3 in a complex interaction depending on behavior performance between TBI animal versus Sham control group. These data demonstrate that simultaneously recording across the hippocampus to identify the network activity and holds significant promise to better understand the link between TBI, hippocampus and memory. Acknowledgements Supported by R37HD059288 and T32HL007713-23.

Keywords: mTBI, hippocampus, theta oscillations, memory

B01-11

REFINING ENVIRONMENTAL ENRICHMENT TO ADVANCE REHABILITATION BASED RESEARCH AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Hannah Radabaugh, Megan LaPorte, Isabel Bleimeister, Michael Helkowski, Gina Bao, Patricia B. de la Tremblaye, Corina O. Bondi, Anthony E. Kline

University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

The typical environmental enrichment (EE) paradigm, which consists of continuous exposure after experimental traumatic brain injury (TBI), promotes behavioral and histological benefits. However, rehabilitation is often abbreviated in the clinic and administered in multiple daily sessions. While recent studies have demonstrated that a once daily 6-hr bout of EE confers benefits comparable to continuous EE, breaking the therapy into two shorter sessions may increase novelty and ultimately enhance recovery. Hence, the aim of the study was to test the hypothesis that functional and histological outcomes will be significantly improved by daily preclinical neurorehabilitation consisting of two 3-hr periods of EE vs. a single 6-hr session. Anesthetized adult male rats received a controlled cortical impact of moderate-to-severe injury (2.8mm tissue deformation at 4m/s) or sham surgery and were then randomly assigned to groups receiving STD housing, a single 6-hr session of EE, or two 3-hr sessions of EE daily for 3 weeks. Motor function (beam-balance/traversal) and acquisition of spatial learning/memory retention (Morris water maze) were assessed on post-operative days 1–5 and 14–19, respectively. Cortical lesion volume was quantified on day 21. Both EE conditions improved motor function and acquisition of spatial learning, and reduced cortical lesion volume relative to STD housing ($p < 0.05$), but did not differ from one another in any endpoint ($p > 0.05$). The findings replicate previous work showing that 6-hr of EE daily is sufficient to confer behavioral and histological benefits after TBI and extend the findings by demonstrating that the benefits are comparable regardless of how the 6-hrs of EE are accrued. The relevance of the finding is that it can be extrapolated to the clinic and may benefit patients who cannot endure a single extended period of neurorehabilitation.

Supported, in part, by NIH grants HD069620, HD069620-S1, NS060005, NS084967 (AEK), NS094950, NS099683 (COB), and the University of Pittsburgh Physicians /UPMC Academic Foundation (COB).

Keywords: Controlled cortical impact, Morris water maze, Environmental enrichment

B01-12

WITHDRAWN

WITHDRAWN

B01-13

GLASGOW OUTCOME SCALE EXTENDED—DIFFERENCES COUNTING DISABILITY FROM ONLY BRAIN INJURY VERSUS BRAIN AND OTHER SYSTEM INJURIES

Nancy Temkin¹, Gabriela Satris², Joan Machamer¹, MaryRose Morrissey², Geoffrey Manley², Sureyya Dikmen¹

¹University of Washington, Seattle, USA

²UCSF, San Francisco, USA

Objectives: To determine the extent scores differ if disability from only the brain injury is counted versus if disability from brain injury and other system injuries occurring in the same event are included.

Method: This is secondary analysis of data from a clinical trial. At 1, 3, and 6 months post-injury, about 450 cases with moderate or severe traumatic brain injury (TBI) were asked the structured interview for the Glasgow Outcome Scale Extended (GOSE) first considering only their brain injury. They were then asked their rating if other system injuries were included. The distribution of scores are summarized by time after injury, brain injury severity (Moderate-Glasgow Coma Scale motor [GCSm] score 5-6 or Severe-GCSm1-4) and other system injury severity (Injury Severity Score excluding the brain (ISSnon)=0-8, 9-15, or ≥16).

Results: At 1 month, differences were slight (<5 percentage points at any dichotomization cutpoint) for almost all cases with mild ISS non; moderate differences of 5 to 10 percentage points became more common with increasing other system injury severity. At 3 months there were only slight differences for ISSnon 0-8, a few moderate differences with ISS non 9-15 and moderate or large differences (≥10 percentage points) for ISSnon ≥16, especially with moderate TBI severity. The pattern was similar at 6 months, with only a small decrease in the differences.

Conclusions: GOSE scores varied considerably depending on whether other system injuries were included in the scoring. This has implications for clinical trials and other studies using GOSE as an

endpoint. Whether to rate GOSE on the brain injury alone or include all injury-related disability should be decided depending on the goals of the study, staff should be trained and data monitored accordingly, and trial sample size should be determined depending on the expected or important differences in the measure as it will be administered.

Keywords: Functional Outcomes, Peripheral injuries, Clinical Trials

B01-14

LONG-TERM NEUROBEHAVIORAL SEQUELAE FOLLOWING REPETITIVE CONCUSSIVE BRAIN INJURIES IN MALE AND FEMALE C57BL/6J MICE

Laura Tucker^{2,1}, Alexander Velosky¹, Amanda Fu^{2,1}, Joseph McCabe^{2,1}

¹Uniformed Services University of the Health Sciences, Department of Anatomy, Physiology & Genetics, Bethesda, MD, USA

²Center for Neuroscience and Regenerative Medicine, Pre-Clinical Studies Core, Bethesda, MD, USA

There is strong evidence to suggest a link between repeated head trauma and subsequent development of chronic traumatic encephalopathy (CTE), a degenerative neurologic condition resulting in motor, cognitive and neuropsychiatric symptoms. Clinical diagnosis of CTE is only definitive post-mortem, but symptomatology and neuropathology can be modeled in pre-clinical settings. In this study, male and cycling female C57BL/6J mice underwent repeated (three) concussive brain injuries (rCBI) delivered via a Leica ImpactOne cortical impact device and were assessed either acutely or chronically (up to one year) on a range of motor, cognitive and neuropsychiatric tests. The day following the final injury, injured mice presented with normal locomotor behavior in the open field (OF) test but were impaired on the rotarod task of motor coordination. Rotarod performance was improved shortly following the injuries, but a state of hyperactivity developed during the month following rCBI as assessed in the OF test. Hyperactivity was especially pronounced in male mice, and persisted for at least one year following the injuries. Injured mice did not show impairments in the y-maze test of working memory shortly after the injuries, but mice tested one year post-rCBI were unable to learn a spatial active place avoidance task. Depressive-like behaviors were not apparent at either an acute or chronic time point. Anxiety-like behaviors assessed in the elevated zero maze were equivalent in injured and sham control mice shortly after injuries, but after one year, injured mice spent greater amounts of time in anxiogenic regions of the apparatus. These data demonstrate that a pathological phenotype with motor, cognitive and neuropsychiatric symptoms can be observed in an animal model of rCBI for at least one year post-injury, providing a pre-clinical setting for the study of the link between multiple brain injuries and neurodegenerative disorders.

Keywords: behavior, mouse, brain injury, sex differences

B01-15

EFFECTS OF FRONTAL TBI ON RISK-BASED DECISION MAKING IN RATS

Kris Martens, Lindsay Veltri, Noah Berdar, Binxing Zhu, Taylor Scott, Trinity Shaver, Cole Vonder Haar

West Virginia University, Psychology, Morgantown, USA

Traumatic brain injury causes severe and long-lasting cognitive consequences. Many individuals exhibit extended impairments and can

even develop *de novo* psychiatric disorders. A large facet of these deficits and component of many psychiatric disorders is altered decision making. Sadly, these symptoms are grossly under-investigated in rodent models of traumatic brain injury. The current study examined one form of decision-making behavior: allocation of choice to probabilistic outcomes, a phenomenon that is most strongly associated with gambling.

Twenty-four rats were trained on the rodent gambling task (RGT) to a stable baseline (~25 sessions). The RGT consists of four options, each associated with a reward size (1-4 sugar pellets) and a probability of winning that reward. Generally, optimal choice involves the low-risk, low-reward options which deliver the highest overall rate of reward as contrasted with the high-reward, but high-risk options. After training, one-half of rats were given a bilateral, frontal controlled cortical impact injury (+3.0, +0.0, -2.5 @ 3 m/s), while the other half received sham procedures. After a week of recovery, rats were re-tested on the RGT until a new stable baseline emerged (~30 sessions). Following the behavioral assessment, rats were transcardially perfused. Brains were sliced and stained with cresyl violet to quantify the lesion.

Rats displayed an initial global disruption in behavioral function, with large increases in omitted trials, and low response rates. Response rates gradually increased over a period of two weeks. In the TBI group, choice was chronically altered, with an increased preference for the high-risk, high-reward options. These data pave the way for using rodent models of TBI to investigate the basis of altered decision making and any treatments that may benefit human patients.

Keywords: decision making, controlled cortical impact, rat, frontal cortex

B02 BLAST

B02-01

ALTERATIONS IN CEREBROVASCULAR RESPONSIVENESS AFTER BLAST CORRELATE WITH CHANGES IN ENDOTHELIAL FUNCTION-RELATED FACTORS

Rania Abutarboush¹, Habib Mullah¹, Margaret Lashoff-Sullivan¹, Ye Chen¹, Francoise Arnaud^{1,2}, Usmah Kawoos¹, Richard McCarron^{1,2}, Stephen Ahlers¹

¹Naval Medical Research Center/ Henry Jackson Foundation, Neurotrauma, Silver Spring, USA

²Uniformed Services University of Health Sciences, Surgery, Bethesda, USA

Converging evidence suggests that changes in the vasculature and perivascular space are contributing factors to traumatic brain injury (TBI) resulting from exposure to blast overpressure (BOP). The injury mechanism is still largely unknown. The aim of this study is to characterize TBI resulting from single 37, 75, or 140 kPa BOP. The functional responses of arterioles in pial microcirculation were assessed in real-time in anesthetized rats using intravital microscopy (IVM). Specifically, cerebrovascular reactivity was studied using hypercapnia (7% CO₂), barium chloride (BaCl₂) and serotonin (5HT) 24 h after blast. To understand the observed changes in pial arteriolar reactivity with blast intensity, the expression of endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS), and vasopressin (AVP) was studied in frontal cortex. At 24 h after BOP, BaCl₂ had an immediate statistically significant vasoconstrictive effect on pial arterioles, while 5HT and CO₂ had a vaso-dilatory effect. The changes in pial arteriolar reactivity varied with blast intensity. The effects of BaCl₂ (but not CO₂ and 5HT) were different from control. Collectively, IVM data

suggest that while pial microvessels remain reactive to vasoactive mediators after blast, their responses tend to be different from those of sham animals. Preliminary RT-PCR data show that blast alters mRNA expression for ET-1, eNOS, and AVP in an intensity-dependent manner 24 h post-BOP. Specifically, the higher the blast intensity, the smaller the observed changes in the ratio of mRNA relative to control. The results suggest the degree of functional changes in cerebrovascular responses may correlate with blast intensity and with molecular changes that affect the function of the endothelium, specifically, the expression of ET-1, eNOS, and AVP. Functional changes from low intensity BOP are influenced by ET-1, eNOS, and AVP but functional changes seen with higher BOP intensities may work through different mechanisms.

Keywords: pial microcirculation, intravital microscopy, vascular function, gene expression

B02-02

COAGULATION AND HUMORAL RESPONSE IN ANESTHETIZED RATS WITH BLAST INJURY

Francoise Arnaud, Ye Chen, Richard McCarron

Naval Medical Research center, NeuroTrauma, SilverSpring, USA

Background: Mild or moderate brain injury can affect cerebral responses and indirectly alter certain functions such as coagulation. The present study focused on the adrenal gland response and clotting ability in a rat model.

Methods: Awake rats were stressed by a 45 minute restraint-immobilization and then instrumented with a femoral catheter under anesthesia for blood sampling (t1). After polytrauma produced by a 75 kPa blast overpressure and a 35% estimated-blood-volume controlled hemorrhage, (t2), the animals were left to recover and were observed over a period of 3 hours. A control group of animals were left uninjured for comparison. Thereafter, some animals in each group were euthanized (t3) and others were recovered and euthanized at 72 h (t4). At t1, t2, t3 and t4, blood count and coagulation profile (thromboelastography and platelet aggregation) were assayed in whole blood, and corticosterone and ACTH levels were measured in plasma samples.

Results: Corticosterone levels were increased in all groups (**198 ± 60 ng/ml**; no intragroup difference) and were significantly higher at t4 than those at preceding time points (**113 ± 54 ng/ml**; p < 0.001), regardless of the presence or absence of injury. ACTH was low in all groups and reached **54 ± 20 pg/ml** at t3, regardless of stress exposure. White blood cells, maximum clot firmness, and platelet aggregation were elevated at t4.

Conclusion: Injury, as produced in this rat model, did not have the expected early effect on the production of stress hormones and change in coagulation while the animals were under anesthesia. However, a delayed effect of stress and elevated platelets activity occurred in most animal groups after a 3 day recovery. This level of injury did not induce an immediate pronounced response. This seems to confirm that anesthesia should be considered a confounding factor that masks any effect of the injury and should be carefully considered in pre-clinical studies.

This work was funded by work unit number 603115HP.2380. 001.A1304. The study protocol was reviewed and approved by the Walter-Reed-Army Institute of Research/Naval-Medical-Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research.

Keywords: Stress, Coagulation, survival, Polytrauma

CHRONIC EFFECTS OF BLAST EXPOSURE: A FUNCTIONAL STUDY IN RATS USING ADVANCED BLAST SIMULATOR

Peethambaran Arun, Donna Wilder, Meghan Benton, Chloe Christoforou, Wafae Driwech, Rodrigo Urioste, Ondine Eken, Sujith Sajja, Steve VanAlbert, Ying Wang, Irene Gist, Joseph Long
Walter Reed Army Institute of Research, Blast-Induced Neurotrauma/Center for Military Psychiatry & Neurosciences, Silver Spring, USA

Anecdotal observations of blast victims indicate that significant neuro-pathological and neurobehavioral defects develop at later stages of life. In this study, we have examined neurobehavioral changes in rats up to 9 months after exposure to single and tightly coupled repeated blasts using an advanced blast simulator. Neurobehavioral changes were monitored at acute, sub-acute and chronic time points till 9 months using elevated plus maze, Y-maze, water maze, open field exploration, rotary pole, rotarod and novel object recognition tests. Single and closely coupled repeated blast exposures resulted in significant functional deficits at acute and chronic time points. In most functional tests, rats exposed to repeated blasts performed more poorly than rats exposed to single blast. Rotating pole and rotarod tests to assess the neuromotor/balance functions revealed significant deficits at acute and sub-acute time points after blast exposures, and all experimental subjects, including sham controls, had difficulties with these tests at chronic time points. Interestingly, several functional deficits were more pronounced in blast exposed rats at 6 months and beyond. The most substantial changes in blast-exposed rats were observed in the center time and margin time legacies in the open field exploration test at 6 and 9 months after blast exposures. Notably, these two outcome measures were minimally altered acutely, fully recovered during sub-acute stages, and were pronounced during the chronic stages after blast exposures. Significant neuromotor impairment occurs at early stages after blast exposure and the severity increases with number of exposures. Water maze test for spatial learning and memory revealed short-term memory impairments at chronic stages, but not at early time points. The pronounced changes in center time and margin time legacies in the open field exploration test after 6 months post-blast implicate development of depressive-like behavior.

Keywords: Chronic effects, Repeated blast, Advanced blast simulator, Overpressure

B02-04

EPIGENETIC MECHANISMS IN BLAST INDUCED NEUROTRAUMA

Zachary Bailey¹, Pamela VandeVord^{1,2}

¹*Virginia Tech, Biomedical Engineering and Mechanics, Blacksburg, USA*

²*Salem Veterans Affairs Medical Center, Salem, USA*

Blast-induced neurotrauma (BINT) is becoming increasingly prevalent in military populations since a majority of combat injuries in current wartime efforts are attributable to explosive mechanisms. Civilian populations are also at risk due to ongoing terrorist events worldwide. BINT often occurs with no external signs of injury, making diagnosis and treatment difficult. Clinical manifestations of BINT include long-term functional and psychological impairments. These symptoms are driven by underlying cellular and molecular sequelae including blood brain barrier disruption, cell death, and sustained glial cell activation. The overall hypothesis of this work is that epigenetic regulatory mechanisms contribute to the progression of the BINT pathology and neurological im-

pairments. Epigenetic mechanisms, including DNA methylation and histone acetylation, are important processes that are capable of regulating cellular function in response to various environmental stimuli. An established rodent model of BINT was employed to assess epigenetic changes in the injury pathology. Relevant clinical outcomes including decreased motor function, increased anxiety and decreased memory were observed. Analysis of DNA methylation levels following blast exposure elucidated time-dependent and overpressure-dependent DNA methylation changes in the hippocampus. Increased DNA methylation was observed at four hours following blast which transitioned to decreased DNA methylation at one week before returning to sham levels at one month. Importantly, expression changes in several enzymes that regulate levels of methylated DNA residues were observed and may contribute to the observed pathological DNA methylation levels. We have previously demonstrated decreased histone acetylation of histones H2b, H3, H4 following blast exposure. H3 hypoacetylation was also observed specifically in astrocytes. Further Western Blot analysis showed increased nuclear localization of signaling molecules including nuclear factor kappa B which is a key mediator of inflammation. Taken together, the observed changes to DNA methylation and histone acetylation have a potentially broad impact on cellular function which may be important to the injury progression. Continuing to increase our understanding of BINT will mitigate current clinical obstacles by making the development of treatment strategies more efficient.

Keywords: Epigenetics, DNA methylation, Histone acetylation, Blast Induced Neurotrauma

B02-05

BLAST-INDUCED TRAUMATIC BRAIN INJURY DISPLAYS A UNIQUE PATTERN OF SPATIAL NEUROPATHOLOGY

Namas Chandra, KV RamaRao, Stephanie Iring, Daniel Younger, Aswati Aravind, Bryan Pfister, Maciej Skotak
New Jersey Institute of Technology, Biomedical Engineering, Newark, USA

Blast-induced Traumatic brain injury (bTBI) is a leading cause of morbidity and mortality in soldiers in the combat and in training sites and is becoming an important public health issue. The bTBI simultaneously causes primary (shockwave), secondary (penetrating), and tertiary (blunt-accelerative forces) injuries causing different loading modalities and pathological outcomes compared to pure blunt injury. The brain injury patterns resulting from primary shockwave propagation across different brain structures has not been carefully investigated. We hypothesize that bTBI has a unique histopathology and pathophysiology different from blunt TBI. In bTBI all the regions of brain from prefrontal cortex to hippocampus to cerebellum to brain stem are all simultaneously biomechanically loaded as the shock wave propagates unimpeded across the brain; this is different from the blunt TBI. Since oxidative stress has been implicated as a major pathogenic factor in TBI, here we examined the brain spatial resolution and cellular distribution of changes in superoxide producing enzyme NADPH oxidase1 (NOX1) as well as neurodegeneration paradigm during the early phase (4h) of moderate blast TBI (180 Kpa) and compared these data with that of moderate blunt TBI (FPI). Immunofluorescence analysis displayed that NOX1 was upregulated in different brain regions and showed a differential vulnerability with the highest increase found in hippocampus and thalamus followed by frontal cortex and cerebellum. Among different cells, neurons exhibit highest amount of basal NOX1 and its increase was higher in hippocampal and cerebellar regions compared to astrocytes and microglia. Interestingly, blunt injury induced in the cortical region did not

display any change in NOX1 expression in cerebellum suggesting that the spread of the injury in blunt TBI is localized. Blast did not display neurodegeneration in any region, while blunt TBI showed focal neurodegeneration in the cortical and hippocampus areas only in ipsilateral side. These studies indicate that the injury pattern and pathological changes in blast TBI are unique compared to blunt injuries.

Keywords: blast TBI, blunt TBI, selective vulnerability

B02-06

CHARACTERIZATION OF NEUROPATHOLOGY RESULTING FROM PRIMARY BLAST EXPOSURE IN A PORCINE TRAUMATIC BRAIN INJURY MODEL

Elizabeth Fievisohn¹, Alexander Kwiatkowski¹, Shaylen Greenberg¹, Timothy Walilko², Laila Zai², Pamela VandeVord^{1,3}

¹Virginia Tech, Biomedical Engineering and Mechanics, Blacksburg, USA

²Applied Research Associates, Littleton, USA

³Salem VA Medical Center, Salem, USA

There is a critical need for a translatable animal model so that human mild blast traumatic brain injury (bTBI) thresholds can be determined and appropriate standoff times can be implemented. Dissimilarities between a rodent and human brain has led to the need to investigate bTBI in a higher-order animal model. The purpose of this study was to characterize the effects of a single blast exposure on male Yucatan minipigs. Animals (5–7 months; n = 10 blast and 5 sham) were exposed to a single blast overpressure (40–72 psi) using a large-diameter mobile shock tube. Ear, eye, and torso protection was used to prevent other injuries. Brains were collected at 72 hours after injury, sectioned, and embedded in paraffin for immunohistochemical staining for Fluoro-Jade C (FJC), α -smooth muscle actin (α -SMA), macrophages/microglia (co-stain of cluster of differentiation 68 (CD-68)/ionized calcium-binding adapter molecule-1 or Iba-1), and β -amyloid precursor protein (β APP). Quantitative analysis of the stained sections showed that FJC was significantly increased in the hippocampus ($p < 0.01$). CD-68, but not Iba-1, was significantly increased in the hippocampus ($p < 0.01$). α -SMA showed significantly increased surface vessel diameter in the frontal cortex, which may be caused by the activation or deactivation of pathways involved in vascular tone ($p = 0.019$). β APP did not reveal any axonal swelling or disconnections. The increase of FJC and microglia/macrophage activation in the hippocampus, as well as lack of elevated β APP found in this study supports previous research in rodent models of primary blast. In summary, a large animal model of primary blast overpressure demonstrated significant neuropathology. Further studies to characterize other brain regions are continuing and will be used to help determine a mild bTBI threshold that can be translated to a human.

The authors would like to acknowledge Dr. Timothy Bentley at the Office of Naval Research for funding this research.

Keywords: Blast, Neuropathology, Traumatic brain injury, minipig

B02-07

PERIPHERAL MONITORING OF BLAST EXPOSURE USING CELL FREE DNA (CFDNA) METHYLATION

Fatemeh Haghighi^{1,2}, Chatterton Zac^{1,2}, Walter Carr³, Matthew LoPresti³, Angela Yarnell³, Yongchao Ge², Natalia Mendelev^{1,2}, Michelle Umali^{1,2}, Brian Choi¹, Jennifer Blaze^{1,2}, Richard McCarron⁴, Gregory Elder¹, Stephen Ahlers³

¹James J Peters VA Medical Center, R&D, Bronx, USA

²Icahn School of Medicine at Mount Sinai, Neuroscience, New York, USA

³Walter Reed Army Institute of Research, Bethesda, USA

⁴Naval Medical Research Center, Silver Spring, USA

Injuries from exposure to explosive blasts rose dramatically during Operations Iraqi Freedom/Enduring Freedom, motivating investigations of blast-related neurotrauma. We have undertaken human studies involving “Breachers”, military personnel who are exposed to repeated blasts as their occupational duty. Breachers report physical, emotional, and cognitive symptoms: headache, sleep issues, anxiety. We aim to identify biomarkers associated with blast exposure. Cell-free DNA, derived from neurological tissue, holds potential as possible indicators of exposure detection. We exploit unique DNA methylation profiles of brain cells to create molecular diagnostic assays, capable of detecting peripheral brain-derived cfDNA. DNA methylation signatures in brain are unique from blood cells. We previously performed whole genome DNA methylation analysis, defining single-base pair maps of neurons (NeuN+) and non-neuronal (NeuN-) cell types. Leveraging from this unique data source, we constructed a targeted next generation sequencing platform to detect brain-derived cfDNA within peripheral blood. Data presented involve a subset of 32 individuals, participating in a 2-week data collection at U.S. Army explosive entry training sites, where blood samples were obtained pre- and post-training for methylation studies. Of these, we profiled brain-derived cell-free DNA within bloods from 6 breachers at days 1 (before training) and at days 7, 8 and 9 (during training). On day 7, the cohort of breachers were exposed to higher (12 psi) blast. Generally, we observed higher amounts of brain-derived cfDNA during training (days 7, 8 and 9) than pre-training (day 1). To establish the presence of brain-derived cfDNA within peripheral blood associated with blast exposure represents an important step towards future biomarker development to diagnose and monitor potential blast-related TBI.

Keywords: Epigenetics, Blast, Inflammation

B02-08

HEAVY WALL BREACHING RESULTS IN IMMEDIATE AND ACUTE NEUROCOGNITIVE DEFICITS

Gary Kamimori, Alejandro Ramos, Arturo Cardenas, Lucas Reilly, Anthony Misistia, Meron Ghebremedhin, Christina LaValle
Walter Reed Army Institute of Research, Blast Induced Neurotrauma Branch, Silver Spring, USA

The original New Zealand breacher study (NZBS) reported significant, quantifiable acute neurocognitive deficits without any diagnosable injury; however, there is very limited data on neurocognitive effects immediately following overpressure exposure. This study measured neurocognitive performance immediately following heavy wall breaching during an Urban Mobility Breaching Course. The Defense Automated Neurobehavioral Assessment (DANA) Rapid test battery was administered to 24 volunteers before exposure (baseline), immediately after detonating two heavy wall breaching charges, and at the end of the day (acute). Blast sensors were used to quantify individual blast exposure. There were significant DANA performance changes ($> 30\%$ slower response time compared to baseline) in 2/22 volunteers for simple reaction time (SRT); 10/22 for procedural reaction time (PRT); and 5/22 for Go/No-Go (GNG) immediately following blast exposure. Significant acute effects were identified in 3/24 volunteers for SRT; 11/24 for PRT; and 7/24 for GNG. Volunteers with acute effects had significantly higher cumulative overpressure

and impulse exposure compared to those who had no performance changes. The relationship between higher overpressure exposure and decrements in acute performance agree with those from the NZBS. The PRT subtask appears to be the most sensitive for quantifying immediate and acute blast exposure effects. SRT may be too effortless while GNG may be too stimulating, or subject recovery from transient blast effects may have occurred amid testing. The current study is one of the first to identify neurocognitive impairments immediately following blast exposure. These effects on higher order mental processes may have a significant impact on operational success and may also be important for determining long term health risk. Additional data from follow-on studies from the same training course will allow for more rigorous statistical analyses. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, Broad Agency Announcement Award No. W81XWH-16-2-0001. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

Keywords: occupational blast exposure, neurocognitive performance, neurotrauma, explosive entry training, military

B02-09

EFFECT OF PHYSICAL PROTECTION AND INCREASED INTRACRANIAL PRESSURE ON THE PROPAGATION OF SHOCK WAVE TO BRAIN IN RATS

Usmah Kawoos¹, Ming Gu¹, Eric Jeronimo¹, Richard McCarron^{1,2}, Mikulas Chavko¹

¹Naval Medical Research Center, Neurotrauma, Silver Spring, USA

²Uniformed Services University of the Health Sciences, Surgery, Bethesda, USA

During blast exposure the shock wave can be transmitted to the brain directly through skull or from the body through blood vessels and cerebrospinal fluid. This study evaluated the contribution of head and/or body in transferring blast to brain by measuring intracranial(ICP), arterial(AP), and venous(VP) pressures. Increasing the cerebral volume(CV) may have a protective effect against blast by limiting the relative motion of intracranial contents. Therefore, the protection conferred by increasing CV by internal jugular vein(IJV) compression was also investigated. Rats were exposed to blast (BOP, ~47 kPa) with their bodies oriented parallel (front) or perpendicular (side) to the blast. Animals were instrumented with pressure probes in the right lateral ventricle, femoral artery, and femoral vein and were randomly assigned to groups with/without protective shielding. Front exposure groups included no protection (F-NP), full body protection excluding tail (F-FP), head protection (F-HP), and body protection (F-BP). Side exposure groups were exposed with no protection (S-NP), or head protection (S-HP). In IJV compression group, compression was applied by tightening a Velcro tape around the animal's neck. Additionally, BOP-induced(front-3x110 kPa, 30 min apart) effects on aquaporin-4 (AQP-4), 3-nitrotyrosine (3-NT), and endothelin receptor A (ETrA) in three groups of animals (control, blast, and blast-IJV) was determined. A significant decrease in shock wave transmission to brain was observed in F-FP, F-HP, and S-HP groups while F-BP offered no significant protection. AP and VP were significantly reduced in F-BP and F-HP. A significant difference was observed in ICP and AP between F-NP and S-NP as well as F-HP and S-HP groups. IJV compression diminished the blast-induced pressure increase, prevented increase in 3-NT and ETrA in cortex, and attenuated the upregulation of AQP-4 and ETrA immunoreactivity in hippocampus. Body protection has limited preventive effect and for brain protection the head and neck should be completely shielded.

Keywords: Blast propagation, Intracranial volume, Shock wave, Protection against blast

B02-10

THE EFFECT OF ADMINISTRATIVE AND POLICY CHANGES ON THE REPORTED INCIDENCE OF TBI DURING THE COURSE OF OEF/OIF/OND

Donald Marion, Lemma Regassa, Yil Agimi, Brian Ivins, Saafan Malik

Defense and Veterans Brain Injury Center, Clinical Affairs, Silver Spring, USA

The Defense and Veterans Brain Injury Center (DVBIC) publishes the incidence of service members (SMs) who have sustained at least one traumatic brain injury (TBI) while serving in the military since the year 2000 on its website (<http://dvbic.dcoe.mil/dod-worldwide-numbers-tbi>). DVBIC obtains quarterly raw data from the Armed Forces Health Surveillance Board (AFHSB), stratified as mild, moderate, severe, penetrating or unclassifiable injuries. Numerous investigators, agencies, and the press have used this resource for current information about the incidence of TBI in the military. However, several changes in the definition of military TBI, the classification of the types of TBI, and the methods of screening for TBI have affected the reported number of SMs with at least one TBI relative to the actual number of SMs with at least one TBI. Key events include a detailed description of ICD-9 codes used for identification of TBIs in 2006, mandatory use of the Military Acute Concussion Evaluation (MACE) and implementation of an incident-based screening protocol in 2010, and redefining most unclassifiable TBIs as mild or moderate in 2015. These changes in TBI definition, screening, and classification over the last 15 years require careful interpretation of cumulative TBI data obtained during that time. Comparisons of the incidence of military TBI from 15 years ago with the current incidence should always include a summary of these changes that affect reporting. To date, no method exists within the administrative data to capture SMs with multiple TBIs. This challenge is currently being evaluated to determine the best method to further improve TBI tracking.

Keywords: Military, concussion, combat, Surveillance, blast

B02-11

CHARACTERIZATION OF A COMBINED MODEL OF BLAST AND BLUNT INJURY

Aswati Arvind, Rama Kakulavarapu, Mathew Long, Namas Chandra, **Bryan Pfister**

New Jersey Inst. of Technology, Dept. of Biomedical Engineering, Newark, USA

Soldiers are often exposed to both primary blast waves and blunt impacts due to falls or vehicle crashes. While the cumulative effects of repeated exposure to a blast or blunt traumatic brain injury (TBI) has been under investigation, a combination of blast and blunt impacts has yet to be examined. Our hypothesis is a blast exposure, while not necessarily leading to a diagnosable injury, will predispose the brain to greater injury upon a second blunt injury to the head. Here, rats were exposed to primary blasts (130 KPa - mild or 180 KPa - moderate) followed by a fluid percussion injury (FPI - 29-32 psi) to examine if a blast exposure would exacerbate the injury outcome of FPI in the rat. The animals were first subjected to the blast then the craniectomy was performed while still under anesthesia and 24 hrs later, the rats were subjected to the FPI. Control groups of blast +craniectomy, FPI only and craniectomy alone were also generated. Time-matched to 4 hr after FPI, coronal sections from all experimental groups were stained with fluoro jade C. Acute neuronal degeneration was observed in the cortex, hippocampus and thalamus regions of the animals subjected to the blunt and blast +blunt group while blast alone

group showed no neurodegeneration. Neurodegeneration was more pronounced in cortical region in both mild and moderate blast +blunt groups than the blunt impact group alone, while respective controls (blast +craniectomy or craniectomy alone) did not show any neuronal degeneration. Hemorrhage was also observed in the moderate blunt and combined impacts than the blast impacts. Potential changes in apnea and the righting reflex are so far inconclusive. Our data indicate that a prior blast impact increases the induced neuronal degeneration from a subsequent blunt impact. Ongoing studies aim to identify the longer term effects on cell death as well as behavioral and biochemical deficits present in the combined model.

Keywords: Combinational injury, fluid percussion injury

B02-12

SPATIAL AND TEMPORAL DEFORMATION PATTERNS OF THE BRAIN UNDER BLUNT AND BLAST TRAUMA

Abdus Ali, Namas Chandra, Bryan Pfister

New Jersey Inst. of Technology, Dept. of Biomedical Engineering, Newark, USA

It is widely accepted that trauma to the head causes the brain to deform inside the skull, creating large strains that cause damage. It is unknown, however, how the brain deforms in many types of trauma such as sports contact, falls and exposure to blasts. We hypothesize that each trauma to the head will lead to distinct magnitudes and rates of deformation that varies spatially and temporally across the brain. The objective of this study was to recreate spatial and temporal deformations that are likely to occur in blast and blunt injuries. For this study a full scale human head surrogate was created from a PVC skull, ballistic gel brain, and hybrid III anthropomorphic neck. Blunt injuries were created with a 2 kg impactor on a drop tower system at velocities of 3&5 mph. For blast injury overpressures of 180 kPa were generated in a field-validated shock tube. Markers in the brain were motion tracked from high speed video and analyzed to compute skull deflections, principal strains and the associated strain rates across the brain. Contour maps were generated to view the spatial and temporal distribution of strains caused by each injury event. In blunt injury, the span and degree of tissue deformation were localized in particular regions and unique to the biomechanical parameters of the blunt injury event (velocity, momentum, direction). 5 mph impacts induced higher strains than 3 mph while crown injuries caused larger skull deflections as well as higher strains and rates than frontal impacts. Preliminary blast experiments produced more widely distributed contour profiles of strain fields within the brain. Maximum principal tensile, compressive, and shear strains were surprisingly high 52–55% that oscillate and decay over several seconds. The data from these experiments may help to identify vulnerable injury sites with more understanding of the relative effects of specific injury loading conditions. This model can act as a platform to build more complex and biofidelic heads as well as a metric to evaluate helmet designs.

Keywords: TBI, Biomechanics, Blast and Blunt trauma, Strain and rate

B02-13

ACTIVATION OF NLRP3 INFLAMMASOME AND ITS IMPACT ON CEREBRAL AUTOPHAGY MECHANISMS IN SEVERE BLAST-INDUCED TRAUMATIC BRAIN INJURY

Rama Rao Kakulavarapu, Stephanie Iring, Daniel Younger, Maciej Skotak, Namas Chandra

New Jersey Institute of Technology, Biomedical Engineering, Newark, USA

Blast-induced traumatic brain injury (bTBI) is the most frequent cause of morbidity and mortality in soldiers in the combat zone as well as in training facilities, which represents a challenging task for the management of patients returning from combat injuries. The bTBI uniquely impacts on the brain by simultaneously causing primary (shockwave), secondary (penetrating), and tertiary (blunt-accelerative forces) injuries that cumulatively exacerbate the pathological outcome. Of several factors that induce brain pathology in TBI, neuroinflammation is an important pathway triggered by upstream factors including the activation of Toll-like receptor4 (TLR4) that transcriptionally activates the synthesis of pro-IL-1 β , a precursor of inflammatory cytokine IL-1 β , resulting in the production of functional IL-1 β by the activation of NLRP3 inflammasome, an assembly of several proteins including apoptosis-associated speck-like protein (ASC1) and caspase-1 (IL-1 β converting enzyme). IL-1 β promotes a variety of neuroinflammatory responses by altering cellular homeostatic events such as autophagy, a physiological process responsible for lysosomal-dependent degradation of senescent cytoplasmic proteins, intracellular organelles as well as toxic protein aggregates. A hallmark of defective autophagy is the accumulation of autophagosome marker LC3, a Microtubule-associated protein that fuses with autophagosome and promotes its degradation. Here we examined regional variations in the brain (hippocampus, thalamus, visual cortex and cerebellum) levels of TLR4, pro-IL-1 β , NLRP3 as well as the levels of autophagy marker LC-3 in acute phase (4h) of severe-blast TBI (240 Kpa) in rats. Immunoblot analysis showed a regional variability in the protein levels of these markers with hippocampus displaying highest increase in TLR4, Pro-IL1 and NLRP3. Additionally, bTBI caused a pronounced accumulation of LC3 protein in hippocampus, which suggests a defective autophagy, process likely mediated by increased neuroinflammation. These data collectively suggest that severe bTBI induces acute neuroinflammatory response by triggering a cascade of events potentially resulting in perturbations in autophagy that may represent an important neuropathological event in bTBI.

Keywords: Autophagy, Blast-TBI, Inflammasome, Interleukin-1-beta, Toll-like Receptor

B02-14

EFFECTS OF BLAST TRAUMATIC BRAIN INJURY ON REGIONAL CELLULAR INJURY

Uyilssa Rodriguez, Margaret O. Parsley, Donald S. Prough, Douglas S. DeWitt

University of Texas Medical Branch, Anesthesiology, Galveston, USA

Neuronal injury in specific brain regions following impact TBI likely contributes to the nature and degree of brain dysfunction. Previous studies that employed the FluoroJade (FJ) fluorochrome stain for labeled identification of blast-induced TBI (bTBI) neuronal injury or degeneration resulted in significantly increased FJ-positive neurons¹ in rat hippocampus,²⁻³ amygdala⁴ and pre-frontal cortices⁵ after 17–19 psi shockwave exposure and in the cerebellum after 62-75 psi exposure.⁶ We examined neuronal injury through FluoroJade-C (FJC) utilization in three brain regions of rats subjected to mild bTBI using an Advanced Blast Simulator (ABS), a compressed air-driven shock tube.

Adult male Sprague-Dawley rats were surgically prepared for bTBI and randomly assigned to receive (n = 6/group) bTBI (20 psi) or Sham bTBI. 24 or 48 hrs after injury, brains were harvested, sectioned on a Cryostat and stained with FJC. FJC-positive cells were counted in the

frontal lobe (F), parietal/temporal lobe (PT) and occipital lobe regions (O) of all bTBI and Sham groups.

FJC-positive cells were significantly greater in both bTBI groups 24 and 48 hrs post-injury compared to Sham ($P < 0.05$, bTBI vs. Sham), with significantly more FJC-positive cells in all bTBI group brain regions ($P < 0.001$, bTBI F vs. Sham F; $P < 0.05$, bTBI PT vs. Sham PT; $P < 0.05$, bTBI O vs. Sham O). FJC-positive cells were significantly higher in the PT than both the F and O regions amongst the bTBI groups ($P < 0.05$, bTBI PT vs. bTBI F; $P < 0.05$, bTBI PT vs. bTBI O) while FJC-positive cells in the F region of both bTBI groups were significantly greater than in their O regions ($P < 0.01$, 24 hr bTBI F vs. 24 hr bTBI O; $P < 0.01$, 48 hr bTBI F vs. 48 hr bTBI O).

Our data indicates that mild bTBI results in significantly acute neuronal injury 24 and 48 hrs post-blast with increased numbers of FJC-positive cells throughout the parietal/temporal cortex compared to the frontal or occipital regions.

Studies were conducted as part of a team supported by The Moody Project for Translational Traumatic Brain Injury Research.

Keywords: blast induced neurotrauma, primary blast injury, acute neuronal injury, FluoroJade-C stain, histopathology

B02-15

RESTORING GM1 GANGLIOSIDE EXPRESSION AMELIORATES AXONAL OUTGROWTH INHIBITION AND COGNITIVE IMPAIRMENTS INDUCED BY BLAST TRAUMATIC

Vardit Rubovitch², Yael Zilberstein³, Joab Chapman^{4,5,6}, **Shaul Schreiber**^{1,6}, Chaim G. Pick^{2,6}

¹Sourasky Medical Center & Tel Aviv University Sackler Faculty of Medicine, Department of Psychiatry, Tel Aviv, Israel

²Sackler Faculty of Medicine, Tel-Aviv University, Department of Anatomy and Anthropology, Tel Aviv, Israel

³Sackler Faculty of Medicine, Sackler cellular & molecular imaging center, Tel Aviv, Israel

⁴The J. Sagol Neuroscience Center, The Chaim Sheba Medical Center, Tel HaShomer, Department of Neurology, Ramat Gan, Israel

⁵Sackler School of Medicine, Department of Neurology, Tel Aviv, Israel

⁶Tel Aviv University, Sagol School of Neuroscience, Tel Aviv, Israel

Objectives: Blast induced traumatic brain injury (B-TBI) may cause various degrees of cognitive and behavioral disturbances but the exact brain pathophysiology involved is poorly understood. It was previously suggested that ganglioside alteration on the axon surface as well as axonal regenerating inhibitors (ARIs) such as myelin associated glycoprotein (MAG) were involved in axonal outgrowth inhibition (AOI), leading to brain damage.

Results: GM1 ganglioside content in the brain was significantly reduced while GD1 ganglioside was not affected. The axonal regeneration was also reduced as seen by the phosphorylated NF-H expression. Moreover, B-TBI induced a significant elevation in MAG expression in the brains of the injured mice. The blast injured mice exhibited a significant decline in spatial memory as seen by the Y-maze test. In addition, the injured mice showed pronounced damage to the visual memory (as evaluated by the Novel object recognition test). A single low dose of GM1 (2mg/kg; IP), shortly after the injury, prevented both the cognitive and the cellular changes in the brains of the injured mice.

Conclusions: These results enlighten part of the complicated mechanism that underlies the damage induced by B-TBI and may also suggest a potential new treatment strategy for brain injuries.

Keywords: Axonal outgrowth inhibition, Regeneration inhibitor

B02-16

IDENTIFICATION OF PATHOLOGICAL CHANGES IN HIPPOCAMPUS INDUCED BY A SINGLE BLAST VIA DIFFERENTIAL PROTEOMICS AND PATHWAY ANALYSIS

Maciej Skotak¹, Bogumila Swietek², Lin Yan³, Tong Liu³, Hong Li³, Vijayalakshmi Santhakumar², Namas Chandra¹

¹New Jersey Institute of Technology, Biomedical Engineering, Newark, USA

²Rutgers New Jersey Medical School, Department of Pharmacology, Physiology and Neuroscience, Newark, USA

³Rutgers New Jersey Medical School, Scientific Research of the Centre for Advanced Proteomics Core, Department of Biochemistry and Molecular Biology, Newark, USA

Proteomics offers unique insight into etiology of brain injury by providing unbiased information about large dataset of proteins involved in variety of cellular processes. Simultaneous label free fingerprinting readily available with an aid of bioinformatics data mining backed by differential expression information allows identification of major biochemical pathways. These attractive traits perfectly suited for cross-sectional characterization were exploited in this work to demonstrate fundamental attributes of blast TBI (bTBI) in hippocampus. Ten weeks old Sprague Dawley rats were exposed to single blast at 130 kPa intensity and sacrificed 24 hours post exposure. Brains were homogenized and thirty micrograms of proteins from each sample were subjected to separation followed by digestion. Peptides were labeled by 8-plex iTRAQ labeling, combined, separated by high pH Reverse-phase Liquid Chromatography (RPLC) and further analyzed by RPLC-MS/MS on Q Exactive™ Hybrid Quadrupole-Orbitrap mass-spec. Protein identification: mass spectra were searched against Uniprot rat database via Mascot 2.4 search engine on Proteome Discovery 1.4 platform. Differential hippocampal proteome analysis in blast exposed rats (n=4) identified 4991 proteins: 219 upregulated and 29 downregulated ($p < 0.05$). Further analysis revealed there are two major canonical pathways involved in pathology of blast TBI: oxidative phosphorylation and mitochondrial dysfunction. These pathways include changes in mitochondrial electron transport/respiratory chain proteins: 1) cytochrome c oxidase (6 fragments), 2) NADH: ubiquinone (16 fragments), 3) ATP synthase (H+) (5 fragments), 4) NADH dehydrogenase (2 fragments) and ubiquinol-cytochrome c complex (2 fragments). These proteins were mostly upregulated suggesting rapid activation of repair mechanism merely 24 hours post injury. Overall pathway analysis identified total of 70+ canonical pathways potential for etiological, therapeutic and diagnostic applications.

Keywords: proteomics, hippocampus, oxidative phosphorylation, mitochondrial dysfunction

B02-17

EXENDIN-4 AMELIORATES BLAST TBI-INDUCED MEMORY DEFICITS, NEURONAL AND SYNAPTIC LOSS, AND GENE EXPRESSION CHANGES IN MOUSE

David Tweedie¹, Lital Rachmany², Vardit Rubovitch², Yazhou Li¹, Dong Seok Kim¹, Barry Hoffer³, Maaad Bader², Chaim Pick², Nigel Greig¹

¹National Institute on Aging, NIH, Drug Design & Development Section; Translational Gerontology Branch, Baltimore, USA

²Sackler School of Medicine, Tel-Aviv University, Department of Anatomy and Anthropology, Tel-Aviv, Israel

³Case Western Reserve University School of Medicine, Department of Neurosurgery, Cleveland, USA

Blast traumatic brain injury (B-TBI), a prevalent form of injury observed in modern warfare, is caused by generation of a blast shock-wave which is induced by the detonation of high explosives. Presently there is no FDA approved medication for the treatment of B-TBI. We investigated the benefits of a FDA approved medicine, Exendin-4 (Exenatide, Ex-4) a long acting glucagon-like peptide 1 receptor agonist, with known neuroprotective/neurotrophic/anti-inflammatory properties (Salcedo et al., *Br J Pharmacol* 166:1586, 2012) in an open field B-TBI model (Rubovitch et al., *Exp Neurol* 232:280, 2011). B-TBI induced lasting cognitive impairments in novel object recognition and less severe deficits in Y-maze behaviors. These deficits were associated with B-TBI-dependent diffuse neuronal loss and reductions in the levels of synaptophysin protein staining in cortical and hippocampal tissues. Changes in gene expressions linked to dementia disorders also were evident. Treatment of animals with a clinically translatable dose of Ex-4 delivered by subcutaneous micro-osmotic pumps to maintain steady-state drug levels for 7 days starting either 48 hours prior to or 2 hours immediately following B-TBI prevented the induction of cognitive deficits, injury-induced neuronal loss, changes in synaptophysin staining, and largely reversed gene expression changes. These data indicate that treatment with the clinically approved drug, Ex-4, represents a viable option for the management of secondary events triggered by blast-induced, mild traumatic brain injury that is commonly observed in militarized zones. Current studies are optimizing Ex-4 administration to support rapid clinical translation.

Keywords: blast injury, novel object recognition, Y maze, synaptophysin, Therapeutics

B02-18

REPEAT LOWER MAGNITUDE TRAUMA INDUCES GREATER AXON DEGENERATION: TREATMENT WITH ANTIOXIDANTS

Victoria Vest, Alexandra Bernardo-Colon, Zhu Li, Adrienne Clark, John Clifton, Tonia Rex
Vanderbilt University Medical Center, Vanderbilt Eye Institute, Nashville, USA

Our goal was to investigate the effects of single vs. repeat blasts on the optic nerve and assess protection by antioxidants. We exposed one eye of an anesthetized C57Bl/6 mouse to one or multiple bursts of overpressure air. Blast and sham mice were given either regular or high vitamin C&E diet ad libitum, beginning one month before blast. At one month post-injury, optic nerves and retinas were collected. Changes in optic nerve histology and the visual evoked potential were quantified. Multiplex ELISAs were performed to assess activation of sterile inflammation. In the C57Bl/6 mouse, axon degeneration and vision loss are first detectable at one month post-trauma in our model, using the single blast paradigm. At one month total axon count and healthy axon count were decreased, and glial area increased, in the repeat blast paradigm as compared to both shams and the single blast paradigm. Markers of sterile inflammation (IL-1a, IL-1b, IL-18) were increased in both blast paradigms at one month post-injury, but not with vitamin C&E treatment. Visual function was also preserved with vitamin C&E treatment. Axon degeneration is increased after repeated blasts as compared to a single blast, even at a lower pressure. Sterile inflammation may contribute to axon degeneration after trauma. Treatment with antioxidants decreases markers of sterile inflammation and mitigates loss of visual function after blast.

Keywords: Repeat Blast, Ocular Trauma, Optic Nerve Injury, Antioxidants

B02-19

A BIOMECHANICAL, HISTOLOGICAL AND COMPUTATIONAL MODELING STUDY OF BRAIN RESPONSE TO FREE-FIELD BLAST

Liyang Zhang, John Cavanaugh, Srinivasu Kallakuri, Ke Feng, Albert King

Wayne State Univ, Biomedical Engineering, Detroit, USA

To date, the underlying mechanisms of primary blast induced traumatic brain injury (pbTBI) are still not fully understood. Much of work on *in vivo* pbTBI has been conducted with rats and swine using a shock tube. The current communication describes a comprehensive study aimed to characterize the blast physics, biomechanical and pathophysiologic features of the operational relevant animal models subjected to open-field blast. The experiments (animal and human cadavers) also provided data for the validation of the computer models and the development of biomechanical correlates at tissue-cellular level. The open-field blast was generated by 3.6 kg of C4 charge to produce incident overpressures (IOP) of three intensities (148.78 ± 3.70 , 278.90 ± 36 , 09.20 ± 18.89 kPa) in frontal blast. One group of anesthetized swine (male Yucatan, n=5) was instrumented with sensors for measuring intracranial pressure (ICP) in various regions of the brain and the head motion. The other group of swine (n=12) was exposed to single blasts of medium or high IOP or no pressure (sham, n=5) for neuropathological analysis. After a 3-day survival period, beta amyloid precursor protein (β -APP), glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule (Iba1) immunohistochemistry was used to assess axonal injury, astrocyte and microglial proliferation. The measured peak ICP in swine brain increased with increasing IOP levels. Peak resultant head acceleration ($< 500g$) correlated well with peak IOP. The arrival of the ICP was almost simultaneous with head motion indicating that it was due to the pressure wave and not the blast wind. Significantly high β -APP, astrocyte and microglial counts were found in the blast groups compared to sham. The human head computer model predicted coup-contrecoup phenomena consistent with the ICP data. The peak maximum principal strain in the brainstem and frontal lobe were comparable and higher than responses in other regions. The correlation of the swine model responses with pathological changes may lead to development of tissue-level criteria for primary blast-induced TBI.

Acknowledgment: MRMC Contract No.W81XWH-12-2-0038, US Army.

Keywords: Open-field blast, Blast induced traumatic brain injury, Swine model, Intracranial pressure measurement, Neuronal injury, Glial change

B02-20

THE ROLE OF ENDOGENOUS AND EXOGENOUS CORTICOSTERONE LEVELS IN THE (MAL)ADAPTIVE RESPONSE TO LOW-PRESSURE BLAST WAVE

Amitai Zuckerman^{1,2}, Omri Ram³, Oren Sadot³, Michael A Matar^{1,2}, Zeev Kaplan^{1,2}, Hagit Cohen^{1,2}

¹Ben-Gurion University of the Negev, Faculty of Health Sciences, Beer Sheva, Israel

²Beer-Sheva Mental Health Center, Department of Anxiety and Stress Research Unit, Beer Sheva, Israel

³Ben-Gurion University of the Negev, Beer Sheva, Department of Mechanical Engineering, Beer Sheva, Israel

Background: In a previous study, we employed a controlled experimental blast-wave paradigm in which rats were exposed to the visual,

auditory, olfactory stimuli and the resultant explosive blast-wave produced by exploding a thin copper wire. Whereas most exposed animals did not demonstrate any behavioral or cognitive abnormalities, in a number of rats exposure did elicit either a PTSD-like phenotype or mTBI-like behavior, or combined mTBI-PTSD-like symptoms. Since dysregulation of the HPA axis is thought to underlie trauma-related (psycho-)pathology, we evaluated the endogenous serum corticosterone levels at different intervals after blast exposure. Subsequently, the efficacy of various doses of exogenous hydrocortisone therapy given immediately after blast-exposure was evaluated.

Methods: Non-anesthetized rats were exposed to low pressure blast-wave. Blood was obtained at a number of pre and post exposure time-points. We subsequently evaluated the behavioral and cognitive effects of various doses of exogenous hydrocortisone to non-anesthetized exposed rats 1 hour after exposure. Validated cognitive-behavioral paradigms were used to assess both PTSD-like and mTBI-like behavior on days 7–14 following the blast.

Results: Retrospective analysis revealed that the PTSD-phenotype group exhibited a blunted corticosterone response to blast exposure compared to all other groups. We found that 125 mg/kg hydrocortisone given 1 h post blast exposure was effective in reducing the prevalence of PTSD-phenotype. No difference in prevalence of the mTBI-phenotype was noted.

Conclusions: Faults in the generation of an adequate and timely endogenous corticosteroid response of the HPA-axis unfavorably alters the trajectory of trauma exposure. Corticosteroid treatment is a feasible avenue for clinical interventions for attenuating PTSD. Our results suggest that mTBI and PTSD may have distinct biological and clinical profiles.

Keywords: HPA-axis, Corticosterone, first aid treatment, Serum

B02-21

CELL-FREE DNA AS A MARKER FOR PREDICTION OF (PSYCHO)PATHOLOGY AFTER BLAST WAVE EXPOSURE IN RAT

Amitai Zuckerman^{1,2}, Omri Ram³, Oren Sadot³, Michael A Matar^{1,2}, Zeev Kaplan^{1,2}, Amos Douvdevani^{4,5}, Hagit Cohen^{1,2}

¹Ben-Gurion University of the Negev, Faculty of Health Sciences, Beer Sheva, Israel

²Beer-Sheva Mental Health Center, Department of Anxiety and Stress Research Unit, Beer Sheva, Israel

³Ben-Gurion University of the Negev, Department of Mechanical Engineering, Beer Sheva, Israel

⁴Ben-Gurion University of the Negev, Department of Clinical Biochemistry and Pharmacology, Beer Sheva, Israel

⁵Soroka University Medical Center, Department of Clinical Biochemistry, Beer Sheva, Israel

Blast wave-induced minimal traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) have become one of the most frequent injuries in the military and civilian health care sectors since the increase in worldwide terrorism and warfare. Those injuries tend to become chronic injuries in the absence of early diagnosis and intervention. Therefore, early identification of the affected population is of great clinical importance for treatment planning, and rehabilitation assessment for patients. In this study, we determined the levels of Cell Free DNA (CFD) in serum at different intervals after low-pressure blast wave exposure to evaluate its potential utility as a sensitive biomarker of blast-induced (psycho)pathology. Non-anesthetized rats were exposed to low pressure blast-wave. Blood was obtained at a number of pre and post exposure time-points.

Simultaneously, combining of cognitive and behavioral paradigms were used in order to determine the rats' performance. The results showed that blast exposure caused significant time-dependent increases in serum CFD. The level of CFD increased significantly at 2 h post-exposure and was independent of the psychopathology or to the blast overpressure. For most of the rats, the maximum increase was recorded at 2 h, and CFD levels returned to baseline by 5 h post exposure. In rats displaying PTSD-phenotype or mTBI-phenotype, the maximum level of circulating CFD was observed at 2 h post exposure and was still significantly elevated at 5 h after blast exposures. CFD levels may be used as a marker to assess psychopathology after blast exposure.

Keywords: Cell Free DNA, First aid treatment, Corticosterone, Blood serum

B03 BLOOD BRAIN BARRIER

B03-01

KPT-350 RESTORES BBB INTEGRITY IN RAT MODEL OF TBI AND MAY REDUCE RISK OF DEVELOPING POST-TRAUMATIC NEUROLOGICAL DISORDERS

Christian Argueta¹, Naoki Tajiri², Sandra Acosta², Sharon Tamir¹, Shelton Cochran¹, Trinayan Kashyap¹, Margaret Lee¹, Sharon Shacham¹, Cesar Borlongan²

¹Karyopharm Therapeutics, Newton, USA

²University of South Florida Morsani College of Medicine, Tampa, USA

Traumatic brain injury (TBI) significantly increases the risk of developing other neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and epilepsy. Simply being part of a high contact activity can increase the likelihood of developing neurodegenerative disorders, e.g., US military veterans are twice as likely as the general population to develop ALS. The connection is strongest with multiple or more severe head injuries and in the years following injury. ALS risk is increased 3-fold within 10 years of a severe head injury and 11-fold within 10 years of multiple head injuries. Epilepsy risk is increased: 10-fold with a skull fracture; 5-fold with a severe TBI; 3-fold with a mild TBI; and 38-fold within the first year of TBI. These correlations are, in part, due to the degradation of the blood brain barrier (BBB), a pathological hallmark of TBI. Karyopharm Therapeutics pioneered the development of selective inhibitors of nuclear export (SINE), which inhibit the nuclear export protein Exportin 1 (XPO1). XPO1 traffics over 200 cargo proteins to the cytoplasm, including critical components of pro-inflammatory pathways. Previous research has demonstrated that the SINE compound KPT-350 significantly reduces post-TBI cortical hyperexcitability (strongly correlated with ALS), as well as epileptiform activity, in a mouse controlled cortical impact (CCI) model of TBI. Here, the effect of KPT-350 on BBB integrity was assessed in a rat CCI model of TBI by administering the compound as a single dose or q.d. for 3 days beginning 2 hours post-injury. Using the Evans Blue extravasation method, it was determined that both groups treated with KPT-350 showed significant improvements in BBB integrity relative to vehicle-treated groups. This set of data suggests that KPT-350 has significant potential as an anti-inflammatory and neuroprotective therapeutic for TBI and associated neurological disorders, possibly by preserving BBB integrity.

Keywords: Amyotrophic Lateral Sclerosis, neuroprotection, anti-inflammatory, neurodegenerative

ROLE OF CASPASE-3 IN CHRONIC CASPASE-3-CLEAVED TAU ACCUMULATION AND BLOOD-BRAIN BARRIER DAMAGE IN THE CORPUS CALLOSUM AFTER TBI

Olena Glushakova¹, Andriy Glushakov², Cesar Borlongan², Alex Valadka¹, Ronald Hayes^{3,1}, Alexander Glushakov⁴

¹Virginia Commonwealth University (VCU), Richmond, USA

²University of South Florida, College of Medicine, Tampa, USA

³Banyan Biomarkers, Inc., Alachua, USA

⁴Single Breath, Inc., Gainesville, USA

Background: Traumatic brain injury (TBI) may be a significant risk factor for development of neurodegenerative disorders such as chronic traumatic encephalopathy, post-traumatic epilepsy, and Alzheimer's and Parkinson's diseases. Chronic TBI is associated with several pathological features that are also characteristic of neurodegenerative diseases, including tau pathologies, caspase-3-mediated apoptosis, neuroinflammation, and microvascular alterations. The goal of this study was to evaluate changes following TBI in cleaved-caspase-3 and caspase-3-cleaved tau truncated at Asp421 and their relationships to cellular markers potentially associated with inflammation and blood-brain (BBB) barrier damage.

Method: We employed immunohistochemistry at different time points from 24 hours to 3 months after controlled cortical impact (CCI) injury in rats, with particular interest in the white matter. We studied astrocytes [glial fibrillary acidic protein (GFAP)], microglia [ionized calcium-binding adapter molecule 1 (Iba1)], BBB [endothelial barrier antigen (EBA)] and activated microglia/macrophages [cluster of differentiation 68 (CD68)].

Results: Our results demonstrated that CCI caused chronic upregulation of cleaved-caspase-3 in the white matter of the corpus callosum. Increases in cleaved-caspase-3 in the corpus callosum were accompanied by accumulation of caspase-3-cleaved tau with increasing perivascular aggregation three months after CCI. Immunofluorescent experiments further showed cellular co-localization of the cleaved-caspase-3 with GFAP and CD68 and its adjacent localization with EBA, suggesting involvement of apoptosis and neuroinflammation in mechanisms of delayed BBB breakdown and microvascular damage, which could contribute to white matter changes.

Conclusion: This study also provides the first evidence that evolving upregulation of cleaved-caspase-3 is associated with accumulation of caspase-3-cleaved tau following experimental TBI, thus providing new insights into potential common mechanisms mediated by caspase-3 and underlying chronic TBI pathologies and neurodegenerative diseases.

Keywords: blood-brain barrier, caspase-3-cleaved tau, chronic TBI, cleaved-caspase-3, GFAP

B03-03

MILD TRAUMATIC BRAIN INJURY LEADS TO WIDESPREAD ACUTE BLOOD BRAIN BARRIER DISRUPTION

Maura Weber¹, William Stewart², D. Kacy Cullen¹, Douglas Smith¹, Victoria Johnson¹

¹University of Pennsylvania, Neurosurgery & Penn Center for Brain Injury and Repair, Philadelphia, USA

²University of Glasgow, Neuropathology, Queen Elizabeth University Hospital, Glasgow, UK

Mild traumatic brain injury (mTBI), or concussion, is a substantial public health problem that can result in persistent and debilitating symptoms in a

subset of patients. Moreover, TBI, including repetitive concussion, has been linked to syndromes of progressive neurodegeneration such as chronic traumatic encephalopathy (CTE). However, the biology of mTBI and its link to late neurodegeneration remain poorly understood. A prominent feature of moderate or severe TBI at acute and late survival time-points is widespread disruption in blood brain barrier (BBB) integrity. However, it is unknown whether a single mTBI alone is sufficient to precipitate altered BBB integrity. Using an established swine model of pure rotational mTBI, animals were subjected to single mTBI or sham conditions (n=3-4 per group) and tissue was prepared for immunohistochemical analyses at 6-72 hours post-injury. Following exposure to mTBI, widespread, abnormal extravasation of blood-borne proteins fibrinogen (FBG) and immunoglobulin-G (IgG) into the brain parenchyma was identified at all time points post-injury, despite an absence of hemorrhage. Typically, this appeared in a stereotyped distribution at structural interfaces, indicating a biomechanical etiology; although, notably, there was incomplete regional overlap between evidence of BBB disruption and axonal pathology. Triple immunofluorescence labeling revealed perivascular cellular uptake of both FBG and IgG in astrocytes (GFAP-positive), but not microglia (Iba1-positive) at these acute post-injury time-points. These data indicate that widespread BBB disruption can be demonstrated as a component of just a single mTBI using this swine model of pure rotational injury. The role of mTBI-induced BBB disruption in the acute presentation and outcomes of concussion, as well as its potential contribution to the late neurodegenerative pathologies of CTE, will be important to examine. Grant Support: DOD: PT110785, NIH: NS056202 and NS038104, The McCabe Fund (Univ. Pennsylvania)

Keywords: TBI model

B04 CELL DEATH

B04-01

ROLE OF TANK-BINDING KINASE IN NEURODEGENERATION ASSOCIATED WITH ABNORMAL EXCITABILITY

Leonard Kaczmarek¹, Yalan Zhang¹, Luis Veralá², Klara Szigeti-Buck², Tamas Horvath²

¹Yale University, Pharmacology, New Haven, USA

²Yale University, Section of Comp. Med, New Haven, USA

Physical trauma to the nervous system produces abnormal patterns of neuronal firing that can eventually lead to neuronal death through mechanisms such as excitotoxicity. Mutations that alter neuronal excitability can also predispose neurons to death. One example of this is Spinocerebellar Ataxia 13 (SCA13), which is caused by mutations in the Kv3.3 voltage-dependent potassium channel, and leads to either early- or late-onset degeneration of the cerebellum. We have found that depolarization of Kv3.3 channels results activation of TANK-Binding kinase-1 (TBK1), an enzyme that controls autophagy and mitophagy. Activation of this enzyme represent a "non-conducting" function of the channel, as it does not require ion flow through the channel itself, and most likely results from conformational changes in cytoplasmic domains of the channel following depolarization. A late-onset mutation in this channel, G952R Kv3.3, leads to greatly potentiated activation of TBK1. Transfection of cell lines with G952R Kv3.3 leads to activation of caspase 7, dense accumulations of multivesicular bodies and presumed autophagosomes that contain the cell survival protein Hax-1, and a significant increase in the rate of cell death. In cerebellar Purkinje cells in the intact nervous system, plasma membrane Kv3.3 channels are closely associated with underlying mitochondria and ER membranes. As in transfected cells, Purkinje cells in mice expressing the G952R Kv3.3 mutation have elevated

levels of TBK1 and enhanced TBK1 activity. Electron immunomicroscopy indicates that this enhanced TBK1 activity is associated with the accumulation of dense multivesicular organelles that contain both TBK1 and Hax-1 and that appear to contain mitochondria destined for degradation. Our results indicate that activation of the Kv3.3 potassium channel directly activates cell survival/death pathways, and suggest that abnormal channel activation, as occurs with channel mutation and may also occur with physical injury, promotes neuronal death.

Keywords: potassium channels, mitochondria, mitophagy, action potential firing

B04-02

EXPRESSION OF SENESENCE MARKERS AFTER TRAUMATIC BRAIN INJURY IN A MOUSE MODEL OF CONTROLLED CORTICAL IMPACT

Kazuhiko Kibayashi, Ryo Shimada, Misato Shibuya

School of Medicine, Tokyo Women's Medical University, Department of Legal Medicine, Tokyo, Japan

Patients' condition may deteriorate after experiencing brain trauma. Edema and hematoma develop at the site of cortical contusion. An increase in inflammation, cell death, and axonal injury is observed in the affected brain regions. To understand the mechanisms of progression of traumatic brain injury (TBI), we hypothesized that cells in the brain acquire senescence, owing to trauma and contribute to the development of TBI. Cellular senescence refers to irreversible arrest of cell division possibly due to stress and damage from exogenous and endogenous stimuli. Senescent cells (SCs) secrete various factors including inflammatory cytokines and proteases that can contribute to tissue dysfunction. The first marker used for identification of SCs is the senescence-associated β -galactosidase (SA- β gal). In addition, p16, an important regulator of senescence, is useful in detecting SCs. We performed histochemical and immunohistochemical analyses for SA- β gal and p16 in brain sections at 1, 4, 7, and 14 days after trauma in a mouse model of controlled cortical impact. On comparing the data from the control and TBI groups, we found that SA- β gal was preferentially expressed in the cortex ipsilateral to the site of injury at 4, 7, and 14 days after trauma. Immunohistochemical analyses showed that macrophages and microglia expressed SA- β gal. We also observed p16 immunoreactivity in the cortex ipsilateral to the site of injury at 4, 7, and 14 days after trauma. p16 immunoreactivity was observed in astrocytes. These findings suggest that SCs are associated with TBI. Since SCs are deleterious to brain tissue, their presence may contribute to the progression of TBI. SA- β gal and p16 are not exclusively expressed in senescence, but they can also be expressed in other types of stress responses. Further studies are required to understand and interpret these results.

Keywords: Cellular senescence, Senescent cells, Senescence-associated β -galactosidase, p16

B04-03

SPINAL CORD INJURY-MEDIATED CPLA2 ACTIVATION CONTRIBUTES TO LYSOSOMAL DEFECTS LEADING TO IMPAIRMENT OF AUTOPHAGY

Yun Li¹, Jace Jones³, Chinmoy Sarkar¹, Shuo Liu¹, Maureen Kane³, Alan Faden¹, Eugene Koh², Marta Lipinski¹, Junfang Wu¹

¹University of Maryland, Baltimore, Department of Anesthesiology & Center for Shock, Trauma and Anesthesiology Research (STAR), Baltimore, USA

²University of Maryland, Baltimore, Department of Orthopedics, School of Medicine, Baltimore, USA

³University of Maryland, Baltimore, Department of Pharmaceutical Sciences, School of Pharmacy, Baltimore, USA

The autophagy-lysosomal pathway plays an essential role in cellular homeostasis and a protective function against a variety of diseases. However, under certain circumstances pathologically increased autophagy can contribute to cell death. This may occur particularly when lysosomal function is impaired and autophagic degradation is not able to proceed to completion, leading to pathological accumulation of dysfunctional autophagosomes. We have previously shown that autophagy is inhibited and contributed to injury after SCI. Here we examine mechanism of autophagy and lysosomal defects following SCI. Expression levels and processing of the lysosomal enzyme cathepsin D (CTSD) were decreased at 2 h after SCI. Enzymatic activity of CTSD and another lysosomal enzyme, alkaline phosphatase, were decreased 24 h post-injury, indicating lysosomal damage. Subcellular fractionation confirmed lysosomal membrane permeabilization (LMP) and leakage of lysosomal content into the cytosol. cPLA2 is an enzyme that cleaves fatty acyl linkage in the phospholipids of cellular membranes and increased activity of cPLA2 may be involved in membrane damage. cPLA2 was activated in the lysosomal fraction, accompanied by increased accumulation of the autophagosomal marker LC3-II and its substrate p62. To directly assess the extent and mechanism of damage to lysosomal membranes, mass spectrometry (MS)-based lipidomics was applied to compare the lipid composition of lysosomal membranes purified from sham or injured spinal cord at 2 h post-injury. Our data demonstrate increases in several classes of lysosomemembranephospholipids- the products of phospholipases (PLAs), as well as accumulation of PLA activator, ceramide. Inhibition of cPLA2 decreased lysosomal damage, restored autophagic flux, and reduced neuronal cell damage. Taken together our data implicate lysosomal defects in the pathophysiology of SCI and further indicate that cPLA2 activation leads to lysosomal damage that causes neuronal autophagosome accumulation associated with neuronal cell death.

Keywords: spinal cord injury, autophagy, lysosomal damage, cPLA2

B04-04

LYSOSOMAL DAMAGE AFTER SPINAL CORD INJURY INHIBITS NEURONAL AUTOPHAGY AND CONTRIBUTES TO NECROPTOSIS

Shuo Liu^{1,2}, Chinmoy Sarkar¹, Yun Li¹, Alan Faden¹, Eugene Koh², Junfang Wu¹, **Marta Lipinski**¹

¹University of Maryland School of Medicine, Anesthesiology & Shock, Trauma and Anesthesiology Research Center, Baltimore, USA

²University of Maryland School of Medicine, Orthopedics, Baltimore, USA

Necroptosis, a form of regulated necrosis mediated by the receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3, respectively), is induced following spinal cord injury (SCI) and thought to contribute to neuronal and oligodendrocyte cell death. However, mechanisms leading to activation of necroptosis after SCI remain unclear. We have previously shown that autophagy, a catabolic pathway facilitating degradation of cytoplasmic proteins and organelles in a lysosome-dependent manner, is inhibited following SCI in the rat. Our current data confirm that inhibition of autophagy is also occurring after T10 contusive SCI in the mouse model, as indicated by accumulation of both the autophagosome marker, LC3-II and autophagy cargo protein,

p62/SQSTM1. This was most pronounced in the ventral horn motor neurons and was caused by rapid inhibition of lysosomal function after SCI. Interestingly, RIPK1, RIPK3 and the necroptosis effector protein MLKL also rapidly accumulated after SCI and specifically localized to motor neurons with disrupted autophagy, suggesting that the two events may be related. To determine if lysosomal dysfunction could contribute to induction of necroptosis, we treated PC12 cells and cultured rat cortical neurons with lysosomal inhibitors, Bafilomycin A and Chloroquine. Both treatments led to rapid accumulation of RIPK1 and RIPK3, confirming that they are normally degraded by the lysosomal pathway. In PC12 cells lysosomal inhibition also sensitized cells to necroptosis induced by TNF α and caspase inhibitor Boc-D. Imaging studies confirmed that RIPK1 partially localized to lysosomes in both untreated and lysosomal inhibitor treated cells. Similarly, we observed accumulation of RIPK1 in both cytosol and lysosomes after SCI *in vivo*. Therefore, lysosomal dysfunction after SCI may contribute to both inhibition of autophagy and sensitize cells to necroptosis by promoting RIPK1 and RIPK3 accumulation.

Support: R01NS094527, R01NS091218

Keywords: necroptosis, autophagy, lysosomal damage, spinal cord injury

B04-05

ACTIVATION OF CPLA2 LEADS TO LYSOSOMAL MEMBRANE DAMAGE AND IMPAIRMENT OF AUTOPHAGY AFTER TBI

Chinmoy Sarkar¹, Nivedita Hegdekar¹, Jace Jones², Julia Peter¹, Shuo Liu¹, Alan Faden¹, Maureen Kane², Marta Lipinski¹

¹University of Maryland School of Medicine, Anesthesiology & Shock, Trauma and Anesthesiology Research Center, Baltimore, USA

²University of Maryland School of Pharmacy, Pharmaceutical Sciences, Baltimore, USA

Autophagy, a lysosome-dependent intracellular degradation pathway, plays an essential role in neuronal homeostasis and is compromised in neurodegenerative diseases. We recently demonstrated that autophagy is inhibited and contributes to neuronal cell death after traumatic brain injury (TBI) in mice. This was caused by lysosomal dysfunction, evidenced by lower protein levels and activity of lysosomal enzymes in day 1 injured cortex. Furthermore, we observed leakage of soluble lysosomal enzymes into the cytosol, indicating lysosomal membrane permeabilization (LMP). This correlated with the activation of cytosolic phospholipase A2 (cPLA2), an enzyme that cleaves fatty acyl linkage in the phospholipids of cellular membranes. At early time points activation of cPLA2 was observed specifically within neurons and co-localized with markers of lysosomal dysfunction. Consistent with the possibility that cPAL2 activity may damage lysosomal membranes, we detected lysosomal damage and LMP *in vitro* in H4 cells and rat cortical neurons treated with cPLA2 activator ceramide-1-phosphate (C1P). This was accompanied by inhibition of autophagy flux, and was prevented by knock-down of cPLA2 or pretreatment with cPLA2 inhibitor, AACOCF₃. To determine if cPLA2 directly injures lysosomal membranes *in vivo* after TBI, we performed MS-based lipidomic analysis of purified cortical lysosomes. We detected specific increase in phospholipase products, lysophospholipids, in lysosomal membranes from injured as compared to naive mice. Confirming involvement of cPLA2, treatment with AACOCF₃ attenuated lysosomal membrane damage and LMP after TBI. This correlated with decrease in accumulation of autophagy markers LC3-II and p62, indicating improved autophagy flux. At 4 weeks after TBI we observed improved motor and cognitive function in mice treated with

AACOCF₃ as compared to vehicle controls. Together these data indicate that activation of cPLA2 mediates lysosomal damage after TBI, causing inhibition of autophagy and contributing to neuronal cell death and poor outcomes.

Keywords: lysosomal damage, autophagy, cPLA2, traumatic brain injury, lipidomics

B04-06

MORPHINE-INDUCED CELL DEATH IN A RODENT MODEL OF SCI

Mabel Terminel, Miriam Aceves, Michelle Hook

Texas A&M Health Science Center, Neuroscience and Experimental Therapeutics, Bryan, USA

Opioids are among the most effective and commonly prescribed analgesics for the treatment of acute pain after spinal cord injury (SCI). We have shown, however, that administration of morphine in the early phase of SCI undermines locomotor recovery in a rodent contusion model. Further, we found that morphine administration is associated with decreased expression of neuronal and astrocytic markers post SCI. Increased neuronal death may lead to further loss of function. Whether morphine increases cell death or decreases the expression of cell specific markers is unknown. To test this, the current study used Caspase-3, as an apoptotic marker, together with specific neuronal and astrocytic markers, and analyzed the temporal sequence of cell loss with morphine administration. Subjects were given a moderate spinal contusion injury or were sham controls. On the day following surgery, half of the subjects in each injury condition were treated with 10 mg of morphine (i.v.) on days 1–2, 20 mg on days 3–4, and 30 mg on days 5–7. The remaining subjects served as controls, receiving an equivalent volume of 0.9% saline across days. To assess the temporal sequence of cell loss, subjects were euthanized on days 2, 4, or 8 (24 hrs after the final dose of morphine). A 1.5 cm section of injured spinal cord was collected and sectioned for immunohistochemistry. We found that morphine-treated subjects had increased co-localization between Caspase-3 and NeuN at 4 days post injury, relative to controls. By contrast, morphine did not increase co-localization between GFAP and Caspase-3, despite the decreased expression of astrocytes at 7 days. These data suggest that while morphine may induce apoptosis in neurons *in vivo*, astrocyte loss might be mediated by an alternate mechanism. Given the clinical utility of opioid analgesics, it is imperative that we identify the molecular mechanisms mediating the adverse effects of morphine in the rodent SCI model. We must develop safe and effective therapeutic strategies for the use of opioids in pain management after SCI.

Keywords: Spinal Cord Injury, morphine, apoptosis, opioids, locomotor recovery

B04-07

EVIDENCE FOR THE IMPORTANCE OF THE RIPK3 SCAFFOLD FUNCTION IN THE PATHOGENESIS OF CELL DEATH, INFLAMMATION, AND FUNCTIONAL OUTCOME

Limin Wu, Sevda Lule, Joonyong Chung, William Edmiston III, Michael Whalen

Massachusetts General Hospital, Pediatrics, Charlestown, USA

Introduction: Receptor-interacting protein kinase-1 and -3 (RIPK1, RIPK3), mediate necroptosis, apoptosis, and inflammation depending

on cell type and injury context. Although the kinase domain of RIPK1/3 is required for programmed necrosis, kinase-independent scaffold functions of RIPKs can initiate apoptosis and inflammation. As master regulators of these pathophysiological processes, RIPKs are compelling therapeutic candidates to inhibit secondary injury after acute brain injury. We used mice deficient in kinase activity of RIPK1 and RIPK3, and RIPK3 knockout mice to inhibit scaffold function, and tested the **hypothesis** that RIPKs mediate acute cell death, interleukin-1 beta (IL-1 β) processing, and functional deficits after controlled cortical impact (CCI) in mice.

Methods: Wild type and RIPK1 and RIPK3 kinase dead mice, as well as RIPK3 KO mice and their littermate controls, were subjected to CCI. HMGB1 release was used to assess necrosis/inflammation, IL-1 β ELISA was used on brain and cerebrospinal fluid samples, immunoprecipitation and Western blot were used to assess RIPK1-RIPK3-MLKL interaction. Microglia and brain macrophages were isolated by fluorescence activated cell sorting, and Nanostring technology was used to assess microglial transcription in RIPK3 KO and WT mice, and behavioral tests were used to assess functional outcome.

Results: RIPK3 and MLKL were induced in brain tissue homogenates and in innate immune cells after CCI. RIPK1-RIPK3-MLKL interaction was observed at 3 h but not at later time points. HMGB1 release was significantly inhibited in RIPK3 KO compared to all other groups. RIPK1 kinase dead mice had reduced wire grip deficits, whereas RIPK3 KO mice had reduced Morris water maze deficits, wire grip deficits, and NORT deficits vs. wild type and RIPK1 kinase dead. RIPK3 KO mice had undetectable CSF IL-1 β but similar transcription profiles as WT in microglia at 48 h.

Conclusions: RIPK3 scaffold function appears to be the predominant mechanism of secondary injury in all of the groups tested. Further studies are needed to elucidate druggable targets associated with RIPK3 scaffold function to improve outcome after contusion TBI.

Keywords: TBI, cell death, inflammation, IL-1 β , HMGB1, behavior

B05 CHRONIC TRAUMATIC ENCEPHALOPATHY

B05-01

LONG-TERM NEUROPATHOLOGIC SEQUELAE OF PEDIATRIC ABUSIVE HEAD TRAUMA

Rebecca Folkerth^{1,2}, Marco Hefti³, Declan McGuone^{1,2}

¹New York City Office of the Chief Medical Examiner, New York, USA

²NYU School of Medicine, Department of Forensic Medicine, New York, USA

³Mt. Sinai Medical Center, Department of Pathology, New York, USA

Neuropathologic descriptions of abusive head trauma (AHT) are limited to short-term survivors, despite the fact that long-term neurologic sequelae are well-described. In particular, the role of tau protein aggregation in pediatric AHT is unclear. From the New York City Office of the Chief Medical Examiner we report a series of five cases of AHT (1 blunt impact, 4 “shaken”) with long-term post-injury survival. The mean age at injury was 9.8 months (range, 2–18 months) and the mean age at death was 16 years (range, 10–25 years). Mean survival was 15.3 years (range, 9.3–23.5 years). The cause of death was certified as long-term sequelae of head trauma in all cases. Manner of death was homicide in three, and undetermined in two. One case had a history of cocaine intoxication at birth; one had neonatal hyperbilirubinemia, then failure to thrive at time of injury. All had severe intellectual disability post-injury and four (80%) had seizures. All cases showed micrencephaly with relative sparing of the posterior fossa. Mean brain weight was 502 g (range, 310–800 g). Two of five

cases (40%) had subdural neomembranes or hygroma at autopsy. All cases showed diffuse parenchymal loss affecting the cerebral cortex and white matter, with relative sparing of the cerebellum and brainstem and secondary degeneration of descending corticospinal tracts. In the one case with blunt impact, the parenchymal loss was ipsilateral to the subdural neomembrane. Tau immunohistochemistry in two cases showed tau-positive threads in both, and neuronal and glial cytoplasmic aggregates in the hypothalamus in one. Beta-amyloid precursor protein immunohistochemistry in two cases showed rare positive axons in the anterior commissure in one. We conclude that long-term neuropathologic sequelae of early-life AHT include evidence of direct trauma (subdural neomembranes), significant hypoxic-ischemic injury, and also focal tau aggregation suggestive of a neurodegenerative component (chronic traumatic encephalopathy).

Keywords: abusive head trauma, shaken infant, tau aggregates, subdural neomembranes

B06 COGNITION / LEARNING / MEMORY

B06-01

STRESS AS A PREDISPOSITION FACTOR FOR PROLONGED COGNITIVE DYSFUNCTION FOLLOWING MILD TRAUMATIC BRAIN INJURY

Coleen Atkins, Nicole Wilson, Julie Freund, David Titus

University of Miami Miller School of Medicine, Neurological Surgery, Miami, USA

Although the majority of people with mild traumatic brain injury (TBI) recover within 10–14 days, a subset of people exhibit symptoms such as cognitive dysfunction for weeks to months after injury. Factors that contribute to developing prolonged cognitive deficits after mild TBI are still unknown. One known predisposition factor for developing psychiatric disorders is an early-life stress event. Exposure to early-life stress primes hippocampal microglia and potentiates the neuroinflammatory response to a subsequent inflammatory challenge. We hypothesized that stress in early life interacts with a mild TBI experienced in young adulthood to result in persistent learning and memory deficits. To test this hypothesis, Sprague Dawley pups were separated from their dams for 3 hr daily from P2-P14. Non-stressed pups remained undisturbed. At 2 months of age, male rats received sham surgery or mild parasagittal fluid-percussion brain injury (1.4–1.6 atm). Animals were assessed behaviorally for 2–6 weeks post-surgery and hippocampal atrophy was evaluated at 2 months post-surgery. Basal corticosterone levels and response to a restraint stress were assayed. Mild TBI did not result in deficits in contextual fear conditioning or water maze performance. In contrast, early-life stress prior to mild TBI resulted in significant impairments in contextual fear conditioning, water maze retention and working memory ability. Hippocampal atrophy was also significantly increased in early-life stressed, mild TBI animals as compared to non-stressed, mild TBI animals. Basal and restraint-induced corticosterone levels were not significantly different between treatment groups. However, corticosterone levels remained significantly elevated after recovery from restraint in early-life stressed, mild TBI animals as compared to non-stressed, mild TBI animals. These results demonstrate that exposure to early-life stress interacts with mild TBI experienced in young adulthood to result in prolonged cognitive deficits and an increased stress response.

This work was supported by The Miami Project to Cure Paralysis and NIH/NINDS R01 NS069721.

Keywords: stress, early life stress, corticosterone, atrophy, hippocampus

MINOCYCLINE PLUS N-ACETYLCYSTEINE RESTORE SYNAPTIC PLASTICITY AND LIMIT GRAY MATTER INJURY WHEN FIRST DOSED 3 DAYS POST-INJURY

Peter Bergold, Elena Nikulina, Andrew Tcherepanov, Kristen Whitney, Todd Sacktor
State University of New York-Downstate Medical Center, Physiology and Pharmacology, New York, USA

The cognitive losses produced by closed head injury (CHI) include impairments in acquiring Barnes Maze, a behavioral task that requires a single functional hippocampus. CHI-injured mice acquire Barnes maze when MINO plus NAC is first dosed as late as 3 days post-injury. Long-term potentiation (LTP) is a form of hippocampal synaptic plasticity needed to acquire Barnes Maze. CHI-injured mice have impaired LTP in both hippocampi, either ipsilateral or contralateral to the impact site. MINO plus NAC treatment beginning 3 days post-injury more readily restored LTP in the contralateral than the ipsilateral hippocampus. The contralateral hippocampus of MINO plus NAC-treated mice retained more neurons, and had greater expression of the dendritic protein MAP2 and the presynaptic protein synaptophysin than saline-treated injured mice. MINO plus NAC treatment also increased expression of PKMzeta, a PKC isoform that is essential for LTP maintenance and memory storage in the hippocampus. Moreover, preliminary studies suggest that neurons in the contralateral hippocampus stained with the rapid Golgi-Cox method have improved dendritic length and arborization, spine density and spine morphology. Together, these data indicate that MINO plus NAC restores the ability to acquire Barnes maze, in part, by limiting gray matter injury and preserving synaptic plasticity in the contralateral hippocampus. These studies also suggest that injury to regions of the brain distal from a head impact can be targeted with drugs with a clinically relevant window even if injuries proximal to the impact site remain.

Keywords: mouse, long-term potentiation, gene expression, time to first dose, neuronal morphology, hippocampus

B06-03

CHRONIC HISTOPATHOLOGICAL AND FUNCTIONAL OUTCOME AFTER CONTROLLED CORTICAL IMPACT TRAUMA IN MICE

Xiang Mao¹, Nicole A. Terpolilli^{1,2}, Farida Hellal¹, Nikolaus Plesnila¹

¹*Institute for Stroke and Dementia Research, University of Munich Medical Center, Munich, Germany*

²*University of Munich Medical Center, Neurosurgery, Munich, Germany*

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. In past decades, most research focused on clarifying the mechanisms of acute brain damage. There is, however, increasing evidence that TBI pathology may continue for years after the initial insult causing progressive histopathological changes; this chronic posttraumatic brain damage has been linked to long term functional deficits that can be observed in trauma patients, including progressive neurocognitive decline and psychological abnormalities. In order to identify putative therapeutic strategies for long term sequelae of TBI we need to know more about the mechanisms and time course of chronic posttraumatic brain damage. The aim of the current study was to characterize functional and histopathological outcome in a mouse

model of controlled cortical impact (CCI) injury, a widely used experimental model of TBI.

Male C57BL/6N mice (n=12 per group) underwent CCI or sham operation and were observed for 15 min, 24 hours, 1 week, 1, 3, 6, or 12 months after TBI. Neurological function was assessed over the whole observation period using test paradigms evaluating motor function (Beam Walk test), depression like behavior (Tail Suspension test), as well as spatial learning and memory (Barnes Maze). Furthermore, histopathological outcome was assessed using immunohistochemistry.

CCI induced significant deterioration of motor function compared to sham-operated or naïve animals. At 3, 6, and 9 months after CCI, TBI animals showed depression like behavior and massive deterioration of spatial learning and memory function. Significant hydrocephalus was observed starting 3 months after CCI.

This study provides comprehensive data about extent and time course of chronic brain damage after experimental CCI trauma and may help to further understand the pathomechanisms of chronic TBI as well as serve as a base for further investigations.

Keywords: Traumatic brain injury, Outcome, Posttraumatic brain damage, Hydrocephalus

B06-04

A NEW RAT MODEL OF REPEAT MILD TRAUMATIC BRAIN INJURY DURING ADOLESCENCE

Katellynn Ondek^{1,3}, Gene Gurkoff^{1,2}

¹*UC Davis, Center for Neuroscience, Davis, USA*

²*UC Davis, Neurological Surgery, Davis, USA*

³*UC Davis, Veterinary Medicine, Davis, USA*

Repeated sports-related injury can lead to cognitive deficits in the absence of motor dysfunction and gross anatomical damage. We hypothesized that impacting a metal disc (helmet) using an electrically-driven piston would produce spatial learning deficits without skull fracture or hippocampal cell death. We designed a 3D-printed plastic implant to permanently secure the helmet to the skull of adolescent rats, allowing for multiple injuries without multiple survival surgeries. Rats received three TBIs (5 m/s, 5 mm depth) separated by 72 hours, beginning on P35. Motor and spatial performance were tested on the Rotarod and Morris water maze, respectively. There were no differences in biological responses (toe pinch reflex, righting time, weight) to the first two injuries; however, toe pinch reflex time (6.7 ± 1.1 s vs. 11.4 ± 1.8 s; $p < 0.05$) and righting time (60 ± 9.9 s vs. 152.1 ± 50.7 s; $p < 0.05$) were significantly longer in injured animals after the final injury, with a trend toward a difference in weight one day post-TBI ($p < 0.1$). There were no differences in motor performance on Rotarod or swim speed in the water maze between repeat- and sham-injured animals. Spatial learning did not differ across five days of training or during three subsequent working memory trials. However, sham animals spent significantly more time searching the platform location during a probe trial 72 hours later ($18.3 \pm 1.8\%$ vs. $7.9 \pm 2.1\%$; $p < 0.05$). Moreover, across three days of working memory trials, sham, but not TBI, animals spent progressively less time searching the original platform location ($p < 0.005$). These data demonstrate a cumulative biological response to three injuries over a 7-day period. In addition, while both sham and TBI animals demonstrate similar spatial memory performance as determined by latency, there is evidence that injured rats utilize alternate search strategies. Therefore, this new model shows relevance for sports-related repeat mild TBI and potential for assessing mechanisms and therapeutic interventions.

Keywords: Animal Model, Spatial Learning, Repeat TBI, Diffuse Injury

DEEP BRAIN STIMULATION TO ENTRAIN THETA OSCILLATIONS AND IMPROVE BEHAVIORAL OUTCOME FOLLOWING LATERAL FLUID PERCUSSION TBI

Aleksandr Pevzner^{1,2}, Kayleen Gimlin^{1,2}, Pedro Hernandez^{1,2}, Ali Izadi^{1,2}, Katelynn Ondek^{1,2}, Kiarash Shahlaie^{1,2}, Gene Gurkoff^{1,2}

¹UC Davis, Neurological Surgery, Davis, USA

²UC Davis, Center for Neuroscience, Davis, USA

We hypothesize that disruption of theta oscillations (5–12 Hz) in the septohippocampal circuit results in cognitive dysfunction following traumatic brain injury (TBI). Furthermore, we propose that entraining oscillations with deep brain stimulation (DBS) will improve cognitive outcome post-injury. We previously reported that 7.7 but not 100 Hz square-wave stimulation of the medial septum (MSN) improved spatial learning on the Barnes maze following lateral fluid percussion injury (LFP). However, there are other theta stimulation paradigms including continuous, cycled and burst stimulation that are proposed to have distinct effects at the neuronal network and behavioral level. Given the relevance to the clinical population we now compare continuous 7.7 Hz to cycled theta square-wave (30 seconds ON/ 150 seconds OFF) and theta burst (50 millisecond trains of 200 Hz, five times a second) to evaluate paradigm specific effects. Prior to stimulation, EEG was collected on days 3 and 7 post-surgery while animals explored a novel environment. Barnes maze was evaluated post-injury days 8–11. LFP injury resulted in significantly longer righting time and weight loss, qualitative observations of hemi-neglect during recovery and structural damage over the injury site. Consistent with previous reports, LFP resulted in altered oscillations over the first week post-injury. However, injury was not associated with worse performance on the Barnes maze compared to sham or to any of the injured stimulation groups. The gross pathology was further investigated by comparing the number of parvalbumin interneurons within the CA1 and MSN. Future experiments include more granular analyses of EEG during behavior (e.g. coherence, correlation with speed) and characterizing the expression profile of multiple interneuron types (e.g. Calbindin) in hippocampal regions. These data are critical in understanding the effect of TBI on neural systems as well as addressing the potential for DBS to enhance recovery post-TBI.

Keywords: TBI, Deep brain stimulation, Hippocampus, Theta, Learning and memory

B06-06

SELECTIVE PDE4D NEGATIVE ALLOSTERIC MODULATION IMPROVES COGNITION AFTER TRAUMATIC BRAIN INJURY

David Titus¹, Nicole Wilson¹, Oscar Alcazar¹, Dale Calixte¹, W. Dalton Dietrich¹, Mark Gurney², Coleen Atkins¹

¹University of Miami Miller School of Medicine, Neurological Surgery, Miami, USA

²Tetra Discovery Partners, Grand Rapids, USA

Alterations in the cyclic AMP (cAMP) signaling pathway is associated with impaired long-term potentiation, memory formation and retrieval after chronic traumatic brain injury (TBI). The cAMP-specific phosphodiesterase 4 (PDE4) is involved in the regulation of cAMP signaling in the brain. The PDE4 subfamily, PDE4D, is of particular interest due to its involvement in learning and memory. Recent advancements in the structural understanding of PDE4 subtypes have led to the development of a novel negative allosteric

modulator for PDE4D (D159687). In the present study, we hypothesized that treating animals with D159687 would rescue cognitive deficits caused by TBI. To test this hypothesis, adult male Sprague Dawley rats received sham surgery or moderate parasagittal fluid-percussion brain injury. After 3 months of recovery, animals were treated with vehicle or D159687 (0.3 mg/kg, 6 ml/kg, intraperitoneally) at 30 min prior to cue and contextual fear conditioning, acquisition in the water maze or during a working memory task. Treatment with D159687 had no significant effect on these behavioral tasks in sham animals. In contrast, D159687 significantly reversed the learning and memory deficits in chronic TBI animals. Assessment of hippocampal slices at 3 months post-TBI revealed that D159687 reversed both the depression in basal synaptic transmission in the Schaffer collateral pathway of area CA1 as well as the expression of hippocampal long-term potentiation. Altogether, these results demonstrate that negative allosteric modulation of PDE4D may be an effective therapeutic strategy to improve chronic cognitive dysfunction following TBI.

Keywords: Traumatic Brain Injury, PDE4D inhibition, Long-term potentiation, Learning and memory

B06-07

BRAIN HISTOPATHOLOGY AND BEHAVIOR CHANGES AFTER FLUID PERCUSSION INJURY OF TAU OLIGOMER TARGETED IMMUNOTHERAPY TREATED RATS

Jordan Wolf¹, Ian Bolding¹, Kathia Johnson¹, Margaret Parslet¹, Clark Anderson², Donald Prough¹, Douglas DeWitt¹, Bridget Hawkins¹

¹The University of Texas Medical Branch, Anesthesiology, Galveston, USA

²The University of Texas Medical Branch, Preventive Medicine and Community Health, Galveston, USA

Evidence suggests that formation of soluble tau oligomers may contribute to the early spread of pathology following traumatic brain injury (TBI). We hypothesized that if an immunotherapy targeted at removal of tau oligomers were given an hour post TBI, it would result in decreased tau oligomers and increased brain tissue sparing, thus improving behavior. Adult male Sprague Dawley rats received either a single fluid percussion or sham injury and the return of righting reflex was assessed. An intracerebroventricular injection of either anti-tau oligomer-specific monoclonal antibody (TOMA) or nonspecific Immunoglobulin G control was administered 1 hour post TBI. Animals underwent cognitive behavior testing and then fifteen days later, the animals were perfused for histology. Neuroscience Associates block sectioned the brains and stained them for myelin (Weill) and inflammation markers (Iba-1 and CD-68). Ventricle volumes, Corpus Collosum volume, and cortical thicknesses were assessed using ImageJ, and volumes ipsilateral and contralateral to the lesion were compared. Preliminary evaluation of TOMA TBI animals show cortical thinning, ventricle enlargement, and decreased white matter volume on the injured side, while the IgG control treated animals showed smaller histological changes. Preliminary comparisons suggest that the animals with more severe brain pathology also show learning and memory impairment. Sham animals did not show this pattern of brain pathology and behavioral deficits. These studies were funded by The Moody Project for Translational TBI Research, Darrell K Royal Research Fund for Alzheimer's Disease, Mission Connect (TIRR Foundation) and the UTMB Technology and Commercialization Program.

Keywords: TOMA, Immunotherapy, Histology, MWM

BRAIN REGION SPECIFIC CHANGES IN GENE EXPRESSION AFTER FLUID PERCUSSION INJURY AND TAU OLIGOMER TARGETED IMMUNOTHERAPY IN RATS

Satoshi Yamamoto¹, Ian Bolding¹, Debbie Boone¹, Kathia Johnson¹, Margaret Parsley¹, Clark Andersen², Donald Prough¹, Douglas DeWitt¹, Bridget Hawkins¹

¹University of Texas Medical Branch, Anesthesiology, Galveston, USA

²University of Texas Medical Branch, Biostatistics, Galveston, USA

Traumatic Brain Injury (TBI) is considered an event that can lead to the development of chronic disease pathology and is thought to induce a predisposition towards dementia. Evidence suggests that formation of soluble tau oligomers may be an early event responsible for the spread of pathology. We designed this study to look at the molecular connections between TBI and Alzheimer's Disease (AD) by using a real-time PCR array following treatment with an immunotherapy designed to reduce the soluble tau oligomers that occur after TBI. Adult male Sprague Dawley rats received either a single fluid percussion or sham injury and the return of righting reflex was assessed. An intracerebroventricular injection of either anti-tau oligomer-specific monoclonal antibody (TOMA) or IgG control was administered 1 hour post TBI. Fifteen days later, brain regions (cortex near the injury site, hippocampus and thalamus) were dissected and homogenized separately. AD pathway-focused PCR arrays for rats, containing 84 genes implicated in AD development, were performed. Normalized gene expression was analyzed by ANOVA for each gene, and differences among treatment groups were assessed by Tukey-adjusted contrasts, followed by Benjamini-Hochberg control of false discovery rate. Results suggest that down-regulation of neurodegenerative genes in the TOMA treated animals was brain region specific, indicating different processes might be occurring and additional time points should be studied. These studies were funded by The Moody Project for Translational TBI Research, Darrell K Royal Research Fund for Alzheimer's Disease, Mission Connect (TIRR Foundation) and the UTMB Technology and Commercialization Program.

Keywords: tau oligomer targeted immunotherapy, fluid percussion TBI, gene expression, Alzheimer's Disease

B06-09

NEUROINFLAMMATION INDUCED TRYPTOPHAN DYSREGULATION AND DEFICITS IN COGNITION AND SLEEP PATTERN IN A RABBIT MODEL OF PEDIATRIC TBI

Zhi Zhang, Sarah Bertrand, Manda Saraswati, Raymond Koehler, Sapna Kudchadkar, Courtney Robertson, Sujatha Kannan
Johns Hopkins School of Medicine, ACCM, Baltimore, USA

Neuroinflammation following TBI is a major player in the secondary response post-injury contributing to widespread cell death and tissue loss. Microglial distribution and function plays an important role in the inflammatory response following TBI in the immature brain. In this study we investigated neuroinflammation-induced changes in brain tryptophan-kynurenine pathway and abnormal cognition and sleep pattern. On postnatal day 5-7 (P5-7), New Zealand white rabbits from the same litter were randomized into three groups, naïve (no injury), sham (craniotomy alone) and TBI (controlled cortical impact). Pro- and anti-inflammatory cytokine mRNA expression was measured at 6h, 1, 3, 7 and 21 days post-injury. Tryptophan, kynurenine, serotonin and melatonin protein levels were measured at 7 and 21 days post-injury. Cognitive function and sleep pattern were measured at 1-2 months post-injury. We found that 1) TBI induced a significant upregulation of pro-

inflammatory cytokines, including TNF- α and IL-1 β that last more than 21 days post-injury, indicating a pro-inflammatory microenvironment. 2) TBI induced a significant upregulation of indoleamine 2,3 dioxygenase 1 (IDO1), the rate limiting enzyme in the tryptophan-kynurenine pathway, in activated microglial and astrocytes at the surrounding area of brain injury up to 21 days post-injury. 3) TBI induced a significant increase in kynurenine level in the brain, along with a significant reduction in plasma melatonin level, suggesting the shift of tryptophan metabolism towards the kynurenine pathway. 4) Pediatric TBI caused significant deficits in cognitive function at two months post-injury. 5) Pediatric TBI induced abnormal sleep EEG pattern, indicated by decreased REM at two month post-injury. This indicates that targeting kynurenine pathway specifically in activated microglia using dendrimer nanodevices may provide a novel therapeutic opportunity to address sleep disturbance and cognitive dysfunction following TBI.

Keywords: melatonin, tryptophan, pediatric TBI, EEG, secondary injury

B07 COMPUTATIONAL / MODELING

B07-01

COMPUTATIONAL MODELING OF APPLIED DC ELECTRIC FIELDS IN THE HUMAN SPINAL CORD

Kavya Cherukuri¹, Jianming Li¹

¹Purdue University, Center for Paralysis Research, West Lafayette, USA

²Purdue University, Biomedical Engineering, West Lafayette, USA

Low level DC electric fields (EF) in the range of 5-1000 mV/mm have been shown to guide axon growth and orientation *in vitro* and *in vivo* during neural development. Our laboratory has exploited this phenomenon by implanting electrodes that deliver weak DC EFs across the lesion to stimulate axonal regeneration after spinal cord injury (SCI). Initial data in human clinical trials (FDA Phase 1) with this stimulator demonstrated higher sensory but not motor recovery versus historical controls. We postulate such differences may arise from spatial variations in the EFs. However, the magnitudes of EFs permeating through the injured region remain largely unknown. The main objective of this study is to model the EFs emanating from the stimulator electrodes and to optimize electrode placement for maximum therapeutic effect. Simulation studies were performed using Sim4Life finite element analysis package. The computational model consisted of a C3-C7 vertebral section obtained from MRI scans, epidural fat, dura, cerebrospinal fluid (CSF), the spinal cord, and torso musculature. Three active electrodes were placed cranial to the site of injury, attached to the spinous process and each of the transverse processes on the C4 vertebra. Three additional ground electrodes were placed caudal to the site of injury on the C6 vertebra. At a 5V electrode input voltage, a cross section of the spinal cord at the level of the injury (C5) showed electric field values toward the lower end necessary for biological effect but varied with electrode placement. There were also differences between EF maps of injured and intact (uninjured) cord. This was due to the CSF, which acted as a shield in the intact cord, but was removed in the injured cord scenario. The computational findings show the importance of considering post-SCI biological consequences that may alter tissue conductivities. The computed results for electric field strength and distribution may also explain the asymmetry in motor vs sensory recovery from the Phase 1 clinical trials and provide insight into field optimization.

Keywords: implantable device, electromagnetics, spinal cord stimulator, finite element analysis

FUNCTIONAL OUTCOME TRAJECTORIES AFTER TRAUMATIC BRAIN INJURY: NOVEL INSIGHTS FROM THE COBRIT STUDY

Raquel Gardner^{1,2}, Adam Ferguson^{2,3}, Jessica Nielson³, Jing Cheng⁴, John Boscardin⁵, Ross Zafonte⁶, Geoffrey Manley³, COBRIT Investigators

¹UCSF, Neurology, San Francisco, USA

²SFVAMC, San Francisco, USA

³UCSF, Neurosurgery, San Francisco, USA

⁴UCSF, School of Dentistry, San Francisco, USA

⁵UCSF, Epidemiology/Biostatistics, San Francisco, USA

⁶Massachusetts General Hospital, Physical Medicine & Rehabilitation, Boston, USA

Introduction: Traumatic brain injury (TBI) is a heterogeneous condition that evolves over time. Current approaches to patient stratification (e.g., Glasgow Coma Scale [GCS]) and outcome assessment (e.g., cross-sectional Glasgow Outcome Scale Extended [GOSE]) may not adequately capture this heterogeneity, thus undermining success of clinical trials. Our aim was to use data-driven analytics to identify functional outcome trajectories after TBI to inform design of improved clinical trial endpoints.

Methods: In this secondary analysis of the failed Citicoline Brain Injury Treatment Trial (COBRIT), we used latent class growth analysis (LCGA) to describe multiple distinct 6-month functional trajectories (using 1-, 3-, and 6-month GOSE) among 1,045 patients across the spectrum of age (18-71y) and TBI severity (mild-complicated to severe). LCGA is a data-driven analytic that identifies groups of patients with similar trajectories allowing for visualization of multiple distinct recovery patterns that best capture heterogeneity in the population. Using chi-square tests, we assessed whether treatment arm (citicoline vs. placebo), age-category (<30 y; 30-54 y; 55+y), or TBI severity (GCS <13; 13-15) predicted GOSE trajectory group membership.

Results: The 4-group model showed the best fit. Visualization of average GOSE trajectories of patients in each group revealed 4 distinct patterns: (1) "good-decliners" with good recovery then marginal decline (n=149), (2) "moderate-improvers" with moderate disability then good recovery (improved >2pts; n=103), (3) "severe-improvers" with severe disability then moderate disability (improved >1pt; n=699), and (4) "severe-maintainers" with severe disability who remained stable (n=94). Trajectory group membership was predicted by TBI severity (p<0.001), but not by treatment (p=0.997) or age-category (p=0.132).

Conclusion: The "severe-improvers" were not captured in COBRIT's endpoints, which dichotomized GOSE at lower-good-recovery. 6-month trajectories or change over time may be more sensitive to clinically relevant recovery after TBI than cross-sectional dichotomized measures.

Keywords: traumatic brain injury, functional outcome, data-driven

B07-03

USING CLASSIFICATION REGRESSIONS TREES TO EVALUATE CLINICAL AND SYSTEMIC BIOMARKER RISK FACTORS FOR TRAUMATIC BRAIN INJURY MORTALITY

Raj Kumar¹, Zhensheng Wang¹, Prerna Ranganathan¹, Amy Wagner^{1,2}

¹University of Pittsburgh, Physical Medicine and Rehabilitation, Pittsburgh, USA

²University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

Mortality rates after severe traumatic brain injury (TBI) are considerable. However, few models accurately predict TBI-associated mortality risk, thereby limiting how to apply reliable baseline risk stratification methods to clinical trials. This knowledge gap may partially explain historical failures with identifying effective TBI neuroprotective agents with previous clinical trials. Classification Regression Trees (CRT) utilize decision-learning to determine optimal variables, and corresponding cut-points that are hierarchically ordered according to their prognostication capacity to discriminate clinical outcomes. We leveraged CRT to determine demographic, clinical (head/non-head injury severity), and acute systemic biomarkers known previously associated with mortality [estradiol (E2), testosterone (T), E2:T ratio, tumor necrosis factor- α (TNF α)], and the CNS marker S100 β , to predict 6-month mortality among N=188 individuals with severe TBI. There was a 26.6% overall 6-month mortality rate. A preliminary CRT showed the strongest mortality predictor was age \pm 42; therefore, primary analyses included two age-stratified CRT for those above (N=73) and below (N=115) age 42. The model for age <42 captured area under the curve (AUC) of 0.91. S100 β was the strongest predictor (cut-point: 10.9pg/mL), with higher levels increasing mortality risk. Among those with high S100 β , high E2 (cut-point: 109.5pg/mL) was associated with greater mortality risk. Whereas those with lower S100 β (<10.9pg/mL), high TNF α (cut-point: 21.2pg/mL) and less non-head injury severity was associated with mortality. The model for age \geq 42 captured an AUC of 0.90. GCS above/below 6 was the strongest predictor. Among those with GCS <6, more men died than women. For those with GCS \geq 6, higher TNF α (cut-point: 11.3pg/mL), followed by less severe non-head injury severity, was associated with mortality. CRT generated strong mortality baseline mortality risk models. However, mortality risk captured by systemic and CNS biomarkers varies considerably by age. Similar methodologies may be considered for early assessment of baseline mortality risk to facilitate pre-randomization cohort stratification with clinical trials. Support: DoD-W81XWH-071-0701; NIDILRR-90DP0041; R49-CCR323155.

Keywords: Statistical Modeling, Hormone Physiology, Mortality Prognostication, Moderate to Severe Traumatic Brain Injury

B07-04

MATHEMATICAL MODELING OF NEUROINFLAMMATION IN SEVERE CLINICAL TRAUMATIC BRAIN INJURY

Leah Vaughan^{1,2}, Prerna Ranganathan¹, Raj Kumar¹, Jonathan Rubin², Amy Wagner^{1,3}

¹University of Pittsburgh, Physical Medicine & Rehabilitation, Pittsburgh, USA

²University of Pittsburgh, Mathematics, Pittsburgh, USA

³University of Pittsburgh, Safar Center for Resuscitation Research, Pittsburgh, USA

Understanding the acute interdependencies of inflammatory mediators following traumatic brain injury (TBI) is essential to providing effective care for TBI patients and minimizing tissue damage. Relative CNS concentrations of cytokines and activated microglia reflect many elements of the complex cascades associated with acute neuroinflammation post-TBI and are often predictive of outcome. However, TBI clinical studies to date have not focused on modeling dynamic temporal patterns of simultaneously evolving inflammatory mediators, which has potential in guiding the design of future immunomodulation studies. We derived a mathematical model of ordinary differential equations (ODE) to represent interactions between pro- and anti-inflammatory cytokines, M1- and M2-type microglia and CNS tissue

damage. We incorporated cytokine variables, interleukin (IL)-1 β , IL-12, IL-10 and IL-4, known to have an active role in microglial activation and phenotype differentiation. The model was fit to cerebrospinal fluid (CSF) cytokine data, collected the first 5 days post-injury from severe TBI individuals. To address the heterogeneity of CSF inflammatory profiles among individuals in the acute recovery phase of TBI, we performed principal components analysis (PCA) on CSF cytokine samples, which identified inflammatory markers that contributed most to this variance. Patients were clustered by PC score into groups with similar CSF inflammatory profiles day 0-3 post-TBI, and then subdivided by 6-month Glasgow Outcome Score (GOS). These classifications identified three patient subgroups – a fair outcome group (GOS=4,5) and poor outcome group (GOS=2,3) with lower inflammatory loads, and a poor outcome group (GOS=2,3) with a higher inflammatory load. Optimal model fits to data showed different microglial and damage responses by subgroup. Significant differences in parameter distributions were identified statistically, suggesting evidence of mechanistic differences underlying the neuroinflammatory responses of each patient subgroup. Through mathematical characterization and data-driven processes, we have created a system for exploring links between acute neuroinflammatory components and patient outcome.

Keywords: mathematical modeling, CSF inflammatory mediators, microglia, TBI outcome

B07-05

DEVELOPING AN AUTOMATED SYSTEM TO PREDICT ICU LENGTH OF STAY FOR TBI PATIENTS WITH SUBDURAL HEMATOMA

S.M. Reza Soroushmeh^{2,3}, Negar Farzaneh^{1,2}, Craig Williamson^{2,4}, Kevin Ward^{2,3}, **Heming Yao**¹, Kayvan Najarian^{1,2,3}

¹University of Michigan, Department of Computational Medicine and Bioinformatics, Ann Arbor, USA

²University of Michigan, Michigan Center for Integrative Research in Critical Care University of Michigan, Ann Arbor, USA

³University of Michigan, Department of Emergency Medicine, Ann Arbor, USA

⁴University of Michigan, Departments of Neurosurgery and Neurology, Ann Arbor, USA

Traumatic brain injury (TBI) is a major cause of death and disability among young adults and a serious public health problem in the U.S. as well as developing countries. Outcome evaluation of traumatic brain injuries and predicting the ICU length of stay (LoS) could be beneficial in reducing complications, examining the efficiency of ICU care and hence improving the quality of care for head trauma patients. Here, we focus on TBI patients who have been diagnosed with subdural hematoma (SDH) and develop an automated system to predict the ICU-LOS. We employ demographic, clinical and injury information to develop our predictive model. Demographic data (e.g. age and gender), injury severity score, imaging information, health history, hemorrhage width, hemorrhage location and dementia are among the information that we used to train a Random Forest model. We used the information of 200 patients admitted to the University of Michigan Hospital and classified these patients to two categories; 1) staying at ICU for less than three days and 2) staying at ICU for at least 3 days. For the purpose of validation we used 11-fold cross validation and achieved the area under curve (AUC), specificity and sensitivity of 0.78, 0.47 and 0.88 respectively.

Keywords: subdural hematoma, ICU length of stay, prediction, machine learning

B08 CONCUSSION / MTBI

B08-01

MILD TRAUMATIC BRAIN INJURY INCREASES HISTAMINE-3-RECEPTOR EXPRESSION

Amarachi Oleru¹, Alexander Edwards¹, Megan Lerner³, **Hibah Awwad**^{1,2}

¹University of Oklahoma Health Sciences Center, Pharmaceutical Sciences, Oklahoma City, USA

²Oklahoma Center for Neuroscience, Oklahoma City, USA

³University of Oklahoma Health Sciences Center, Surgery, Oklahoma City, USA

Following a TBI, shear force and stress to brain tissue releases a plethora of neurotoxic factors in addition to glutamate that contribute to TBI pathology and a worse prognosis, if left untreated. However, histamine release in cerebral ischemia alleviates neuronal apoptosis and improves neurological function and histamine-3 receptors (H₃R) are implicated in neuronal disorders to modulate memory, pain and sleep. The specific objective of this study was to quantify changes in H₃R and to correlate these changes with pain and vestibulomotor deficits following a mild TBI (mTBI) in Sprague-Dawley male rats compared to sham rats. Brains were extracted from rats euthanized on day 1 or day 8 post-TBI using the controlled cortical impact model. Levels of H₃R and injury proteins were quantified in the ipsilateral (left) and contralateral (right) sensory cortex (SC) using immunoblotting analysis. H₃R levels were significantly increased in the ipsilateral SC compared to the contralateral SC of mTBI rats and sham rats (n=3-4). Rotarod performance, tactile allodynia and thermal hyperalgesia were assessed up to day 8 post-injury/sham. mTBI-induced vestibulomotor deficits were transient and rats showed complete recovery within 7 days of injury, whereas the same animals did not recover from mTBI-induced neurological deficits, tactile allodynia and thermal hyperalgesia during that same post-injury period. Reactive astrogliosis and degranulated mast cells were detected in mTBI rats at day 1 post-injury using immunohistochemistry. In conclusion, changes in H₃R levels seem to correlate with the histopathology of reactive astrogliosis and neuroinflammation as well as increased pain sensitivity following a mild TBI.

Funding was provided by: University of Oklahoma College of Pharmacy seed grant (HOA), American Association of Colleges of Pharmacy (AACP) New Investigator award (HOA) and summer INBRE funds by the National Institutes of Health, National Institute of General Medical Sciences (Grant 1U54GM104938), an IDeA-CTR to the University of Oklahoma Health Sciences Center.

Keywords: mild traumatic brain injury, histamine receptor, rotarod, pain, rats

B08-02

AMYGDALA CIRCUIT DYSFUNCTION WITH STRUCTURAL AND MOLECULAR PATHOLOGY COINCIDE WITH LATE-ONSET TBI-INDUCED AFFECTIVE SYMPTOMS

Joshua Beitchman^{1,2,3}, Daniel Griffiths^{1,2}, Ann Hoffman⁴, Yerin Hur², P. David Adelson^{1,2}, Jonathan Lifshitz^{1,2}, Theresa Currier Thomas^{1,2}

¹BARROW Neurological Institute at Phoenix Children's Hospital, Phoenix, USA

²University of Arizona-COM-Phoenix, Phoenix, USA

³Midwestern University, Glendale, USA

⁴UCLA, Los Angeles, USA

Affective symptoms, including anxiety and post-traumatic stress disorder, are reported in up to 50% of traumatic brain injury (TBI)

survivors. The etiology and persistence of these affective symptoms remains unknown which often makes treatment options ineffective. However, the amygdala processes affective behaviors and demonstrates increased hyperexcitability post-injury. We therefore hypothesized experimental diffuse TBI produces amygdala circuitry dysfunction coinciding with structural and molecular pathology as explanations for late-onset affective symptomatology. Adult, male Sprague-Dawley rats were subjected to midline fluid percussion injury or sham surgery. Anxiety-like behavior was assessed using open-field at 1wk or 1mo post-injury. Immediately following testing, micro-electrode arrays placed in the basolateral amygdala (BLA) and central nucleus of the amygdala (CeA) quantified real-time glutamatergic neurotransmission as an index of amygdala circuit function. Circuit structure was evaluated histologically by Golgi, silver, and GFAP stains to assess neuronal complexity, neuropathology, and astrogliosis. We found brain-injured rats spent significantly less time in the center of the open-field compared to shams at 1mo but not 1wk post-injury. This late-onset, anxiety-like behavior coincided with a significant decrease in CeA glutamate release and significantly slower CeA extracellular glutamate clearance in brain-injured rats compared to shams at 1mo post-injury. Pyramidal neurons of BLA-CeA circuitry significantly increased dendritic complexity over 1mo post-injury compared to shams. Increased complexity occurred without neuropathology and transient astrogliosis at 1wk post-injury, but not 1mo post-injury, compared to shams. Together, diffuse TBI resulted in late-onset, anxiety-like behavior coinciding with dysfunctional neurotransmission in the amygdala circuit comprised of hypertrophied pyramidal neurons and limited pathology. Subsequent studies of glutamate receptors and transporter levels in the CeA may identify molecular mechanisms. These novel findings demonstrate TBI-induced dysregulation of the amygdala circuit may be a plausible therapeutic target to alleviate affective symptoms for TBI survivors. Funding-ABRC(ADHS14-00003606)-VRP(P1201607)-PCH Director's Fund

Keywords: Diffuse TBI, amygdala, anxiety, glutamate, neuron morphology

B08-03

MILD TRAUMATIC BRAIN INJURY-INDUCED ALCOHOL USE: A ROLE FOR CLASS IIA HISTONE DEACETYLASES

Amanda Bolton Hall^{1,2}, Brandy Schneider^{1,2}, Jennifer Charlton^{1,2}, Laura Susick^{1,2}, Patrick O'Shea^{1,2}, Shane Perrine³, Alana Conti^{1,2}

¹Wayne State University, Neurosurgery, Detroit, USA

²John D. Dingell VAMC, Research and Development, Detroit, USA

³Wayne State University, Psychiatry and Behavioral Neurosciences, Detroit, USA

Traumatic brain injuries (TBIs) increase the risk of developing alcohol use disorders. Using a mouse model of mild TBI (mTBI), we demonstrate that mTBI increases binge and chronic ethanol consumption and chronic ethanol preference compared to sham animals. The mechanism by which TBI escalates drinking behaviors is unknown. However, histone hypoacetylation has been observed following either contusive TBI or ethanol consumption, suggesting a key role for the epigenetic modulators, histone deacetylases (HDACs). Class IIA HDACs are robustly expressed in the brain and regulate synaptic signaling through phosphorylation-induced nuclear-cytoplasmic translocation. Disruption of this balance alters the response to abused substances. Thus, we hypothesized that mTBI will alter Class IIA HDAC cellular localization and that mTBI-induced alcohol use will be significantly reduced following treatment with MC1568, a Class IIA specific inhibitor. Here we show that HDAC4, a specific Class IIA HDAC, is increased in the anterior striatum, a critical region of the

reward system, 30d following mTBI compared to sham-injured mice (n=4/group). However, the ratio of cytoplasmic HDAC4 (pHDAC4) to total HDAC4 was decreased in mTBI mice compared to sham-injured mice. Chronic intermittent ethanol exposure (CIE) mTBI mice (n=7) decreased expression of total HDAC4 compared to CIE sham-injured mice (n=8). Yet, the ratio of pHDAC4:total HDAC4 was similar between CIE groups and mTBI alone, suggesting an increase in nuclear HDAC4 versus cytoplasmic HDAC4 after mTBI and/or ethanol exposure. Next, naïve mice were injected with increasing doses of MC1568 (10–40 mg/kg) or vehicle (n=7–8/group) 2h prior to and/or just before binge ethanol exposure. Daily ethanol consumption was decreased in MC1568-treated mice compared to vehicle-treated mice. Future studies will evaluate the therapeutic efficacy of MC1568 in mitigating increased ethanol consumption following mTBI. Supported by the John Dingell VAMC, Detroit, MI.

Keywords: Mild TBI, Alcohol, Epigenetics, Histone Deacetylase, MC1568

B08-04

SHORT- AND LONG-TERM COGNITIVE DEFICITS IN PEDIATRIC MILD TRAUMATIC BRAIN INJURY

Andrew Dodd¹, Christopher Wertz¹, Richard Campbell², Ronald Yeo², Keith Yeates³, Grace Park⁴, John Phillips^{1,2}, Scott Oglesbee⁴, Jason Cromer⁵, Benjamin Wasserott¹, Sephira Ryman^{1,2}, Andrew Mayer^{1,2}

¹The Mind Research Network/LBERI, Albuquerque, USA

²UNM, Psychiatry, Psychology, or Neurology, Albuquerque, USA

³University of Calgary, Psychology, Calgary, Canada

⁴UNM Hospital, Pediatric Emergency Medicine, Albuquerque, USA

⁵Yale University Child Study Center, New Haven, USA

Although cognitive skills are known to increase as a function of maturation during adolescence, the impact of pediatric mild traumatic brain injury (pmTBI) on cognitive trajectories is still actively debated. Clinical and neuropsychological data were collected from 41 youth (12–18 years old [y.o.]) with sub-acute (6.5 ± 2.4 days post-injury) pmTBI and 37 age- and sex-matched healthy controls (HC). Data have also been collected for 21 pmTBI and 22 HC at early chronic phase (i.e., four months post-injury) in this ongoing study, with additional follow-up planned at one year post-injury. Participants completed computerized (CogState Brief Battery) and traditional neuropsychological tests, including effort and estimated pre-morbid functioning (single-word reading). Participants and their parents also completed current and retrospective versions of the Post-Concussive Symptom Inventory. Premorbid function was higher in HC compared to pmTBI. Relative to HC, pmTBI exhibited impaired performance on the CogState attention task and on composite measures of working memory and executive function (p 's < 0.05), with trends for decreased processing speed (p 's < 0.10). Both pmTBI and their parents endorsed increased post-concussive symptoms following injury relative to HC when compared to retrospective recollection (p 's < 0.05). Reduced attention and working memory in pmTBI were associated with parental report of symptom burden (p 's < 0.05). Follow-up analysis at four months indicated attention deficits persisting into the early chronic phase ($p = 0.002$), with working memory reduced to a trend ($p = 0.080$). Patient-reported post-concussive symptoms remained elevated in pmTBI ($p = 0.047$), albeit at much lower magnitude of difference, whereas parental report fell to trend level ($p = 0.053$). In summary, current results suggest cognitive dysfunction in the sub-acute injury phase that decreases, but does not fully resolve, at four months post-injury.

Keywords: pediatric mild traumatic brain injury, post-concussive symptoms, neuropsychological battery, longitudinal

PAIRED SUPPORT VECTOR MACHINE CLASSIFICATION SHOWS PERSISTENT LONGITUDINAL CHANGES IN FUNCTIONAL CONNECTIVITY FOLLOWING MTBI

Jason Druzgal¹, Amanda Stanton¹, Christina Gancayco¹, Bryson Reynolds², Thomas Eluvathingal Muttikkal¹

¹University of Virginia, Radiology and Medical Imaging, Charlottesville, USA

²Vanderbilt University Medical Center, Institute of Imaging Science, Nashville, USA

Multiple studies have shown mTBI causes functional brain abnormalities detectable by resting state functional MRI (rs-fMRI), with some suggesting imaging abnormalities outlast clinical recovery. These studies generally utilize mass-univariate analysis of case-control data, an approach that limits sensitivity for heterogeneous findings across individuals. We hypothesized that delayed imaging recovery after mTBI would be more evident in longitudinally acquired rs-fMRI data analyzed with multivariate methods. This hypothesis was tested using the Maryland MagNeTS prospective cohort available through FITBIR. MagNeTS collected MRI data on TBI patients ranging from GCS 3–15 at four time points (within 10 days of injury, 1-month, 6-month, and 18-month). Our analysis examined the 30 patients with GCS ≥ 13 , no MRI evidence of intracranial hemorrhage, and rs-fMRI data at all time-points. For each rs-fMRI dataset, we calculated six voxelwise connectivity metrics progressing from short-to-long range: amplitude of low frequency fluctuation (ALFF), fractional ALFF (fALFF), regional homogeneity (ReHo), voxel mirrored homotopic connectivity (VMHC), binarized degree centrality (bDC), and weighted degree centrality (wDC). Paired support vector machine (SVM) classification compared individuals across time-points. Comparing injury to 18-month, significant SVM classification accuracy was found for all connectivity metrics (ALFF=93%, fALFF=73%, ReHo=77%, VMHC=70%, bDC=73%, wDC=73%). Significant classification accuracy was also found for all metrics when comparing 1-month to 18-month (ALFF=93%, fALFF=73%, ReHo=83%, VMHC=73%, bDC=73%, wDC=73%). However, comparing injury to 1-month performed at chance, and injury to 6-month showed a non-significant trend toward successful classification for all metrics. The results suggest that rs-fMRI connectivity changes related to mTBI are detectable in individuals when longitudinal individual reference data is available. In most patients, the rs-fMRI connectivity changes persist beyond 1 month, with partial normalization by 6 months, and complete normalization by 18 months. These findings demonstrate how the timeline of imaging recovery from mTBI extends beyond the typically described timeline for clinical recovery.

Keywords: fMRI, machine learning, resting state, connectivity, longitudinal, FITBIR

B08-06

BIOMECHANICS OF CONCUSSION, TRAUMATIC BRAIN INJURY, AND CHRONIC TRAUMATIC ENCEPHALOPATHY IN A MOUSE MODEL OF CLOSED-HEAD IMPACT

Erich Franz¹, Chad Tagge¹, Andrew Fisher¹, Olga Minaeva¹, Mark Wojnarowicz², Juliet Moncaster², Andrew Anderson⁴, Robin Cleveland⁵, William Moss⁴, Lee Goldstein^{1,2,3}

¹Boston University, College of Engineering, Boston, USA

²Boston University, School of Medicine, Boston, USA

³Boston University, Alzheimer's Disease Center, CTE Program, Boston, USA

⁴Lawrence Livermore National Laboratory, Livermore, USA

⁵University of Oxford, Institute of Biomedical Engineering, Oxford, UK

The mechanisms underpinning concussion, traumatic brain injury (TBI), and chronic traumatic encephalopathy (CTE), and the relationships between these disorders, are poorly understood. To investigate causal mechanisms, we developed a mouse model of closed-head impact injury that uses momentum transfer to induce traumatic head acceleration. Unanesthetized mice subjected to unilateral impact exhibited abrupt onset, transient course, and rapid resolution of impaired arousal, contralateral hemiparesis, truncal ataxia, locomotor and balance impairments, and neurobehavioral deficits resembling human concussion. Acute neuropathological neuroexamination of brains from impact-injured mice demonstrated acute and chronic neuropathologies, including phosphorylated tau proteinopathy, that recapitulates early CTE pathologies in humans. Impact-induced CTE brain pathologies are also similar to brain pathology in blast-exposed mice and humans. Moreover, acute neurobehavioral signs of concussion were observed only after impact injury but not after blast exposure under conditions matched for head kinematics. Experimental and computational modeling showed that impact generated ipsilateral point loading on the head and seven-fold greater peak shear stress in the brain compared to blast. By comparison, blast induced distributed force loading on the head and diffuse, lower magnitude shear stress in the brain. We conclude that fast-acting, high-amplitude cortical shear stress triggers experimental concussion, whereas longer duration, lower amplitude shear stress associated with head motion induces structural brain damage and neuropathological sequelae. These results suggest that closed-head impact injuries, independent of concussive signs, can induce TBI as well as early pathologies and functional sequelae associated with CTE. These findings elucidate the origins of concussion and differentiate this condition from TBI and sequelae. Funding: NIH, DoD, WWE, Concussion Legacy Foundation, private foundation.

Keywords: Impact, Chronic Traumatic Encephalopathy, Modeling, Blast, Neurobehavior

B08-07

SPORTS-RELATED CONCUSSION COMMON DATA ELEMENTS VERSION 1.0 RECOMMENDATIONS

Kristen Joseph¹, Claudia Moy², Sherita Ala'i¹, Joy Esterlitz¹, Patrick Frost Bellgowan², Sports-Related Concussion CDE Working Group²

¹The EMMES Corporation, Rockville, USA

²National Institutes of Health, NINDS, Rockville, USA

Objective: The DOD and NINDS initiated the development of TBI Sports-Related Concussion (SRC) CDEs as part of the NINDS project to develop data standards for funded clinical research in neuroscience. The purpose of CDEs is to increase the efficiency and effectiveness of clinical research studies and treatment, facilitate data sharing across studies, significantly reduce study start-up time, and more effectively aggregate information into significant metadata results.

Background: In 2016 the Sports-Related Concussion (SRC)-specific CDE working group (WG) was created to identify CDEs, template case report forms (CRFs), data dictionaries and guidelines to assist investigators who are initiating and conducting SRC clinical research studies.

Design/Methods: The SRC CDE WG consists of 34 worldwide experts with varied fields of related expertise divided into three Subgroups: Acute, Subacute and Persistent/Chronic. The WG met regularly to reviewed various existing CDE domains, selecting CRFs and field-tested data elements from national registries and funded research studies.

Results: Following an internal WG review along with public comments received, Version 1.0 of the SRC CDEs will be available on the NINDS CDE website in May 2017. The recommendations included are required and strongly recommended CDEs or instruments for cognitive measures and symptom checklists, as well as, other outcomes and endpoints and sample case report forms for domains typically included in clinical research studies.

Conclusion: The NINDS CDEs are a continually evolving resource, requiring updates as research advancements indicate. The NINDS encourages the use of the SRC Version 1.0 CDE recommendations by the clinical research community in order to standardize data collection across studies.

Support: This material is based upon work supported by the U.S. Army Medical Research and Materiel Command's Combat Casualty Care Research Program and also funded by HHSN271201200034C.

Keywords: Common Data Elements, Sports-related, Concussion, CDE

B08-08

CHRONIC NEUROBEHAVIORAL AND NEUROPATHOLOGICAL OUTCOMES FOLLOWING REPETITIVE HEAD IMPACTS IN A TRANSGENIC MOUSE MODEL

Mihika Gangolli¹, Theodore Floros², Thomas J. Esparza², Emeka M. Fountain², David L. Brody²

¹Washington University in St. Louis, Biomedical Engineering, St. Louis, USA

²Washington University in St. Louis, Neurology, St. Louis, USA

Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disease that has been increasingly linked to patients who have sustained one or more head impacts. The unique neuropathological features of CTE, including phosphorylated tau tangles and tau positive astrocytes in sulcal depths and perivascular regions, that distinguish it from other tauopathies such as Alzheimer's disease and frontotemporal dementia are currently identified using immunohistochemistry, making CTE a post-mortem diagnosis. An animal model that reliably recapitulates the long term tau pathology of CTE would be highly beneficial not only in finding noninvasive diagnostic biomarkers, but also in evaluating the efficacy of potential therapeutics. We therefore have implemented the CHIMERA injury paradigm in a transgenic mouse line expressing all six isoforms of human tau.

Four month old mice were exposed to daily subconcussive (2.1 psi) or concussive (3.0 psi) head impacts for twenty consecutive days, with loss of consciousness measured by the loss of righting reflex (LRR). Neurobehavioral measures of sociability, anxiety, depression, learning and memory were then evaluated acutely, three months, and one year post-injury. Cerebrospinal fluid, plasma and brain tissue were then collected from the mice and analyzed for chronic markers of tau, axonal injury, microgliosis, and astrogliosis. Compared to uninjured shams, mice in the 3.1 psi group showed an initial increase in LRR following the second impact, which decreased and plateaued by the final day of injury. There were no differences related to time or injury when assessing changes in social activity or anxiety related activity at acute or chronic time points. Interestingly, mice exposed to repetitive concussive injuries showed progressive deficits in Morris Water Maze during acquisition and probe trial sessions. These preliminary data may provide improved understanding of the relationship between repetitive head impacts, progressive behavioral deficits, and underlying neuropathology.

Keywords: Non Surgical Repetitive TBI, Chronic phosphorylated tau pathology, Astrocytosis, White matter injury

B08-09

HOSPITALIZATION RATES IN THE YEAR FOLLOWING CONCUSSION

Seth Seabury¹, **Etienne Gaudette**¹, Dana P. Goldman¹, Khristina Ipapo¹, Geoff Manley², the TRACK-TBI Investigators

¹University of Southern California, Schaeffer Center, Los Angeles, USA

²University of California San Francisco, Department of Neurological Surgery, San Francisco, USA

We assessed hospitalization rates and expected medical costs in the 6-12 months following a concussion. We identified patients aged 18 to 65 with an inpatient or emergency department visit with a primary or secondary diagnosis of concussion from 2011–2013 in a 25% sample of the Optum Clinformatics Data Mart (n=1,872). We matched these to uninjured control patients according to age, gender, and insurance plan type (n=15,427). We then compared hospitalization rates of concussed patients and controls 6 and 12 months following the index date, defined as the date of discharge for the index trauma event. To control for differences in trauma and control patients, we modeled hospitalizations using multivariate logistic regression. Other covariates included age, sex, race, education, household income, the prevalence of chronic health conditions and total medical spending in the 6 months prior to the index event. In the 6 months following the index event, 6.1% of concussion patients experienced a hospitalization compared to 2.3% of control patients without a traumatic brain injury. After adjusting for patient characteristics using logistic regression, concussed patients had a higher probability of being hospitalized 6 months (OR 2.18, 95% CI [1.73–2.73]) and 12 months (OR 2.53, 95% CI [1.90–3.38]) after the index trauma event compared to uninjured control patients. We conclude that concussions are associated with significantly higher rates of hospitalization in the year following injury, even after accounting for differences at baseline. This implies that patients should be closely monitored after discharge.

Keywords: Concussion, Rehospitalization, Health Care Utilization

B08-10

SECONDARY INFLAMMATION AND NEUROPATHOLOGY AFFECT CEREBELLAR FUNCTION FOLLOWING DIFFUSE TBI

Katherine Giordano^{1,2}, Murtaza Akhter^{1,2,3}, Jonathan Lifshitz^{1,2}, Rachel Rowe^{1,2}

¹BARROW Neurological Institute at Phoenix Children's Hospital, Phoenix, USA

²University of Arizona-COM-Phoenix, Phoenix, USA

³Maricopa Integrated Health System, Phoenix, USA

Survivors of diffuse traumatic brain injury (TBI) have impairments in postural control, balance, and motor coordination, which impede rehabilitation efforts and lower quality of life. While primary injury to the cerebellum impairs acute movement, few studies have investigated cerebellar vulnerability to secondary consequences of TBI. Here, we hypothesize that injury-induced inflammation and neuropathology contribute to acute cerebellar functional deficits. Adult male rats were subjected to midline fluid percussion injury (mFPI) or sham surgery and assessed for motor function and coordination (1, 2, 7days post-injury; DPI). Tissue was collected and stained with Iba1 to assess microglial ramification and silver stain for neuropathology. Diffuse TBI resulted in motor and coordination deficits assessed by the rotarod and bilateral tactile adhesive removal task. Brain-injured rats had a significantly

decreased latency to fall off the rotarod at 1 and 2DPI and a decreased distance traveled at 1, 2, and 7DPI compared to uninjured shams. The time to make contact and remove the adhesive was significantly increased in injured rats at 1 and 2DPI compared to uninjured shams but was resolved by 7DPI. Semi-quantitative histological assessment for neuropathology revealed 'stripes' of activated microglia in the molecular layer of the cerebellar cortex at 1, 2, and 7DPI. Stripes were defined as four or more activated cell bodies clustered perpendicular to the cortical surface. Regions with microglial stripes corresponded to regions of silver-stained neuropathology in the same brain-injured rats at identical time points post-injury. The results support cerebellar vulnerability as a secondary consequence of diffuse TBI. From this pathology, acute motor and coordination deficits track the temporal profile of acute injury-induced neuroinflammation representing a plausible therapeutic target to improve postural, balance, and motor impairments in TBI survivors. Funding: PCH Mission Support

Keywords: diffuse traumatic brain injury, microglia, cerebellum, motor impairment

B08-11

TRAUMATIC BRAIN INJURY AND CONCUSSION: TIME FOR BRAIN FOOD

Bri Goslin

University of Southern California, Nutrition, Los Angeles, USA

Introduction: Given combat casualties, sports-related injuries, and motor vehicle accidents, traumatic brain injury (TBI) is the leading cause of death under age 44. TBI deficits linger in concentration, depression, sleep-wake cycle, behavior and motor skills. Basic science has long instructed that omega-3 fatty acids, specifically docosahexaenoic acid (DHA), improve cell membrane fluidity and signaling. B-complex vitamins provide nerve myelin substrate and neurotransmitter cofactors. The presence of all 20 amino acids is critical to cell membrane and neurotransmitter protein production.

Hypothesis: This systematic review hypothesizes that given ample substrates of DHA, B-complex vitamins, and elemental amino acids, the brain will promote tissue restoration.

Methods: A literature search of the PUBMED database identified current research using the keywords "omega-3-fatty acid", "B-complex vitamin", "elemental amino acids" with "TBI", and "MTHFR C67T".

Findings: DHA deficiency in the population may be as high as 80%. DHA, having more double bonds than vegetable oil, provides greater brain tissue strength. Researchers have found that DHA increases cognitive ability, motor function, membrane fluidity, cell signaling, and is neural protective. DHA enhances neuronal growth, transmission speed, dendritic formation, and synaptic maintenance. The American Society for Parenteral and Enteral Nutrition now suggests fish oil for trauma patients. Studies have found B-complex vitamin deficiencies in spinal cord injury patients. B-complex vitamins improve sensorimotor skills, cognitive function, memory, vision, and DNA methylation. Folate's role in DNA methylation may be reduced by genetic polymorphisms. Amino acids mediate cell damage, are neuro-protective, and decrease hippocampal deficits. Amino acids demonstrate increased cerebral blood flow and DNA methylation.

Conclusion: DHA, B-complex vitamins and elemental amino acids show evidence of improving TBI prognosis. These nutrients are a safe, effective manner to apply basic science to rebuild brain tissue and improve patient outlook.

Keywords: DHA, B-Complex Vitamins, Elemental Amino Acids, MTHFR C67T

B08-12

ANTI-INFLAMMATORY PROPERTIES OF NANO-PULSED LASER THERAPY (NPLT) AFTER TRAUMATIC BRAIN INJURY

Auston Grant¹, June Guptarak¹, Margaret Parsley¹, Ian Bolding¹, Kathia Johnson¹, Irene Petrov², Yuriy Petrov², Rinat Esenaliev^{2,3}, Donald Prough¹, Maria Micci¹

¹*University of Texas Medical Branch, Anesthesiology, Galveston, USA*

²*University of Texas Medical Branch, Center for Biomedical Engineering, Galveston, USA*

³*University of Texas Medical Branch, Department Neuroscience/Cell Biology, Galveston, USA*

Background: Each year in the US an estimated 1.7 million people are afflicted with a Traumatic Brain Injury (TBI). Neuro-inflammation is a common consequence of TBI in humans that can persist for years following the initial impact. We designed and built a novel, medical grade, Nano-Pulsed Laser Therapy (NPLT) system that combines near-infrared laser light (808 nm) and laser-generated, low-energy optoacoustic waves. In this study, we tested the ability of NPLT to reduce the activation of microglia (which play a crucial role in long term inflammation) in a rat model of TBI.

Methods: Adult male rats were randomly assigned to receive fluid percussion injury (FPI) or Sham surgery. NPLT was applied transcranially 1 hour after injury. The rats were euthanized 2 weeks after TBI and the brains were harvested and sent to NeuroScience Associates (NSA) for CD68 staining (a marker of activated microglia). CD68 staining was quantified using ImageJ area analysis. Total volumes of the cortex and of the cortical lesion (commonly seen in our FPI model) were also measured using ImageJ.

Results: NPLT significantly decreased CD68 staining in the thalamus. The volume of the cortex was significantly decreased in FPI rats compared to FPI rats treated with NPLT. NPLT also significantly reduced the cortical lesion volume. CD68 staining in the region surrounding the cortical lesion was significantly decreased in NPLT treated FPI rats compared to untreated FPI rats.

Conclusion: Our data show that NPLT reduces the activation of microglia and prevents brain volume loss in a rodent model of TBI, further supporting its therapeutic value for the treatment of brain injury survivors.

Funding: These studies were funded by The Moody Project for Translational Traumatic Brain Injury Research.

Keywords: Light, Optoacoustic, Neuroinflammation

B08-13

VASCULAR STRUCTURE OF TRAUMATIC MICROBLEEDS IN MTBI PATIENTS IMAGED AT 7 TESLA

Allison Griffin¹, Christine Turtzo², Martin Cota¹, Anita Moses¹, Neekita Jikaria¹, Lawrence Latour²

¹*CNRM/HJF/NIH, NINDS/SB, Bethesda, USA*

²*NIH, NINDS/SB, Bethesda, USA*

Traumatic microbleeds (TMBs) are small foci of hypointensity seen on T2* weighted MRI. TMBs were originally equated to diffuse axonal injury (DAI), in association with focal shearing of axons. Recent pathological evidence indicates that TMBs may be vascular in origin and indicative of broader microvascular injury. This study's purpose was to determine if 7T MRI could improve visualization of the vascular injury underlying TMBs. Subjects were enrolled in an arm of the

“THINC” study, with 3T MRI within 48 hours of injury and 7T MRI at 3 months and 1 year post-injury. Subjects met ACRM criteria for mTBI with evidence of injury on baseline MRI. A T2* weighted GRE (TR/TE = 12/700, resolution = $3.5 \times 3.5 \times 3.0$ mm, 4 minutes) and 3D SWI were acquired at 3T. A T2* weighted GRE (TR/TE = 32 & 15/1300, resolution = $1.0 \times 1.0 \times 1.0$ mm, 10 minutes) sequence was acquired at 7T. Two independent raters scored both 3T and 7T MRI sequences separately, for conspicuity and location. Over a period of 28 months, thirty-three patients were 7T arm eligible, and twelve patients (median GCS = 15, median age = 52.5) agreed to 7T, three female and nine male. Eight patients with punctate and/or linear microbleeds at baseline 3T were confirmed to have microbleeds on 7T. Clusters with multiple punctate TMBs across axial slices on 3T were visualized on 7T as connecting in branching structures most similar to vascular trees. These branching hypointensities were not visualized on 7T in any of the 4 patients who had no visible microbleeds on 3T. This study provides evidence that TMBs represent traumatic vascular injury rather than isolated axonal damage. Multiple punctate TMBs seen at 3T were visible in connected branching structures when visualized at 7T. These patterns likely represent traumatic injury to the microvasculature, resulting in extravasation of erythrocytes into perivascular spaces along vascular trees.

Keywords: Microbleeds, 7T MRI, Vascular Injury, mTBI

B08-14

ACUTE MITOCHONDRIAL DYSFUNCTION AFTER MILD TRAUMATIC BRAIN INJURY

Brad Hubbard¹, Anna Juras¹, Binoy Joseph¹, Hemendra Vekaria¹, Kathryn Saatman^{1,2}, Patrick Sullivan^{1,3}

¹University of Kentucky, Spinal Cord and Brain Injury Research Center, Lexington, USA

²University of Kentucky, Department of Physiology, Lexington, USA

³University of Kentucky, Department of Neuroscience, Lexington, USA

Traumatic brain injuries (TBIs) are a major public health concern with over 1.7 million TBIs reported each year in the US. Mild TBIs (mTBIs), accounting for over 80% of TBIs, can cause cognitive impairment. While it is known that mTBI does not cause widespread neuronal death, the mechanisms underlying neurological impairment and increased cellular susceptibility to subsequent head impacts are unknown. To investigate the hypothesis that altered mitochondrial bioenergetics following mTBI underlie cellular vulnerability to repeated insults, we employed a mouse model of closed head injury (CHI) to examine mitochondrial function after mTBI. A single CHI was produced by a pneumatically controlled impact device with a silicone tip at midline to model a bilateral diffuse injury. A novel object recognition (NOR) test was performed at 48 hours post-injury. Mitochondrial function was assayed from ventral (including entorhinal) cortex and hippocampus homogenates collected at 24 and 48 hours post-injury (n = 3-6/group). Oxygen consumption rates (OCRs) were measured from isolated mitochondria using a Seahorse XF24 Flux Analyzer. At 48 hours post-injury, recognition memory was significantly impaired in the CHI group compared to sham (p < 0.05). State III (ADP-mediated) respiration OCRs were significantly decreased in the hippocampal mitochondria of the CHI group compared to sham at 24 hours (p < 0.01) but not at 48 hours post-injury. Conversely, ventral cortex-derived mitochondria exhibited a delayed decrease in State III OCRs at 48 hours post-injury (p < 0.0001). No significant differences were observed in other respiration states. This study establishes that mTBI associated with

cognitive impairment results in early mitochondrial dysfunction which may have region-specific temporal characteristics. Future directions will expand the acute time course of mitochondrial bioenergetics.

Funding Sources: This project was supported by NSF EPSCoR Seed Grant 4978/111315, VA Merit Award 1101BX003405-01A1 and Kentucky Spinal Cord and Head Injury Research Trust Grant 14-13A.

Keywords: Mild traumatic brain injury, Mitochondrial dysfunction, Closed head injury, Cognitive impairment

B08-15

APPLICATION OF AN ACTIVITY TRACKER AND MOBILE APPLICATION TO TRACK ACTIVITY VERSUS REST FOLLOWING SPORT-RELATED CONCUSSION

Daniel Huber, Michael McCrea, Lindsay Nelson

Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

Clinical management guidelines for sport-related concussion (SRC) emphasize initial rest followed by a gradual return to activities. How to operationalize “rest,” however, is unclear, because the relationship between activity and SRC recovery is complex and dynamic. The aim of this pilot study was to develop and implement procedures for tracking activities after SRC and to explore associations between activities and recovery. High school and collegiate football players (25 concussed, 15 uninjured contact-controls) enrolled within 48 hours of injury and provided data daily for two weeks through a survey embedded into a smartphone application and a commercial fitness tracker (Fitbit). Metrics collected included magnitude and duration of physical activities, mental activities, sleep, and recovery. Completion rate of the daily data was 85%. As expected, concussed athletes reduced their activity levels (relative to controls) acutely after injury and gradually increased them to normal over the first two weeks post-injury, with somewhat different metrics for the Fitbit and mobile survey showing this pattern. Furthermore, there was significant variability across athletes in activities and recovery rates. For example, about 1/3 of athletes endorsed doing an activity that they believed would not be approved by their athletic trainers or doctors in the first week, and 3/4 avoided an activity that they believed could harm their recoveries. Endorsement rates of activity-related symptom exacerbation were extremely high in the first several days post-injury and diminished over time. However, endorsement of an unapproved activity or activity-related symptom exacerbation was not associated with recovery time. The results support the use of these technologies to remotely track athletes’ activities and suggest that activity that follows a graded exertion protocol or athletes’ judgment may be safe for clinical recovery after SRC. Future studies will evaluate the subset of athletes (nearly 1/3) who engaged in vigorous activity while symptomatic and will gather more information about the types of avoided and unapproved activities.

Keywords: concussion, sport, activity

B08-16

CONNECTIVITY DOMAIN ANALYSIS ALLEVIATES CROSS-CENTER DIFFERENCES AND IMPROVES DIAGNOSIS OF MILD TBI

Armin Iraj¹, Jiachen Zhuo², Rao Gullapalli², Natalie Wiseman³, E. Mark Haacke^{1,4}, Zhifeng Kou^{1,3,4}

¹Wayne State University, Department of Biomedical Engineering, Detroit, USA

²University of Maryland, Diagnostic Radiology and Nuclear Medicine, Baltimore, USA

³Wayne State University, Department of Psychiatry and Behavioral Neurosciences, Detroit, USA

⁴Wayne State University, Department of Radiology, Detroit, USA

Identification of biomarkers for mild traumatic brain injury (mTBI) diagnosis and outcome prediction is challenging due to the heterogeneity of mTBI patients. Multi-center studies help to alleviate this, if we can reduce the between-center difference. This is particularly true of functional MRI studies. In this study, we demonstrated the advantage of our recently-developed approach, the connectivity domain analysis (CDA), in reducing susceptibility to cross-center heterogeneity and improving prediction accuracy of classification models using two datasets, from Wayne State University (WSU) and University of Maryland School of Medicine (UMD), with a total of 75 controls and 63 mTBI patients. Independent component analysis (ICA) was applied to the time-domain data and the transformed connectivity domain data to extract the intrinsic brain network features, which were then used to classify patients and controls using five different methods. For all investigated classifiers, CDA significantly improved classification accuracy for single-center, combined, and between-center analyses compared to the conventional time domain analysis (TDA). For instance, for an artificial neural network (multilayer perceptron) classification with 10-fold cross-validation, the prediction accuracies for **CDA** and **TDA** were as follows: for WSU (**75.89 ± 1.68%**) and (**69.05 ± 3.45%**); for UMD (**72.25 ± 2.49%**) and (**70.00 ± 3.73%**); and for the combined data (i.e. WSU+UMD): (**73.70% ± 3.48%**) and (**65.33 ± 3.45%**), respectively. For between-center analysis, the prediction accuracies for **CDA** and **TDA** were (**75%** and **65%**) when the training data was WSU and test data was UMD and (**69.47%** and **54.74%**) when the training data was UMD and test data was WSU. In conclusion, CDA significantly improved accuracy of classification of mTBI patients and maintained it in a multi-center analysis. Multi-center analyses with large numbers of subjects may benefit from use of CDA for generation of outcome prediction models.

Keywords: multi-center analysis, connectivity domain analysis (CDA), classification

B08-17

BLOOD-CSF BARRIER DISRUPTION IN MTBI PATIENTS VISUALIZED USING DELAYED POST-CONTRAST MRI

Neekita Jikaria¹, Martin Cota¹, Anita Moses¹, Victoria Uche¹, Cristina Lawrence¹, Tara Davis², Alexa Tabackman¹, Ana Tinoco Martinez², L. Christine Turtzo³, Lawrence Latour^{3,1}

¹Center for Neuroscience and Regenerative Medicine, Bethesda, USA

²Johns Hopkins Suburban Hospital, Bethesda, USA

³NINDS/NIH, Bethesda, USA

Injury to meninges and vasculature occurs frequently following head trauma. The meninges conspicuously enhance on post-contrast FLAIR MRI in approximately half of mild traumatic brain injury (mTBI) patients imaged acutely. Enhancement in the falx and convexity typically is limited to the dura or subdural space; however, early, subtle enhancement has been detected in adjacent subarachnoid (SA) space. Here we study delayed contrast dispersion into SA following mTBI. Patients presenting to the emergency department were imaged within 6 hours of head injury on 3T MRI. Following administration of single-dose Gd-DTPA, FLAIR sequence (TR/TE/TI = 9000/120/2500, 0.47x0.47x3.5 mm voxels, 1:21 min) was acquired. Patients with en-

hancement of dura were reimaged with FLAIR within ~3 hours without additional contrast. Images were reviewed by two expert raters, blinded to order, for presence/absence of contrast enhancement in SA along falx. Values are n(%) or median(IQR). Over 15 months, 15 subjects with baseline enhancement of falx were studied; 9 (60%) male, median age 58 (40.5-63) years, GCS = 15 in 13 and 14 in 2. Time from injury to baseline MRI 4.4 (4.0-5.4) hours; time from contrast to second scan 2.8 (2.4-3.5) hours. From the blinded read, 2 (13%) patients had detectable enhancement in the SA adjacent to falx on baseline FLAIR and 7 (47%) on SA adjacent to the falx on delayed FLAIR. Extravasation of contrast with propagation/dispersion into SA was found in approximately half the patients studied, for an estimated prevalence of 1 in 4 mTBI patients. This phenomenon seen early after mTBI may be indicative of blood-CSF barrier disruption that permits substances in plasma to reach the pial surface of the brain, and as such, has the potential to be deleterious. Understanding the prevalence, pathological basis, and evolution may provide insight into a target for acute treatment.

Keywords: FLAIR, Acute, Delayed post-contrast imaging, BBB Disruption, Contrast Enhancement

B08-18

DETECTING SUBTLE COGNITIVE IMPAIRMENT IN RATS AFTER MODERATE FLUID-PERCUSSION INJURY

Kathia Johnson, Margaret Parsley, Ian Bolding, Bridget Hawkins, Donald Prough, Douglas DeWitt, Stacy Sell
University of Texas Medical Branch, Anesthesiology, Galveston, USA

Detecting cognitive impairment in rats after moderate traumatic brain injury can be problematic. Although there is histological evidence of damage produced by moderate fluid-percussion injury (FPI) in specific brain regions (e.g. hippocampus), corresponding cognitive dysfunction may not be detectable. Procedures that increase sensitivity of the Morris water maze, a widely used test of cognitive function, would be useful to overcome this challenge. In tests of working memory, increasing the delay time (inter-trial interval) between trial 1 (learning) and trial 2 (recall) typically increases the difficulty of the test. Here we tested the hypothesis that increasing the inter-trial interval in the working memory version of the Morris water maze would make this test more sensitive to FPI-induced working memory deficits. We compared two delay times (15-sec and 45-sec) in the working memory test between Trial 1 (finding the platform) and Trial 2 (remembering the platform location). Male Sprague-Dawley rats were anesthetized and intubated before a craniotomy was trephined lateral-right of the sagittal suture. Rats were randomly assigned to receive a moderate (2.0 atm) FPI (n = 13) or SHAM injury (n = 12) before the water maze testing including 15-sec or 45-sec inter-trial delay on post-injury days 11 – 15. Using a mixed model ANOVA, comparisons were made between FPI and SHAM for Trial 1, Trial 2, and Delta (Trial 1 – Trial 2) for each delay time. We also compared SHAM 15-sec to SHAM 45-sec and FPI 15-sec to FPI 45-sec. The results indicated that the longer inter-trial delay time of 45 sec did not produce a greater difference between SHAM and TBI than the standard 15-sec delay. This may be due to the poorer performance of SHAM rats tested with the 45-sec delay. Based on these findings, a longer inter-trial interval in this version of the working memory water maze was not more sensitive to FPI-induced deficits. These studies were funded by The Moody Project for Translational TBI Research

Keywords: Cognitive function, TBI, behavioral measure, water maze

PRE-INJURY PERSONALITY TRAITS PREDICT INCIDENCE OF CONCUSSION IN HIGH SCHOOL AND COLLEGIATE FOOTBALL PLAYERS

Anna Klotz, Jana Ranson, Brian D. Stemper, Alok S. Shah, Michael A. McCrea, Lindsay D. Nelson

Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

An estimated 300,000 sport-related concussions (SRCs) occur annually in the U.S., with the highest incidence in high school and collegiate football. About 25% of football SRCs are due to unsafe playing styles, making behavior—and the motives, attitudes, and personality traits that drive it—a credible SRC risk factor that is largely overlooked. The current study explored associations between self-report personality measures, head impact telemetry system (HITS) data, and SRC incidence in a sample of high school and collegiate football athletes. Data were retrieved from Project Head-to-Head II, a large (N=917), prospective SRC study conducted over the 2015 and 2016 seasons. SRC incidence was operationalized as “concussed” (those who completed baseline testing and were concussed during the evaluation period; n=83) or “nonconcussed” (matched controls from the same teams; n=73). Playing style was operationalized as cumulative risk metric (CRM), a cumulative head impact severity estimate. Results of binomial logistic regression showed that concussion group membership was significantly ($p \leq .05$) predicted by negative emotionality, meanness, psychoticism, and alienation to complement related findings linking such traits to potentially hazardous playing styles. Although broad aggression predicted concussion incidence only marginally ($p = .065$), aggression operationalized as physically expressed anger was strongly predictive (OR=3.09, $p = .004$). Non-concussion group membership was significantly predicted by more years of sports participation and communal positive emotionality, and marginally by social closeness and resilience, suggesting that maturity, playing experience, and having a prosocial temperament may promote on-field behaviors that minimize risk of high-impact contact. There was a great deal of consistency in the personality traits that predicted concussion incidence and the traits that predicted playing style (CRM). These findings accentuate the potential role of personality in concussion risk and can be used by coaches and athletic trainers to develop intervention and prevention strategies that encourage on-field safety and reduce SRC risk.

Keywords: sport-related concussion, high school football, collegiate football, HITS, personality, athletic trainers

B08-20

OPERATION BRAIN TRAUMA THERAPY: LEVETIRACETAM TREATMENT FOR TRAUMATIC BRAIN INJURY IN THE MICRO PIG

Audrey Lafrenaye¹, Kevin Wang², Stefania Mondello³, Ronald Hayes⁴, Karen Gorse¹, Susan Walker¹, Yasuaki Ogino¹, Patrick Kochanek⁵, John Povlishock¹

¹Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

²University of Florida, Center for Neuroproteomics & Biomarkers Research, Departments of Psychiatry & Neuroscience, Gainesville, USA

³University of Messina, Messina, Italy

⁴Banyan Biomarkers Inc., Alachua, USA

⁵University of Pittsburgh, Department of Critical Care Medicine, Pittsburgh, USA

Traumatic brain injury (TBI) is a highly prevalent disease with devastating costs. The unsuccessful clinical translation of many promising therapeutics has triggered the rigorous standardization and refinement of TBI models and the use of higher-order animals before moving to large scale clinical trials. Levetiracetam (LEV) is an antiepileptic agent that was the fifth drug tested by Operation Brain Trauma Therapy (OBTB) in three independent rodent models of moderate to severe TBI. To date LEV is the most promising drug tested by OBTB and was therefore advanced to testing in the micro pig model. Adult male micro pigs weighing 22 ± 5 kg were subjected to a mild (1.7 ± 0.2 atm) central fluid percussion brain injury followed by a 15-min post-injury intravenous infusion of either 170 mg/kg LEV or equal volume of saline (n=7 pigs for each treatment group). Systemic physiology was assessed throughout the post-injury period. Serial blood samples were obtained pre-craniotomy and post-craniotomy, as well as at 1 min, 30 min, 1 h, 3 h and 6 h post-injury. All blood samples were processed to obtain serum following OBTB standard operations for detailed analysis of the astroglial biomarker glial fibrillary acidic protein (GFAP). Animals were killed 6 h following injury for histological assessment of cellular damage, using hematoxylin and eosin, and diffuse axonal injury, using antibodies against amyloid precursor protein (APP). Group analysis showed no differences in post-injury serum GFAP levels between saline and LEV treated micro pigs. There were also no differences in the numbers of APP+ axonal swellings or cellular damage within the thalamic region of the micro pig brain. However, a significant alteration in the morphological properties of the APP+ axonal swellings, including reduced swelling area and increased swelling roundness was observed, suggesting potentially beneficial effects of LEV in the micro pig that warrant further investigation at later survival time points. Support: USArmy W81XWH-10-1-0623.

Keywords: Traumatic brain injury, Micro pig, Axonal injury, Levetiracetam, Biomarker

B08-21

EFFECTS OF BLAST EXPOSURE AND CAMPYLOBACTER INFECTION ON ANXIETY AND PTSD-LIKE BEHAVIORS IN A RODENT MODEL

Melissa Mehalick¹, Frederic Poly¹, Michael Prouty¹, Richard McCarron^{1,2}, Stephen Ahlers¹

¹Naval Medical Research Center, Neurotrauma Department, Silver Spring, USA

²Uniformed Services University of Health Sciences, Department of Surgery, Bethesda, USA

The brain and the enteric system are intricately connected and together form the “gut-brain axis” (GBA). When the brain is exposed to a stressor there can be physiological changes within the gut bacteria (microbiome). Likewise, a stressor to the gut, can contribute to changes in brain function. This bidirectional relationship between the brain and gut can influence anxiety and PTSD-like behavior. Blast-induced mild traumatic brain injury (mTBI) and enteric infections are common health concerns among military members in theatre. Together, they influence the GBA, and may influence behavior, especially the anxiety and PTSD-like behavior that is often reported in service members returning from deployment. The goal of the current study was to assess how a physical trauma to the brain and an enteric infection may impact long-term anxiety-like behavior. To simulate a blast-induced mTBI, rats were anesthetized and exposed to 75 Kpa (~10 psi) of blast overpressure from a blast tube once a day for three consecutive days. Approximately 24 hours after the final blast exposure, half of the animals were given a gavage of *Campylobacter jejuni*

to induce an enteric infection. About 4 months after the blast exposure and infection, the presence of anxiety behavior was assessed using the elevated zero maze and the light/dark emergence test. Rats exposed to both blast and *Campylobacter* infection were expected to show the greatest amount of anxiety compared to control animals or animals exposed to only blast or only *Campylobacter*. Contrary to hypotheses, animals exposed to both blast and *Campylobacter* demonstrated the least amount of anxiety and showed significantly reduced levels of corticosterone compared to control animals. These results indicate that there may be an interaction between the physical trauma of TBI and enteric infection that contributes to a decrease in anxiety-like behaviors, and/or possibly a dampening of the stress response.

Keywords: campylobacter jejuni, gut brain axis, anxiety behavior, blast TBI

B08-22

INTER-NETWORK RESTING STATE FUNCTIONAL CONNECTIVITY ASSOCIATED WITH MOOD SYMPTOMS FOLLOWING SPORT-RELATED CONCUSSION

William McCuddy¹, Lezlie España¹, Rasmus Birn², Andrew Mayer³, Timothy Meier^{1,4}

¹Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

²University of Wisconsin-Madison, Psychiatry, Madison, USA

³The Mind Research Network, Albuquerque, USA

⁴Laureate Institute for Brain Research, Tulsa, USA

The clinical presentation of sport-related concussion (SRC) is heterogeneous and can include a combination of cognitive, somatic, and mood-related symptoms. Identifying subtypes related to specific symptom domains may reveal unique prognostic trajectories, informing clinical treatment. The present study investigated patterns of functional connectivity associated with mood symptoms following SRC. We hypothesized that a) SRC would be associated with disrupted resting state connectivity in regions associated with emotional processing and that b) concussed athletes with more mood symptoms would have greater disruption of functional connectivity in regions associated with emotional processing. Resting state fMRI and structured interviews for the Hamilton Depression Rating Scale (HAM-D) were collected in collegiate athletes at approximately one day ($n=28$), one week ($n=29$), and one month post-concussion ($n=26$). Non-concussed contact sport athletes served as healthy controls (HC; $n=50$). Twenty-three regions-of-interest (ROI) were meta-analytically derived using the Neurosynth neuroimaging database based on the term "emotion." A connectivity matrix of Fisher-Z transformed correlation coefficients between each ROI was calculated for each subject and time point. Concussed athletes reported significantly higher HAM-D scores at one day and one week relative to one month post-concussion, and relative to controls ($p's < 0.001$). There was no difference in connectivity between concussed and healthy athletes. However, there was a multivariate effect of HAM-D scores on resting state connectivity in concussed athletes at one day and one week post-concussion ($p's < 0.01$). Follow-up analyses indicated that this multivariate effect was driven by an inverse relationship between HAM-D scores and functional connectivity of regions of the default mode network (e.g., medial prefrontal cortex) to regions of the salience and dorsal attention networks (e.g., anterior insula and superior parietal lobule). Results suggest that mood symptoms following SRC are associated with attenuated inter-network connections between regions commonly associated with both depression and cognitive impairment.

Keywords: concussion, resting state fMRI, mood symptoms, functional connectivity

B08-23

PROLONGED KING-DEVICK TIME IN CONCUSSION PATIENTS

Briana Meyer, Meeryo Choe, Constance Johnson, Philip Rosenbaum, Sonal Singh, Zhe Wang, David McArthur, Christopher Giza

University of California, Los Angeles, Pediatric Neurology, Los Angeles, USA

The King-Devick (KD) test is a vision-based rapid number-naming task used to detect impairment in concussed individuals, primarily assessed on the sideline assessment. We tested the following hypotheses: (1) acute and chronic concussion will affect total KD time and (2) KD time will correlate with symptom recovery. KD was administered as recommended to three groups: High school student athletes (13–18 years, 234 females, 149 males) at preseason baseline evaluations during the 2016–2017 school year, patients diagnosed with an acute concussion ≤ 28 days (12–18 years, 21 females, 29 males) and patients diagnosed with a chronic concussion > 28 days (12–18 years, 26 females, 33 males). [GC1] Each participant completed Graded Symptom Checklist (GSC) providing a total score and four symptom subtypes: somatic, cognitive, sleep and emotional. In hypothesis 1, we examined group differences and found group (baseline, acute and chronic) had a significant effect on KD time ($F_{2,489} = 18.91$, $p < 0.001$). KD time was significantly greater in the acute group than both the baseline and chronic groups ($p < 0.01$). Though not statistically significant, KD time was greater in the chronic group compared baseline ($p = 0.06$) [GC2]. In hypothesis 2, we evaluated the robust correlation between GSC total, GSC subtype and KD time in each group. No significant correlations were reported in the baseline or chronic groups ($r_{pb} = [GC3] 0.04$ and 0.08). KD time. In the acute group, GSC total and KD were significantly correlated ($r_{pb} = 0.51$). Somatic and cognitive symptom subsets were most strongly correlated to KD ($r_{pb} = 0.89$ and 0.80). Slower KD times in the acute concussion group show a possible injury-related deficit in KD performance that correlates with concussion symptoms (GSC). Potentially, KD can be an objective measure of concussion and/or concussion related symptoms in the acute clinic setting. GSC and KD did not correlate in the chronic group, suggesting persistent symptoms may be due to other factors. High variability of KD time in all three groups supports the need for an individual baseline. Supported by: UCLA BIRC, UCLA Steve Tisch BrainSPORT Program

Keywords: King-Devick, Vision, Clinical Setting, Recovery

B08-24

NILVADIPINE AMELIORATES MEMORY IMPAIRMENT IN AGED MICE WITH REPETITIVE MILD TBI

Alex Morin^{1,2}, Benoit Mouzon¹, Scott Ferguson¹, Daniel Paris¹, Fiona Crawford¹

¹Roskamp Institute, Sarasota, USA

²Open University, Milton Keynes, UK

Mild TBI (mTBI) is the most common form of brain trauma worldwide. The effect of mTBI is not well-studied within the elderly population, yet it constitutes a significant part of all mTBI patients. The rate of hospitalization for the mTBI population aged over 65 is two times higher than it is for younger adults, with ground falls and motor vehicle accidents as primary reasons. Age alone is known to slow neurorecovery and exacerbate cognitive decline but also dramatically worsens mTBI outcomes. However, preclinical studies focusing on geriatric TBI and its treatment are scant and are limited to

moderate and severe injuries only. We hypothesize that mTBI pathophysiology in the aged brain has distinct characteristics from mTBI in younger adults due to pre-existing age-related deteriorations and might require different therapeutic interventions. Herein, we present a study of 23 months old aged hTau mice after sham injuries or repetitive mTBI (r-mTBI, 5x), modeling forces common in human mTBI with or without nilvadipine treatment. Furthermore, we investigated the effects of three weeks of treatment with Nilvadipine, a spleen tyrosine kinase inhibitor which has been shown to block amyloid production, tau hyperphosphorylation and inflammation in mouse models of Alzheimer's Disease and Tauopathy. Nilvadipine is currently under investigation to treat AD in a Phase III trial in Europe. In our r-mTBI mice, we observed that nilvadipine reversed memory impairments and showed a trend in the reduction of inflammation and tau pathology. To our knowledge, this is the only preclinical study focusing on the treatment of r-mTBI in the aged and these results highlight the therapeutic potential of nilvadipine in this situation. Additional studies are ongoing to investigate the effects of nilvadipine on other outcomes and in other injury paradigms.

Keywords: Repetitive mild TBI, Geriatric TBI, Treatment, Nilvadipine

B08-25

ACUTE TEMPORAL PROFILE OF NEUROINFLAMMATION IN THE HIPPOCAMPUS FOLLOWING REPEATED MILD TRAUMATIC BRAIN INJURY

Jessica Nichols, Candace Floyd

University of Alabama at Birmingham, Physical Medicine and Rehabilitation, Birmingham, USA

Repeated mild traumatic brain injuries (rmTBI) are major health concern as they can result in lasting cognitive and neuropsychological complications. These functional alterations are likely due to changes in the neurochemical, cellular, and metabolic environment of the brain elicited during the secondary injury cascade. In moderate to severe TBI, neuroinflammation plays a key role in secondary injury with reactive species (RS), glial activation, and activation of inflammatory signaling pathways, such as nuclear factor kappa B (NF- κ B), being major contributing factors. However, the pathophysiological changes that result from rmTBI are less characterized and vary between models. Additionally, many studies focus on sub-acute and chronic time periods post-rmTBI due to the association with neurodegenerative diseases, but the early inflammatory response is also important to understand. Therefore, the purpose of this study was to examine different aspects of neuroinflammation acutely following rmTBI. An impact-acceleration model was utilized to deliver rmTBIs (3) to adult C57BL/6J mice with an inter-injury interval of 24 hours. At 6 or 72 hours following the last mTBI, mice were euthanized and hippocampal tissue was collected for biochemical analysis. Immunoblotting was performed to assess markers of oxidative/ nitrosative stress (protein carbonyls, 4-HNE, 3-NT), glia response (GFAP, YM1, CD68), and NF- κ B activation. Additionally, a panel of 25 chemokines/cytokines was examined with a multiplex assay. At 6 hours post-injury, rmTBI resulted in a significant reduction in the anti-inflammatory M2 microglia marker (YM1) and a significant increase in IL-10. At 72 hours, IL-10 remained elevated and there was also a significant increase in IL-1 β after rmTBI. There were no differences observed in oxidative/ nitrosative stress or NF- κ B pathway markers. Taken together, our results demonstrate that neuroinflammation has a dynamic role acutely following rmTBI and highlights the need to gain a better temporal resolution of the pathophysiological events occur-

ring following rmTBI. Supported by NIH/NINDS R01NS075162 and F31NS093717, Civitan International Research Center (CIRC) Emerging Scholar Award, and NFL Charities Medical Research Grant.

Keywords: repeated mild TBI, inflammation, oxidative stress, NF- κ B

B08-26

MULTIVARIATE APPROACH TO STUDYING THE EFFECTS OF SUBCONCUSSION ON FUNCTIONAL CONNECTIVITY

Bryson Reynolds¹, Amanda Stanton², Sauson Soldozy², Howard Goodkin³, Max Wintermark⁴, Jason Druzgal²

¹*Vanderbilt University Medical Center, Institute of Imaging Science, Nashville, USA*

²*University of Virginia, Radiology and Medical Imaging, Charlottesville, USA*

³*University of Virginia, Neurology, Charlottesville, USA*

⁴*Stanford University, Radiology, Stanford, USA*

Concerns about the effects of subconcussive head impacts in sport and the effects of subconcussion on brain connectivity are not well understood. We hypothesize that college football players experience changes in brain functional connectivity that are not found in athletes competing in lower impact sports (soccer and lacrosse) or healthy controls. Functional connectivity changes are likely to be spatially heterogeneous across participants, requiring analytical methods that go beyond mass-univariate methods commonly used in functional MRI (fMRI). To test these hypotheses, resting-state fMRI data was acquired from 31 college football players, 18 college men's lacrosse players, 14 college men's soccer players, and 30 male controls at preseason and postseason time points. Regional homogeneity and degree centrality were calculated as measures of local and long-range functional connectivity, respectively. We utilized paired support vector machine (SVM) classification to probe subconcussion's effects on local and long-range connectivity. SVM classification had high accuracy for regional homogeneity (87%, $p=0.009$) in college football, but not in any other metric or group. This finding suggests that subconcussion results changes in local functional connectivity that may be detectable with multivariate analyses. Across the published findings for regional homogeneity in concussion, our regional trends matched 9 of 13 regions from previously published findings, suggesting that subconcussion in football may produce local functional connectivity changes similar to concussion. Lastly, while SVM classification for degree centrality maps in college football players did not reach statistical significance ($p=0.084$), a ranking distance comparison of regional trends for ReHo and DC demonstrated a high degree of correspondence between the measures ($p=0.007$). Overall, this research suggests that functional connectivity metrics combined with multivariate analyses may be sensitive to functional changes in the brain in response to subconcussive head impacts.

Keywords: subconcussion, fmri, multivariate

B08-27

ACUTE CORTISOL LEVELS MAY AID IN PREDICTING RECOVERY FOLLOWING PAEDIATRIC SPORT-RELATED CONCUSSION

Evan Ritchie¹, Carolyn Emery^{3,4}, Chantel Debert^{1,2}

¹*University of Calgary, Clinical Neurosciences, Calgary, Canada*

²*Hotchkiss Brain Institute, Calgary, Canada*

³*University of Calgary, Kinesiology, Calgary, Canada*

⁴*Alberta Children's Hospital Research Institute, Calgary, Canada*

Background: Acute neuroendocrine response has received little attention in paediatric sport related concussion (SRC). This study aimed to determine whether there may be alterations in acute cortisol levels following paediatric SRC and to compare cortisol levels to outcome measures of symptom burden and length to return to sport.

Methods: Ice hockey players ages 11–12 were recruited during the 2013/2014 season. If players sustained a SRC they were assessed by a sports medicine physician and completed a childSCAT3. Serum cortisol samples were collected and compared to length of time to return to sport, and symptom burden.

Results: Of 636 ice hockey players enrolled, 41 sustained a sport concussion. In total, 22 serum cortisol samples were collected, with 15 (62.5%) meeting inclusion criteria. Four players presented with abnormally low cortisol levels. Players with abnormally low cortisol levels experienced more symptoms (17.7 ± 1.9 vs. 8.5 ± 6.6) and more severe symptoms on the symptom portion of the ChildSCAT3 (28.5 ± 5.8 vs. 11.9 ± 10.1) and took longer return to sport (23 ± 13.6 vs. 16.7 ± 11.8).

Conclusions: Paediatric ice hockey players following SRC with abnormally low acute cortisol levels may be more susceptible to experiencing increase symptom burden and take longer to return to sport than players with population-based normal cortisol levels.

Keywords: Cortisol, Athletes, Neuroendocrine

B08-28

REPETITIVE MTBI CAUSES FUNCTIONAL CHANGES IN THE ABSENCE OF SYNAPSE LOSS, NEURONAL CELL DEATH AND NEUROINFLAMMATION

Stephanie Sloley, Evan Wicker, Charisse Winston, Bevan Main, John Partridge, Jian-Young Wu, Mark Burns
Georgetown University, Neuroscience, Washington, USA

Mild traumatic brain injury (mTBI) is defined as a trauma-induced alteration in brain function in the absence of structural damage. Recently we described an experimental model of mTBI that resulted in a temporary loss of synapses following a single mTBI. In this study, we examined how 30 mTBI delivered over 6 days (5 mTBI per day) affected outcomes in C57Bl/6 mice. Mice were exposed to either daily isoflurane (3 minutes per day for 6 days) or daily isoflurane followed by 5 rapid succession mTBI for 6 consecutive days (30 mTBI total). Immunohistochemical analyses revealed that 30 mTBI over 6 days did not cause grey matter inflammation, cell death, or inflammation. Golgi staining for dendritic spines indicated that the mouse brain adapted to repeat mTBI, with no excitatory synapse loss detectable compared to sham mice. Despite the normal synapse number, behavioral testing at one-month post-injury showed that repeat mTBI mice have deficits in the acquisition and probe phases of the Morris water maze, indicating impaired learning and memory. Furthermore, repeat mTBI mice displayed enhanced risk-taking behavior on the elevated plus maze. Finally, EEG monitoring revealed chronic disruptions in brain wave activity and in the sleep cycles of repeat mTBI mice. We also examined the effect of mTBI on hippocampal circuitry using extracellular field recording electrophysiology. Input/output curves for repeat mTBI mice were identical to sham mice, indicating that the Schaffer collateral pathway remained undamaged after repeat mTBI – however there were significant impairments in long-term potentiation (LTP). We conclude that high-frequency repeat mTBI causes impaired learning, memory, and overall brain function in the absence of structural damage or a decrease in the number of excitatory synapses. We hypothesize that synaptic adaptation occurs after repeat mTBI causing chronic behavioral changes including impairments in sleep,

judgment, and cognition. Future experiments will determine the biochemistry of the synaptic adaptation, and assess strategies to reverse the loss of brain function.

Keywords: Repetitive mTBI, Function, Inflammation, Oscillations

B08-29

LONGITUDINAL RESOLUTION OF TRAUMATIC MENINGEAL ENHANCEMENT ON POST-CONTRAST FLAIR MRI

Ana Tinoco Martinez^{1,2}, L. Christine Turtzo³, Martin Cota², Andrew van der Merwe², Leighton Chan^{2,4}, Lawrence Latour^{2,3}

¹John Hopkins Suburban Hospital, Bethesda, USA

²CNRM, Bethesda, USA

³NIH, NINDS, Bethesda, USA

⁴NIH, CC, Bethesda, USA

Introduction: Traumatic meningeal enhancement (TME) is a novel imaging marker characterized as hyperintensity in the meninges on post-contrast FLAIR MRI. TME was first characterized on acute MR imaging but the implications and temporal evolution are not yet understood. This study represents an initial effort to characterize the longitudinal resolution of TME in a cohort of traumatic brain injury (TBI) patients.

Methods: Included subjects originally presented with suspected TBI to a Level 1 or Level 2 trauma center and prospectively enrolled in the THINC protocol within 48 hours of injury. After completion, subjects were re-contacted 2 years post-injury and enrolled in a study at NIH. For both studies subjects were imaged on 1.5T/3T MRI with administration of one dose of Gd-DPTA. Two raters blinded to scan time points independently scored de-identified pairs of pre- and post-contrast FLAIR images for presence and conspicuity of TME, with inter-rater reliability assessed by Cohen's Kappa and consensus reads to classify discrepant ratings. Data were analyzed in 2x2 contingency tables with Fisher's exact test and Relative Risk(RR). Age and times reported as medians.

Results: 30 subjects (21 male; age 43.5 years) were imaged acutely and longitudinally (2.1 years). Raters demonstrated moderate agreement for presence of TME ($k=0.72$). 18 subjects positive for TME at acute time point, with 12 remaining TME positive at follow-up ($(RR)=2.7$ (95%CI 1.1-7.8) $p=0.06$). Of the 13 subjects rated as positive for both TME and conspicuous TME on acute scan, 9 had continued TME at longitudinal imaging ($RR=2.0$ (95%CI 1.0-4.3) $p=0.14$).

Conclusions: The continued presence of TME in 40% of subjects at 2 years post-injury suggests persistent injury to the meninges after TBI. Further longitudinal research is necessary to determine whether TME resolves at later time points and if differences in outcomes exist in groups of patients with persistent versus resolving TME.

Keywords: FLAIR, MRI, Contrast Enhancement, Chronic Blood Brain Barrier Disruption

B08-30

BEHAVIORAL AND MOLECULAR ASSESSMENT FOLLOWING BLAST-EXPOSURE IN A RAT MODEL

Anna Tschiffely¹, Jessica Goodrich^{1,2}, Jonathan Statz^{1,2}, Richard McCarron^{1,3}, Amanda Glueck⁴, Peter Walker¹, Stephen Ahlers¹

¹Naval Medical Research Center, Neurotrauma, Silver Spring, USA

²Henry M. Jackson Foundation, Bethesda, MD, USA

³Uniformed Services University, Surgery, Bethesda, MD, USA

⁴University of Kentucky, Rehabilitation Sciences, Lexington, KY, USA

Traumatic brain injury (TBI) has been a frequent injury in recent conflicts, Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF). A common mechanism of injury is exposure to overpressure waves from incendiary devices, such as improvised explosive devices (IEDs). Recent literature indicates high rates of posttraumatic stress disorder (PTSD) and post concussive symptoms in military personnel following mTBI. It is currently unclear whether mTBI predisposes an individual to PTSD, or if the two develop independently. If mTBI predisposes an individual to PTSD, the brain structures involved in PTSD should be impacted by mTBI. Two areas involved in the “fear circuitry” are the amygdala and the medial prefrontal cortex (mPFC). When the fear circuitry is disrupted, a potential outcome may be PTSD. Evidence indicates that exposure to blast-overpressure (BOP) damages or causes alteration to other regions of the brain’s fear circuitry, but it is currently unknown if the mPFC is also damaged. Our study examines the involvement of the mPFC and the amygdala through behavioral tests and molecular assessments. Two such behavioral assessments are cued-fear conditioning and the elevated zero maze (EZM). Long-Evans rats were exposed to a BOP of 75 kPa once daily over 3 consecutive days. Following the final BOP exposure, animals were assessed for fear and anxiety via cued-fear conditioning and EZM. Rats exposed to BOP exhibited greater freezing during extinction compared to controls in the fear-conditioning paradigm. Additionally, rats exhibited altered Corticosterone and Stathmin levels, indicating the stress pathway and amygdala protein expression may be affected. Taken together these findings indicate blast mTBI may exacerbate the development of anxiety related behaviors such as fear. These changes may be the result of underlying hormonal or protein alterations however; further research is needed to further elucidate the specific mechanisms involved.

Keywords: amygdala, PTSD, TBI, animal model, blast

B08-31

NOVEL DRUGS THAT AUGMENT KCNQ (KV7, M-TYPE) POTASSIUM CHANNELS AS A POST-EVENT TREATMENT FOR TRAUMATIC BRAIN INJURY

Fabio Vigil¹, Eda Bozdemir², Rafael J. Veraza¹, Vladislav Bugay¹, Liliana Espinoza¹, Deborah M. Holstein², Shane M. Sprague³, MaryAnn Hobbs¹, Isamar Sanchez¹, Jose Cavazos⁴, Robert Brenner¹, James Lechleiter², Mark S. Shapiro¹

¹UTHSCSA, Cell and Integrative Physiology, San Antonio, USA

²UTHSCSA, Cell & Structural Biology, San Antonio, USA

³UTHSCSA, Neurosurgery, San Antonio, USA

⁴UTHSCSA, Neurology, San Antonio, USA

Traumatic brain injury (TBI) is a risk factor for the development of epilepsy. Here, we are testing the hypothesis that reducing neuronal excitability before the process of epileptogenesis commences, moderates or prevents post-TBI deleterious effects. Using a model of blunt TBI, we pharmacologically enhanced currents from M-type K⁺ channels, which play a dominant role in limiting neuronal excitability. We tested such effects with the anti-convulsant drug, Retigabine (RTG), which enhances M current. Mice subjected to TBI were administered RTG or vehicle 30 minutes after injury. Twenty-four hours later, occurrence of spontaneous seizures was monitored by EEG and video recording. To assay for seizure susceptibility, animals were challenged with pilocarpine (3X, 75 mg/kg, 5 days after TBI), monitored for 24h by video and EEG recording, and the percentage of animals displaying seizures or abnormal EEG spike activity quantified. An independent cohort of

mice were sacrificed 24 h after TBI and brain slices Nissl-stained to quantify TBI-induced changes in cell soma areas. A third dual cohort of animals were sacrificed 6 days after TBI and used for immunoblotting analyses of CD40L levels. After TBI ~33% of vehicle only-treated mice displayed spontaneous seizures, while no animal treated with RTG had seizures. In addition, RTG treatment significantly reduced abnormal spike activities after pilocarpine injection. RTG also significantly reduced TBI-induced neuronal soma swelling and TBI-induced increases in CD40L levels. Preliminary results indicate that phosphorylation of TAU and total TAU levels were affected by TBI and RTG. Long-term behavioral effects of TBI with or without RTG will be assayed in future experiments. *Supported by DoD CDMRP grants W81XWH-15-1-0284 and W81XWH-15-1-0283.*

Keywords: Potassium channels, KCNQ, Spikes, CD40L

B08-32

HELMET DESIGN AND HITS TO THE HEAD: ANALYSIS OF NFL TACKLING 1951-PRESENT

David Wang, Taylor Polk, Douglass Johnson, Brian McCormick, Alex Webb, Andrew Horn, Jordan Murphy, Eric Wang, Rand Floyd, Alex Oster, Nikhil Chervu, Aiden Neustadt, Dave Milzman
Georgetown University School of Medicine, Washington, USA

Purpose: Non-scientific reports claim increased helmet technology has led to more dangerous tackling and thus, concussive injuries in the National Football League (NFL). This study evaluates the change in tackling style based on helmet technological changes over time and whether the rise in concussive injuries is brought about by an increase in dangerous helmet-to-helmet hits. There are no such current studies for the NFL.

Methods: Investigators were trained in the NFL definition of “helmet hits” and game-film analysis. Three independent investigators analyzed all 67 NFL Championship game obtained from the NFL Hall of Fame since 1951. Helmet involvement of offensive and defensive players was determined for each hit. Players’ behavior after a hit was labelled “concussive” as defined by a neurotrauma-trained emergency physician based on gait steadiness and delay on getting up.

Results: From the 67 games, there was an average 107.1 (IQR: 102.5–112) “hits” per game, 4.3 (IQR: 2–7.5) were helmet-to-helmet. The percentage of helmet-to-helmet hits increased 5.44-fold from the 50s to the 10s with statistically significant jumps after the 70s (p=0.004, t-test) and again after the 80s (p=0.001). Overall hits determined to involve the helmet of either the defensive or offensive player also made jumps after the 70s (p=.002) and again after the 80s (p=.009). Dangerous “launching” helmet hits increased after the 80s (p=.001). The number of players who exhibited concussive symptoms after a hit increased directly corresponding to the number of helmet-to-helmet hits with the greatest change occurring after the 80s (p=.001)

Conclusions: Dangerous tackling styles involving the helmet, particularly helmet-to-helmet have increased in frequency corresponding with helmet innovation in the NFL, with the largest jumps corresponding with the introduction of the two most significant helmet technology changes in the time studied, energy absorbing plastic helmets in the 1970s and molded polycarbonate helmets in the late 1980s. That helmets with better technology potentially leads to more dangerous hits suggests that stronger helmets may be a detriment to player safety.

Keywords: Football, Protective Helmets

NEUROSENSORY DEFICITS VARY AS A FUNCTION OF POINT OF CARE IN PEDIATRIC MILD HEAD TRAUMA

Christopher Wertz¹, Christina Master², Sephira Ryman¹, Eileen Storey², Grace Park³, John Phillips^{1,3}, Andrew Dodd¹, Scott Oglesbee³, Richard Campbell³, Ron Yeo³, Benjamin Wasserott¹, Nicholas Shaff¹, John Leddy⁴, Rebekah Mannix⁵, Kristy Arbogast², Timothy Meier¹, Matthew Grady², Andrew Mayer^{1,3}

¹MRN, Albuquerque, USA

²CHOP, Surgery or Pediatrics, Philadelphia, USA

³UNM, ED., Neurology, Psychiatry or Psychology, Albuquerque, USA

⁴UB, Orthopaedics, Buffalo, USA

⁵BCH, Medicine, Boston, USA

Deficits in neurosensory functioning are increasingly recognized as a key component of pediatric mild traumatic brain injury (pmTBI). However, no study has examined how neurosensory dysfunction varies as a function of point of care, or how symptom provocation during neurosensory testing distinguishes patients from controls and predicts recovery. Patients were recruited from either the emergency department at University of New Mexico Hospital (UNMH; N=35) or a concussion clinic at Children's Hospital of Philadelphia (CHOP; N=41) along with healthy controls (HC; N=42). Participants completed a standardized battery examining vestibular, oculomotor and visual functioning. Symptoms were measured pre-exam and immediately following each task. There were no differences in demographics or number of prior concussions ($p > 0.10$) between CHOP and UNMH patients, whereas mean days post-injury was higher for CHOP patients ($p < 0.001$). Both pmTBI cohorts had higher pre-exam symptomatology ($p < 0.001$) relative to HC but not different from one another ($p > 0.10$). Symptom provocation (i.e., relative to pre-exam ratings) was significantly higher for pmTBI patients relative to HC, with greater provocation observed for CHOP compared to ED patients. Symptom provocation marginally increased total classification accuracy for ED patients relative to HC compared to pre-test symptoms alone ($\approx 5\%$ change), but had a much larger effect on accuracy in CHOP sample ($\approx 17\%$ change). Magnitude of symptom provocation also classified recovered versus non-recovered patients with moderate accuracy (73.7%) as defined by pre-test symptomatology. These results highlight the importance of neurosensory testing and symptom provocation in the clinical management of pmTBI and suggest that point of care represents an important outcome variable.

Keywords: Recovery, Vestibular, Ocular Motor, Neurosensory

B08-34

DIFFUSION TENSOR IMAGING IN COLLEGIATE ATHLETES SHOWS EFFECTS OF BOTH CONCUSSION AND EXPOSURE TO CONTACT SPORT

Elisabeth Wilde^{1,2}, Ashley Ware^{1,3}, Brian Biekman¹, Brian Taylor^{1,2}, Laura Hollowell¹, Trevor Wu⁶, Holly Paxton⁶, Leslie Pritchep^{5,4}, Sylvain Bouix⁷, Kenneth Podell⁸, David Schnyer⁹, Roger Newman-Norlund¹⁰, Doug Casa¹¹, Susan Yeargin¹⁰, Harvey Levin^{1,2}

¹Baylor College of Medicine, Physical Med & Rehab, Neurology, Radiology, Houston, USA

²Michael E. DeBakey VA Medical Center, Houston, USA

³University of Houston, Psychology, Houston, USA

⁴BrainScope, Inc., Bethesda, USA

⁵NYU School of Medicine, Psychiatry, New York, USA

⁶Hauenstein Neurosciences of Mercy Health/Michigan State University, Department of Translational Science & Molecular Medicine, Grand Rapids, USA

⁷Brigham and Women's Hospital/Harvard Medical School, Psychiatry, Boston, USA

⁸The Neurological Institute, Houston Methodist, Houston Methodist Concussion Center/Neuropsychology Section, Houston, USA

⁹University of Texas - Austin, Psychology, Austin, USA

¹⁰University of South Carolina, Exercise Science, Columbia, USA

¹¹University of Connecticut, Storrs, USA

¹²SUNY Upstate Medical University, Physical Medicine and Rehabilitation, Syracuse, USA

Prior imaging studies using DTI in sports-related concussion have demonstrated group differences in several brain regions at both sub-acute and chronic post-injury intervals, but many imaging studies are limited by small sample sizes and a single comparison group. This study investigates DTI-detectable differences in a multi-site study of collegiate athletes in the following groups: 1) athletes sustaining sports-related concussion at day of injury (TBI; n=49), 2) non-concussed contact sports athletes (CC; n=27), and 3) non-concussed, non-contact sports athletes (NCC; n=20). Groups did not differ in age, sex, race/ethnicity, or handedness. Multiple regression analyses including age and sex indicated that group assignment predicted fractional anisotropy (FA) for the genu, body, and splenium of the corpus callosum, right and left frontal white matter, and right and left cingulum bundles, but not for the uncinate fasciculi. In these models, the TBI and CC groups both generally differed from the NCC group, with the concussed group demonstrating lower FA. Thirty-seven of the 49 participants in the TBI group returned 45 days after return to play for repeat MRI assessment. Repeated measure ANCOVAs disclosed decreased FA in the corpus callosum splenium in concussed athletes between Day 0 and 45 days after return-to-play, but not in other tracts, though trend-level decreases were noted in the right cingulum bundle and the right and left uncinate fasciculi.

DTI-related metrics suggested the possibility of brain tissue alteration that is both related to contact sport exposure and concussion-level events. Future work will focus on the relation of DTI to EEG-based findings as well as other symptom and outcome measures.

This work was supported by the United States Department of the Navy (N6311614MPX083) and Brainscope, Inc.

Keywords: sports-related concussion, diffusion tensor imaging

B08-35

THE DISCRIMINATIVE ABILITIES OF COMMONLY USED SIDELINE CONCUSSION ASSESSMENT TOOLS

David Wright¹, Lindsay Lee¹, Sidi Zhao², Timothy Moran¹, Russell Gore³, Tamara Espinoza¹, Kristopher Hendershot¹, Kelsey Evans¹, Brian Liu², Nicole Kosoris², Alessio Medda², Shean Phelps², Michelle LaPlaca²

¹Emory University, Atlanta, USA

²Georgia Tech, Atlanta, USA

³Shepherd Center, Atlanta, USA

The sports medicine community strongly advocates testing multiple neurological domains to assess sports-related concussion, yet data supporting the combination of different assessment tools is lacking. The purpose of this study was to evaluate the relative abilities of the King-Devick Test (KD), the Balance Error Scoring System (BESS), and the Standardized Assessment of Concussion (SAC) (individually and in combination) to correctly discriminate between concussed and uninjured athletes. Athletes who sustained a concussion were paired with healthy controls and underwent testing at 3, 10, and 30 days post-injury. The ability of the tests to differentiate between concussed and

control athletes at each testing day was determined by comparing the area under ROC curves (AUC) generated using logistic regression models. The discriminative abilities of each test varied by testing day. When assessed individually, the BESS showed higher discriminative ability at testing days 3 (AUC: 0.85) and 10 (AUC: 0.72), while SAC had the highest discriminative ability at day 30 (AUC: 0.76). On testing day 3, backward elimination logistic regression procedures resulted in a model that included only the BESS firm score and the total symptom score as predictors of concussion (AUC: 0.85). On day 10, the SAC and the BESS firm score remained in the model (AUC: 0.76), although the BESS firm score alone was not a significant predictor. On day 30, only the SAC remained in the model (AUC: 0.76). The discriminative ability was highest when all three tests were used together on testing day 3 (AUC: 0.88). Multiple domains and tests need to be utilized to improve concussion detection. Additionally, individual tests have different discriminatory abilities depending on when they are deployed after concussion, which should be considered when implementing a recovery/return-to-play program.

Keywords: concussion, Sports concussion, Diagnostics, Assessment tools

B08-36

SUBCONCUSSIVE OCULOMOTOR DEFICIT IN AMERICAN FOOTBALL AND CORRELATION WITH HEAD IMPACT EXPOSURE

Lyndia Wu¹, Maria Malone², Calvin Kuo², Wei Zhao³, Songbai Ji³, Gerald Grant⁴, David Camarillo^{1,2}

¹Stanford University, Bioengineering, Stanford, USA

²Stanford University, Mechanical Engineering, Stanford, USA

³Worcester Polytechnic Institute, Biomedical Engineering, Worcester, USA

⁴Stanford University, Neurosurgery, Stanford, USA

Background: Studies have shown that subjects with exposure to subconcussive impacts without diagnosed concussions may also be at risk for long term brain changes. The King-Devick (KD) test, which is an oculomotor test, may be a promising sensitive test to detect mild neurological deficits. In this study, we investigated subconcussive neurological deficit in football players using the KD test, and correlated test performance with head impact exposure measured using an instrumented mouthguard.

Methods: Baseline KD scores were gathered from 34 collegiate football players and 18 control volleyball players. We gathered post-athletic-event KD scores over 55 player-events in football and 18 player-events in volleyball. We defined a test completion time increase of over 5 seconds without a clinically diagnosed concussion to be subconcussive neurological deficit. For a subset of the players (n=9), we gathered head impact data using instrumented mouthguards.

Results and Discussion: Over 55 player-events, there were 2 occurrences of subconcussive neurological deficit in football players and none in control volleyball players. This occurrence in football was higher than the previously reported collegiate football concussion incidence of 3.02 per 1000 player-events. Among the nine instrumented players, one showed subconcussive neurological deficit, while most players showed an improvement in test performance. The changes in test completion time linearly correlated with number of head impacts with a Pearson's correlation coefficient of 0.75 (p=0.02).

Conclusion: In this pilot study, we showed that the occurrence of subconcussive neurological deficit as measured by an oculomotor test was higher than reported concussion incidence. In addition, oculomotor deficit may correlate with the number of head impacts sustained. Further studies instrumenting a greater number of players can

help examine the mechanism of how impact exposure accumulates to induce neurological deficit.

Keywords: Neurological Testing, Head Impact Exposure, Oculomotor Function

B09 DEPRESSION

B09-01

ELEVATED BASELINE LEVELS OF INTERLEUKIN-6 ARE ASSOCIATED DEPRESSION AFTER SPINAL CORD INJURY

Kiralyn Brakel^{1,2}, Alejandro Aceves¹, Miriam Aceves^{1,2}, Michelle Hook^{1,2}

¹Texas A&M Health Science Center, Neuroscience and Experimental Therapeutics, Bryan, USA

²Texas A&M Institute for Neuroscience, College Station, USA

Previously, we have shown that approximately one-third of spinally injured rats exhibit behavioral, physiological and immunological correlates of depression. This incidence corresponds with the clinical population. Following SCI, 18–26% of patients are diagnosed with depressive disorders, compared to 8–12% in the general population. As increased inflammation strongly correlates with depression in both animal and human studies, we hypothesized that the immune activation inherent to SCI could foster future depression. Thus, we proposed that reducing immune activation with minocycline, a microglial inhibitor, would decrease depression-like behavior following injury. Male Sprague-Dawley rats were given access to minocycline (0, 0.33 or 1 mg/ml) in their drinking water for 14 days following a moderate, mid-thoracic (T12) spinal contusion. An array of depression-like behaviors (social activity, sucrose preference, forced swim, open field activity) were examined prior to injury as well as on days 2, 9, 19, and 30 post-injury. Peripheral cytokine levels were analyzed in serum collected prior to injury and on days 10 and 32 post-injury. Hierarchical cluster analysis divided subjects into two groups based on behavior: depressed and not-depressed. Depressed subjects displayed lower levels of open field activity and social interaction relative to their not-depressed counterparts. Minocycline did not appear to reduce depression; equal numbers of minocycline versus vehicle-treated subjects appeared in both phenotypic groups. Further, minocycline dose did not affect serum cytokine levels, but depressed subjects showed significantly greater expression of pro-inflammatory cytokines (IL-6, TNF α , IL-2, IL-13, IL-5) at 10 days post-injury. Interestingly, depressed subjects also exhibited elevated pro-inflammatory cytokines prior to injury. Subjects who later showed depressive behaviors had higher baseline levels of IL-6, which persisted throughout the duration of the experiment. While others have found that elevated IL-6 alone does not produce depressive behavior, our data suggest that it could predict the development of depression after a physical stressor. Funding: Gillson Logenbaugh Foundation, Mission Connect

Keywords: Spinal Cord Injury, Depression, Proinflammatory cytokines, Interleukin-6, Rat model SCI

B09-02

S100A1 IS ESSENTIAL FOR PEDIATRIC TRAUMATIC BRAIN INJURY (PEDTBI) INDUCED DEPRESSIVE BEHAVIOR

Chirayukumar Pandya, Sumbul Fatima, Bhaumik Pandya, Kumar Vaibhav, Krishnan Dhandapani, Md Nasrul Hoda
Augusta University, MLIRS, Augusta, USA

Pediatric traumatic brain injury (PedTBI) is a major public health problem due to its highest rate of TBI (~40%), especially in males

aged 0–4 years. PedTBI is also associated with increased long term psychiatric disorders including depression in children that they can carry to their adulthood. However, the molecular mechanisms underlying these signaling, morphological alterations and their association with depression have not yet identified. Here we show that chronic PedTBI increases levels of S100A1, an isoform of S100 Ca⁺⁺-binding proteins in the brain is associated with a multitude of intracellular functions like learning and memory in mice frontal cortex. This concurrent with a decrease levels of brain derived neurotrophic factor (BDNF), plays important roles in the development and plasticity of neural circuits that is known to be altered in depression. We also found significant decrease in BDNF, but increase in S100A1 mRNA expression in PFC of PedTBI mice. We found that mice genetically deficient in S100A1 showed improved behavioral activities and BDNF signaling deficits caused by chronic PedTBI. These data implicate S100A1 in the development of PedTBI induced depression. Using S100A1^{-/-} mice as a novel resource, functional studies at the cellular and molecular level would reveal more about the involvement of S100A1 in depressive phenotypes. Identifying novel regulatory mechanisms of BDNF, a critical mediator of neuroplasticity, by S100A1 may provide avenues to develop newer therapeutics for depression and psychiatric disorders.

Keywords: S100A1, Pediatric traumatic brain injury (PedTBI), Depression, BDNF

B09-03

THROMBIN INDUCES DOWN-REGULATION OF GLAST AND GLT-1 AND CONTRIBUTES TO DEPRESSION AFTER TRAUMATIC BRAIN INJURY VIA PAR-1

Chunshu Piao, Ashley Holloway, Sue Hong, Mark Wainwright
Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Department of Pediatrics, Chicago, USA

Background: Depression is a major chronic complication after traumatic brain injury (TBI) but the mechanism by which TBI causes depression is not known. Human autopsy and preclinical studies implicate loss of astrocyte glutamate transporters and dysregulation of glutamate signaling in the mechanisms of depression. We have previously shown in primary astrocytes that thrombin causes a decrease in glutamate transporter via activation of Rho-kinase. Here we tested the hypothesis that TBI activates thrombin, resulting in down-regulation of glutamate transporters, which contributes to the depression after TBI.

Methods: We used a mouse closed skull TBI model, measured the activation of thrombin, expression of glutamate transporters and the depressive-like behaviors. To elucidate whether thrombin/PAR-1 activation results in down-regulation of glutamate transporters, we administered SCH79797, a selective PAR-1 antagonist, after TBI, and evaluated the expression of GLAST and GLT-1. To investigate whether the down-regulation of glutamate transporters are via PAR-1 activation, we infused PAR-1 agonist, and examined the expression of GLAST and GLT-1. We also measured in vivo real-time glutamate changes after TBI.

Results: Following TBI there was a significant decrease in cortex and hippocampal GLAST and GLT-1 levels at 7-day recovery. Depressive behaviors measured by tail suspension, forced swim, and sucrose preference tests were also increased compared to sham controls. Thrombin was activated after TBI, and inhibition of PAR-1 by SCH79797 ameliorated the down-regulation of GLAST and

GLT-1 in TBI mice compared to controls. Activation of PAR-1 induced down-regulation of GLAST and GLT-1 and depressive behaviors. Changes in glutamate levels are significantly elevated in mice subjected to TBI.

Conclusions: These data suggest that thrombin contributes to the down-regulation of hippocampal and cortical astrocyte transporters caused by TBI. This response is mediated by the PAR-1 receptor. These results also provide indirect evidence linking decrease in glutamate transporter expression and dysregulation of glutamate signaling to depression, a major cause of morbidity following moderate and severe TBI.

Keywords: Glutamate transporters, thrombin, Par-1, depression

B10 DIAGNOSTICS

B10-01

THE INCIDENCE, ETIOLOGY, AND RECORDED DIAGNOSTIC CRITERIA OF TRAUMATIC BRAIN INJURY IN THE COUNTY OF KAINUU, FINLAND 2004-12

Samuli Kemppainen¹, Asla Pitkänen^{2,3}, Olli Tenovuori⁴, Matti Sillanpää⁵

¹*Kainuu Social and Health Care Joint Authority, Laboratory of Clinical Neurophysiology, Kajaani, Finland*

²*A.I.Virtanen Institute for Molecular Sciences, UEF, Dep of Neurobiology, Kuopio, Finland*

³*Kuopio University Hospital, Dep of Neurology, Kuopio, Finland*

⁴*Turku University Hospital and University of Turku, Dep of Rehabilitation and Brain Trauma, Turku, Finland*

⁵*University of Turku, Deps of Public Health and Child Neurology, Turku, Finland*

Traumatic brain injury (TBI) is one of the leading causes of disability and disablement in the Western World. The aim of the study is to provide data on incidence, demography and etiology in adults with TBI.

Patients with an ICD-10 diagnosis code indicating TBI or a high probability of TBI were collected from the medical files of Kainuu Central Hospital 2004-12 and Sotkamo Municipality Health Center 2005-12 (67.774 and 8.824 adult inhabitants, respectively). Demographic features, etiology, and clinical findings for the diagnosis of TBI were reviewed.

The first 657 potential TBI patients were reviewed. Twentysix patients were excluded because of TBI before year 2004, 85 patients were children, and 92 patients were living outside the County of Kainuu. Of the remaining 454 patients, 193 were diagnosed non-TBI and 89 uncertain TBI. The remaining 172 had definite TBI, of which 5 TBI incidents were duplicates, making up final study sample. The annual crude incidence per 100.000 adult inhabitants of TBI with medical attendance was 135.47 (365/72/9*100.000/67.774*163) in the Kainuu Central Hospital and 28.73 (365/72/8*100.000/8.824*4) in the Sotkamo Municipality Health Center summing 164 new TBI incidents in the County of Kainuu annually. Falls were the leading cause of TBI (63%) and alcohol consumption was involved in 24% of cases. Evidence of loss of consciousness was found in 40% of patients. Acute CT scan was acquired in 60% of patients; one tenth of patients had acute TBI related findings.

Eighty-three % of newly diagnosed TBI cases are recognized in a hospital and 17% in a basic health care.

Keywords: Traumatic brain injury, incidence, etiology, clinical findings

B11 ELECTROPHYSIOLOGY

B11-01

ELECTROPHYSIOLOGICAL PROPERTIES OF PARVALBUMIN-EXPRESSING INTERNEURONS IN THE DENTATE GYRUS AFTER LATERAL FLUID PERCUSSION INJURY

Kaitlin Folweiler^{2,3}, Hannah Metheny², Akiva Cohen^{1,2}

¹University of Pennsylvania, Anesthesiology and Critical Care Medicine, Philadelphia, USA

²Children's Hospital of Philadelphia, Anesthesiology and Critical Care Medicine, Philadelphia, USA

³University of Pennsylvania, Neuroscience Graduate Group, Philadelphia, USA

Inhibitory interneurons and their functional integration into the network are thought to play a key role in regulation of cortical excitatory input to the dentate gyrus (DG). After traumatic brain injury, GABAergic inhibition is reduced onto dentate granule cells, and is associated with DG network hyperexcitability. Within the DG, parvalbumin-positive (PV+) interneurons (i.e., fast-spiking basket cells and axo-axonic cells) exert strong perisomatic inhibition onto dentate granule cells. While it is known that PV+ interneuron loss occurs after injury, it is unknown how the intrinsic and synaptic properties of the surviving DG PV+ population is affected. In this study, we examined both the intrinsic membrane properties and excitatory synaptic input onto DG PV+ interneurons one week after lateral fluid percussion injury (LFPI) or sham surgery in transgenic mice expressing the fluorescent marker tdTomato in PV+ interneurons. Preliminary data from whole-cell patch clamp recordings reveal no change in the intrinsic membrane properties—including resting membrane potential, action potential threshold and input resistance—of PV+ interneurons after LFPI. However, voltage clamp recordings demonstrate a reduction in spontaneous and miniature EPSC frequency and amplitude in PV+ neurons from LFPI mice compared to sham controls. These findings suggest that while membrane excitability of PV+ interneurons is left intact after LFPI, excitatory drive onto this inhibitory population may be diminished. Less excitatory activation of PV+ interneurons could provide a mechanism of reduced inhibition onto granule cells, and contribute to shifts in DG network excitability after injury.

Funding provided by: NICHD R37 HD059288

Keywords: lateral fluid percussion injury, dentate gyrus, interneurons, parvalbumin

B11-02

TERMINAL DEPOLARIZATION FOLLOWS ELECTRICAL SILENCE IN DEATH OF THE HUMAN CEREBRAL CORTEX

Jed Hartings¹, Sebastian Major³, Vince DiNapoli¹, Maren Winkler³, Jason Hinzman¹, Brandon Foreman¹, Johannes Woitzik³, Norberto Andaluz¹, Andrew Carlson², Jens Dreier³

¹University of Cincinnati, Neurosurgery and Neurology and Rehabilitation Medicine, Cincinnati, USA

²University of New Mexico, Neurosurgery, Albuquerque, USA

³Charité University Medicine Berlin, Neurology, Experimental Neurology, and Neurosurgery, Berlin, Germany

A spreading 'wave of death', known as terminal depolarization, is hypothesized to mediate the death of cerebral cortex after survivable ischemic insults and at the end-of-life. To determine whether this phenomenon occurs in the human brain, here we performed electrocorticographic recordings with either subdural electrode strips (n = 3) or intraparenchymal electrode arrays (n = 5) in patients with devastating

brain injury from ruptured aneurysms (n = 3) or trauma (n = 5) that resulted in activation of a Do Not Resuscitate-Comfort Care order followed by terminal extubation. Systemic physiology and multimodal brain monitoring were performed. Cortical brain function was measured by spontaneous synaptic activity (0.5–50 Hz) and terminal depolarization was measured by direct-current potentials (<0.02 Hz) from intracranial electrodes. The dying process began with declining cardiac function and brain partial pressure of oxygen. This first resulted in the silencing of cortical synaptic activity (i.e., isoelectricity) when mean arterial pressure fell to ~20 mm Hg. In every case, isoelectricity developed simultaneously on intracranial as well as scalp EEG electrodes. After a delay of 1 to 5 min, a large-amplitude (2 to 9 mV) negative shift of direct-current cortical potential then developed on intracranial electrodes in all patients, indicating terminal depolarization. In 5 patients, the depolarization was observed to spread across cortex at 3 to 31 mm/min. Terminal depolarizations followed cardiac arrest with median delay of 8.2 min. These results provide fundamental insight into the neurobiology of dying and have further implications for survivable cerebral ischemic insults such as cardiac arrest and stroke.

Keywords: spreading depolarization, spreading depression, cardiac arrest

B11-03

EFFECT OF IMMEDIATE RESIDUAL COMPRESSION FOLLOWING A RAT DISLOCATION SPINAL CORD INJURY ON SOMATOSENSORY EVOKED POTENTIALS

Stephen Mattucci^{1,2}, Jason Speidel^{1,2}, Jie Liu², Matthew Ramer², Wolfram Tetzlaff², Thomas Oxland^{1,2}

¹University of British Columbia, Orthopaedics and Mechanical Engineering, Vancouver, Canada

²International Collaboration On Repair Discoveries, Vancouver, Canada

The timing of surgical decompression following a spinal cord injury (SCI) is controversial, as clinical studies have yet to show strong evidence for the effectiveness of early decompression, despite positive preclinical study results. However, there are no previous preclinical studies which employ the most clinically common SCI mechanism: dislocation. Additionally, most preclinical residual compression studies involve slow force application or a time period after insult when the spinal cord is decompressed before residual compression is applied – both being counter to what happens in real-world injuries. The objective of this study was to determine the acute effect of immediate residual compression following a traumatic dislocation spinal cord injury in a rat using somatosensory evoked potentials. A 1.0 mm dislocation spinal cord injury was performed on twelve rats at C5/C6, then either reduced completely or held in residual compression at 0.8 mm. SSEP signals reached steady state prior to injury, and were recorded for 30 minutes post-injury. When the injury was immediately reduced to 0 mm following dislocation, the SSEP amplitude typically dropped immediately. In four cases the signal was absent for at least 12 minutes, and the average never recovered to above 50% baseline. When the spinal cord was held in compression following injury, in each instance the signal remained steady, or increased. The two groups were significantly different at each time point post injury ($p < 0.05$). Intuitively, one would expect added compression to the spinal cord would reduce signal conduction, however the results demonstrated the opposite. Two potential hypotheses are: compression activates Piezo2 stretch channels, effectively sensitizing the axons, and also restrict tonic descending inhibitory axons, increasing the probability of ascending axon potentials firing. These results empha-

size the need for preclinical studies to replicate the pathophysiology of residual compression immediately following a traumatic spinal cord injury.

Keywords: Dislocation, Residual Compression, Animal Model, Pathophysiology, Decompression, Acute

B11-04

IN VIVO ELECTROPHYSIOLOGICAL ALTERATIONS OF THE HIPPOCAMPUS IN A PORCINE MODEL OF DIFFUSE AXONAL INJURY

Alexandra Ulyanova^{1,2}, Paul Koch¹, Michael Grovola², Robin Russo¹, Douglas Smith¹, Victoria Johnson¹, D. Kacy Cullen^{1,2}, John Wolf^{1,2}

¹University of Pennsylvania, Neurosurgery, Philadelphia, USA

²Philadelphia VA Medical Center, Philadelphia, USA

Memory disruption is often an indication of neurological deficit due to traumatic brain injury and/or post-traumatic epilepsy. Pigs have been demonstrated as an ideal model of inertial injury given the gyrencephalic aspects of the porcine brain and significant white matter composition that produce diffuse axonal injury. We performed *in vivo* extracellular recordings in order to investigate changes in hippocampal function post injury. To precisely place silicone in-depth probes into porcine hippocampus, we have implemented MRI-free stereotaxis and single-unit functional mapping. Oscillatory field potentials and single-unit neuronal activity were recorded in the dorsal hippocampus of miniature swine in sham and injured animals. Pathophysiological changes were studied at 7 days following a model of closed-head rotational acceleration that produces diffuse axonal injury. Male Yucatan miniature pigs (6 months) underwent coronal rotational acceleration (220–260 rad/sec) with little or no loss of consciousness and minimal subdural bleeding. Paired pulse facilitation in CA1 was deficient, potentially due to changes in neurotransmitter release probability. Theta burst stimulation in entorhinal cortex used to alter baseline activity induced sub-clinical epileptiform activity in a subset of animals. Pyramidal cells and interneurons in CA1 layer were affected differentially based on waveform and firing rate analysis. These alterations suggest an increased post-synaptic excitability in response to deafferentation, or a shift in the excitation-inhibition balance of the local circuitry. Over time post injury these changes may lead to circuit-level changes in the hippocampus that may elicit sub-clinical epileptiform activity and potentially lower seizure thresholds, as well as disrupting network level memory encoding. This large-animal methodology has since been expanded into awake chronic recordings which are being utilized to evaluate the progression of epileptogenesis post injury, as well as investigating how disruptions in limbic circuitry may contribute to post traumatic stress disorder as a co-morbidity.

Keywords: In-Vivo, Stereotaxis, Silicone Probes, Single-Unit Activity, Oscillatory Field Potentials, Hyperexcitability

B12 ENDOCRINE

B12-01

SEX-DEPENDENT NEUROENDOCRINE STRESS RESPONSES AFTER MILD BLAST TBI

Ashley Russell^{1,2}, Elizabeth Shupe³, Robert Handa⁴, John Wu^{1,2,3}

¹Uniformed Services University, Program in Neuroscience, Bethesda, USA

²Uniformed Services University, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

³Uniformed Services University, Department of Obstetrics and Gynecology, Bethesda, USA

⁴Colorado State University, Department of Biomedical Sciences, Neuroscience Division, Fort Collins, USA

Of the 1.5 million people diagnosed with TBI annually, 15–30% develop neuroendocrine dysregulation, leading to subsequent diagnosis of neuropsychiatric disorders. Among military personnel, mild blast (mb)TBI is the leading diagnosis in active war zones. Currently, little is known on how mbTBI disrupts the limbic-HPA axis differentially among males and females, resulting in a dysregulated neuroendocrine stress response. This study sought to examine the sex-dependent hormonal, neuroanatomical and behavioral responses after mbTBI. Animals were exposed to mbTBI (15.5 psi) utilizing the ORA Advanced Blast Simulator and tested either short- (1 day) or long-term (7–12 days) post-injury. To assess the HPA axis after mbTBI, serum corticosterone (CORT) was measured. mbTBI enhanced the diurnal CORT rise ($p < 0.05$). In response to restraint, mbTBI diminished short-term CORT in males and long-term CORT in females ($p < 0.05$). We then examined the central and peripheral components in the limbic-HPA stress response. mbTBI did not alter peripheral adrenal 11β -OHase and MC2R or pituitary POMC and CRFR1 expression. DEX suppressed CORT regardless of injury, pointing to a central dysregulation of the HPA axis. Utilizing a transgenic model, we visualized stress reactive CRF neurons in the stress-relevant limbic-PVN circuitry. cFOS labeling was used to detect restraint-induced CRF neuronal activation after mbTBI. In non-injured animals, proestrus females had greater PVN CRF neuronal activation than males ($p < 0.05$). mbTBI increased PVN CRF neuronal activation in injured compared to sham females ($p < 0.05$). Preliminary data examining limbic regions suggest increased PFC CRF neuronal activation after mbTBI. Full analysis on CRF activation in all stress-relevant limbic regions is underway. Exposure to mbTBI increased anxiety-like behaviors in all animals ($p < 0.05$). Understanding the limbic-HPA disruption can begin to develop a targeted approach to treatment for the long-lasting TBI-induced neuroendocrine dysregulations.

Keywords: sex differences, HPA axis, stress, glucocorticoids

B13 EXERCISE

B13-01

ACTIVITY RESTRICTION AFTER SCI SIGNIFICANTLY ALTERS VASCULAR STRUCTURE AND FUNCTION

Fiona Brabazon^{1,4}, Kathryn M. DeVeau^{2,4}, Sujata Saraswat Ohri^{1,4}, A. Gabrielle Hamilton³, B. Danni Wood¹, David S. K. Magnuson^{1,4}

¹University of Louisville, Department of Neurological Surgery, Louisville, USA

²University of Louisville, Anatomical Sciences & Neurobiology, Louisville, USA

³University of Louisville, Biomedical Engineering, Louisville, USA

⁴University of Louisville, KSCIRC, Louisville, USA

Cardiovascular (CV) dysfunction is arguably the most deleterious secondary outcome of SCI and is one of the leading causes of morbidity in the chronic SCI population. CV decline may be further exacerbated by reduced activity following injury. The objective of the present study was to investigate the cardiac changes induced by limiting an animals' ability to move by reducing their cage size. Adult

female Sprague Dawley rats received a T2 25 g-cm SCI, then were housed in either large cages (LC; n=16) or tiny cages (TC; n=16). Vascular function from the carotid (CA) and femoral arteries (FA) were assessed at baseline, week 1 post-SCI and every two weeks for 10 weeks using ultrasound. All animals showed a reduced CA diameter at 2 weeks post-injury which recovered significantly by 8 weeks for the LC group only. Interestingly, there were no differences in CA blood flow velocity between the TC and LC animals. In contrast, there were differences in FA peak and mean velocity at weeks 6 and 8 post-SCI between the TC and LC groups. Quantitative PCR analysis showed increased expression of markers of angiogenesis including elastin, ACE (Angiotensin-Converting Enzyme), and CD31 (Platelet Endothelial Cell Adhesion Molecule-1), in LC animals compared to TC. Additionally, there was an increase in markers of adaptive ER stress including Atf4, Chop, Grp78, Gadd3 and Xbp1 in the LC animals compared to TC. These findings show that simply limiting the amount of spontaneous activity after SCI influences vascular structure and function above and below the lesion level.

*Dr. Brabazon and Dr. DeVea contributed equally to this work.

Keywords: SCI, vascular remodeling, exercise, ultrasound

B13-02

EFFECTS OF TRAUMATIC BRAIN INJURY ON EXERCISE CAPACITY

Lisa Kreber, Charan Singh, Grace Castillo, Megan Schafer, Grace Griesbach

Centre for Neuro Skills (CNS), Clinical Research and Education, Bakersfield, USA

Introduction: Following traumatic brain injury (TBI), resultant physical deficits compounded by autonomic dysregulation significantly impact capacity to exercise and fitness. The objective of this study was to assess changes in cardiovascular fitness and exercise tolerance in response to treadmill ambulation in individuals with TBI.

Methods: Cardiovascular fitness was assessed via cardio pulmonary exercise testing (CPET) in five, adult males (M age=38; M chronicity=385 days) with TBI every 2-weeks until discharge. CPET physiological measurements included maximum respiratory oxygen uptake (VO₂ max), carbon dioxide production (VCO₂), heart rate (HR), body temperature, and blood pressure during a modification of the Bruce Treadmill Test. In addition, actigraphy was also obtained on these patients. Patients were randomly assigned to an aerobic exercise (3 times per week for 30 minutes) or non-exercise group. Functional, cognitive and quality of life were assessed in each participant at admission and discharge from the study.

Results: Preliminary findings of this ongoing clinical study indicate that 80% of patients fell below published norms for predicted maximum HR at initial CPET testing. At each successive CPET testing time point, more patients were able to reach their predicted maximum HR. Eighty percent of patients had VO₂ max levels below age and gender matched published normative data from uninjured adults at initial CPET testing. Over time, the percentage of patients who were below published norms for VO₂ max decreased (40% at 2-week testing and 25% at 4-week testing). Patients who were in the aerobic exercise group, increased their VO₂ max over time at a faster rate compared to patients in the non-exercise group.

Conclusions: Patients with chronic TBI have an abnormally low VO₂ max and maximum HR indicating exercise intolerance. This intolerance is less pronounced across time in patients who engage in higher amounts of aerobic exercise.

Keywords: Actigraphy, traumatic brain injury, exercise capacity, heart rate, VO₂ max

B14 FREE RADICALS

B14-01

MITOCHONDRIAL REDOX REGULATION OF NEUROPATHIC PAIN AFTER SPINAL CORD INJURY

Lonnie Schneider

University of Alabama at Birmingham, Physical Medicine and Rehabilitation, Birmingham, USA

Spinal cord injury (SCI) results in sudden life changes such as paralysis, loss of bowel and bladder control, spasticity and neuropathic pain (NP). Spinal cord-induced neuropathic pain (SCI-NP) has a massive impact on the patients' quality of life following injury. Of the people who suffer from SCI, 60%–80% experience SCI-NP and 1/3 of those describe the pain as debilitating. Although some of the biological underpinnings of neuropathic pain (NP) have been studied, *there are no effective treatments for SCI-NP*. Major factors in the pathophysiology of SCI-NP are reactive oxygen species (ROS) and reactive nitrogen species (RNS). These oxidative stress molecules are acutely increased after SCI and work in concert to modify proteins. Nitric oxide (NO) production is thought to be a major contributor of oxidative stress following SCI. NO induced protein thiol-nitrosylation (SNO) represents an understudied redox mechanism for the formation of NP. It is known that SNO is dependent on the redox state of the cell which is a function of mitochondrial bioenergetics. Mitochondrial bioenergetic dysfunction is involved in chemotherapeutic and inflammatory induced NP; however its role in SNO and SCI-NP has not been thoroughly evaluated. Surviving cells in the spinal cord are maintained in a chronic state of inflammation and oxidative stress and are subject to altered bioenergetics. The purpose of this study is to understand how mitochondrial bioenergetics alters SNO during the pathological changes post-SCI. This project will test the hypothesis that sub-lethal bioenergetic changes alter SNO and contribute to SCI-NP. Here we found that SCI leads to acute and chronic decreases in glutathione levels, alterations in mitochondrial oxygen consumption and changes in SNO. These changes in the oxidative environment correspond to increased pain states. Identification and validation of SNO and specific bioenergetic changes in the acute and chronic stages post-SCI will provide a basis in which multi-modal and synergistic therapies can be developed for ameliorating SCI-NP.

Keywords: Nitric Oxide, Neuropathic pain, Spinal Cord Injury

B15 GENETIC FACTORS

B15-01

VARIATION IN GENES ENCODING CANDIDATE BIOMARKERS ARE ASSOCIATED WITH NEUROLOGICAL OUTCOMES AFTER SEVERE TBI

Ava Puccio, Nicole Osier, Yvette Conley, David Okonkwo

University of Pittsburgh, Neurosurgery, Pittsburgh, USA

Objectives: Diagnostic and prognostic biomarkers of traumatic brain injury (TBI) are being actively pursued, with promising candidates including glial fibrillary acid protein (GFAP), S100 calcium-binding protein B (S100B), and Ubiquitin C-Terminal Hydrolase L1 (UCHL1). The relationship between biomarker-encoding genes and TBI outcomes remains unknown. The purpose of this pilot study was to explore variation in 18 single nucleotide polymorphisms (SNPs) in biomarker-encoding genes as predictors of neurological outcome in a population of adults with severe TBI.

Methods: Severe TBI participants (n=305) were consented through an IRB-approved protocol by proxy. DNA was extracted and SNPs were genotyped using iPLEX MassARRAY multiplex assay platform. Glasgow Outcomes Scale (GOS) score was assessed at 3-, 6-, 12-, and 24-months post-injury by a trained neuropsychological technician. Multinomial logistic regression was used to calculate the Odds Ratio (OR) and determine the odds of having a lower score on the Glasgow Coma Scale (GCS 3–5 vs. 6–8) and GOS (= 1–2 vs. 3–5) based on variant allele presence, while controlling for confounders.

Results: Possessing the variant allele of one GFAP SNP (rs3785891; OR=0.58; $p=0.03$); was associated with higher admission GCS score as was the variant allele of one S100B SNP (rs2839365; OR=0.52; $p=0.01$). Possessing the variant allele of a second S100B SNP (rs1051169) was associated with higher scores on the GOS at 3 months (OR=0.39; $p=0.04$), 6 months (OR=0.34; $p=0.02$), 12 months (OR=0.32; $p=0.02$), and 24 months (OR=0.30; $p=0.02$) post-severe TBI.

Conclusions: The relationship between polymorphisms, protein levels, and biomarker utility, merits examination, especially in promising candidates following a TBI. This information may be relevant to enhancing interpretation of existing biomarker data, identifying novel biomarkers, and ultimately harnessing this information to improve clinical outcomes and personalize care.

Keywords: severe TBI, brain trauma, genotype, neurological outcomes

B16 HEMORRHAGE

B16-01

HEMIN IS PROTECTIVE WITH A CLINICALLY-RELEVANT TIME WINDOW AFTER INTRACEREBRAL HEMORRHAGE

Jing Chen-Roetling, Raymond Regan

Thomas Jefferson University, Emergency Medicine, Philadelphia, USA

Hemorrhage is the primary event in about 15% of strokes, and accompanies most severe traumatic CNS injuries. Blood-brain barrier (BBB) disruption is a key feature of hemorrhagic injuries and may contribute to peri-hematoma cell loss. The authors' previous study demonstrated that treatment with the heme oxygenase-1 (HO-1) inducer hemin improved barrier function and neurological outcome after experimental intracerebral hemorrhage (ICH). Since hemin is already in clinical use to treat acute porphyrias, the present study was designed to test the hypothesis that its protective efficacy on BBB function after ICH was sustained through a clinically-relevant time window. ICH was induced in Swiss-Webster mice by an infusion of autologous blood or bacterial collagenase into the right striatum. Mice were randomly assigned to hemin (4 mg/kg i.p.) or vehicle groups; treatment was initiated at intervals up to 12 hours after the induction of ICH, and was repeated daily for a total of four doses. Blood-brain barrier disruption was assessed by Evans blue assay and perihematoma cell viability by MTT assay. A total of 77 mice were used in this study. Two mice died within 24 hours of ICH; both had been randomized to the vehicle group. Consistent with prior observations, systemic hemin therapy increased striatal HO-1, with expression localized to perivascular cells that were predominantly astrocytes. In the blood injection model, hemin reduced parenchymal extravasation of Evans blue by over 60% when therapy was initiated at up to 6 hours; this was associated with a 40% increase in striatal cell viability at four days. The therapeutic time window was narrower in the collagenase model, with no effect on BBB integrity or perihematoma cell vi-

bility when treatment was initiated 6 hours or later after ICH. These results suggest that hemin is protective in mouse ICH models when treatment is initiated within 3–6 hours of hemorrhage. In light of the established safety profile of this drug over decades of clinical use, further investigation as a primary therapy for hemorrhagic CNS injuries seems warranted.

Keywords: intracerebral hemorrhage, stroke, heme oxygenase-1, subarachnoid hemorrhage

B16-02

PAIN INDUCED HEMORRHAGE AFTER SPINAL CORD INJURY IS BLOCKED BY PENTOBARBITAL ANESTHESIA

Jacob Davis, Yung-Jin Haung, Misty Strain, Joshua Reynolds, James Grau

Texas A&M, Psychology, College Station, USA

We have shown that nociceptive stimulation after a contusion injury increases tissue loss and impairs long-term recovery. We recently showed that the local application of an anesthetic (lidocaine; Turtle et al., 2017, *J Neurotrauma*, 34, 1200–1208), but not systemic morphine, has a protective effect that counters the adverse effect of nociceptive stimulation. Other work implies that this local effect depends, in part, on communication with rostral (brain-dependent) neural systems. Supporting this, nociceptive stimulation has no effect on tissue sparing in rats that received a rostral spinal transection after a contusion injury (see presentation by Reynolds et al.). This suggests that experimental treatments that generally inhibit brain function may reduce the adverse effects of nociceptive input. The present study explored this possibility by testing the effect of systemic pentobarbital. Rats received a contusion injury at T12 and, 24 hrs later, were given an anesthetic dose of pentobarbital (30 mg/Kg). Subjects then received 6 min of intermittent tailshock or nothing. Three hours after shock, rats were sacrificed and one cm of tissue was collected enveloping the injury site. The extent of hemorrhage was assessed by measuring the absorbance of light at 420 nm. As expected, nociceptive stimulation increased the extent of hemorrhage in vehicle treated (awake) rats. It appears that this effect was attenuated by pentobarbital anesthesia. This is consistent with prior work showing that anesthesia also reduces the adverse effect of nociceptive stimulation on recovery. Further work is exploring the generality of these results.

Supported by the Craig H Neilsen Foundation and the National Institute of Health under award number R21465891-00001 to JW.

Keywords: Pentobarbital, Contusion, Hemoglobin, anesthesia, shock

B16-03

ENDOTHELIOPATHY OF TRAUMA AFTER TRAUMATIC BRAIN INJURY

Erika Gonzalez Rodriguez, Sisse Ostrowski, Jessica Cardenas, Ernesto Lopez, Lisa Baer, Jeffrey Tomasek, Bryan Cotton, Paar Johansson, John Holcomb, Charles Wade

University of Texas Health Science Center Houston, Center for translational injury research, Houston, USA

Introduction: Bleeding prompts endothelial cell and glycocalyx (EGL) disruption that can lead to traumatic endotheliopathy (EOT) and worse outcomes. It is unknown if traumatic brain injury (TBI) can

trigger EOT. We aimed to determine the frequency of EOT, as measured by syndecan-1 (a main component of the EGL), and its association with worse outcomes in TBI patients. We hypothesized that TBI could trigger EOT that would result in poorer outcomes.

Methods: we included patients admitted with the highest level of trauma team activation. Demographics, clinical data, mechanism of injury, injury severity scores (ISS), and outcomes were obtained. We quantified biomarkers of EGL and endothelial cell disruption (syndecan-1 and soluble thrombomodulin (sTM), respectively) from plasma with ELISAs. Based on our previous work, we defined EOT+ as syndecan-1 level ≥ 40 ng/ml, determined its frequency in the general TBI population (Head AIS >2), and in polytrauma-TBI vs. isolated-TBI (other AIS regions <3). We stratified by EOT, and compared. With multifactorial analysis, we identified factors associated with mortality.

Results: Out of 410 patients, 179 (43.6%) had TBI. We found that i) the frequency of EOT+ (syndecan-1 ≥ 40 ng/ml) was similar in the Non-TBI and TBI groups; ii) EOT+ was significantly more frequent in polytrauma-TBI patients than in isolated-TBI patients; and, iii) survivors were less likely to have EOT+. After stratifying by EOT, TBI-EOT+ patients had about two-fold higher rates of mortality (TBI-EOT+35.6% vs. 20.0% TBI-EOT-) and blood transfusions than TBI-EOT- patients (71.2% vs. 34.2% respectively). Increased sTM levels (median EOT+5.2 vs. EOT- 4.7 ng/ml) was further confirmation of endothelial dysfunction; all $p < 0.05$. In multivariable logistic regression, EOT+ was associated with mortality (adjusted-OR 2.23 95%CI 1.00-4.80).

Conclusions: We demonstrated the presence of EOT+ in TBI patients. Polytrauma and TBI result in a higher frequency of EOT+, suggesting a synergic deleterious effect that facilitates EOT progression. Vascular leakage and endothelial dysfunction resulting from TBI may explain why EOT+ is associated with higher mortality and an increased need for blood transfusions.

Keywords: hemorrhage, endothelial damage, traumatic brain injury, syndecan-1, endothelial glycocalyx breakdown

B16-04

TUMOR NECROSIS FACTOR ALPHA (TNF-A) IS ASSOCIATED WITH HEMORRHAGE PROGRESSION AFTER TRAUMATIC BRAIN INJURY

Holly Hinson¹, Kelly Fair², Beth Rick², Elizabeth Dewey², Susan Rowell²

¹Oregon Health and Science University, Neurology, Portland, USA

²Oregon Health and Science University, Surgery, Portland, USA

Objectives: To determine if specific pro-inflammatory cytokine levels are associated with progression of intracranial hemorrhage (ICH) after traumatic brain injury (TBI).

Methods: A subset of patients with isolated TBI were analyzed from a prospective observational cohort of adult trauma patients. Progression of ICH was defined as $>30\%$ increase in lesion volume, worsened traumatic SAH, or presence of a new hemorrhagic lesion. Plasma cytokine levels were measured on admission and at 6 hours after injury, and analyzed using a multiplex fluorescent immunoassay. Samples were run in duplicate for confirmation and medians (pg/dL) were reported. Univariate ANOVA informed the construction of a multivariate model predicting ICH progression. Multivariate logistic regression models were constructed. Wald tests at $\alpha = .05$ were used to indicate significance. The primary outcomes were odds ratios for a 1-fold change (doubling of cytokine levels).

Results: 108 patients met criteria for inclusion in the analysis. Of the cytokines investigated (IL-6, IL-8, IL-10, IFN-g, TNF-a), only

TNF-a levels were significantly different between patients with and without ICH progression (admission: 1.03 v. 1.59, $P=0.045$) (6 Hours: 1.40 v. 1.43, $P=0.02$). Two models were constructed to avoid co-linearity between TNF-a at admission and 6 hours. For both models, lower values of TNF-a were significantly associated with ICH progression ($p=.02$ at admission, $p=.01$ at 6 hours) after accounting for GCS, age, and AIS head score. For each doubling of the value of TNF-a, the odds of ICH progression was 29% lower (OR=0.71, 95% CI: 0.53 to 0.94) at admission and 31% lower at six hours (OR=0.69, 95% CI: 0.52 to 0.91).

Conclusion: Lower serum TNF-a levels on admission and at 6 hours are associated with ICH progression in patients with isolated TBI. Future studies should be performed to determine if lower TNF-a values are associated with impaired hemostasis after TBI.

Keywords: TNF-alpha, Hemorrhage Progression, Isolated TBI

B16-05

TRIGEMINAL NERVE STIMULATION IMPROVES SURVIVAL AFTER TRAUMATIC BRAIN INJURY COMPLICATED BY HEMORRHAGIC SHOCK

Chunyan Li, Adil Afridi, Amrit Chiluwal, Raj Narayan

Feinstein Institute for Medical Research, Neurosurgery, Manhasset, USA

Introduction: Traumatic brain injury (TBI) and hemorrhagic shock (HS) often occur concomitantly, leading to severe morbidity and mortality. Patients with hypotension after severe TBI have twice the mortality rate of normotensive patients. Stimulation of trigeminal nerve induces a vasopressor response by activating rostral ventrolateral medulla (RVLM). We investigated the use of electrical trigeminal nerve stimulation (TNS) to temporize the effects of hemodynamic collapse with the aim of improving survival and attenuating end-organ damage.

Methods: A controlled cortical impact (CCI) model was used to create severe TBI in male Sprague-Dawley rats (250–400g). Immediately after CCI, 50% of the total blood volume was removed over 25 ± 5 minutes to induce polytrauma. Trigeminal Nerve Stimulation (TNS) was performed by introducing two needles (26G) placed subcutaneously bilaterally over the ophthalmic branch of the trigeminal nerve. Specific parameters were used for the electrical stimulation. Mean arterial blood pressure (MAP) was measured via a pressure transducer connected to the femoral artery.

26 rats were randomized to one of three study groups: (1) sham ($n=5$); (2) polytrauma ($n=10$); (3) polytrauma with TNS ($n=11$). TNS was delivered for 30 seconds every 10 minutes for 2 hours. The survival time, plasma levels of lactate dehydrogenase (LDH), aminotransferase (AST) and creatinine were quantified using commercially available assay kits according to the manufacturer's specifications after 90 minutes polytrauma.

Results: Polytrauma produced significant drops in MAP from 103.6 ± 12.7 to 34.2 ± 4.6 mm Hg ($n=12$). During the 30-second TNS, MAP significantly increased by $45.9 \pm 5.6\%$ compared to the baseline ($n=3$). As compared to polytrauma group, the survival time was significantly improved in animals with polytrauma who received trigeminal stimulation (86 ± 28 vs. 227 ± 99 minutes; $n=11$, $p < 0.05$; polytrauma vs. polytrauma+TNS). TNS also produced significant decreases in organ damage (LDH: 89.2 ± 18.9 vs. 38.7 ± 10.1 U/L; AST: 70.4 ± 11.9 vs. 37.5 ± 5.7 IU/L; Creatinine: 0.784 ± 0.009 vs. 0.69 ± 0.03 mg/dl; $n=5$ /group, $p < 0.05$) after 90 minutes of polytrauma.

Conclusion: TNS significantly increased the survival time and attenuated organ injury in an animal model of severe TBI complicated

by HS. TNS may serve as a novel resuscitation approach to boost the ability of organs and tissues to endure prolonged period of hemodynamic instability.

Keywords: traumatic brain injury, hemorrhagic shock, trigeminal nerve stimulation, organ damage

B16-06

TRIGEMINAL NERVE STIMULATION ATTENUATES SECONDARY BRAIN INJURY AFTER TRAUMATIC BRAIN INJURY AND HEMORRHAGIC SHOCK

Chunyan Li, Chiluwal Amrit, Adil Afridi, Raj Narayan

Feinstein Institute for Medical Research, Neurosurgery, Manhasset, USA

Introduction: Ischemia plays an early role in the development of secondary brain injury after traumatic brain injury (TBI). Stimulation of the trigeminal nerve has been shown to increase cerebral blood flow (CBF) by increasing arterial blood pressure (ABP) and reducing cerebrovascular resistance (CVR). In this study, we explore TNS as a novel neuromodulation therapy in attenuating secondary brain injury after severe TBI complicated by hemorrhagic shock.

Methods: A controlled cortical impact (CCI) model was used to create severe TBI in male Sprague-Dawley rats (275–375 g). Immediately after CCI, 40% of the total blood volume was removed over 20 minutes to induce polytrauma. Trigeminal Nerve Stimulation (TNS) was performed by introducing two needles (26G) subcutaneously bilaterally over the V1 distribution. Rectangular cathodal pulses (3V, 0.5 ms) were delivered by an electrical stimulator at 25 Hz. Arterial blood pressure (ABP) and CBF were measured simultaneously and CVR was calculated as a ratio between ABP and CBF and expressed as percentage of change relative to the baseline.

In the first study, 3 rats were spinalized at the C6 level and CVR was measured during TNS. In the second study, 40 rats were randomized to one of three groups: (1) sham (n=4); (2) polytrauma (n=18); (3) polytrauma with TNS (n=18). TNS was delivered for 1 minute every 15 minutes for 1 hour. Brain edema (wet-dry-weight method), lesion volumes (cresyl violet staining) and blood-brain barrier permeability (Evans blue dye) were measured at 24 hour after injury.

Results: In spinalized rats, the ABP remained at 42 ± 2 mm Hg (n=3). During TNS, CVR decreased to a minimum of $33.1 \pm 2.3\%$. In rats with polytrauma, the animals that received TNS demonstrated a significant decrease in brain edema ($80.1 \pm 0.7\%$ vs. $82.7 \pm 0.5\%$; n=6, p<0.05), lesion volumes (9.2 ± 1.7 vs. 14.3 ± 0.9 mm³; n=6, p<0.05) and blood-brain barrier permeability (1.7 ± 0.6 vs. 2.6 ± 0.4 µg/g; p<0.05; n=6) as compared to the control group.

Conclusion: TNS increases CBF by increasing ABP and decreasing CVR, and can mitigate ischemia after TBI. These data provide strong early evidence that TNS could be an effective neuroprotective strategy following brain injury.

Keywords: traumatic brain injury, hemorrhagic shock, trigeminal nerve stimulation, secondary brain injury

B16-07

ALTERATIONS IN PERIPHERAL REDOX SYSTEMS IN A RAT MODEL OF TRAUMATIC BRAIN INJURY ASSOCIATED WITH POLYTRAUMA

Bernard Srmbical Wilfred, Hye Mee Huang, Katherine Cardiff, Xiaofang Yang, Sarah Buttles, Shear Deborah, Lai Yee Leung
WRAIR, BTNN, Silver Spring, USA

Traumatic brain injury (TBI) commonly occurs in polytrauma with concomitant organ dysfunction. However, the pathophysiology of peripheral organs following TBI/polytrauma is poorly understood. Here we examined the status of redox systems, glycogen content, creatinine clearance and mitochondrial health in the peripheral organs following penetrating ballistic-like brain injury (PBBI) combined with hypoxemia and hemorrhagic shock (HH). Rats were assigned into four groups: sham, HH, PBBI, and PBBI combined with HH (PHH). In PHH or HH group, a 30-minute hypoxemia (PaO₂<40mmHg) was initiated 5 minutes following TBI followed by a 30-minute hemorrhagic shock (MAP ~40 mm Hg). Urine output, food and water intake were recorded at baseline and end points. Liver, kidney and heart tissues were collected at 1, 2, 7, 14 and 28 days post-injury (DPI). Reduced/oxidized Glutathione (GSH/GSSG) and glycogen content were quantified in the peripheral organs. Superoxide dismutase (SOD) and cytochrome C oxidase activities were measured using enzyme activity kits. Urine/serum creatinine levels were analyzed. Although polytrauma did not change the mitochondrial cytochrome C oxidase activity, a modest decrease in SOD activity was observed following PBBI on 2DPI. The PHH and sham groups exhibited similar trend over time, except that the kidney SOD activity in the PHH group was lower than the other groups on 14DPI. Hepatic glycogen levels were reduced acutely following polytrauma. Urine/serum creatinine ratio in PHH group was significantly elevated on 7-28DPI. Polytrauma induced a delayed disruption of the GSH/GSSG ratio in the liver and kidney, which resolved within 1–2 weeks post-injury. The increase in ratio was primarily due to the augmented GSH level which might imply a defensive mechanism against oxidative damage in these organs following TBI/polytrauma. Increased liver glycogenolysis in the PHH group might imply the increased energy demand following polytrauma. High urine/serum creatinine ratio in PHH group point to elevated renal clearance. Collectively, these specific stress marker results reflect temporal cytological/tissue level damage to the peripheral organs due to TBI/Polytrauma.

Keywords: Polytrauma, Systemic injury, Oxidative stress

B17 HYPOXIA / ISCHEMIA

B17-01

DELAYED HYPOXEMIA FOLLOWING TRAUMATIC BRAIN INJURY EXACERBATES LONG-TERM BEHAVIORAL DEFICITS

McKenzie Davies, Addison Jacobs, David Brody, **Stuart Friess**
Washington University at St. Louis, Pediatric Critical Care Medicine, Saint Louis, USA

Preclinical models of TBI have rarely shown cognitive or behavioral deficits beyond six weeks post injury, whereas outcome measures in clinical trials of therapeutics for TBI are typically assessed 6–12 months after injury. The absence of detectable behavioral deficits at longer time points in preclinical models may be a contributing factor to the lack of success in translating the relatively short-term efficacy of therapeutics in pre-clinical models to human trials. We have previously developed a murine model of TBI followed by delayed hypoxemia to model the secondary insult of hypoxemia and brain hypoxia occurring in the intensive care setting. Understanding long-term effects of delayed hypoxemia following TBI in our murine model is critical for future testing of candidate therapeutics targeting secondary brain hypoxia. Forty 5 week old male mice (C57BL/6J) were randomized to controlled cortical impact (CCI) (N=24) or sham surgery (N=16). One day later, awake animals were randomized to 60 minutes of hypoxemia or normoxemia. Six months after initial injury,

animals underwent behavior testing (Morris Water Maze (MWM), social interaction, and tail suspension) before euthanasia for immunohistochemical assessments. At six months post injury, mice experiencing CCI and hypoxemia (CCI+H) had longer swim distances to the hidden platform and spent less time in the target quadrant during probe testing compared with CCI alone or sham animals. During social interaction assessments, CCI + H mice spent less time interacting with novel stimulus mice than CCI alone or sham animals. CCI+H had larger lesion volumes compared with CCI alone (14.0% vs 9.9%, $P < 0.003$). GFAP immunohistochemistry at six months post injury demonstrated increased astrogliosis in the ipsilateral white matter of CCI+H compared with CCI alone. A clinically relevant model of delayed hypoxia following TBI resulted in long-term behavioral deficits and evidence of white matter injury.

Keywords: hypoxemia, hypoxia, secondary injury, preclinical, long-term outcomes, behavior

B17-02

PERFLUOROCARBON NVX-108 REDUCED SECONDARY BRAIN INJURY IN A SWINE MODEL OF TRAUMATIC BRAIN INJURY

Anke Scultetus^{1,2}, Lam Thuy Vi Tran Ho¹, Ashraful Haque¹, Biswajit Saha¹, Brittany Hazzard¹, Evan Unger³, Charles Auker¹, Paula Moon-Massat¹, Richard McCarron^{1,2}

¹Naval Medical Research Center, NeuroTrauma Department, Silver Spring, USA

²Uniformed Services University, Department of Surgery, Bethesda, USA

³NuvOx Pharmaceuticals, Tucson, USA

Objective: Hypoxia is an important cause of secondary brain damage after traumatic brain injury (TBI). Therapeutic agents such as perfluorocarbons (PFCs) can transport 50 times more oxygen than human plasma which may be advantageous in treating patients with TBI. We hypothesized that administration of the PFC emulsion NVX-108 (NuvOx Pharmaceuticals, Tucson, AZ) after TBI could improve neurological damage caused by hypoxia in a Fluid Percussion (FP)TBI swine model.

Methods: Anesthetized Yorkshire swine received FP-TBI at Time 0 (T0), followed by a bolus of NVX-108 (1 ml/kg) at T15 (TBI-NVX) or no treatment (TBI-NON). Animals were euthanized at the end of a 6 hour observation period, and the brain was immersion fixed for 48 hours. Brain slices were sectioned, mounted and stained using hematoxylin and eosin, cresyl violet, MAP2 and GFAP for light microscope examination. The scoring scale was ranked and quantified by a veterinarian pathologist who was blinded to the study groups. The scoring scale ranked from 0 (absent) to 5 (significant) for hemorrhage, spongiosis, inflammation and ischemic neurons. Histopathology scores between the groups were analyzed using an independent sample T-Test. For all parameters, P-values ≤ 0.05 were considered statistically significant.

Results: Spongiosis, ischemic neurons and hemorrhage in the overall brain regions were lower in TBI-NVX animals incompared to TBI-NON ($P > 0.005$), and was significantly less in the cerebellum [average score of 2.75 compared to TBI-NON animals (average score of 1.71 ($P = 0.04$)).

Conclusion: In this swine model of TBI, administration of NVX-108 resulted in lower injury scores for spongiosis in the cerebellum compared to the TBI-NON animals. This data suggests that NVX-108 may play a role in mitigating secondary brain damage by transporting oxygen to the injured areas of the brain especially the cerebellum.

Keywords: TBI, swine, brain tissue oxygenation

B17-03

PREHOSPITAL ADVANCED AIRWAY MANAGEMENT IN TRAUMATIC BRAIN INJURY AND ITS RELATION TO OUTCOME

Rebecka Rubenson Wahlin¹, David W. Nelson², Bo-Michael Bellander³, Mikael Svensson³, **Eric Thelin**³

¹Karolinska Institutet, Department of Clinical Science and Education, Södersjukhuset, Stockholm, Sweden

²Karolinska Institutet, Department of Physiology and Pharmacology, Section of Anesthesiology and Intensive Care, Stockholm, Sweden

³Karolinska Institutet, Department of Clinical Neuroscience, Stockholm, Sweden

Prehospital intubation in traumatic brain injury (TBI) focuses on limiting the effects of harmful secondary insults, such as hypoxia and hypotension, but has not shown indisputable evidence to be beneficial for patient outcome. The aim of this study was to explore the characteristics of patients that undergo pre-hospital intubation and, in turn, what factors that affect long-term functional outcome. All TBI patients ≥ 15 years admitted to the Department of Neurosurgery in Stockholm, Sweden from 2008 through 2014 were included. Data was extracted from prehospital and hospital charts, including prospectively collected Glasgow Outcome Score (GOS) after 12 months. Univariate and multivariate logistic regression models were employed to examine which parameters were independently correlated to prehospital intubation and outcome. A total of 458 patients ($n = 178$ unconscious) were included. Multivariate analyses indicated that high energy trauma, prehospital hypotension, pupil unresponsiveness, mode of transportation and distance to the hospital were independently correlated with pre-hospital intubation (model explained 0.393 adjusted pseudo- R^2), and among them only pupil responsiveness was independently associated with outcome. Pre-hospital intubation was neither associated with outcome in unconscious TBI patients in univariate regression ($p = 0.296$), nor did it add independent information in a step-up model of independent parameters versus GOS ($p = 0.154$). In summary, we showed a distinct discrepancy between factors correlated with pre-hospital intubation and parameters associated with outcome. We believe this indicates that the decisions to intubate or not at the scene are based on judgments that are multifactorial and difficult to assess in a retrospective analysis, but could be beneficial for the individual patient. Prospective studies with structured protocols are necessary to determine potentially favorable effects of pre-hospital intubation, but will be affected by logistic and ethical considerations.

Keywords: Traumatic brain injury, Advanced airway management, Pre-hospital trauma care, Human, Emergency medical services

B17-04

COLONIC REMODELING AND DECREASED MESENTERIC BLOOD FLOW ACCOMPANY EXPERIMENTAL SPINAL CORD INJURY

Amanda White, Gregory Holmes

Pennsylvania State University College of Medicine, Neural and Behavioral Sciences, Hershey, USA

Bowel dysfunction, often referred to as neurogenic bowel, is one of the most prevalent and clinically recognized comorbidities associated with spinal cord injury (SCI), yet significant knowledge gaps persist regarding the mechanisms contributing to colonic dysfunction after SCI. Following SCI, there is an immediate loss of vascular symp-

thetic tone resulting in circulatory hypotension. The gastrointestinal (GI) system is highly dependent upon adequate blood flow for proper functioning. Other models of GI inflammation report even brief periods of visceral hypoxia leads to GI dysmotility, inflammation and neuromuscular remodeling. We hypothesized that decreased blood flow and inflammation accompanies colonic remodeling in adult male Wistar rats following a 300kdyn T3-SCI. Three days or three weeks post-injury, both injured and age-matched controls underwent *in vivo* experimentation to quantify mesenteric blood flow. Following experimentation, tissue was harvested for histological evaluation. Muscularis propria thickness of the proximal colon was significantly increased in the 3-week SCI rats, while the distal colon demonstrated a significant increase in thickness in both 3-day and 3-week SCI rats. Collagen content within the muscularis propria of both the proximal and distal colon was significantly increased in both groups of rats. Colonic mucosal crypt depth and width was significantly reduced within the proximal colon of both groups but only the 3-week SCI rats displayed significant changes in the distal colon. Mean arterial pressure (MAP) and inferior mesenteric artery (IMA) blood flow were significantly lower in T3-SCI rats. Derangements in collagen networks and shortening of mucosal crypts are hallmarks of animals with colitis, and are associated with bowel motor dysfunctions. Our previous reports of diminished colonic motility, enteric oxidative stress, and our present data suggests that bowel dysfunction following T3-SCI may reflect a rapid, multifactorial loss of extrinsic supraspinal innervation, colonic hypoxia in response to decreased systemic blood pressure, and colonic neuromuscular remodeling that may affect the normal physiology and reflect long-term colonic motor dysfunctions as seen in other disease models.

Keywords: Neurogenic bowel, Gastrointestinal smooth muscle, Fibrosis, Dysmotility

B17-05

PROGESTERONE ATTENUATES STROKE-INDUCED BRAIN INJURY VIA INHIBITION OF ENDOPLASMIC RETICULUM STRESS: ROLE OF VITAMIN D DEFICIENCY

Seema Yousuf, Fahim Atif, Claudia Espinosa-Garcia, Donald G. Stein
Emory, Emergency Medicine, Atlanta, USA

Objective: We investigated whether vitamin D deficiency (VDH^{def}) or sufficiency (VDH^{suf}) affects behavioral outcome in a transient middle cerebral artery occlusion (tMCAO) model of stroke followed by lipopolysaccharide (LPS)-induced post-stroke inflammation. We also evaluated the effect of progesterone (PG) treatment on inflammation-mediated endoplasmic reticulum (ER) stress after stroke, and whether PG treatment is neuroprotective in this kind of injury.

Method: Male Wistar rats were kept on a VitD^{def} or VitD^{suf} diet, subjected to tMCAO or sham operation, and assigned to: Sham, tMCAO, tMCAO+LPS and tMCAO+LPS+PG groups. Post-stroke systemic inflammation was induced by injections of LPS. PG was administered 1h after occlusion, then daily for 3 days. Behavioral testing was conducted on day 4 post-stroke. After testing, brains were harvested for immunostaining and Western blots.

Results: The VDH^{suf} groups showed significant decrease in all behavioral deficits after tMCAO group. The tMCAO+LPS group showed even greater behavioral impairments compared to Shams. The VDH^{def} animals were worse than the VDH^{suf} group; and the ischemic injury groups ($p < 0.05$) were worse in all behaviors compared to shams and the MCAO+LPS group was worse than either Sham+LPS or tMCAO alone. Stroke significantly increased ($p < 0.05$) in the neuroinflammatory response as evidenced by expression of GFAP and

Iba-1 in VDH^{def} ischemic animals compared to sham control animals. This neuroinflammatory response is known to induce ER-stress. Increased expression of ER-stress markers was seen in the tMCAO and tMCAO+LPS groups. PG treatment inhibited stroke-induced neuroinflammatory response by suppressing ER stress activation.

Conclusion: VDH^{def} exacerbates behavioral deficits following post-stroke inflammation, and PG is neuroprotective against post-stroke LPS-induced inflammation and ER stress activation under VDH^{def}. It may be concluded that PG treatment is beneficial against stroke and post-stroke systemic inflammation via inhibition of ER stress.

Acknowledgments: This work was supported by gifts from Allen and Company, the Marcus Foundation, and the Stein Family Research Fund. There are no conflicts of interest associated with this work.

Keywords: Stroke, Vitamin D, ER Stress, Behavior

B17-06

QUANTIFICATION OF IMMUNOHISTOCHEMISTRY ON ADJACENT SECTIONS COMPARING FLUORESCENT AND DAB MARKERS

Cody Zurhellen

NeuroScience Associates, Neurohistology, Knoxville, USA

The location of antibody binding sites in brain tissue sections is commonly detected with fluorescent markers or with colored reaction products such as diaminobenzidine. We sought to answer the question: Do both types of detection schemes reveal the antibody binding sites to the same extent as judged by density of staining?

Adjacent sections of tissue were stained with several antibodies, including GFAP for astrocytes and Iba1 for microglia from rat brains that were unilaterally rendered ischemic by middle cerebral artery occlusion. Sets of adjacent sections were immunohistochemically stained free-floating using standard IHC methods. For one set, the antibody binding site was detected using a secondary antibody conjugated with a fluorescent molecule. The second set was stained using the sequence of a secondary antibody conjugated with a biotin molecule, followed by an avidin-HRP complex and then reacting with diaminobenzidine and H₂O₂.

Two areas of interest were digitally captured for each detection scheme/stain: An ischemic area in one hemisphere and an unaffected area in the other hemisphere. The same region of interest for each stain was analyzed on adjacent sections to rule out any random differences in brain areas. Images of both the DAB and fluorescent stains were converted to 8-bit grayscale. The 8-bit grayscale images of fluorescent-stained tissue were inverted to mimic the 8-bit grayscale images of the DAB images. All 8-bit grayscale images were converted to a binary image for densitometric analysis. Densitometry (measured as the percent area occupied by signal) was performed to determine any differences between the DAB and fluorescent images. The percent area in both the ischemic and normal areas of interest were analyzed in both DAB and fluorescent images for each stain.

There were dramatic differences observed between the two staining methods. In both the GFAP and Iba1 stains there were obvious variations in staining intensity in both the affected and normal brain regions. This is not surprising as there is considerably greater amplification of the antibody presence using the DAB multiple stage sequence vs. the "single" stage fluorophore-secondary antibody sequence. Nonetheless, the degree of hypertrophied astrocytes and microglia seen with the fluorescent method is meager compared to the images using the DAB sequence.

Keywords: IHC Development Method Comparison, Fluorescent vs DAB, Densitometry, IHC Staining Methods

B18 INFLAMMATION / IMMUNE FUNCTION

B18-01

THE SEVERITY OF EARLY POST-TBI FUNCTIONAL IMPAIRMENT IS ASSOCIATED WITH THE MAGNITUDE OF T CELL INFILTRATION INTO THE BRAIN

Xavier Nnode-Ekane, Liz Matthiesen, Ivette Banuelos Cabrera, Catia Alexandra Pegas Palminha, Asla Pitkänen

A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland

T-lymphocyte (T cell) invasion of the brain parenchyma is a major consequence of traumatic brain injury (TBI). T cells are potent inflammatory mediator, regulating the function of other immune cells including microglial cells. Restricting T cell invasion into the brain improves the function outcome in several brain injury models. We tested the hypothesis that the magnitude of T cell invasion into the cortex is associated with recovery from early post-TBI functional impairment in rats. TBI was induced in adult male Sprague Dawley rats using the lateral fluid percussion injury (FPI) model. The acute functional impairment was assessed based on the neuroscore test. The recovery index was estimated from 2 d to 7 d post-injury. Animals were killed at different time points and the brains processed for immunohistochemical detection of T cells. The total number of T cells was estimated in different brain areas using unbiased stereology. There was a dramatic surge in the total number of T cells in brain at 1 d post-injury ($69\,146 \pm 8\,710$), peaked at 2 d ($106\,757 \pm 19\,342$) and then drops progressively to $53\,409 \pm 20\,308$ at 4 d and $6983 \pm 1\,150$ at 7d. At all time-points, the number of T cells was higher in the cortex than in the hippocampus and thalamus. High number of T cells in the cortex was associated with poor recovery index. Furthermore, animals with poor acute functional impairment continued to show high inflammatory response at the chronic phase. These data demonstrates that T-cell infiltration was most prominent during the first few days after lateral fluid-percussion injury, and a high infiltration magnitude, particularly in the cortex, was associated with severe functional impairment.

Keywords: Lateral fluid-percussion brain injury, T lymphocytes, Functional impairment

B18-02

GENDER DIFFERENCES IN THE PERIPHERAL IMMUNE RESPONSE TO SPINAL CORD INJURY

Carlos Ayala, Noelle Messina, Shambhavi Metgud, Meher Pandher, Wise Young

Rutgers University, W.M. Keck Center for Collaborative Neuroscience, Piscataway, USA

Four times as many men are paralyzed from spinal cord injury (SCI) than women worldwide. Although the difference is often credited to male propensity to take risks, female gender may be neuroprotective as females recover motor function better than males. The mechanism is not known. Recently, Dr. Charlotte Hsu, in her PhD thesis, found Fischer 344 (F344) female rats recovered faster with better locomotion than male F344 rats. She found no gender differences in spinal cord anatomy, lesion volume or lipid peroxidation at 24 hours after injury. We next investigated neuroimmune response, a possible contributor to secondary spinal cord injury not well studied in both genders. We performed a T11 spinal cord contusion in F344 rats. Blood was sequentially collected in the same animal cohort pre-injury, 24 hours, 3 days, 7 days and 14 days after injury. White blood cells (WBCs) were manually counted with Hoechst stain followed by differentiation of blood smears and flow

cytometry. We found gender differences in the peripheral immune response to SCI. Blood neutrophils and monocytes increased in males and females at 24 hours but continued to peak only in males 3 days after injury. Neutrophils returned to baseline 7 days after injury. In contrast, blood monocytes returned to baseline at 7 days but increased 14 days after injury in both genders. Blood T cells significantly declined in females 24 hours after injury while there was no change in males. T cells increased at 3 days and 7 days, returning to baseline by 14 days. B cells declined at 24 hours, slightly increased at 3 and 7 days but significantly declined to the lowest counts 14 days after injury. Total circulating WBCs declined in both genders 14 days after injury. Our findings demonstrate significant and gender specific changes in the peripheral blood response to SCI. Significantly lower blood neutrophils and monocytes in females may be neuroprotective acutely after SCI.

Keywords: Gender, Blood, Neutrophil, Monocyte, Lymphocyte

B18-03

NOX2 DEFICIENCY ALTERS MACROPHAGE PHENOTYPE THROUGH AN IL-10/STAT3 DEPENDENT MECHANISM: IMPLICATIONS FOR TRAUMATIC BRAIN INJURY

James Barrett¹, Rebecca Henry¹, Sonia Villapol², Bogdan Stoica¹, Alok Kumar¹, Mark Burns², Alan Faden¹, David Loane¹

¹*University of Maryland, Department of Anesthesiology and Shock, Trauma and Anesthesiology Research Center, Baltimore, USA*

²*Georgetown University, Department of Neurosciences, Washington, DC, USA*

NOX2 is an enzyme that generates ROS in microglia and macrophages. ROS production is linked with neuroinflammation and chronic neurodegeneration following traumatic brain injury (TBI). NOX2 inhibition following moderate-to-severe TBI markedly reduces pro-inflammatory activation markers and results in concomitant increases in anti-inflammatory responses. Here, we report the signaling pathways that regulate NOX2-dependent macrophage/microglial phenotype in the TBI brain. Bone marrow-derived macrophages (BMDMs) prepared from wildtype (C57Bl/6) and NOX2 deficient (NOX2^{-/-}) mice were treated with combined lipopolysaccharide/ interleukin-4 (LPS/IL-4; both 10 ng/ml), to investigate signal transduction pathways associated with macrophage activation. Signaling pathways and activation markers were evaluated in ipsilateral cortical tissue obtained from adult male wildtype and NOX2^{-/-} mice that received moderate-level controlled cortical impact (CCI). A neutralizing anti-IL-10 approach was used to determine the effects of IL-10 on NOX2-dependent changes. LPS/IL-4-stimulated BMDMs mimic the pro- and anti-inflammatory responses observed in the injured cortex, we show that NOX2^{-/-} significantly reduces markers of proinflammatory activation. In addition, NOX2^{-/-} results in significantly increased anti-inflammatory marker expression, which seems to be dependent on IL-10-mediated STAT3 signaling. Following moderate-level CCI, IL-10 is significantly increased in microglia/macrophages in the injured cortex of NOX2^{-/-} mice. These changes are associated with increased STAT3 activation and a robust anti-inflammatory response. Neutralization of IL-10 in NOX2^{-/-} BMDMs or CCI mice blocks the anti-inflammatory response, thereby demonstrating a critical role for IL-10 in regulating NOX2-dependent changes. These studies indicate that following TBI NOX2 inhibition promotes a robust anti-inflammatory response that is mediated by the IL-10/STAT3 signaling pathway. Thus, therapeutic interventions that inhibit NOX2 activity may improve TBI outcomes by not only limiting pro-inflammatory, but also enhancing IL-10-mediated anti-inflammatory responses that are neuroprotective.

Keywords: Macrophage, NOX2, Neuroinflammation, Interleukin-10, Traumatic brain injury

ACTIVATION OF CANNABINOID RECEPTOR 2 ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED INFLAMMATION

Molly Braun¹, Zenab Khan^{1,2}, Nancy Saad³, M Badruzamman Khan⁵, Lin Yin³, David Hess⁵, Md Nasrul Hoda⁴, Babak Baban³, Krishnan Dhandapani¹, Kumar Vaibhav¹

¹Augusta University, Neurosurgery, Augusta, USA

²Augusta University, College of Nursing, Augusta, USA

³Augusta University, Oral Biology, Augusta, USA

⁴Augusta University, Department of Medical Laboratory, Imaging, and Radiological Sciences, Augusta, USA

⁵Augusta University, Neurology, Augusta, USA

Inflammation is an important component of secondary neurological injury after TBI. Immune cells, particularly macrophages, aid in clearance of cellular debris and tissue repair, but sustained release of pro-inflammatory mediators from infiltrating immune cells may exacerbate neuronal death, increase neurovascular injury, and contribute to long-term loss of white matter. Although the precise mechanisms underlying the dual beneficial and detrimental roles of macrophages after CNS injury remain poorly defined, it has been well established that macrophages polarize along a continuum from a classical pro-inflammatory (M1) state to an alternative anti-inflammatory (M2) state. Thus, modulation of macrophage polarization may identify novel opportunities for therapeutic intervention after TBI. Non-psychoactive cannabinoid receptor 2 (CB2R) is predominantly expressed on immune (lymphocytes, monocytes, macrophages) and endothelial cells in both rodents and humans. In the present study, we hypothesize that activation of CB2R attenuates inflammation post-TBI. Using a moderate controlled cortical impact (CCI) model of murine TBI, we assessed the endogenous upregulation of CB2R after TBI and the effects of CB2R agonist and antagonist post-injury. We observed acute upregulation of CB2R up to 3 days post TBI and moderate elevated expression persisted at 3 weeks. Furthermore, CB2R co-localized with infiltrated myeloid cells, specifically macrophages, after TBI. Finally, administration of selective CB2R agonist GP1a attenuated neuroinflammation, while treatment with antagonist AM630 worsened the injury. Interestingly, attenuation of inflammation by GP1a was associated with increased M2 polarization of macrophages. Our findings suggest that CB2R may play an important role in immune cell regulation; therefore, the development of selective CB2R agonists may provide clinical benefits for brain injury patients, without the psychoactive effects of CB1R activation.

Keywords: traumatic brain injury (TBI), cannabinoid receptor 2 (CB2), inflammation, macrophage

B18-05

ACTIVATION OF MYELOID TLR4 MEDIATES T LYMPHOCYTE POLARIZATION AFTER TRAUMATIC BRAIN INJURY

Molly Braun¹, Kumar Vaibhav¹, Md Nasrul Hoda², Babak Baban³, Krishnan Dhandapani¹

¹Augusta University, Neurosurgery, Augusta, USA

²Augusta University, Medical Laboratory, Imaging, and Radiological Sciences, Augusta, USA

³Augusta University, Oral Biology, Augusta, USA

Traumatic brain injury (TBI) is a major public health issue, producing significant patient mortality and poor long-term outcomes. Increasing evidence suggests an important, yet poorly defined, role for the immune system in the development of secondary neurologic injury over the days and weeks following a TBI. In this study, we tested the hypothesis that

peripheral macrophage infiltration initiates long-lasting adaptive immune responses after TBI. Using a murine controlled cortical impact model, we used adoptive transfer, transgenic, and bone marrow chimera approaches to show increased infiltration and proinflammatory (M1) polarization of macrophages for up to 3 wk post-TBI. Monocytes purified from the injured brain stimulated the proliferation of naive T lymphocytes, enhanced the polarization of T effector cells (TH1/TH17), and decreased the production of regulatory T cells in an MLR. Similarly, elevated T effector cell polarization within blood and brain tissue was attenuated by myeloid cell depletion after TBI. Functionally, C3H/HeJ (TLR4 mutant) mice reversed M1 macrophage and TH1/TH17 polarization after TBI compared with C3H/OuJ (wild-type) mice. Moreover, brain monocytes isolated from C3H/HeJ mice were less potent stimulators of T lymphocyte proliferation and TH1/TH17 polarization compared with C3H/OuJ monocytes. Taken together, our data implicate TLR4-dependent, M1 macrophage trafficking/polarization into the CNS as a key mechanistic link between acute TBI and long-term, adaptive immune responses.

Keywords: traumatic brain injury (TBI), inflammation, macrophage polarization, T lymphocyte

B18-06

KNOCKDOWN OF $\alpha 9/\alpha 10$ NICOTINIC ACETYLCHOLINE RECEPTORS AMPLIFY INFLAMMATION AND SLEEP DISRUPTIONS

Daniel Griffiths^{1,2}, Ronald Lukas³, Barbara Morley⁴, Jonathan Lifshitz^{1,2}, Rachel Rowe^{1,2}

¹Barrow at Phoenix Children's Hospital, Phoenix, USA

²University of Arizona-COM-Phoenix, Phoenix, USA

³Barrow Neurological Institute, Phoenix, USA

⁴Boys Town National Research Hospital, Omaha, USA

Effects of traumatic brain injury (TBI) are not isolated to the central nervous system, but can lead to excessive systemic inflammation, exacerbating neurological symptoms such as sleep disruptions which complicate recovery. In the absence of a pathological inflammatory event, peripheral inflammatory cytokines are regulated by the innate immune response. Subsequent to a pathological inflammatory event, the adaptive immune response then attenuates uncontrolled systemic inflammation, with evidence for action through the cholinergic anti-inflammatory pathway. We show that $\alpha 9/\alpha 10$ nicotinic acetylcholine receptors (nAChR) are widely expressed on peripheral immune cells, suggesting that nAChR activation may affect inflammatory cell (e.g., macrophage) function. Specifically, $\alpha 9/\alpha 10$ -nAChR activation may amplify the migration and production of inflammatory cytokines. Inflammatory cytokines can have dual roles as sleep regulatory substances (IL-1 β , IL-6) and we previously showed prolonged sleep may be a physiological indicator of inflammation. Here, we hypothesize that a peripheral injection of lipopolysaccharide (LPS), a trigger of inflammation, significantly increases peripheral cytokines and sleep in wildtype mice, but not in $\alpha 9/\alpha 10$ -nAChR double knock-out mice. Mice were acclimated to non-invasive piezoelectric sleep cages and baseline sleep was recorded. Wildtype or transgenic mice were administered intraperitoneal injections of LPS (3 μ g) or saline and returned to sleep cages. Following 6 hours of sleep recording, blood was collected for plasma analysis of peripheral cytokines. Results indicated LPS significantly increased sleep in wildtype-LPS mice compared to $\alpha 9/\alpha 10$ -nAChR-LPS mice and saline-treated mice. As expected, LPS significantly increased IL-1 β and IL-6 compared to saline-mice and current analyses comparing cytokine levels in $\alpha 9/\alpha 10$ -nAChR are underway. Ongoing experiments evaluating sleep and cytokines in $\alpha 9/\alpha 10$ -nAChR knock-out mice following diffuse TBI may identify a mechanism for the peripheral amplification of central inflammation. The role of $\alpha 9/\alpha 10$ -nAChR in regulating the immune

response is unknown and remains a potential underlying mechanism of action and therapeutic target for uncontrolled inflammation after TBI. Funding: Sleep-Medicine Foundation; BNI@PCH.

Keywords: peripheral, cholinergic, nicotinic

B18-07

INVESTIGATING THE ACUTE INFLAMMATORY RESPONSE TO MIDLINE AND LATERAL FLUID PERCUSSION INJURY

Kristina Witcher, Daniel Eiferman, Jonathan Godbout, **Olga Kokiko-Cochran**

The Ohio State University, Neuroscience, Columbus, USA

Fluid percussion-induced traumatic brain injury (TBI) is one of the most popular experimental models available. Typically, fluid percussion injury (FPI) is applied at midline or parasagittal to midline in rodents. Midline FPI is broadly defined as a diffuse model of TBI in which both hemispheres are equally injured. Lateral FPI is a mixed model of TBI in which the ipsilateral hemisphere receives a focal injury resulting in diffuse damage to subcortical structures. The present study investigated the acute inflammatory response to midline and lateral FPI, with specific emphasis on leukocyte recruitment and cytokine expression within the brain during the first week post-injury. We hypothesized that the acute inflammatory response to TBI would be enhanced following lateral FPI compared to midline FPI. To test this hypothesis, lateral or midline FPI or sham injury was administered to equal numbers of male and female C57BL6 mice at two months of age. By four hours post-injury, flow cytometric analysis revealed a TBI-induced increase in circulating neutrophils following midline and lateral FPI. Unexpectedly, sham injured mice receiving a midline or lateral craniectomy displayed a higher percentage of patrolling monocytes compared to TBI. Furthermore, both sham and TBI mice in the lateral FPI group displayed a higher percentage of circulating inflammatory monocytes. Brain neutrophils and inflammatory monocytes increased following midline and lateral FPI and occurred in conjunction with an increase in inflammatory cytokines IL-1 β , TNF- α , and CCL2 at 4 hours post-injury. By 72 hours post-injury, the percentage of circulating neutrophils and monocytes declined in midline and lateral TBI mice. Similarly, brain leukocytes and inflammatory cytokine expression decreased by 72 hours post-injury, although CCL2 expression remained increased in the ipsilateral hemisphere following lateral FPI. Taken together, these data indicate that the acute inflammatory response to midline and lateral FPI is quite similar with regard to leukocyte recruitment and cytokine expression soon after injury. Further studies are underway to determine differences in behavior and leukocyte recruitment at sub-acute time points.

Keywords: leukocytes, fluid percussion injury

B18-08

NADPH OXIDASE-2 REGULATES NLRP3 INFLAMMASOME ACTIVATION AFTER TRAUMATIC BRAIN INJURY

Merry Ma^{1,2}, Jing Wang^{1,2}, Krishnan Dhandapani^{2,3}, Darrell Brann^{1,2}

¹*Medical College of Georgia - Augusta University, Neuroscience and Regenerative Medicine, Augusta, USA*

²*Charlie Norwood VA Medical Center, Augusta, USA*

³*Medical College of Georgia - Augusta University, Neurosurgery, Augusta, USA*

Traumatic brain injury (TBI) contributes to over 30% of injury related deaths. A complex secondary injury cascade follows the initial me-

chanical injury and exacerbates the primary injury. Oxidative stress and inflammation play key roles in this TBI pathology. NADPH oxidase-2 (NOX2) and inflammasomes have been reported as major contributors to oxidative stress and inflammation in the injured brain, respectively, and deletion of NOX2 is neuroprotective. In particular, the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome can become activated in response to oxidative stress, but little is known about this mechanism following TBI. In this study we utilize NOX2 knockout mice to study the role of NOX2 in mediating NLRP3 inflammasome expression and activation in a controlled cortical impact model of focal TBI. Expression of NLRP3 inflammasome components (NLRP3, apoptosis speck-like protein containing a CARD (ASC), and caspase-1) was robustly increased in the injured cerebral cortex following TBI. Deletion of NOX2 attenuated the expression and assembly of the NLRP3 inflammasome components, which was coupled to reduced cleavage of the pro-inflammatory factor, interleukin-1 β (IL-1 β). Further work revealed that NOX2 regulation of NLRP3 inflammasome may be mediated by thioredoxin interacting protein (TXNIP), a sensor of oxidative stress. In conclusion, the results of the current study provide novel evidence that NOX2-dependent inflammasome activation contributes to TBI pathology.

Keywords: NADPH Oxidase 2, NLRP3, Inflammasome, Traumatic Brain Injury, Oxidative Stress, Controlled Cortical Impact

B18-09

L-SELECTIN MEDIATES NEUTROPHIL RECRUITMENT AND ACTIVATION FOLLOWING SPINAL CORD INJURY

Dylan McCreedy^{1,3}, Steven Rosen², Linda Noble-Haesslein¹

¹*UCSF, Neurological Surgery, San Francisco, USA*

²*UCSF, Anatomy, San Francisco, USA*

³*J. David Gladstone Institutes, GICD, San Francisco, USA*

The acute inflammatory response following spinal cord injury (SCI) is marked by extensive invasion of myeloid lineage leukocytes that exacerbate the injury site by releasing neurotoxic substances. L-selectin, a C-type lectin adhesion receptor, is broadly expressed on myeloid cells and mediates their recruitment to sites of inflammation. Recent studies have shown that L-selectin is involved in additional functions including the activation of integrins, tethering of myeloid cells along the endothelium, and potentiation of neutrophil effector functions. We have previously demonstrated reduced acute pathology and improved long-term recovery in L-selectin knockout mice (KOs). However, the early pathogenic activities of L-selectin and the specific myeloid subtypes involved have yet to be identified. To address this, we examined L-selectin (CD62L) levels on myeloid lineage cells, as well as accumulation of these cells, in uninjured and acutely injured mice by flow cytometry. We found that L-selectin is dynamically regulated after SCI on circulating inflammatory monocytes (Ly6C^{hi}/Ly6G⁻), non-classical monocytes (Ly6C^{low}/Ly6G⁻) and neutrophils (Ly6C^{low}/Ly6G⁺). Reduced neutrophil accumulation was observed in the injured spinal cords of L-selectin KO mice compared to wildtypes (WTs) at 24 hours post-SCI, but not at 72 hrs post-SCI. Furthermore, activation of circulating neutrophils, as shown by CD11b levels, was reduced in KOs compared to WTs at 24 hours post-SCI. Administration of diclofenac, an FDA-approved non-steroidal anti-inflammatory drug (NSAID) which is a potent inducer of L-selectin shedding (mediated by cell surface metalloproteinases), reduced L-selectin on circulating non-classical monocytes and neutrophils. Diclofenac did not affect the number of monocytes or neutrophils that accumulated in the injured spinal cord, but did reduce L-selectin levels on infiltrated non-classical monocytes and neutrophils, suggesting other activities for L-selectin beyond

recruitment. These findings implicate L-selectin in early pathogenesis and elucidate a novel mechanism (L-selectin shedding) by which NSAIDs attenuate acute inflammatory damage after SCI. Supported by: Department of Defense SCIRP W81XWH-12-1-0563 and National Institutes of Health NINDS F32NS096883.

Keywords: neutrophil, l-selectin, shedding, diclofenac, NSAID

B18-10

THE DELTA OPIOID RECEPTOR AGONIST DADLE MITIGATES BRAIN INFLAMMATION IN A RAT MODEL OF HEAD TRAUMA

Michael Ogier^{1,2,3}, Beatrice Georges^{2,3}, Sandrine Bouvard^{2,3}, Laurent Bezin^{2,3}

¹French Armed Forces Biomedical Research Institute, Bretigny-sur-Orge, France

²Lyon Neuroscience Research Center, TIGER Team, Villeurbanne, France

³Institute for Epilepsy IDÉE, Bron, France

We tested whether [D-Ala², D-Leu⁵]-Enkephalin (DADLE), a synthetic delta-opioid receptor agonist, could mitigate brain inflammation following head trauma in adult rats. DADLE (1 and 5 mg/kg) was intraperitoneally injected to rats 15 minutes, 2 and 5 hours after being exposed to left-side parietal fluid percussion injury. Three hours after the last DADLE injection, rats were euthanized, then, their brain was harvested. Cortical and hippocampal tissues were microdissected from the left (ipsilateral, injured) and right (contralateral) sides of each brain and processed for qPCR quantitation of the expression of several prototypical inflammatory markers: Interleukin-1 beta (IL-1b), IL-6, Tumor necrosis factor alpha (TNFα), Macrophage inflammatory protein 1 alpha (MIP1a) and Monocyte chemoattractant protein 1 (MCP-1). As predicted, the expression of all of the inflammatory markers was increased in the ipsilateral cortex and hippocampus after TBI. To a lower extent, exposure to TBI also increased the expression of IL-1b, TNFα, MIP1a and MCP-1 in the contralateral cortex and of IL-1b, IL-6, TNFα and MCP-1 in the contralateral hippocampus. Treatment with the highest dose of DADLE decreased the expression of IL-1b in the contralateral cortex by 39%, and in the ipsilateral and contralateral hippocampus by 31 and 33%. Low dose DADLE did not influence the expression of IL-1b but rather increased the expression of IL-6 in the contralateral cortex and of TNFα and MIP1a in the contralateral hippocampus. Thus, treatment with a high dose of DADLE can mitigate acute brain inflammatory processes following head trauma. By contrast, treatment with a low dose of DADLE tends to increase post-traumatic brain inflammation. It is possible that the differential effects of DADLE are due to recruitment of different subtypes of opioid receptors. This work was supported by a grant from the Direction Générale de l'Armement (French Ministry of Defense).

Keywords: traumatic brain injury, animal model, delta opioid agonist, inflammation

B18-11

TERIFLUNOMIDE TO TREAT TRAUMATIC BRAIN INJURY

Karthik Prabhakara¹, Kota Daniel², Gregory Jones¹, Scott Olson¹

¹University of Texas Medical School at Houston, Pediatric Surgery, Houston, USA

²Sanford Research Institute, Sioux Falls, USA

Traumatic brain injury (TBI) remains a serious public health problem in the United States. According to the World Health Organization (WHO), TBI is a permanent, chronic disease process, caused by non-reversible pathological alterations, requiring special rehabilitation, and/or a long period of observation, supervision, or care. However, despite intensive investigative endeavors, there are no FDA-approved drugs designed to reduce morbidity and mortality associated with TBI.

Neuroinflammation, which been detected as lasting in humans for up to 17 years, plays a pivotal role in the pathogenesis of TBI. In the acute stage of TBI, inflammation activates and mobilizes immune cells toward the site of injury that interfere with the endogenous capacity of the brain to repair itself, exacerbating neuronal death. In the chronic stage, an inflammatory microenvironment caused by an excessive activation of immune cells further contributes to secondary neuronal death and a subsequent positive feedback inflammatory loop.

Teriflunomide (Aubagio) has been approved by the US Food and Drug Administration (FDA) for use in the treatment of multiple sclerosis since 2012. Teriflunomide has been shown to act as an inhibitor of dihydroorotate-dehydrogenase (DHODH), a key mitochondrial enzyme involved in the de novo synthesis of pyrimidines in rapidly proliferating T lymphocytes and B lymphocytes. Moreover, teriflunomide has been shown to affect immunological responses outside of its ability to inhibit pyrimidine synthesis in rapidly proliferating cells, suggesting Teriflunomide may have additional therapeutic mechanisms.

In here, we studied the treatment effect of teriflunomide in severe/moderate and mild injury models of rats. Our results show that in both the rat models the drug could recover the blood-brain barrier integrity and ameliorate inflammation in brain both short term and long term. Consequently, we saw improvement in memory and special learning abilities after treatment in severe/moderate injury. However, our mild injury model didn't have any behavioral deficits.

This project was funded by Sanofi Genzyme.

Keywords: teriflunomide, neuroinflammation, Traumatic Brain Injury, Aubagio,

B18-12

DIACYLGLYCEROL LIPASE- β KNOCKOUT MICE: SURVIVAL PROTECTIVE PHENOTYPE FROM TRAUMATIC BRAIN INJURY

Lesley Schurman (nee O'Brien)¹, Terry Smith², Linda Phillips², Thomas Reeves², Aron Lichtman¹

¹Virginia Commonwealth University, Pharmacology & Toxicology, Richmond, USA

²Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

The endogenous cannabinoid 2-arachidonyl glycerol (2-AG) serves as a rate-limiting precursor for arachidonic acid (AA), a precursor for the production of pro-inflammatory eicosanoids in brain. *In vitro* work shows that inhibiting the 2-AG biosynthetic enzyme diacylglycerol lipase- β (DAGL- β), which is highly expressed on CNS resident microglia and macrophages in the periphery, reduces inflammatory responses. Here, we examine whether DAGL- $\beta^{-/-}$ mice display a protection phenotype from the consequences of experimental traumatic brain injury. *Experiment 1*: Male DAGL- $\beta^{-/-}$ and $^{+/+}$ mice were subjected to a left lateral moderate Fluid Percussion injury (FPI) (1.94 \pm 0.1 atm), and then assessed for spatial memory performance in a Morris water maze Fixed Platform task as well as for neurological motor impairments using the Neurological Severity Score (NSS) and the Rotarod assay. Unexpectedly, DAGL- $\beta^{-/-}$ mice demonstrated a

significant survival protective phenotype (100% survival) in response to brain injury compared to DAGL- $\beta^{+/+}$ mice (77% survival), despite equal injury severities between groups. However, DAGL- $\beta^{-/-}$ mice displayed similar magnitudes of FPI-induced cognitive impairments in the Fixed Platform task and neurological motor deficits in the NSS and Rotarod assays as DAGL- $\beta^{+/+}$ mice. *Experiment 2:* We increased the magnitude of FPI to 2.0 ± 0.1 atm and 2.17 ± 0.1 atm in male and female DAGL- $\beta^{-/-}$ and $+/+$ mice, and assessed mortality. Male DAGL- $\beta^{-/-}$ mice demonstrated a significant survival protective phenotype at both 2.0 atm (100% survival) and 2.17 atm (90% survival) compared to DAGL- $\beta^{+/+}$ mice (75% survival at 2.0 atm, and 60% survival at 2.0 atm). In contrast, female DAGL- β mice generally survived both injury magnitudes regardless of phenotype, suggesting sex differences in survival from FPI. These findings suggest the provocative possibility that DAGL- β activity contributes to TBI-induced mortality, but not to the evolution of TBI cognitive or motor impairments in mice. Accordingly, DAGL- β inhibition represents a potential strategy to ameliorate post-traumatic fatality.

Keywords: Endogenous Cannabinoid, Mortality, Diacylglycerol lipase- β , 2-arachidonylglycerol (2-AG), Spatial Memory, Sex Differences

B18-13

TARGETING NLRP3 INFLAMMASOME TO TREAT TRAUMATIC BRAIN INJURY WITH A NOVEL PHARMACOLOGICAL COMPOUND

Dong Sun, Andrew Rolfe, Long Di, Melissa Powell, Ram Kuwar, Liu He, Shijun Zhang
Virginia Commonwealth Univ., Anatomy and Neurobiology, Richmond, USA

Inflammation is a prominent secondary event following traumatic brain injury (TBI). Inflammation is mediated by inflammatory cells and inflammatory cytokines/chemokines. Among pro-inflammatory cytokines, interleukin-1 β (IL-1 β) plays a pivotal role in triggering inflammatory cascade. Another cytokine, interleukin-18 (IL-18) is a potent inflammation mediator that initiates/amplifies many inflammatory processes. Recent studies have found that release of IL-1 β and IL-18 is mediated by inflammasomes. Inflammasomes are intracellular multiprotein complexes which regulate innate immune response, production of pro-inflammatory cytokines such as IL-1 β and IL-18, activation of caspase-1 and induction of cell death (pyroptosis). Among the known inflammasomes, NLRP3 is the most extensively studied and its activation is induced following TBI. As a novel target, drug development targeting formation/activation of inflammasomes could be a prospective therapy for TBI. We have recently developed a small molecule GA3 with specificity on NLRP3 inflammasome. GA3 can selectively inhibit NLRP3 inflammasome formation, activation of caspase-1, and production of IL-1 β . In this study, we explored the therapeutic value of GA3 for TBI. Adult male Sprague-Dawley rats were subjected to a moderate cortical impact injury. Following TBI, animals received 4 doses of GA3 treatment 6 hrs in between with the first dose starting at 30 minutes following injury. Sensorimotor functions and cognitive functions were assessed. Animals were sacrificed at 2 or 15 days post-injury. Brain tissues were processed for histological examination to assess degenerative neurons, lesion volume, and inflammatory cell response. We found that post-injury treatment with GA3 significantly decreased the number of injury-induced degenerating neurons in the injured cortex and hippocampus. GA3 treatment also reduced cortical brain tissue damage and inflammatory cell response. Moreover, injured animals treated with GA3 had sig-

nificant improvement in cognitive functional recovery. Further studies assessing the dose response and therapeutic time window of GA3 treatment in this focal injury model are ongoing. Our data suggest that our novel NLRP3 inhibitor has neuroprotective effects for TBI. Supported by CHRB #236-14-15 (Sun).

Keywords: traumatic brain injury, inflammasomes, neuroinflammation, neuroprotection, cognitive function

B18-14

TARGETING THE LIVER ACUTE PHASE RESPONSE FOLLOWING TRAUMATIC BRAIN INJURY REDUCES BRAIN INFLAMMATION

Leah Benton¹, Evan Wicker², Seham Alaiyed², William Mualem¹, Kershina George¹, William Furlow¹, Mark Burns¹, **Sonia Villapol¹**
¹Georgetown University, Neuroscience, Washington, USA

²Georgetown University, Pharmacology & Physiology, Washington, USA

Traumatic brain injury (TBI) triggers a loss of tissue followed by a strong and acute inflammatory response. Serum amyloid A1 (SAA1) is an acute phase protein that is mainly produced in the liver in response to inflammatory events such as TBI. SAA1 is released into the bloodstream, establishing a channel of communication between the liver and extra-hepatic organs. To date, no *in vivo* studies have investigated the role of SAA1 release in the central inflammatory response after TBI. We performed a mild and moderate/severe controlled cortical impact (CCI) injury in male C57BL/6J mice. One week before CCI, we injected antisense oligonucleotides (ASOs) (25 mg/kg/week by i.p.) to suppress mRNA SAA1 production. We wanted to explore the potential application of ASOs by modifying the inflammatory response. Our results revealed that the highest grade of TBI severity was correlated with high levels of SAA1 in plasma at 1 day post-injury, and with a larger lesion volume and inflammation. We also found that SAA1 colocalizes with activated macrophages/microglial cells in the injured cortex and hippocampus; blocking SAA1 production reduced microglia activation and macrophage infiltration into the brain. Finally, we demonstrated that ASOs reduced SAA1 levels by 60–80% in plasma at 1 and 3 days after brain injury. ASO-treated mice displayed better motor skills on the rotarod 1 day post-injury. Collectively, our findings demonstrate that SAA1 levels in plasma after brain damage depend on damage severity, acting as a potential biomarker. Also, we found that SAA1 binds to macrophages/microglial cells in the injured brain, and that ASO treatment reduces the inflammatory response in the damaged brain. Altogether, this suggests that blocking SAA1 production could provide an advantageous environment for repair and functional recovery after brain trauma.

Keywords: peripheral inflammation, liver, microglia, macrophages, acute phase proteins

B19 INFORMATICS

B19-01

DEVELOPING BIG-DATA APPROACHES FOR DIVERSE PRECLINICAL TRAUMATIC BRAIN INJURY RESEARCH

Jessica Nielson^{1,2}, J. Russell Huie¹, Jordan Wolf³, Clark R. Andersen³, Heidi M. Spratt³, Douglas S. DeWitt³, Adam R. Ferguson^{1,2}, Bridget E. Hawkins³

¹University of California San Francisco, Weill Institutes for Neurosciences, Brain and Spinal Injury Center, Department of Neurosurgery, San Francisco, USA

²San Francisco VA Medical Center, San Francisco, USA

³University of Texas Medical Branch, The Moody Project for Translational Traumatic Brain Injury Research, Department of Anesthesiology, Galveston, USA

The objective of this collaborative study is to provide a proof-of-concept of modern data science tools applied to existing high-density data repositories of preclinical animal studies in traumatic brain injury (TBI) for novel translational discoveries. Multi-modal data from graded TBI injuries with varied treatment conditions were gathered from Moody Project for Translational TBI Research studies in rodents. In a chronic time course study, long term learning and memory deficits persist up to one-year post TBI. To convert these big-data to knowledge and to elucidate connections between behavior and histopathologic, genomic and proteomic changes post TBI, the Moody Project PIs launched a collaboration with data scientists with expertise in preclinical and clinical neurotrauma data. The main purpose of this effort is to create an example repository of completed studies in preclinical TBI, and develop syndromic analytical workflows that 1) capture both the complexity of the study design and 2) render outcome testing through multidimensional machine learning approaches to increase the effect size resolution for hypothesis testing of treatment trials. Prior applications of these methodologies to preclinical TBI and clinical TBI have demonstrated utility of multiscale 'omics (syndromics) for TBI and SCI. The current study aims to expand upon these efforts, capturing the full spectrum of bio-behavioral and molecular changes that result from TBI, and help resolve treatment approaches that reproduce results across diverse endpoints, increasing potential for translation into clinical trials in humans. These studies were supported in part by the Moody Project for Translational TBI Research and NIH BD2K RoAD-Trip Fellowship.

Keywords: Syndromics, Statistics, Multimodal, Rats

B19-02

CHANGES IN BRAIN PROTEIN LEVELS FOLLOWING TRAUMATIC BRAIN INJURY: A LONGITUDINAL PROTEOMIC STUDY

James Sowers¹, Bridget Hawkins³, Douglas Dewitt³, Donald Prough³, Kangling Zhang⁴, Ping Wu²

¹UTMB, Neuroscience Graduate Program and MD/PhD Program, Galveston, USA

²UTMB, Department of Neuroscience and Cell Biology, Galveston, USA

³UTMB, Department of Anesthesiology, Galveston, USA

⁴UTMB, Department of Pharmacology and Toxicology, Galveston, USA

The purpose of this study was to determine the global protein changes in the acute, subacute and chronic stages of traumatic brain injury (TBI). Rats were injured using a fluid percussion injury (FPI) model. Brain tissues were collected at 24 hours, 2 weeks, 3 months, 6 months, 1 year after injury. Ipsilateral rat cortex and hippocampi were isolated and analyzed separately for each time point. Tissues were homogenized and then trypsin digested. Protein fragments were analyzed using an orbitrap mass spectrometer and the relative changes in protein levels were determined by comparison with sham operated and naïve animals of the same age. Numerous protein changes were found at the five different time points and they differed among the two brain

regions. There was a dramatic change in the cortex neurotransmitter receptor composition at 3 months that resolved by 6 months. Furthermore, significant changes in the hippocampus were observed at 6 months which resolved at 1 year. Finally, proteins associated with multiple metabolic pathways were found to be altered at the 1 year time point. Changes observed indicate substantial modifications in energy metabolism, calcium homeostasis, neurotransmitter receptor composition and immune response. This study has given us greater insight as to what changes occur in the brain after TBI and is the most extensive study of its kind to date. Most significantly, we find changes at the later time points that could help explain the increased risk for neurological disorders in patients with TBI. We acknowledge the financial support of the Moody Project for Translational Traumatic Brain Injury Research.

Keywords: Longitudinal Proteomics, Chronic TBI

B20 METABOLISM / ENERGETICS

B20-01

FOOD FOR THOUGHT: OPTIMAL ALTERNATIVE FUEL SUPPLEMENTATION IS DEPENDENT ON METABOLIC STATE AFTER TBI

Tiffany Greco¹, Alyssa Warth¹, Daniel Braas², Tom Glenn¹, Mayumi Prins¹

¹UCLA, Neurosurgery, Los Angeles, USA

²UCLA, Molecular and Medical Pharmacology, Los Angeles, USA

Decreases in energy metabolism following traumatic brain injury (TBI) are attributed to impairment in utilizing glucose as a primary fuel source and supplementation with alternative fuels improves outcomes. Unanswered is when and what fuel to provide? Changes in energy metabolism following TBI happen in two phases; a brief period of hyper-metabolism followed by extensive hypo-metabolism. A fuel may function through different mechanisms depending on timing of delivery. It is hypothesized that outcome measures will differ based on a fuel's timing of delivery. Adult male rats were given sham or controlled cortical impact (CCI) injury, then intravenously infused with either glucose, lactate or beta-hydroxybutyrate for 3 hr (early) immediately after or beginning 6hr (late) after injury. Animals were euthanized 24 hr post-injury; ipsilateral-cortex and blood were collected for analyses. Injury alone significantly impaired mitochondrial respiration and citrate synthase (CS) activity and increased reactive oxygen species (ROS) production. Mitochondrial respiration was significantly improved by both early-lactate and late-BHB. ROS production was decreased by both early- and late-BHB and lactate in part by their scavenging properties and upregulation of antioxidant and uncoupling proteins by BHB. Late-glucose also decreased ROS production and may be due to increased pentose phosphate pathway (PPP) activity. Glucose is a known stimulator of mitochondrial respiration and CS activity was normalized. As expected, BHB decreased CS activity via inhibition by acetyl-CoA production. Both early and late-lactate normalized activity of CS. Untargeted metabolomics found significant group differences in PPP, TCA and glycolytic metabolites. An additional cohort of sham, CCI and CCI + early-lactate animals were euthanized at 1hr post-injury and glycogen was quantified. CCI significantly decreased glycogen, while early-lactate increased glycogen to sham amounts indicating early-lactate may improve lactate shuttle activity between astrocytes and neurons. These data stress there is no one optimal alternative fuel, but rather the fuel type used should be guided by metabolic state. This also suggests combination therapy of fuels may be necessary to ensure best outcome in TBI patients.

Acknowledgments: NFL Charities, UCLA Brain Injury Research Center, Marilyn and Austin Anderson Fellowship, NS058489-01, NS27544, UCLA Easton Neurotrauma Laboratories, UCLA Steve Tisch BrainSPORT program.

Keywords: mitochondria, metabolomics, alternative fuel, timing of delivery, outcome

B20-02

BIOENERGETIC FAILURE TIME-COURSE FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

Todd Kilbaugh^{1,2}, Michael Karlsson¹, Melissa Byro¹, Susan Margulies²

¹Children's Hospital of Philadelphia, Philadelphia, USA

²University of Pennsylvania, Philadelphia, USA

Damage to the mitochondrial bioenergetics, including the electron transport chain function (ETC) and substrate oxidation and phosphorylation (OXPHOS), exacerbates traumatic brain injury (TBI) and impedes repair. To identify the time-course of mitochondrial bioenergetics after diffuse TBI, which is critical to defining therapeutic targets and treatment windows. Piglets (N=15), toddler 4-wk olds 8–9 kg, were randomized to 3 groups: sagittal rapid nonimpact rotations (RNR) with terminal end-points: 6 hours (N=5) or 7 days (n=5), and Sham (N=5). At terminal time-points, cortical and hippocampal tissue were harvested rapidly. Fresh tissue homogenates were assayed utilizing high resolution respirometry (Oroboros) for real-time analysis of ETC function and OXPHOS, via titration of substrates, toxins or uncouplers. Respiration values were normalized by mitochondrial content: citrate synthase (rmol/s*mg/CS) and reported as mean ± SEM. Complex I (CI) respiration was significantly (p<0.05) decreased in cortex and hippocampus, at 6 hours and 7 days compared to shams. Specifically, cortical CI was 1.98 ± 0.12 at 6 hours, 2.12 ± 0.11 at 7 days, and 4.00 ± 0.23 in sham. Hippocampal CI was 1.55 ± 0.10 at 6 hours, 1.77 ± 0.20 at 7 days, and 3.09 ± 0.20 in sham. Complex II respiration (CII) was significantly decreased compared to sham in the cortex (3.12 ± 0.08 vs. 4.1 ± 0.27) and hippocampus (3.39 ± 0.34 vs. 4.4 ± 0.25) at 6 hours; by 7 days there was no significant difference in CII in either injured region. Maximal OXPHOS CI+CII was significantly decreased at 6 hours and 7 days in both injured brain regions versus sham (cortex: 4.88 ± 0.01 and 5.27 ± 0.10 versus 6.28 ± 0.17; hippocampus: 5.00 ± 0.34 and 5.16 ± 0.06 versus 6.64 ± 0.21). Persistent CI dysfunction is implicated in the pathogenesis of neurodegenerative disease and in murine animal models of focal TBI and ischemia reperfusion injury. Interpreted with our previous reported data at 24 hours, diffuse TBI triggers a tri-phasic response of CI: early decrease at 6 hours, transient recovery by 24 hours, with significant CI dysfunction again at 7 days, contributing to decreased OXPHOS. Future interventions should target CI dysfunction after diffuse TBI.

Acknowledgments: NINDS U01NS069545.

Keywords: mitochondria, time-course, complex I, porcine, diffuse TBI

B20-03

LASTING DYSREGULATION OF BRAIN METABOLISM IN CHRONIC LATERAL FLUID PERCUSSION INJURY

Jennifer McGuire¹, Erica DePasquale², Fatima Anwar³, Gowtham Atluri⁴, Robert McCullumsmith⁵, Nathan Evanson⁶

¹University of Cincinnati, Neurosurgery, Cincinnati, USA

²CCHMC, Bioinformatics, Cincinnati, USA

³Washington University, Psychology, St Louis, USA

⁴University of Cincinnati, Electrical Engineering and Computing Systems, Cincinnati, USA

⁵University of Cincinnati, Psychiatry, Cincinnati, USA

⁶CCHMC, Pediatric Rehabilitation Medicine, Cincinnati, USA

Traumatic brain injury (TBI) is a leading cause of accidental death in individuals under the age of 45, affecting 2.8 million people in the United States annually. Acute changes in brain metabolism after TBI are associated with changes in neurologic function in animal models and human subjects. Less is known about chronic changes in brain metabolism after TBI. NMR-metabolomics measures small molecule metabolites that are the substrates and products of biochemical reactions. These metabolites support diverse processes including energy production, protein, nucleotide, and transmitter synthesis, and post-translational modification that are critical to cell function. We hypothesize that metabolic changes in chronic TBI are not uniformly distributed throughout the brain and that different brain regions have unique metabolic signatures after TBI. As brain metabolism is tightly regulated, we further hypothesize that these changes are subtle, reflecting reprioritization of resources rather than changes in tissue concentrations of individual metabolites. We examined metabolic profiles in 5 brain regions and plasma in adult rats 60 days after lateral fluid percussion injury or in naïve animals. We found changes in metabolite concentrations between naïve and TBI animals only in hippocampus. Changes in metabolite levels in hippocampus suggest a bias towards excitatory>inhibitory neurotransmission, with increases in glutamate and glutamine, but not GABA. These data also suggest that in hippocampus molecules involved in processes that span neurons and glia (the glu/gln cycle, the lactate shuttle) may be preferentially altered in chronic TBI. Using a correlation-based analyses, we found both significantly increased and decreased correlations between metabolites in chronic TBI in every brain region looked at. We interpret these findings as alterations in how these molecules are utilized and regulated within cells and between cells types in each brain region.

Keywords: chronic TBI, metabolomics, rat, lateral fluid percussion

B20-04

ALTERATIONS IN METABOLIC PATHWAYS FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN IMMATURE RAT

Susanna Scafidi, Caitlyn Bowman, Munanchu Poudel, Michael Wolfgang

Johns Hopkins University School of Medicine, Division of Pediatric Anesthesiology and Critical Care Medicine, Baltimore, USA

Traumatic brain injury (TBI) is the leading cause of permanent life-long disability in children and is characterized by deficits in learning and memory, cognition, attention and sensory-motor integration. Metabolic crisis caused by impaired oxidative glucose metabolism following TBI further contributes to cell death. Although glucose is the primary substrate for brain energy and metabolism, brain is also equipped with the ability to oxidize fatty acids for energy and metabolism. This study aimed to examine alterations in both brain glucose and fatty acid metabolism after TBI. Postnatal day 21–22 male rats were anesthetized with isoflurane and TBI was administered using a controlled cortical impact to the left parietal cortex. Harvested brains were dissected into ipsilateral cortex, ipsilateral hippocampus, contralateral cortex, and hippocampus. Using RT-PCR (Qiagen) we analyzed the expression of genes involved in regulation and enzymatic pathways of glucose and glycogen metabolism, as well as genes involved in fatty acid transport and metabolism at 24 hr, 72 hr and 7 days after TBI. We found that both astrocytic (Glut1) and neuronal

(Glut3) transporters RNA were peaked at 24hrs in all regions studied, while RNA for pyruvate dehydrogenase E1 α – a key enzyme, which links glycolysis and Krebs's cycle, was decreased at 24 hrs and 72 hrs post injury. In contrast, Fabp7 (brain specific fatty acid transporter) was increased following TBI and peaked at 72 hrs, while mitochondrial fatty acid metabolism genes (CPT1A, CPT2) were not changed after TBI. TCA cycle genes remained unaffected by TBI. To determine the function, we measured glucose and fatty acid oxidation. Glucose oxidation was significantly decreased in ipsilateral cortex and hippocampus, while fatty acid oxidation was increased after TBI in the ipsilateral hippocampus but not in the cortex. This study provides evidence that brain following TBI is capable to use endogenous substrates (fatty acids) to meet the metabolic demands.

Keywords: glucose

B20-05

ASSOCIATING THE ALTERED GLUCOSE UPTAKE WITH CHANGES IN GLUCOSE TRANSPORTERS, INFLAMMATION AND PATHOLOGY IN DIFFUSE TBI

Tsang-Wei Tu^{1,2}, Jacklyn Witko^{1,2}, Neekita Jikaria^{1,2}, Wael Ibrahim¹, Dima Hammoud¹, Joseph Frank¹

¹National Institutes of Health, Radiology and Imaging Sciences, Bethesda, USA

²Henry Jackson Foundation, Center for Neuroscience and Regenerative Medicine, Bethesda, USA

Objectives/Methods: Altered glucose uptake and metabolism has been reported in the TBI patients posing a high risk for neurodegenerative diseases. The current study correlated changes in glucose transporters and other pathology associated with such metabolic alterations observed with TBI. 36 female 10-week-old rats were induced diffuse TBI by 2m/450g weight drop, and imaged at baseline, 24hr and 4-weekly scans by 9.4T/MRI with DTI and glucoCEST weighted imaging (GWI). 3-rats per time-point were picked for 2DG autoradiography for glucose uptake, and immunohistochemistry (IHC) for axon/SMI31, neuron/NeuN, glucose transporters/Glut1/Glut3, microgliosis/Iba1, astrogliosis/GFAP and apoptosis/TUNEL.

Results/Conclusion: After TBI, diffuse axonal injury was clear seen in corpus callosum by DTI and SMI31. Both GWI and 2DG showed an immediate increase of glucose uptake in 24hrs in cortex (+10% of baseline level, $p=NS$), a decrease at week2 (−19%, $p<0.05$) and then normalized at week 4 ($p=NS$). A biphasic increase of Glut1 was observed in 24 hr (+500%, $p<0.01$) and between week 2–3 (+300%, $p<0.01$). A two-fold increase in Glut3 ($p<0.01$) appeared in 24 hr. Triple staining of Glut3-NeuN-GFAP showed a dramatic decrease of Glut3 on neurons, instead expressed primarily on reactive astrocytes, which normally utilized 45kDa Glut1 for glucose transport. Significant increases ($p<0.01$) of microglia and astrocyte were seen after 24 hr and peaked at week 2. TUNEL also exhibited a biphasic increase of apoptosis in 24 hr (+300%, $p<0.01$) and week 2–3 (+200%, $p<0.01$).

These results suggest that the injured brain required immediate energy to restore neurological function within 24 hr after injury, where the increased astrocyte-to-neuron lactate shuttle (ANLS) appeared to support the high-energy demands by neurons. This was associated with acute increase expression of Glut1/Glut3. However, the brain then entered a hypometabolism state in week 2–3, decreased glucose uptake was dissociated from the expression of Glut1/Glut3, potentially related to the increased inflammation and apoptosis in this secondary injury phase. The non-invasive GWI affords the sensitivity to identify the glucose level for treatment window to increase neuronal survival in TBI.

Keywords: CEST, 2DG, Astrocyte, DTI, Glucose Metabolism, Neuron

B20-06

ASSESSING THE ORGAN-DEPENDENT ROLE OF MITO-NEET, A MODULATOR OF MITOCHONDRIAL BIOENERGETICS AND TARGET FOR NEUROPROTECTION

Hemendra Vekaria^{1,2}, Amber Cloud¹, Malinda Spry¹, William Hubbard¹, Sara Ngo Tenlep⁴, Kevin Pearson⁴, Patrick Sullivan^{1,2,3}

¹University of Kentucky, Spinal Cord and Brain Injury Research Center, Lexington, USA

²University of Kentucky, Department of Neuroscience, Lexington, USA

³Lexington VAMC, VAMC Research, Lexington, USA

⁴University of Kentucky, Department of Pharmacology and Nutritional Sciences, Lexington, USA

Mitochondrial dysfunction has been shown to occur following traumatic brain injury (TBI) and spinal cord injury (SCI). Trauma-induced intracellular Ca^{2+} influx leads to increased mitochondrial Ca^{2+} buffering and reduced membrane potential. The link between Ca^{2+} buffering and shut down of mitochondrial respiration seems to be modulation of mitoNEET, an outer mitochondrial membrane protein. Pioglitazone, which binds mitoNEET, has shown neuroprotective effects following TBI and SCI possibly through its interactions with mitoNEET in a peroxisome proliferator activated receptor (PPAR γ) independent manner. Furthermore, NL-1, a pioglitazone derivative lacking PPAR γ binding, has been demonstrated to increase cortical tissue sparing following TBI, an effect lost in mitoNEET knock out (KO) mice. These results signify mitoNEET as an important mitochondrial target for the treatment of traumatic CNS injuries. To further elucidate organ-specific mitoNEET-mediated mitochondrial function, we utilized mitoNEET KO mice to assay mitochondrial bioenergetics in multiple organs (brain, heart, liver, muscle, brown and white adipose tissue) and characterize mitoNEET-mediated changes in metabolic profiling and behavioral testing. A significant reduction in cognitive function, measured with a novel object recognition test, was observed in KO mice compared to wild-type (WT) mice. It was observed that mitochondrial respiration in mitoNEET KO mice was altered in a tissue specific manner. Brown adipose tissue showed a significant drop in mitochondrial function whereas heart and brain also showed lowered respiration in mitoNEET KO animals. No changes were observed in glucose metabolism but fat deposition was significantly lowered in KO mice. The current results show unique mitochondrial-mediated phenotyping of mitoNEET KO mice and support the role of mitoNEET as a central modulator of mitochondrial bioenergetics and a novel target for intervention following CNS injury.

Keywords: Mitochondrial dysfunction, Bioenergetics, Traumatic Brain Injury, neuroprotection

B21 MICROGLIA

B21-01

INHIBITION OF MIR-155 LIMITS NEUROINFLAMMATORY EFFECTS AND IMPROVE FUNCTIONAL OUTCOMES AFTER EXPERIMENTAL TBI

Rebecca Henry, David Loane, James Barrett, Sarah Doran, Boris Sabirzhanov, Bogdan Stoica, Faden Alan

UMB, STAR, Baltimore, USA

Recent data suggest that the small noncoding microRNAs (miR)-155 and −124 control the microglial activation state following CNS injury.

The present study examined the expression profiles of these miRs following traumatic brain injury (TBI), and investigated the effects of acute or delayed administration of a selective miR-155 antagomir on neuroinflammation and neurological recovery following injury. *Experiment 1:* Male C57Bl/6 mice underwent moderate controlled cortical impact (CCI) and cortical tissue was isolated at 6h, 24h, 72h and 7d post-injury for miR expression analysis. *Experiment 2:* Mice received a single icv injection of miR-155 or control antagomir (0.5nmol) immediately after CCI. Cognitive function (Y maze) was tested at post-injury day (PID) 6, and hippocampal tissue was collected for mRNA analysis of inflammatory genes and miR expression, at PID7. *Experiment 3:* Mice received a delayed 7 day icv infusion of miR-155 or control antagomir (0.5 nmol/day), beginning at 24 h following CCI. Hippocampal tissue was processed as per experiment 2. *Experiment 4:* Mice received a delayed 7 day icv infusion of miR-155 or control antagomir as per experiment 3. Motor function was assessed using the beam walk test at PID 1, 3, 7, 14, 21 & 28. CCI increased miR-155 and decreased miR-124 expression at PID7. Acute administration of a miR-155 antagomir, attenuated CCI-induced increases in gene expression of several pro-inflammatory mediators including TNF- α , gp-91 and p22^{phox}, at PID7 and reversed injury-induced impairments in the Y maze. Delayed infusion of the miR-155 antagomir attenuated expression of pro-inflammatory mediators including TNF- α , gp-91 and p22^{phox} and improved motor function at PID 14 and 21. These data demonstrate that TBI causes an altered miR-155/miR-124 expression profile up to 7 days post-injury, and that pharmacological inhibition of miR-155 limits expression of inflammatory markers and improves functional recovery. Thus, inhibiting miR-155 may offer a novel therapeutic approach for targeting neuroinflammatory responses after TBI.

Acknowledgments: Supported in part by NINDS R01 NS037313-15.

Keywords: TBI, neuroinflammation, micro-RNA

B21-02

THE IMMUNOPROTEASOME MODULATES MULTIPLE FEATURES OF THE MICROGLIAL INFLAMMATORY RESPONSE

Kasey Moritz¹, Nikki McCormack¹, Mahlet Abera², Coralie Violette³, Young Yauger¹, Gauthaman Sukumar³, Clifton Dalgard^{1,2,3}, Barrington Burnett¹

¹Uniformed Services University of the Health Sciences, Anatomy, Physiology and Genetics, Bethesda, USA

²Uniformed Services University of the Health Sciences, Collaborative Health Initiative Research Program, Bethesda, USA

³Uniformed Services University of the Health Sciences, The American Genome Center, Bethesda, USA

Microglia respond to disruption of brain homeostasis following traumatic brain injury (TBI) by transitioning to a reactive state, characterized by morphological and functional changes to promote immune defense and a robust inflammatory response. Immunoproteasomes, an inducible form of the constitutive proteasome, are elevated in reactive microglia following TBI, stroke and infection, however the exact role of the immunoproteasome in neuropathology remains poorly characterized. Here we report that the proteasome undergoes rapid reorganization in microglia following interferon-gamma exposure, converting the constitutive proteasome to the inducible immunoproteasome. Stranded mRNA sequencing analyses indicate that interferon-gamma-dependent transcriptome changes, which are key features of the immune defense and inflammation activities of microglia, are reversed when the immunoproteasome is inhibited. We show in cultured microglia that the immunoproteasome regulates nitric oxide production, phagocytosis and

priming consistent with the transcriptome data. These results imply that targeting immunoproteasome function following brain injury may attenuate select microglial activity to improve the pathophysiology of neurodegenerative conditions or the progress of inflammation-mediated secondary injury following neurotrauma.

Keywords: Immunoproteasome, RNA Sequencing, Proteasome, Priming

B21-03

NEUROPATHOLOGY OF THE HIPPOCAMPAL MEMORY CIRCUITRY IN DAYS FOLLOWING EXPERIMENTAL DIFFUSE BRAIN INJURY

Neet Shah^{1,2}, Loren Law^{1,2}, Theresa Thomas^{1,2}, Jonathon Lifshitz^{1,2}, H Morrison^{1,2}

¹University of Arizona College of Medicine - Phoenix, Phoenix, USA

²Barrow Neurologic Institute, Phoenix Children's Hospital, Phoenix, USA

Traumatic brain injury (TBI) is not a transient event from which all people recover; the resulting damage can evolve into neurological disease, with significant cognitive symptoms. Injury stretches and shears axons that disrupt neurons within dedicated neural circuits. Further, microglia are activated in response to neuronal injury, representing histological hallmarks of neuroinflammation and chronic neuropathology following TBI. Microglial activation follows morphological change from an inactive ramified state to an active, deramified or amoeboid state the represents the inflammatory process. Memory circuits throughout the hippocampus, in particular, are vulnerable to pathology and underlie impaired cognitive function 28 days after experimental TBI. Therefore, we evaluated the progression of neuropathology and microglia activation within the hippocampal dentate gyrus (DG), CA1, and CA3 sub-regions following midline fluid percussion injury. At time intervals of 1, 7, and 28 days post-injury, significant increases in neuropathology (Silver stain) and decreases in microglia ramification (Iba-1 immunohistochemistry) were quantified compared to control. The dentate gyrus and CA3 showed significant increases in silver pathology at 1 and 7 days post-injury, which decreased by 28 days. The CA1 region showed a significant overall increase in pathology, but not significant at any one time point. The CA3 and CA1 showed significant increases in microglia number at day 7, which decreased by day 28. The DG showed a significant decrease in microglia number on day 1 followed by a significant increase on days 7 and 28. Microglia process length and end points also significantly decreased following injury in all 3 regions suggesting a conversion from an inactive to an active state following TBI. Thus, TBI harbors cellular pathology along the hippocampal memory circuit, which may contribute to memory deficits at 28 days post-injury. Additionally, injury-induced microglia recruitment and activation suggests sustained inflammation associated with the TBI disease process.

Keywords: Dentate Gyrus, CA1, CA3, microglia, neuroinflammation

B21-04

EFFECTS OF NICOTINAMIDE UPON THE INFLAMMATORY RESPONSE IN A MODEL OF JUVENILE TRAUMATIC BRAIN INJURY

Aidan Smith, Michael Hylin

Southern Illinois University, Psychology, Carbondale, USA

Following a traumatic brain injury (TBI), a series of neurochemical and inflammatory events occur to repair and promote healing of neural

tissue. Prolonged inflammation however, results in neurodegeneration of healthy tissue and can result in cognitive and behavioral deficits. Juvenile brains are more susceptible to the damaging effects of inflammation after a TBI. Microglia play a crucial role in the inflammatory response demonstrating both pro and anti-inflammatory properties. Chronic activation of pro-inflammatory microglia has been associated with poor outcomes. Nutraceutical treatments have been used to help mediate against the negative events following a TBI. In adult models of TBI, nicotinamide (NAM, vitamin B₃) has been shown to decrease behavioral deficits and reduce inflammation. These nutraceutical treatments however, have not been studied in the developing brain following injury. The current study explored the effects of NAM on the microglial response over the course of time following a TBI in juvenile animals. Following a bilateral controlled cortical impact at PND-28, animals were given either 500 mg/kg of NAM or 1 mL/kg of 0.9% saline. NAM was administered at 15 minutes, 24 hours, and 48 hours post-injury. Subjects were euthanized at 3 hours, 72 hours, 1 week, and 1 month after injury. Microglia levels were sampled in the cortex lateral and medial to the injury, the dentate gyrus, CA1, and CA3 of the hippocampus, and the dorsal thalamus. Microglia were detected using anti-Iba1 antibodies. Analysis found NAM treated animals had significantly fewer microglia in all regions of the hippocampus and cortex, and dorsal thalamus when compared to saline treated animals. This NAM-induced reduction was observed following the injury at 3 days. These findings suggest that NAM treatments alone have neuroprotective properties and reduce microglial activation. This data corresponds with previous behavioral data showing NAM treated animals having improved performances in Morris water maze testing compared to saline treated animals. These results, in conjunction with previous behavioral data demonstrate potential clinical use of NAM in juvenile populations following TBI.

Keywords: Juvenile, Nutraceutical, Time Course, Recovery

B21-05

INHIBITION OF NOX4 REDUCES OXIDATIVE STRESS AND NEURONAL DAMAGE INDUCED BY IRON ACTIVATED MICROGLIA

Young Yauger, Alexandra Yaszemski, Sara Bermudez, Kimberly Byrnes

Uniformed Services University, Bethesda, USA

Iron contributes to the pathophysiology of secondary central nervous system injury, as it can accentuate reactive oxygen species (ROS) production among microglia, leading to increased neuronal damage. However, the mechanism by which iron accentuates ROS production among microglia is unknown. NADPH oxidases (NOX) are a family of enzymes known for ROS production within microglia; NOX4 is one isoform that is constitutively active in microglia and directly produces hydrogen peroxide. We hypothesize that NOX4 inhibition can ameliorate iron induced ROS accentuation among microglia, leading to improved neuronal survivability. We now show, using the microglial BV2 cell line activated with pro-inflammatory stimulants including lipopolysaccharide (LPS) or interferon (IFN) γ , that iron induces a significant increase in ROS release. We also demonstrate that the NOX4 specific inhibitor GKT137831 significantly reduced this ROS production. Co-culture of BV2 with either primary cortical neurons or the neuronal-like PC12 cell line showed that iron in combination with LPS significantly reduced neuronal viability and induced caspase-3 activation. Inhibition of NOX4 in microglia blocked this neuronal toxicity. These findings support the use of GKT137831 as an effective ROS reduction strategy following CNS

injury, which may increase neuronal survivability and translate into improved functional outcomes *in vivo*.

Keywords: NADPH oxidase, cytokine, neurotoxicity, oxidative stress, iron

B22 NEURODEGENERATION

B22-01

SIDESTREAM SMOKE AFFECTS DENDRITIC COMPLEXITY AND ASTROCYTES AFTER MODEL MILD CLOSED HEAD TRAUMATIC BRAIN INJURY

Bruce Citron^{1,2}, Whitney Ratliff^{1,2}, Kristen Keeley¹, Chaim Pick³, Maja Okuka⁴, John Tsibris⁴, Ronald Mervis⁵, Jessica Chang^{1,2}

¹Bay Pines VA Healthcare System, R&D, Bay Pines, USA

²Morsani College of Medicine, Univ. of S. Florida, Molecular Medicine, Tampa, USA

³Sackler School of Med., Tel Aviv Univ., Anatomy and Anthropology, Tel Aviv, Israel

⁴Morsani College of Medicine, Univ. of S. Florida, Obstetrics and Gynecology, Tampa, USA

⁵NeuroStructural Research Labs., Neurosurgery and Brain Repair, Tampa, USA

Mild traumatic brain injuries have been experienced by at least 15% of recently deployed military personnel and the worldwide prevalence is approximately 0.5% per year. Mild injuries can have long-term consequences that interfere with the quality of life and our goal is to identify therapeutic targets that will reduce neurodegeneration and improve outcomes. We have previously identified pathways responsive to oxidative attack, e.g., transcription factor Nrf2, as regulators of the consequences of mild TBI. An improved understanding of the changes in dendritic complexity in the brain and reactive astrocytosis could provide a context for the effects of tobacco smoke exposure on traumatic brain injury recuperation and whether increasing antioxidant signaling affected plasticity following injury. To examine the potential influences of second hand smoke during recovery from TBI, we exposed mice to cigarette smoke for three 30 min periods each day for 12 days. The dendritic arbor of neurons was determined by Golgi staining, Sholl, and branch point analysis. Astrocytes were examined by immunohistochemical staining for GFAP. We found that neither the mild injuries nor the smoke exposure produced axonal damage detectable with amino cupric silver staining. However, there was significantly reduced complexity in the dendritic arbors in the after mild TBI plus smoke exposure. In the hippocampus, there was an appearance of reactive astrocytosis after the injury and tobacco smoke insult. Although traumatic brain injuries will not be fully preventable, it is important to understand preventative strategies and lifestyle choices, such as cigarette smoking, that could affect postinjury prognosis.

Keywords: Dendrites, Hippocampus, Smoke, GFAP, Golgi Staining, Oxidative Stress

B22-02

RETROGRADE DEGENERATION OF CORTICOTHALAMIC CIRCUITS AFTER TRAUMATIC CONTUSIONS IN VENTRAL FRONTAL LOBES

Nikolaos Ziogas, Merrysha Castillo, Jiwon Ryu, Olga Pletnikova, Juan Troncoso, Vassilis Koliatsos

Johns Hopkins University, School of Medicine, Pathology, Division of Neuropathology, Baltimore, USA

Traumatic contusions are generally viewed as circumscribed brain lesions, without considering their neocortical, limbic or subcortical connections and related circuits. In the era of parallel/distributive theories of information processing in the brain and the Human Connectome Project it is important to understand these lesions as a network problem according to which a node is eliminated, leading to anterograde or retrograde degeneration of interconnected areas. Post-mortem human brains with ventral frontal (orbitofrontal) contusions or diffuse axonal injury (DAI) were examined using immunohistochemistry for phosphorylated epitopes of heavy and medium-molecular weight neurofilament proteins (SMI 310 antibody) as a marker of retrograde degeneration of neuronal cell bodies in connected neocortical, limbic, and subcortical sites. Some cases with frontal also had temporopolar contusions. Maps of afferent and efferent connections of orbitofrontal cortex were drawn based on existing tract-tracing studies in non-human primates, with input by older reports of retrograde degeneration after leucotomy in humans and data from the human connectome project. We focused on major sources of afferent inputs and identified hippocampus, thalamus and interconnected frontal cortices as target regions for investigation. Here we present initial data from 4 patients with TBI (3 with OFC contusions, 1 with DAI). Large numbers of phosphorylated NF-H/NF-M-immunoreactive neuronal cell bodies were found in the ventral anterior (VA) and ventral lateral (VL) nucleus in all cases. In certain cases, p-NF immunoreactivity was also found in cell bodies of hippocampal neurons. In contrast, frontal neocortex did not show any p-NF (+) cell bodies. In the DAI case that had hemorrhagic lesions in the genu of corpus callosum, frontal sections showed robust p-NF (+) cells throughout cortex. Our findings indicate that ventral frontal contusions lead to retrograde degeneration in thalamus, less so in hippocampus, and none in interconnected frontal cortices. Labeling in thalamus is robust and corresponds nicely to known afferents to primate orbitofrontal cortex from tract-tracing and retrograde degeneration studies. In contrast, DAI leads to extensive retrograde changes in prefrontal cortical regions. These studies demonstrate the power of modern molecular tools to update or “rewrite” the neuropathology of traumatic contusions and DAI based on impaired neocortical and limbic circuits, with implications for understanding the diverse and severe neuropsychiatric symptoms of such patients.

Keywords: Human TBI, Transsynaptic Degeneration, Thalamus, Orbitofrontal Cortex

B22-03

DIFFUSION BASIS SPECTRUM IMAGING DETECTS AXONAL LOSS AND DEMYELINATION IN CHRONIC TRAUMATIC BRAIN INJURY

Jie Zhan^{1,2}, Peng Sun², Ajit George², Michael Wallendorf², Zezhong Ye², Tsen-Hsuan Lin², Jeff Viox², Honghan Gong¹, Sheng-Kwei Song²

¹The First Affiliated Hospital, Nanchang University, Radiology, Nanchang, China

²Washington University in St. Louis, Radiology, St. Louis, USA

Traumatic brain injury (TBI) frequently results in neurodegenerative injuries lasting for months or years post-injury. To investigate white matter changes at the chronic stage of TBI, we examined whole-brain white matter tracts using diffusion basis spectrum imaging (DBSI) and tract-based spatial statistics (TBSS). In this preliminary report, we present DBSI findings from corpus callosum (CC), correlating with Mini-Mental State Examination (MMSE) and Beck Depression Inventory (BDI). Eighteen chronic TBI patients (post-injury >3 months; 9 mild, 2 moderate and 7 severe) and twenty healthy control subjects

were included in this study. Diffusion-weighted images were collected with a multi-b value scheme (99 directions, maximum b-value = 2000 s/mm²) at 3T (Trio; Siemens, Erlangen, Germany) with voxel size of 2.5 × 2.5 × 2.5 mm³. DBSI were computed using the in-house software developed using Matlab. DBSI pathological metrics were projected onto the skeleton generated by TBSS for statistical analyses. Student *t*-test was applied for group comparison. Pearson correlation was tested between DBSI metrics and clinical assessments. Compared with control subjects, significantly decreased ($p < 0.05$) fiber fraction and increased ($p < 0.05$) fiber radial diffusivity in TBI were found in CC, indicating the predominant role of axonal loss and demyelination at the chronic stage of TBI. In addition, strong correlation was observed in the splenium between fiber fraction and clinical assessments (MMSE: $r = 0.60$, $p < 0.01$; BDI: $r = -0.72$, $p < 0.01$). Our results were consistent with previous reports that CC is sensitive to shear strain, especially the splenium. In conclusion, DBSI-derived pathological metrics could be novel imaging biomarkers to monitor the course of white matter abnormalities in TBI.

Acknowledgments: Supported by National Natural Science Foundations of China (grants 81460263, 81660286), NIH P01-NS059560, U01-EY025500, National Multiple Sclerosis Society (NMSS) RG 4549A4/1, and Department of Defense Idea Award W81XWH-12-1-0457.

Keywords: Traumatic Brain Injury, Diffusion MRI, Corpus Callosum, TBSS

B23 NEUROGENESIS

B23-01

CHRONIC IMPAIRMENT OF NEUROGENESIS IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

Elizabeth Bishop, Debbie Boone, Ian Bolding, Kathia Johnson, Margaret Parsley, Douglas Dewitt, Donald Prough, Maria-Adelide Micci

UTMB Galveston, Anesthesiology, Galveston, USA

Background: Traumatic brain injury (TBI) results in a range of cognitive dysfunctions that significantly affect the quality of life for post-traumatic brain injury survivors. Using a rat fluid percussion injury (FPI) model, we have previously shown that, two weeks following injury, increased gliogenesis is coupled with reduced maturation and integration of new neurons in the hippocampus dentate gyrus (DG). The aim of this work was to build on our short-term studies and determine the chronic effect of TBI on the genetic and epigenetic regulation of neurogenesis in the rat FPI model.

Methods: Adult male Sprague-Dawley rats were randomized to receive FPI or sham surgery. The brains were collected 6 months later and the DGs were laser capture microdissected before gene expression analysis with the “Neurogenesis RT² Profiler” PCR array. Further analysis of miRNAs known to regulate neurogenesis was performed by qRT-PCR.

Results: We found that following moderate FPI, several genes known to regulate neuronal differentiation, migration and survival were significantly up-regulated or down-regulated in the hippocampus DG as compared to sham rats. Further analysis showed that miRNAs known to regulate neurogenesis (miR9, miR24, miR124, miR132, miR134, miR184) were significantly up-regulated in the DG of FPI rats as compared to sham rats. Interestingly, these same miRNAs were up-regulated in the DG in our previous study performed two weeks after FPI.

Conclusions: Our data suggest that FPI produces genetic and epigenetic changes in hippocampal neurogenesis that persist up to 6 months after injury. Understanding the mechanisms underlying impaired

neurogenesis after TBI will aid the development of novel therapeutic interventions for the treatment of TBI survivors.

Funding: These studies were completed as part of an interdisciplinary research team funded by The Moody Project for Translational Traumatic Brain Injury.

Keywords: Chronic, Laser Capture Microdissection, Dentate Gyrus, miRNA, Fluid Percussion Injury

B23-02

PENETRATING BALLISTIC-LIKE BRAIN INJURY ACTIVATES NEUROGENIC NICHE IN ADULT RATS

Sindhu Kizhakke Madathil, Ying Deng-Bryant, Sarah Urnkar, Janice Gilsdorf, Shear Deborah
WRAIR, BTNN, Silver Springs, USA

Active proliferative response is a common occurrence following traumatic brain injury (TBI) and contributes to both gliogenesis and neurogenesis. While post-injury neurogenesis stimulates neurorepair, gliogenesis may cause neuroinflammation that may be detrimental to reparative processes. To design strategies that promote neurorepair, we need to understand the changes in post-traumatic neurogenesis. Here we examined the effects of penetrating ballistic-like brain injury (PBBI) on immature neuronal population in two important neurogenic niches of the adult brain viz., forebrain sub-ventricular zone (SVZ) and hippocampal sub-granular zone (SGZ). Rats were subjected to PBBI (n=6/time point) or sham craniotomy (n=6/time-point). To capture proliferating cells, rats were injected with BrdU (50mg/kg) for 7 days and were euthanized at 10 day post-injury. Doublecortin (DCX) immunolabeling was used to identify immature neurons and BrdU/DCX double-labelling was performed to identify newborn immature neurons. Quantification of immunolabelled cells was done using a fluorescence microscope equipped with multi-channel filter set. To understand forebrain neurogenesis, total immature neurons and BrdU positive newborn immature neurons were counted separately in ipsilateral SVZ, striatum, subcortical white matter (SCWM) and cortex. In the hippocampus, DCX positive and BrdU/DCX co-labelled cells were counted from SGZ. DCX positive neurons and BrdU/DCX co-labelled newborn immature neurons were found in both SVZ and SGZ, regardless of the injury status. However, PBBI appeared to enhance the production of newborn neurons in both SVZ and SGZ areas. Furthermore, PBBI significantly enhanced the migration of newborn neurons from SVZ towards striatum, SCWM and cortex ($p < 0.05$ compared to sham). In the hippocampal SGZ, PBBI did not alter the total number immature neurons however, increased the number of BrdU/DCX co-labelled newborn neurons ($p < 0.05$ compared to sham). Overall, our results indicate an activation of neurogenesis in two major neurogenic niches of the adult rat brain following PBBI. Our findings also show that these newborn neurons can migrate to areas of injury, purportedly to modulate neurorepair. Further studies will characterize the anatomical integration of newborn neurons and their role in mediating functional recovery.

Keywords: PBBI, Neurogenesis, Doublecortin, Proliferation

B23-03

INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION ENHANCES NEUROGENESIS AND ACTIVATES THE MTOR PATHWAY AFTER MODERATE TBI

Erica Littlejohn, Danielle Scott, Anthony DeSana, Kathryn Saatman
University of Kentucky, SCoBIRC/Physiology, Lexington, USA

Nearly 5 million people in the United States are living with TBI related disabilities, in part because of the brain's limited capacity to replace lost and damaged neurons. Immature neurons in the hippocampus are highly vulnerable to trauma, but can be replaced through proliferation and differentiation of neural stem cells in the sub-granular zone. The extent of injury-induced neurogenesis, however, may be injury severity dependent. Insulin-like Growth Factor 1 (IGF1) modulates basal and injury-induced hippocampal neurogenesis. Using a transgenic mouse model with IGF1 overexpression restricted to astrocytes (IGF Tg) to raise brain levels of IGF1 by means of injury-induced astrogliosis, we previously showed that IGF1 enhances recovery of the immature neuron population and morphology after severe TBI. Mammalian target of rapamycin (mTOR), a signaling molecule downstream of IGF1, has been identified as a potential target for TBI interventions because of its regulatory role in plasticity and cell survival. We hypothesized that increased IGF1 would stimulate mTOR activity following moderate injury, resulting in improved neurogenesis. To this end three cohorts of IGF Tg and wild-type (WT) mice received moderate controlled cortical impact (CCI, n=8-11/genotype) and survived 1, 3 or 10d or received sham injury (n=3/genotype; 72 h survival). At 1 and 3d following moderate injury, immunohistochemical labeling of pS6, a well characterized downstream effector of mTOR, was quantified in the granule cell layer, molecular layer, and the hilus of the dentate gyrus. Analysis of pS6 at the injury epicenter (3 sections/animal) suggests that IGF1 stimulates activity of the mTOR pathway following moderate TBI in a region-specific manner. At 10d after moderate injury, IGF1 overexpression enhances recovery of immature neurons.

Funding Sources: Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT) 14-12A and NIH R01 NS072302-02S1, R01 NS0072302, T32 NS077889, and P30 NS051220.

Keywords: IGF1, Neuronal Survival, Moderate Injury, CCI

B23-04

MODULATION OF ADULT NEUROGENESIS BY NANOPULSED LASER THERAPY IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

Emanuele Moccia^{1,3}, Rinat Esenaliev^{2,4}, Irene Petrov², Yuriy Petrov², Kathia Johnson¹, Ian Bolding¹, Margaret Parsley¹, Ashley Untereiner¹, Csaba Szabo¹, Donald Prough¹, Maria-Adelaide Micci¹
¹UTMB, Dept. of Anesthesiology, Galveston, USA

²UTMB, Center for Biomedical Engineering, Galveston, USA

³University of Palermo, Dept. of BIONEC, Palermo, Italy

⁴UTMB, Dept. of Neuroscience and Cell Biology, Galveston, USA

Background: Traumatic Brain Injury (TBI) is a chronic disease that occurs after a head trauma. One of the brain regions most affected by TBI is the hippocampus, which plays a pivotal role in learning and memory and is one of the only two regions in the brain where neurogenesis occurs throughout life. Here we tested the ability of Nanopulsed Laser Therapy (NPLT), that combines near-infrared laser light (NIL; 808 nm) and laser-generated, low-energy optoacoustic waves (OA), to modulate hippocampal neurogenesis in rat fluid percussion injury (FPI) TBI.

Methods: Adult male rats were treated with NPLT 1 hour after FPI or sham surgery. Proliferation of neural stem cells (NSC) in the hippocampus was studied using BrdU incorporation 2 weeks later. *In vitro*, we analyzed the effect of the main components of NPLT, NIL and OA, on the proliferation (EdU uptake) and differentiation (immunofluorescence) of NSC isolated from adult rat

hippocampus (hipp-NSC). The expression of miRNAs known to regulate neurogenesis and mitochondrial bioenergetics (known to play a critical role in the regulation of stem cell differentiation) were also assessed.

Results: NPLT stimulated NSC proliferation in the hippocampus of uninjured rats and prevented their aberrant proliferation induced by FPI. *In vitro*, OA and NIL+OA increased Hipp-NSC proliferation, while NIL alone did not. NIL+OA also increased the expression of miR9, miR25, and miR29 (known to stimulate proliferation), while OA treatment decreased their expression. Both NIL+OA and NIL treatments stimulated mitochondrial bioenergetic in hipp-NSC.

Conclusion: NPLT selectively regulates neurogenesis by modulating the expression of specific miRNAs and by stimulating mitochondrial bioenergetics.

Funding: These studies were funded by The Moody Project for Translational Traumatic Brain Injury Research.

Keywords: miRNA, Gene Expression, Proliferation, Differentiation, Therapy, Hippocampus

B23-05

ASSESSING NEURONAL ABLATION METHODS TO STUDY NEUROGENESIS IN MICE

Je'Vana Pickens, Tracy Niedzielko, Candace Floyd
Univ. of Alabama at Birmingham, Dept of Phys Med. & Rehab., Birmingham, USA

Traumatic brain injury (TBI) affects nearly 2.8 million people in the United States per year and often leads to lasting cognitive impairment. A strategy to promote recovery after TBI centers on enhancing or controlling neurogenesis in the dentate gyrus region of the hippocampus. We hypothesize that to directly evaluate the role of neurogenesis in recovery after TBI, experimental methods to selectively ablate neurogenesis are necessary. Thus, the goal of this study was to optimize ablation of dentate gyrus neurogenesis in mice using a transgenic strategy to conditionally express diphtheria toxin receptors (DTR) and then crossing these mice with nestin-Cre^{ERT2} mice in which a tamoxifen (TAM)-inducible Cre recombinase is expressed under control of a nestin promoter (Nestin-Cre iDTR). It has been reported previously that TAM administration induces permanent expression of the DTR in neural progenitor cells which targets these cells for subsequent ablation in Nestin-Cre iDTR mice. To optimize this "tag-and-ablate" protocol, we compared two injection methodologies for TAM, comparing daily intraperitoneal (i.p.) injection versus delivery in matrix-driven delivery pellets. The main outcome measure was percentage ablation of nestin positive cells assessed via immunohistochemistry and stereology in brains extracted acutely after completion of diphtheria toxin administration. Mice were genotyped to determine if they were Cre+ or Cre-, then they were randomly assigned to either the TAM+ or TAM- groups. Mice were checked daily for body weight during the duration of the experiment. There were no significant differences in nestin positive cells between Cre+ and Cre- animals at any assessed time points for animals receiving the i.p. injection of TAM. There were also no significant difference in number of nestin positive cells in Cre+ and Cre- animals after administration of TAM via pellet. This work suggests that Nestin-CRE iDTR mice may not be a robust tool for studying the role of neurogenesis in neurotrauma under these conditions. Work supported by NS075162.

Keywords: transgenic mice

B24 PAIN

B24-01

DEVELOPMENT AND INTER-RATER RELIABILITY OF A PIG EVOKED PAIN SCALE (PEPS) FOR USE IN A PORCINE MODEL OF SCI

Kathryn Henley¹, Betty Pat², Candace Floyd²

¹*University of Alabama at Birmingham, Rehabilitation Science, Birmingham, USA*

²*University of Alabama at Birmingham, Physical Medicine and Rehabilitation, Birmingham, USA*

Spinal cord injury (SCI) often leads to central neuropathic pain. Unfortunately, the pathobiological mechanisms of SCI-induced neuropathic pain are complex and treatment options are few. Thus, translational research utilizing animal models of SCI-induced neuropathic pain is needed. One limitation to the evaluation of pain in animals is the reliance on withdrawal responses. We therefore hypothesize that pain assessments should include both withdrawal and supraspinal responses. The objective of this study was to develop a numerical scale to classify evoked pain in pigs which incorporates both reflexive and supraspinal responses. The scale was adapted from previous work on piglets and other domesticated animals. We applied graded stimuli to able-bodied pigs and simultaneously video-recorded their behavioral responses. Stimuli were selected to model quantitative sensory testing as used in clinical practice and research. Four raters scored the responses according to the scale. Operational definitions of individual behaviors were iteratively revised to refine the scale. Next, graded stimuli were applied to four groups of pigs: able-bodied, laminectomy, mid-thoracic SCI, and formalin-injected to induce a local inflammatory response. Recorded responses were randomized and evaluated by raters who were not informed of the force level of the stimuli. The inter-rater reliability and the scores of responses to the stimuli were evaluated. We found that in general, responses to lower force stimuli were consistently rated on the lower end of the scale and that the higher force stimuli were rated at the higher end. We also found that the inter-rater reliability of the scale was 0.924 for five raters with varying scientific backgrounds and experience scoring animal behavior. These data suggest that the PEPS can effectively classify behavioral responses to graded sensory stimuli with a high degree of inter-rater reliability. Support: Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development Service (121RX002200).

Keywords: supraspinal pain, porcine animal model, behavioral assessments

B24-02

NOX2-MEDIATED MICROGLIAL/MACROPHAGE ACTIVATION CONTRIBUTES TO HYPERESTHESIA AFTER SCI THROUGH MODULATION OF IL10/MIR155 PATHWAYS

Jessica Matyas, Marina Coll-Miro, Boris Sabirzhanov, Yun Li, Alok Kumar, Jingwen Yu, Alan Faden, Junfang Wu

University of Maryland Baltimore, Anesthesiology, Baltimore, USA

After spinal cord injury (SCI), approximately 80% of individuals develop chronic, severe, unrelenting pain (SCI-pain) that is often resistant to conventional pain medication. Thus, there is critical need to identify new therapeutic targets to reduce or eliminate SCI-pain. NADPH oxidase (NOX2) is an enzyme that induces reactive oxygen

species (ROS) and serves as a switch between the pro-inflammatory and neurorestorative microglial/macrophage phenotypes; such changes play an important role in chronic neuropathic pain associated with a variety of conditions including SCI-Pain. Increased NOX2 expression and activity after SCI has been demonstrated and pharmacological inhibition of NOX2 improves motor functional recovery. However, the underlying mechanisms of NOX2 in post-traumatic pain remain unexplored. In the present study we report that genetic deletion of NOX2 in NOX2^{-/-} mice or pharmacological inhibition of NOX2 using NOX2ds-tat administrated intraperitoneally significantly reduced mechanical/thermal hyperesthesia and motor dysfunction after moderate contusion SCI in mice. Western blot (WB) and immunohistochemistry (IHC) showed that SCI elevates NOX2 expression predominantly in microglia/macrophages. Deletion of NOX2 significantly reduced CD11b⁺/CD45⁺/F4/80⁺ macrophage infiltration detected by flow cytometry and 8-OHG⁺ ROS production by IHC in both lesion area and lumbar enlargement at 24h post-injury. NOX2 deficiency also altered microglial/macrophage M1-/M2-like balance towards the M2-like response. The latter was validated by WB showing robust increased protein expression of Arginase1 and YM1 in NOX2^{-/-} mice. Furthermore, qPCR analysis showed that NOX2 deficiency significantly up-regulates anti-inflammatory cytokine IL10 mRNA levels, associated with reduced microRNA155 expression in injured spinal cord tissue 1d after SCI. These findings were confirmed in CD11b-selective microglia/macrophages isolated from adult spinal cord tissue at 3 days post-injury. Taken together, our data suggest an important role for IL-10/miR155 pathway in regulating NOX2-mediated SCI-pain. Thus, specific targeting of NOX2 enzyme may provide an effective strategy for treating chronic pain in SCI patients.

Keywords: Spinal Cord Injury, NOX2, IL-10, Microglia/Macrophage, MiR155

B24-03

COMPARISON OF DEFICITS FOLLOWING SPINAL CORD INJURY IN A DORSAL ROOT AVULSION VERSUS CONTUSION/COMPRESSION MODEL IN PIGS

Tracy Niedzielko, Betty Pat, Candace Floyd

University of Alabama Birmingham, Physical Medicine and Rehabilitation, Birmingham, USA

Neuropathic pain (NP) is a chronic and debilitating condition affecting many patients with spinal cord injury (SCI). Since chronic pain is the leading cause of disability in the United States, there is a strong impetus to develop efficacious treatments for NP and animal models are key elements in the therapeutic development process. However, paralysis can make evaluation of reaction to a sensory stimulus more complex. Thus, the goal of the current study is to compare motor and sensory deficits in two models of SCI. One model, the dorsal root avulsion model (DRA), is typically associated with little locomotor deficit. In contrast, the mid-thoracic contusion/compression model induces incomplete hind limb paralysis. Adult, female mixed breed pigs (N=5/group, 36±2 kg) were randomized to receive either a mid-thoracic contusion/compression SCI at T10 (T10-SCI) or a unilateral DRA at the last thoracic and first lumbar nerve roots. For 8-13 weeks post-injury, animals were evaluated with weekly locomotor and quantitative sensory testing for NP in 8 dermatomes located at or below or the level of injury. In addition, animals that developed NP were administered 3 analgesics, randomized with washout to include: intravenous lidocaine (5 mg/kg/day), oral baclofen (60 mg/day) and oral pregabalin (300 mg/day) for 7 days to determine their analgesic efficacy. All T10-SCI animals showed significant motor impairment compared to DRA ani-

mals. Interestingly, NP developed below the level of injury in only T10-SCI animals. This occurred 5 weeks post-SCI and persisted to the end of the study. Lidocaine had the most robust analgesic effect. In summary, T10-SCI animals develop below-lesion NP. This was not observed in the DRA model. Intravenous lidocaine was the most effective analgesic in the treatment of SCI-NP. Further investigation is required to more thoroughly characterize the profile and mechanisms of SCI-NP development in the T10-SCI model. Supported by the UAB TJ Atchison Spinal Cord Injury Research Program.

Keywords: spinal cord injury, pain, model, behavior

B24-04

A BRIEF PERIOD OF NOCICEPTIVE STIMULATION AFTER SPINAL CORD INJURY CAN EXPAND THE REGION OF SECONDARY INJURY

Misty Strain-Malamakal, Yung-Jen Huang, Joshua Reynolds, Rachel Baine, Jacob Davis, Melissa Brumley, Gizelle Leal, Victoria Golub, James Grau

Texas A&M University, Institute for Neuroscience, San Antonio, USA

Spinal cord injuries are often accompanied by injuries to other parts of the body (polytrauma). These injuries can lead to the activation of pain fibers (C-fibers). Research has shown that the activation of C-fibers using electrical stimulation after a spinal cord injury hinders behavioral recovery and increases signs of hemorrhage and inflammation (Grau et al., 2014). From this work we know that stimulation at an intensity of 1.5 mA or greater, and for a duration of 6 minutes or more [for shocks given an average of 2 s apart (0.5 Hz)], induces a form of maladaptive plasticity. In the current study, we examine whether less intense, and briefer, periods of stimulation adversely affect recovery after a contusion injury. The present study examined whether less severe stimulation has an adverse effect and the generality of this finding across age and sex. Twenty-four hours after receiving a moderate spinal cord contusion at T12, Sprague Dawley rats received uncontrollable electrical stimulation at either a lower intensity (0, 0.17, 0.5 or 1.5 mA) or a shorter duration (0, 14.5s, 72, or 360s). Locomotor performance was assessed prior to and 3 hrs after stimulation. The tissue was then collected to examine the extent of hemorrhage. We found that relatively mild (0.5 mA) shocks acutely disrupted locomotor performance and increased the extent of hemorrhage (secondary injury). Likewise, just 72 s of shock impaired performance and increased secondary injury. The adverse effect of nociceptive stimulation on spinal function was greatest in older male rats. On-going experiments are examining whether these effects are regulated by behavioral control and the physiological factors that trigger increased hemorrhage. The work suggests that even brief nociceptive input after injury can augment the extent of injury and undermine long-term recovery.

Keywords: nociception, pain, spinal cord injury, hemorrhage, sex, age

B24-05

EXAMINING RELATIONSHIP BETWEEN PRE-INJURY PAIN AND FUNCTIONAL STATUS FOLLOWING TRAUMATIC BRAIN INJURY

Chieh Cheng¹, Nai-Ching Chi¹, Ellita Williams¹, **Hilaire Thompson**^{1,2}

¹*The University of Washington, Biobehavioral Nursing and Health Informatics, Seattle, USA*

²*Harborview Injury Prevention and Research Center, Seattle, USA*

Objectives: To assess if pre-injury pain is related to functional status and disability in persons following traumatic brain injury (TBI) and if this relationship differs based on age.

Methods: Observational cohort study of adults (N=34) with mild-moderate TBI who were enrolled from a level one trauma center. Pre-injury pain was self-reported using a 5 point Likert-type scale, with lower scores indicate more pain interference. Functional status was assessed at 3, 6, and 12-months post-injury using the Functional Status Examination (FSE). Relationship of pain to functional status was conducted using regression analyses.

Results: The sample included 19 younger (aged 21-64) and 15 older (65 and above) adults, 65% were male. There was no difference in mean baseline pain interference scores between younger (2.6 ± 1.5) and older adults (2.6 ± 1.6). For the full sample, more pain at baseline was associated with impaired overall function (as measured by the total FSE) at 3-, 6- and 12 months. In examining functional domains, home maintenance was more impaired in persons with pain across all time points examined while ability to perform personal care was effected only at 3 months, standard of living at 6 months, and social integration at 12 months post-injury. When separating by age groups to examine the relationship between baseline pain score and functional outcomes, baseline pain significantly influenced only younger adults when controlling for baseline MCS.

Conclusion: Pain prior to injury contributes to poorer functional status to one-year post injury in younger adults. The lack of association of baseline pain to functional status in older adults may be due, in part, to expectations of pain with aging or compensatory strategies learned prior to injury. Clinicians should include assessment of pain prior to injury in younger adults as this may be a potential area for attention during recovery to optimize function. Supported, in part, by NIH/NCRR KL2RR025015.

Keywords: functional status, quality of life, pain

B25 PEDIATRIC

B25-01

SEVERITY-ASSOCIATED GLIOVASCULAR CHANGES IN WHITE MATTER IN A NEW JUVENILE TBI MODEL

Jerome Badaut^{1,2}, Beatriz Rodriguez-Grande¹, Aleksandra Ichkova¹, Justine Aussudre¹, Elodie Barse¹, Bassem Hiba¹, Gwaenelle Catheline¹, Gregory Barriere¹, Andre Obenaus^{3,4,5}

¹CNRS, UMR 5287-Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

²Loma Linda University, Basic Science Department, Loma Linda, CA, USA

³Loma Linda University, Department of Pediatrics, Loma Linda, CA, USA

⁴UC Riverside, Center for Glial-Neuronal Interactions, Riverside, CA, USA

⁵UC Irvine, Department of Pediatrics, Irvine, CA, USA

Pediatrics mild-TBI is a leading cause of hospital visits and has been shown to lead to long-term disability. The underlying pathomechanisms are often assumed to depend on the type of injury and it is generally considered that increased severity would lead to increased pathology. We hypothesized that different severities could lead to different pathomechanisms, even in the same type of TBI. Therefore, we aimed to identify early severity-associated difference within the corpus callosum (CC). In a new jTBI mouse model, Closed Head Injury with Long-term Disorders (CHILD), a kinematic analysis showed a longer-lasting head acceleration and displacement

in grade-2 (G2) compared to grade-1 (G1) severity. T2 weighted-imaging (T2WI) showed a significant increased T2-values in G2 (suggesting edema) whereas decreased T2-values were observed in G1 at day1. Higher levels of the astrocyte water channel-AQP4 were observed in G1 compared to G2 mice in both in astrocyte end-feet and processes at day1. G2 mice displayed increased BBB breakdown evidenced by more IgG-extravasation staining compared to G1. Despite these acute differences between severities, one month after CHILD both G1 and G2 mice showed anxiety-like behavior in the open field test, whereas only G2 mice had decreased fractional anisotropy in the CC in DTI.

Mild-TBI (G1) exhibited a distinct acute gliovascular phenotype of damage that was unique in that it was not simply a milder form of the same pathology seen in a more severe impact (G2). Astrocytic AQP4, which had the highest expression in the milder phenotype of TBI, could be a key target for differential treatments based on severity.

Keywords: DTI, Behavior, aquaporin, closed head injury

B25-02

MODELING HEMISPHERIC HYPODENSITY: WIDESPREAD HYPOXIC-ISCHEMIC DAMAGE IN THE HEMISPHERE IPSILATERAL TO A SUBDURAL HEMATOMA

Beth Costine^{1,2}, Declan McGuone³, Eleanor Crawford¹, Jacqueline Andrews¹, Madeline Perlewitz¹, John Shen¹, Ann-Christine Duhaime^{1,2}

¹Massachusetts General Hospital, Neurosurgery, Boston, USA

²Harvard Medical School, Neurosurgery, Boston, USA

³Office of the Chief Medical Examiner, New York, USA

Hemispheric hypodensity (HH) is a striking injury pattern in young children typically associated with subdural hematoma (SDH) and is characterized by widespread hypodensity on CT scan spanning multiple vascular beds. In the unilateral form, the entire hemisphere or large patches of parenchyma underlying the SDH are damaged with relative sparing of the contralateral hemisphere. The objective of this study is to determine the pattern of tissue damage after abusive head trauma (AHT) focal injuries (cortical impact, mass effect, subdural hematoma, seizures) and global insults (apnea, hypoventilation, hypercapnia). Building upon our previous work in a model of HH, we used an anesthetic regimen that avoided the GABA_A receptor, induced seizures with kainic acid applied to the ipsilateral hemisphere, and lengthened survival to 24 hours. Areas of damage were identified by red neurons and/or perineuronal or perivascular vacuolization in serial sections spanning each hemisphere in male, 1 month old piglets receiving the AHT insult/injury (n=6) or sham surgery (n=2). In injured piglets, the percentage of tissue damage was greater in the ipsilateral vs. the contralateral hemisphere ($41.6 \pm 9.9\%$ vs. $11.2 \pm 4\%$; $P=0.017$) and greater than either hemisphere of sham piglets (ipsilateral: 1.1 ± 0.19 , contralateral 4.8 ± 4.2). A piglet with AHT insult/injury that only had a 5-minute seizure failed to display hemispheric tissue damage. In piglets receiving injuries, the proportion of the damaged hemisphere was positively correlated with SDH area ($P=0.03$). Hypoxic-ischemic injury encompassed approximately half of the hemisphere adjacent to the SDH including most of the cortical ribbon with relative sparing of the gyral white matter and deep gray matter. The extent of tissue damage may depend upon seizure duration and subdural blood. We will continue to use this model to investigate the pathophysiological cascades leading to hemispheric demise after AHT injuries, and, ultimately, to determine prevention strategies or interventions to limit damage.

Keywords: Hemispheric hypodensity, abusive head trauma

PROLONGED WHITE MATTER DEGENERATION IN A SUBSET OF PEDIATRIC TBI PATIENTS

Emily Dennis¹, Faisal Rashid¹, Monica Ellis², Talin Babikian², Julio Villalon-Reina¹, Roza Vlasova³, Yan Jin¹, Alexander Olsen⁴, Richard Mink⁵, Christopher Babbitt⁶, Jeffrey Johnson⁷, Christopher Giza⁸, Paul Thompson¹, Robert Asarnow²

¹USC Keck SOM, IGC, INI, Marina del Rey, USA

²Semel, UCLA, Psychiatry Biobehav Sciences, Los Angeles, USA

³CHLA, CIBORG Lab, Radiology, Los Angeles, USA

⁴NTNU, Psychology, Trondheim, Norway

⁵Harbor-UCLA Medical Center, Torrance, USA

⁶Miller Children's Hospital, Long Beach, USA

⁷LAC+USC Medical Center, Pediatrics, Los Angeles, USA

⁸Mattel Children's Hospital, UCLA, BIRC, Dept. Neurosurgery, Div. Pediatric Neurology, Los Angeles, USA

Post-injury outcome varies considerably in children who have sustained a moderate/severe traumatic brain injury (msTBI), with acute injury factors only accounting for some of this variance. In our study, we found that interhemispheric transfer time (IHTT), a measure of corpus callosum (CC) functional integrity, divided patients into two groups a few months post-injury. We studied 21 children (16M/5F) with msTBI, assessed 2-5 months and 13-19 months post-injury, and 20 well-matched healthy control children. CC function was assessed through IHTT, measured using event-related potentials, and white matter (WM) microstructural organization was modeled using diffusion-weighted magnetic resonance imaging (dMRI). Half of the TBI patients had significantly slower IHTT at the first time-point (TBI-slow-IHTT, N=11), and half were in the normal range (TBI-normal-IHTT, N=10). The TBI-normal-IHTT group did not differ significantly from healthy controls in WM organization and showed longitudinal improvements in WM organization. In contrast, the WM organization of the TBI-slow-IHTT group was significantly poorer than healthy controls, and longitudinal analyses showed a progressive decline in WM integrity throughout the brain from 2-5 to 13-19 months post-msTBI. These groups did not differ significantly in demographic or clinical variables. IHTT is a potential biomarker for identifying patients with continuing and progress WM degeneration, although the causative factor is unknown at present. Identifying patients at risk for poorer outcomes will help clinicians know which patients might benefit from targeted treatment.

Keywords: Longitudinal, Multimodal, moderate/severe TBI, dMRI, IHTT

B25-04

SEX DIFFERENCES FROM 20-HETE INHIBITION AFTER PEDIATRIC TRAUMATIC BRAIN INJURY IN IMMATURE RATS

Nagat El Demerdash, Shiyu Shu, Manda Saraswati, Ray Koehler, Courtney Robertson

John Hopkins, ACCU, Baltimore, USA

Introduction: Emerging evidence suggests that metabolites of the arachidonic acid pathway play important roles in brain signaling. Previous work has shown that inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) formation by cytochrome P450 (CYP) omega-hydroxylation of arachidonic acid can protect immature and mature brain from ischemia. However, the majority of studies have been performed in

adult, male animals. We tested the hypothesis that post-treatment with the 20-HETE synthesis inhibitor N-hydroxy-N-4-butyl-2-methylphenylformamidinium (HET0016) can be neuroprotective after pediatric TBI in both male and female rat pups.

Methods: Male and female Sprague Dawley rats (postnatal day 9-10) were subjected to controlled cortical impact (CCI; 3 mm impactor; velocity 5.5 m/s; depth 1.5 mm), and studied in 3 groups: 1) vehicle-treated sham, 2) vehicle-treated TBI, and 3) HET0016-treated TBI (1 mg/kg, ip, at 5 min and 3 h post-injury). At 30d after CCI, rats underwent neurologic testing (foot fault, novel object recognition/NOR), and percent tissue loss in the ipsilateral hemisphere was measured. Female rats had additional neurologic testing at 90d after CCI.

Results: In male rats, the number of contralateral hindlimb foot faults were reduced (TBI+veh=8.9±1.1; TBI+HET=4.2±0.9) and the discrimination index on NOR was improved (TBI+veh=0.37±0.08; TBI+HET=0.50±0.07) after treatment with HET0016. Also, HET0016 reduced the tissue loss after TBI in male rats (TBI+veh=18.2±0.8%; TBI+HET=8.8±0.9%). In contrast, there were no differences between groups in performance on foot fault or NOR testing in female rats, and HET0016 did not change the tissue loss in female rats.

Conclusions: The 20-HETE synthesis inhibitor HET0016 improved both histologic and neurologic outcome after pediatric TBI in male rats, but not in female rats. This suggests potentially important sex differences in the response to treatment after pediatric TBI. However, direct comparisons are limited due to different timepoints tested in male and female rats in this study. Future studies evaluating sex differences in response to 20-HETE inhibition will be conducted prospectively with identical outcome assessments.

Keywords: arachidonic acid, HET0016, 20-Hete

B25-05

THE IMPACT OF TREATMENT WITH THE PROBIOTIC, LACTOBACILLUS REUTERI, UPON NEUROPROTECTION AND NEUROINFLAMMATION FOLLOWING JTBI

Michael Hylin¹, Aidan Smith¹, Shawn Roller¹, Rebecca Key², Madison Cox³, Garret Suen³, Bethany Rader²

¹Southern Illinois University, Psychology, Carbondale, USA

²Southern Illinois University, Microbiology, Carbondale, USA

³University of Wisconsin - Madison, Bacteriology, Madison, USA

Heightened neuroinflammation and cell loss following juvenile traumatic brain injury (jTBI) frequently results in impaired cognitive function that can last into adulthood. Modification of the microbiome using dietary probiotics has been shown to impact behavior and inflammation. The probiotic bacterial strain *Lactobacillus reuteri* impacts the brain through modification of the gut-brain axis and has been associated with the attenuation of anxiety deficits. In the current study, *L. reuteri* was introduced starting immediately following jTBI at postnatal day 28 and continued daily for one month following injury. Subjects were tested in a battery of neurocognitive tasks over the next few weeks following injury. Although *L. reuteri* treated subjects didn't show improvement in the Morris water maze; they spent significantly more time with a novel object 24 hours following object recognition training suggesting improved object but not spatial long-term memory. Treated subjects spend an increased amount of time freezing compared to uninjured shams, suggesting a potential impact of *L. reuteri* upon contextual fear memory. Treatment with *L. reuteri* resulted in a significant reduction in lesion size and reduced number of IBA-1+ microglia, suggestive of increased neuroprotection and attenuated neuroinflammation following jTBI. Fecal samples were collected weekly following injury and throughout treatment to

examine changes in the gut microbiome. Using 16s sequence analysis, the trajectories of intestinal bacterial communities from injured and non-injured rats separate by injury status with the community trajectory being more similar between individuals receiving injury than shams. Several Operational Taxonomic Units, revealed differences in abundance in injured versus shams with most bacteria being *Firmicutes* specifically *Lachnospiraceae* and *Ruminococcaceae*. Taken together, these data show that TBI impacts the composition of the gut microbial community, and that introducing a probiotic *Lactobacillus* strain post-injury can impact recovery following jTBI.

Keywords: Juvenile, Microbiome, Probiotic, Inflammation, Cell Loss

B25-06

VALIDATION OF THE PILOT SCORE FOR PEDIATRIC TBI

Margaret Jackson, Jessica Yeary, Charles Cox, Jr.

UTHealth McGovern Medical School, Department of Pediatric Surgery, Houston, USA

The Pediatric Intensity Level of Therapy (PILOT) scale was published by Shore *et al.* in 2006 and has been adopted as a means of measuring ICU therapeutic intensity in pediatric TBI. However, it has never been validated in another institution. We performed an IRB-approved retrospective chart review examining patients admitted to the pediatric ICU from July 2015 to March 2016. Critical care patients were included in the study if they were admitted due to trauma and were not deemed futile care by neurosurgery on arrival. Patients were broken down into 3 groups depending on their injuries: (1) severe TBI, (2) mild/moderate TBI, (3) acute trauma but no TBI. Those patients who received hypertonic saline were also scored with a Revised PILOT score that gave additional points for daily peak sodium: 150-159 = 2 additional points; 160-169 = 4 additional points; ≥ 170 = 6 extra points. 44 patients were divided into 3 groups: severe TBI (16), mild/moderate TBI (18), and extracranial trauma (10). The mean PILOT score in the severe TBI group was 35.5 ± 20.4 , the mild/moderate group was 9.61 ± 9.04 , and the extra-cranial trauma group was 8.4 ± 7.05 . ANOVA showed that the severe TBI group was statistically different from each of the other groups ($p < 0.001$). Of 16 patients in the severe TBI group, 15 received hypertonic saline. No patient in the extracranial trauma group received hypertonic saline. Regression analysis shows that treatment with hypertonic saline increases the 7-day PILOT score by 33 points (95%CI 24-41, $p < 0.0001$) with an R^2 of 0.67. The Revised PILOT score was applied to those 15 patients who received hypertonic saline, and increased the 7-day score by 4.76 points ($p < 0.001$). This small cohort validates the original findings by Dr. Shore that the PILOT score successfully discriminates severe TBI patients from other trauma patients in the ICU. Additionally, our data show a ceiling effect in the original scoring, which is ameliorated by our revised score which takes into account the daily sodium values.

Keywords: Therapeutic Intensity, Clinical Scores

B26 REGENERATION AND PLASTICITY

B26-01

EFFECTS OF CONTINUOUS GABAPENTIN ADMINISTRATION ON THE INCIDENCE AND SEVERITY OF AUTONOMIC DYSREFLEXIA

Khalid Eldahan, David Cox, Samir Patel, Alexander Rabchevsky
University of Kentucky, Physiology/SCoBIRC, Lexington, USA

Spinal cord injury (SCI) can have profound effects on the autonomic and cardiovascular systems, notably with injuries above high-thoracic levels frequently resulting in the development of autonomic dysreflexia (AD) characterized by volatile hypertension in response to exaggerated sympathetic reflexes triggered by afferent stimulation below the injury level. We have reported that in rats with complete T4-transection SCI, the antiepileptic drug gabapentin (GBP) significantly reduced the magnitude of induced AD shortly after administration (50 mg/kg), but that once daily treatment did not significantly reduce the frequency of spontaneous AD events. Since daily GBP treatment is reported to prevent excitatory synapse formation by binding to the $\alpha_2\delta_1$ subunit of voltage-gated calcium channels, we investigated the effects of more prolonged and higher GBP dose administration on the incidence and severity of AD, notably in relation to plasticity within the circuitry underlying AD development. Female Wistar rats implanted with telemetric probes for continuous hemodynamic monitoring underwent T4-transection SCI and immediately received 100 mg/kg (i.p.) of GBP every six hours (400 mg/kg/day) for 4-weeks. Results show that this regimen reduces the frequency of daily spontaneous AD events, as well as the magnitude of weekly-induced AD. Such GBP dosages did not affect daily activity measurements and, unlike reports in the mouse AD model, spleen weights were unaltered weeks after T4 SCI, with or without treatment. Assessments of CGRP+ nociceptive afferent fiber sprouting and synapse formation (VGlut2, PSD-95) in the thoracolumbar spinal cord demonstrated established injury effects, but GBP amelioration of AD did not correlate with reduced measures of plasticity. We are increasing the power of these hemodynamic findings while refining our histological assessments to establish whether prolonged high-dose GBP administration can abrogate AD development, possibly by preventing maladaptive intraspinal plasticity. Funding: SCoBIRC Chair Endowment (AGR), 5T32 NS077889 (KCE).

Keywords: Autonomic Dysreflexia, Cardiovascular, Sprouting, Sympathetic

B26-02

DEVELOP A NOVEL STRATEGY TO ENHANCE AXON REGENERATION AND NEUROPLASTICITY AFTER CNS INJURY

Shuxin Li¹, Yosuke Ohtake¹, Lena Ma¹, Makoto Horiuchi¹, Jason Berke¹, Shin-ichi Muramatsu²

¹Temple University School of Medicine, Dept. of Neurology, Philadelphia, USA

²Jichi Medical University, Division of Neurology, Shimotsuke, Japan

Axons in adult mammalian CNS fail to regenerate and there are no effective regenerative strategies to treat patients with CNS injuries. Several genes, including PTEN/mTor, Kruppel-like factors and insulin/insulin-like growth factor 1 receptor, partly regulate axon regeneration failure in adult CNS of mammals. Here we report the crucial role of liver kinase B1 (LKB1) in mediating CNS regeneration after CNS injury. LKB1 is a key serine/threonine kinase required for maintaining cell metabolism, energy homeostasis and cell polarity by activating a number of kinases. As the downstream effector of cAMP/PKA and PI3 kinase pathways, LKB1 is a major determinant for migration and differentiation of various cells, including neurons. We thus evaluate the role of LKB1 in regulating regenerative capacity of neurons in adult mammals using transgenic and adeno-associated virus (AAV) vector approaches. Upregulation of LKB1 significantly enhanced neurite extension of adult neurons cultured on inhibitory substrates CNS myelin and aggrecan. Transgenic overexpression of LKB1 stimulates robust regeneration of corticospinal tract (CST) axons in adult mice with mid-thoracic spinal cord injury. Local injections of AAV2-LKB1 into

sensorimotor cortex or systemic application of mutant AAV9-LKB1 promote long distance regeneration of injured CST axons into the caudal spinal cord in adult mice. Systemic injection of mutant AAV9-LKB1 also enhances regrowth of descending serotonergic and tyrosine hydroxylase fibers. Importantly, LKB1 upregulation by either transgenic or viral approaches improves recovery of locomotor function after CNS injury. Therefore, LKB1 is critical for regulating growth capacity of mature neurons and may become an important target for designing highly effective therapies for CNS injury.

Keywords: Liver kinase B1, spinal cord injury, axon regeneration, Functional recovery, growth capacity

B26-03

IMMEDIATE AND DELAYED HYPERBARIC OXYGEN THERAPY AS A NEUROPROTECTIVE TREATMENT FOR TRAUMATIC BRAIN INJURY IN MICE

Renana Baratz-Goldstein¹, Shlomi Toussia-Cohen¹, Aviya Elpaz¹, Vardit Rubovitch¹, **Chaim Pick**^{1,2,3}

¹Sackler School of Medicine, Tel-Aviv University, Anatomy and Anthropology, Tel Aviv, Israel

²Sagol School of Neuroscience, Tel Aviv, Israel

³The Dr. Miriam and Sheldon G. Adelson Chair and Center for the Biology of Addictive Diseases, Tel Aviv, Israel

Traumatic brain injury is the most common cause of death and chronic disability among people under-35-years-old and without an effective pharmacological treatment currently existing. Hyperbaric oxygen therapy (HBOT) is defined as the inhalation of pure oxygen in a hyperbaric chamber that is pressurized greater than 1 atmosphere. HBOT offers physiological and mechanical effects by inducing a state of increased pressure and hyperoxia. HBOT has been proposed as an effective treatment for mTBI, yet the exact therapeutic window and mechanism that underlies this effect is not completely understood. HBOT was administered for 4 consecutive days, post a mouse closed head weight drop mild TBI (mTBI) in 2 different time lines: immediate-initiated 3 hours post injury and a delayed treatment - initiated 7 days post injury. Behavioral cognitive tests and biochemical changes were assessed. The results were similar for both the immediate and the delayed treatments. mTBI mice exhibited impairment in learning abilities, whereas mTBI mice treated with HBO displayed significant improvement compared with the mTBI group, performing similar to the sham groups. mTBI mice had a decline in the myelin basic protein, an increase in neuronal loss (NeuN staining), and an increase in the number of reactive astrocytes (GFAP). The HBO treated mice in both groups did not exhibit these changes and remained similar to the sham group. The delay HBOT has a potential to serve as a neuroprotective treatment for mTBI with a long therapeutic window. Further research is needed for fully understanding the cellular changes.

Keywords: hyperbaric oxygen, mice, neuroprotection, initiated and a delayed treatments

B26-04

TRANSCRANIAL DIRECT CURRENT STIMULATION FACILITATES WORKING MEMORY AFTER MILD-MODERATE TRAUMATIC BRAIN INJURY

Davin Quinn

University of New Mexico, Center for Brain Recovery and Repair, Albuquerque, USA

Objectives: Working memory (WM) deficits after mild-moderate traumatic brain injury (mmTBI) result in significant disability, yet there are few effective treatments. One intervention that may improve WM by modulating neuroplasticity is transcranial direct current stimulation (tDCS). The authors hypothesized that active tDCS to left dorsolateral prefrontal cortex (DLPFC) paired with cognitive training in patients with mmTBI would result in greater improvement in WM reaction time (RT) and accuracy compared to sham tDCS.

Methods: Thirteen patients with mmTBI within 5 years and cognitive post-concussive symptoms (PCS) were recruited (7M, 6F, mean age=40.6 yrs, SD=12.6). All subjects received 10 daily cognitive training sessions over 2 weeks, consisting of 10 minutes of a continuous performance task and 20 minutes of a dual auditory-visual WM n-back task. Active (n=6) or sham tDCS (n=7) was administered for 30 minutes during training through 5 cm × 5 cm saline-soaked sponges placed at F3 (anode) and right deltoid (cathode). Active tDCS was delivered at 2.0 mA while sham consisted of 1 min of 2.0 mA followed by intermittent 0.2 mA for 29 minutes. Hit rate and RT for auditory, visual, and dual stimuli in the 2-back condition were calculated for each session and averaged across subjects within each stimulation group.

Results: There were no significant differences in session 1 between active and sham in accuracy and RT on the 2-back task ($p > 0.5$). By session 10, the active group performed significantly better in visual RT compared to sham ($t(11)=3.5008$; $p=0.005$). In all other variables, the active group outperformed the sham group at session 10 but these differences did not reach significance ($p > 0.17$).

Discussion: In this preliminary study, anodal DLPFC tDCS paired with cognitive training in mmTBI patients led to significant improvements in visual WM RT compared to sham, and may represent an efficacious treatment for chronic cognitive PCS. Further analysis with a larger sample size is expected to reveal more significant WM effects.

Keywords: brain stimulation, postconcussive symptoms, executive function, transcranial direct current stimulation

B26-05

IN VIVO REPROGRAMMING OF NG2 GLIA INTO FUNCTIONAL NEURONS IN THE INJURED SPINAL CORD

Wenjiao Tai^{1,2}, Lei-Lei Wang^{1,2}, Chun-Li Zhang^{1,2}

¹University of Texas Southwestern Medical Center, Molecular Biology, Dallas, USA

²University of Texas Southwestern Medical Center, Hamon Center for Regenerative Science and Medicine, Dallas, USA

Spinal cord injury (SCI) results in irreversible loss of neurons and disruption of neural circuits, with a consequence of motor and sensory dysfunction. The goal of the present study is to regenerate functional neurons after SCI. A key SCI-induced pathological feature is the formation of glial scars surrounding the injury site. NG2 glia constitute a major component of the glial scars and their presence at the injury sites is inhibitory for axon regrowth. Reprogramming these cells into functional neurons may provide a regeneration-based therapeutic strategy for SCI repair. We established a lentiviral approach to specifically target endogenous NG2 glia by using a human NG2 promoter. SCI was introduced by contusion in the adult mice. Our preliminary data showed that neurogenesis can be induced through injection of SOX2-expressing lentivirus under this promoter in both healthy and injured spinal cord. Immunohistochemistry revealed that induced DCX+ new neurons pass through an ASCL1+ neural progenitor and proliferation phase. Through genetic lineage mappings, we confirmed parenchymal NG2 glia as a cellular source for the newly reprogrammed neurons. We also excluded nestin+ neural stem cells or FOXJ1+ ependymal cells along the central canal as a cellular origin. Importantly, these NG2 glia-derived new neuron

can mature into synapse-forming cells *in vivo*. Neuronal maturation can be further promoted by neurotrophic factors. Our results demonstrate that reactive NG2 glia can be robustly reprogrammed into neurons *in situ*, opening up the possibility of using endogenous cells for spinal cord regeneration after injury.

Keywords: *in vivo* reprogramming, neurogenesis, spinal cord injury, NG2 glia

B26-06

SELECTIVE LOSS OF GASTRIN RELEASING PEPTIDE IN SPINAL EJACULATION GENERATOR FOLLOWING CHRONIC SPINAL CORD INJURY IN RATS

James Wiggins, George Wilson, Lique Coolen

University of Mississippi Medical Center, Department of Physiology, Jackson, MS, USA

Chronic spinal cord injury (SCI) causes ejaculatory dysfunction in men. Ejaculation is a reflex mediated by a spinal ejaculation generator in the lumbosacral spinal cord. A principle component of this generator is a neuronal population located in spinal levels L3-4 and named for their projections to the thalamus (lumbar spinothalamic cells: LSt cells). In rat, LSt cells co-express the neuropeptides galanin, gastrin releasing peptide (GRP), enkephalin, and cholecystokinin. LSt cells integrate sensory inputs during sexual activity to coordinate autonomic and motor outputs required for ejaculation, via interspinal connections and the release of neuropeptides onto spinal target neurons. We recently showed that SCI caused long term changes in the rat spinal ejaculation generator. Specifically, contusion injury ablated ejaculatory reflexes triggered by sensory stimulation, 6 weeks after injury. Moreover, these effects were independent of supraspinal influences, indicating long term changes within the spinal ejaculation generator itself. Here, we tested the hypothesis that SCI caused long term reduction in the expression of GRP and galanin in LSt cells, thereby disrupting ejaculatory reflexes. Male Sprague Dawley rats received either a contusion injury at spinal levels T6-7 or sham surgery. Six weeks following contusion or sham injury, animals were perfused with paraformaldehyde and spinal cords were immunohistochemically processed for galanin and GRP. Quantitative analysis of numbers of cells single or double labeled for galanin and GRP showed that SCI did not cause a significant reduction in galanin-immunoreactive cells. In contrast, SCI significantly reduced labeling for GRP, and fewer galanin-labeled cells co-localized GRP. These results demonstrate that SCI did not reduce the numbers of LSt cells per se, nor the localization of galanin in LSt cells. However, SCI resulted in a selective loss of GRP in LSt cells. Since our prior studies have shown GRP to be a powerful facilitative neuropeptide for control of ejaculation, such selective loss could contribute to the disruption of ejaculation seen in male SCI rats and human patients.

Keywords: Ejaculation, Gastrin Releasing Peptide, Sexual Reflexes, Spinal Cord Injury, Lumbar Spinal Cord, Urogenital

B27 SECONDARY INJURY

B27-01

THE SPLEEN IS AN IMPORTANT SITE OF MESENCHYMAL STROMAL CELL-MEDIATED IMMUNOMODULATION FOLLOWING TRAUMATIC SPINAL CORD INJURY

Anna Badner^{1,2}, Justin Hacker², James Hong^{1,2}, Mirriam Mikhail², Michael Fehlings^{1,2}

¹*University of Toronto, Institute of Medical Science, Toronto, Canada*

²*Krembil Research Institute, Genetics and Development, Toronto, Canada*

The spleen plays an important role in erythrocyte turnover, adaptive immunity, antibody production and, more recently revealed, the mobilization of monocytes/macrophages (M ϕ) following tissue injury. In response to trauma, the spleen initiates production of inflammatory cytokines, by resident M ϕ , which in turn influences the recruitment of immune cells to the inflamed tissue and exacerbates damage. Our previous work has shown that intravenous mesenchymal stromal cell (MSC) infusion has potent immunomodulatory effects following traumatic spinal cord injury (SCI), associated with the transplanted cells homing to and persisting within the spleen. Therefore, this work aimed to characterize the relationship between the splenic inflammatory response and SCI pathophysiology, with an emphasis on splenic involvement in the therapeutic use of MSCs post-SCI. A rodent model of SCI (C7-T1, 35gram clip) was used to compare secondary tissue damage and 8-week functional recovery (including grip strength, inclined plane and BBB locomotor rating) between splenectomized rodents versus those with a sham procedure. Subsequently, 2.5 million MSCs derived from the term human umbilical cord matrix (HUCMCs) were infused via tail vein at 1 hour post-SCI and immunomodulatory effects assessed in the presence or absence of a spleen. Splenectomy alone had no effect on acute spinal cord lesion volume, hemorrhage or inflammation. There was also no significant difference between the groups in functional recovery and 8-week lesion morphometry. Yet, while the infusion of HUCMCs reduced spinal cord hemorrhage and lesion volume in the presence of a spleen, these effects were lost with splenectomy. The HUCMC-mediated rise in systemic levels of *interleukin-10* was also absent in splenectomized rodents, suggesting that the spleen is an important target and site of MSC effects post-SCI. Altogether, our results provide a link between MSC function and splenic inflammation, a finding that can help tailor the cells/transplantation approach to enhance therapeutic efficacy and clinical deployment.

Keywords: Mesenchymal Stromal Cells, Spleen, IL-10, Spinal Cord Injury

B27-02

DIFFERENTIAL CSF CYTOKINE PROFILE OF PATIENTS WITH POST-TRAUMATIC HYDROCEPHALUS

Jordan Brooks, Christopher Deibert, David Okonkwo, Xiaoran Zhang, Ava Puccio

University of Pittsburgh, Neurological Surgery, Pittsburgh, USA

Post-traumatic hydrocephalus (PTH) is a secondary neurological insult resulting in the derangement of cerebrospinal fluid (CSF) dynamics ensuing moderate to severe traumatic brain injury (sTBI). Given the high risk of clinical deterioration and documented worse outcomes, the identification of biomarkers indicating the onset of PTH is imperative to allow early clinical detection, and improve neurological outcomes in afflicted patients. This study examined CSF cytokine profile with PTH to elucidate the pathogenesis and aid in the early diagnosis of PTH. We conducted a matched case-control study on 50 patients who sustained a sTBI at a level 1 Trauma facility from 2002-2015. All patients were treated with five days of continuous CSF drainage via an extraventricular drain. CSF research samples were collected on post-trauma days 1, 3 and 5. Patients who incurred CNS infection or died within 6 months were excluded. 25 patients who incurred PTH were matched by age, sex, and initial Glasgow Coma Scale with 25 patients who did not incur PTH. The CSF concentrations of 36 different inflammatory markers were analyzed via a Luminex Array Scanner. There were no PTH differences detected between the groups in CSF RBC, WBC. Across all time points, IL-15 ($p=0.007$), IL-5 ($p=0.038$) and CX3CL1 ($p=0.031$) were significantly lower among PTH patients. CCL4 was significantly higher in the PTH group ($p=0.029$). IL-2 levels increased at a significantly

slower rate in patients with PTH ($p=0.037$). No other statistically significant differences were found in any other of the cytokines assayed. Overall, our data suggests potential differences in the immune responses in patients who develop PTH, which may impede the clearing of debris following sTBI. Lower levels of IL-15 and IL-5 suggest the decreased recruitment and proliferation of natural killer cells, T-cells, B-cells, and eosinophils in PTH patients. High levels of CCL4 may indicate a more macrophage rich environment. A slower increase in IL-2 may be indicative of a global impairment of immune function, particularly T-cell function and B-cell differentiation.

Keywords: Post Traumatic Hydrocephalus, Cerebrospinal Fluid, Biomarkers, severe TBI

B27-03

CHRONIC UPREGULATION OF CLEAVED-CASPASE-3 AND MYELIN PATHOLOGY IN THE THALAMUS AFTER TRAUMATIC BRAIN INJURY IN RATS

Andriy Glushakov¹, Olena Glushakova², Tetyana Korol³, Cesar Borlongan¹, Alex Valadka², Ronald Hayes^{2,4}, Alexander Glushakov⁵

¹University of South Florida College of Medicine, Neurosurgery, Tampa, USA

²Virginia Commonwealth University, Neurosurgery, Richmond, USA

³Uppsala University, Uppsala, Sweden

⁴Banyan Biomarkers, Inc., Alachua, USA

⁵Single Breath, Inc., Gainesville, USA

Traumatic brain injury (TBI) is associated with long-term disabilities and devastating chronic neurological complications including problems with cognition, motor function, sensory processing as well as behavior and mental health including depression, anxiety, personality changes and social unsuitability. Clinical data suggests that disruption of corticothalamic and thalamocortical networks and acute and chronic anatomical and metabolic changes in the thalamus following TBI might be responsible for chronic neurological deficits and altered brain function. The detailed mechanism of the pathological processes is not completely understood. The goal of this study is to evaluate changes following TBI focusing on cleaved-caspase-3, a specific effector of caspase pathway activation, and myelin and microvascular pathologies using immuno- and histochemistry at different time points from 24 hours to 3 months after controlled cortical impact (CCI) in rats. The results of the study for the first time demonstrate a significant chronic upregulation of cleaved-caspase-3 in selected thalamic regions associated with cortical regions affected by CCI injury at the chronic stages of TBI. The significant increases of cleaved-caspase-3 immunoreactivity in the thalamus were observed starting one month and persisting at least for three months following experimental TBI. Further, the study demonstrated the association of the cleaved-caspase-3 with the demyelination of the neuronal processes and the tissue degeneration in the gray matter of thalamus. However the immunofluorescent experiments did not reveal direct association of cleaved caspase-3 with blood brain barrier damage. In conclusion, this study provides new insights into potential mechanisms involving corticothalamic and thalamocortical networks in the etiopathology of chronic neurological disorders associated with TBI. Moreover, this study for the first time suggests that this upregulation of activated caspase-3 and delayed degeneration of myelinated neurons and nerve fibers in the thalamus might be representative of reciprocal pathological processes affecting neuronal connectivity and overall brain function at the chronic stages of TBI.

Keywords: chronic TBI, cleaved-caspase-3, thalamus, myelin pathology

B27-04

EXPRESSION AND ROLE OF THE PRO-INFLAMMATORY CHEMOKINE CCL3 AND ITS RECEPTORS AFTER SPINAL CORD INJURY (SCI) AND ITS INFLUENCE ON SECONDARY DAMAGE

Antje Kroner-Milsch

Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

Spinal cord injury (SCI) is a very severe condition which has far-reaching effects on health and quality of life. While the primary tissue damage after SCI occurs from the trauma itself, secondary damage is caused by a cascade of subsequent events, including hemorrhage and inflammation. It is well established that this secondary reaction contributes significantly to the pathology and thereby to the severity of the functional deficits. Inflammation after SCI is exacerbated and prolonged. The main objective of my research is to investigate and modulate the inflammatory response after SCI in order to minimize tissue damage and to promote an environment that is permissive for healing and repair.

The pro-inflammatory chemokine CCL3 plays an important role in various pathological conditions of the nervous system by initiating and maintaining the pro-inflammatory response. Conditions in which CCL3 has a negative impact include neuropathic pain, autoimmune neuropathy and Multiple Sclerosis and its model Experimental Autoimmune Encephalomyelitis.

This project is aimed at investigating and dissecting the roles of CCL3 and its receptors after SCI in C57BL/6 mice, using a model of moderate contusion injury. Preliminary results suggest that CCL3 mRNA is upregulated early and peaks at 6 hours after SCI. It stays elevated for at least 14 days after injury. The main receptor for CCL3, CCR1, is also upregulated after SCI, as is another receptor (CCR5). CCR4, in contrast, is not changed in response to SCI.

Furthermore, CCL3 knockout mice showed improved functional recovery compared to wild type mice, using the BMS score for locomotor recovery. Ongoing and future experiments will confirm expression patterns and behavioral results and assess the impact of CCL3 neutralization after SCI. Our preliminary data suggest a potential role of CCL3 in SCI.

Funding: Wings for Life.

Keywords: Chemokine, CCL3, microglia, macrophage

B27-05

DISRUPTION OF NNOS-PSD95 INTERACTION AS A NEUROPROTECTIVE STRATEGY FOR TRAUMATIC BRAIN INJURY

Wenrui Qu¹, **Nai-Kui Liu**¹, Xiangbing Wu¹, Ying Wang¹, Yongzhi Xia¹, Yan Sun¹, Yvonne Lai², Anantha Shekhar³, Xiao-Ming Xu¹

¹IUSM, Neurological Surgery, Indianapolis, USA

²IU, Psychological and Brain Sciences, Bloomington, USA

³IUSM, Psychiatry, Indianapolis, USA

Excessive activation of N-methyl-D-aspartate receptors (NMDARs) and the resulting neuronal nitric oxide synthase (nNOS) activation have been shown to play a crucial role in the pathogenesis of traumatic brain injury (TBI). However, directly inhibiting NMDARs or nNOS produces adverse side effects because these substances have key physiological functions in the central nervous system. Interaction of nNOS-PSD95 is a key step in NMDAR-mediated excitotoxicity. In this study, we examined whether ZL006, a small-molecule that disrupts the nNOS-PSD95 interaction, would be neuroprotective in *in vitro* cortical neuronal cultures and an *in vivo* mouse model of controlled cortical impact (CCI). *In vitro*, ZL006 treatment (0.1, 1.0,

and 10 μ M) significantly reduced glutamate-induced neuronal death in a dose-dependent manner as measured by propidium iodide staining and lactate dehydrogenase release assays. *In vivo*, administration of ZL006 (10 mg/kg) at 30 min post-injury in mice significantly inhibited nNOS-PSD95 interaction in the cortex as compared with the vehicle-treated control. Western blot analysis showed that ZL006 treatment significantly reduced CCI-induced expression of apoptotic markers of active caspase-3 and PARP-1. Additionally, administration of ZL006 at 30 min post-injury followed by daily treatment of ZL006 for 7 days significantly improved neuroscores and sensorimotor performance on a rotarod device after mouse CCI. An adhesive removal test showed significant reduction of somatosensory and motor deficits after CCI and ZL006 treatment. Morris water maze test showed that ZL006 significantly reversed CCI-induced memory deficits. Administration of ZL006 also attenuated cognitive impairment in the direct contact social test and the social activity and novelty test. Histological analysis showed a reduction of brain lesion volume in the ZL006-treated group as compared to the control group. These findings collectively suggest that the glutamate-NMDAR-PSD95-nNOS cascade may mediate secondary TBI and that blocking nNOS-PSD95 interaction may represent an attractive strategy for ameliorating TBI.

Keywords: TBI, nNOS-PSD95 interaction, Neuroprotection, Apoptosis

B27-06

BIDIRECTIONAL BRAIN-GUT INTERACTIONS AFTER CHRONIC TRAUMATIC BRAIN INJURY IN MICE

Elise Ma¹, Allen Smith², Neemesh Desai¹, Lumei Cheung², Marie Hanscom¹, Bogdan Stoica¹, David Loane¹, Terez Shea-Donohue¹, Alan Faden¹

¹University of Maryland School of Medicine, Baltimore, USA

²United States Department of Agriculture, Beltsville, USA

Traumatic brain injury (TBI) has complex effects on the gastrointestinal tract that are associated with TBI-related mortality and morbidities, including susceptibility to peripheral infections and septicemia. Although human and animal studies have established a link between TBI and intestinal dysfunction during acute stages after injury, long-term consequences of TBI in the gut are vastly unknown. We examined changes in mucosal properties and enteric glial cells in the gut after experimental TBI in mice, as well as effects of *Citrobacter rodentium* (Cr)—an enteric murine analog of pathogenic human *E. coli*—on both gut and brain after chronic TBI. Moderate-level TBI in adult male C57BL/6 mice was induced by controlled cortical impact (CCI; 6 m/sec, 2 mm depth). CCI led to delayed, chronic changes in colon morphology, including increased mucosal depth and smooth muscle thickening. At day 28 post-CCI, increased paracellular mucosal permeability associated with decreased claudin-1 mRNA and protein expression was observed in the absence of inflammation in the colon. Activated enteric glial cells (Sox10+/GFAP+) in the colon were significantly increased 28 days after CCI. When challenged with a Cr infection at 28 days post-CCI, host Th1/Th17 immune response in the colon and clearance of Cr were unaffected by CCI; however, colonic paracellular flux and enteric glial cell reactivity were significantly increased. Importantly, Cr infection in chronically-injured mice worsened TBI neuropathology, as measured stereologically by lesion volume, and increased neuroinflammatory responses (GFAP+ astrocytes, CD68+ microglia/macrophages) in the cortex. These enteric microbial-induced changes in the gut and brain were associated with increased circulating IP-10 levels and decreased serum miR-223 expression. This work demonstrates long-term consequences of TBI on the brain-gut axis, and the impact of gut dysfunction on resolution of chronic brain injury. Future studies aim to

elucidate centrally- and intestinally-derived mechanisms of brain-gut modulation during injury and immune responses after chronic TBI.

T32DK067872

Keywords: brain-gut axis, intestinal mucosal barrier, enteric glial cells, *Citrobacter rodentium*, serum biomarkers

B27-07

EFFECTS OF TBI ON MICRORNA ASSOCIATION WITH MITOCHONDRIA AND INTERVENTION USING A NOVEL NANOPARTICLE MIRNA DELIVERY STRATEGY

Wang-Xia Wang¹, Hemendra Vekariab^{2,3}, Malinda Spry^{2,3}, Amber Cloud^{2,3}, Seth Batten⁴, Joshua Beckmann⁴, Patrick G. Sullivan^{2,3}, Joe E. Springer^{2,3}

¹University of Kentucky, Sanders Brown Center on Aging, Lexington, USA

²University of Kentucky, Spinal Cord and Brain Injury Research Center, Lexington, USA

³University of Kentucky, Neuroscience, Lexington, USA

⁴University of Kentucky, Psychology, Lexington, USA

Traumatic brain injury (TBI) is a leading cause of long-term impairments in higher cognitive function. Ongoing destructive secondary injury events occur minutes to days after the initial insult characterized by a cascade of pervasive biochemical and pathophysiological stressors. A rapid and sustained phase of mitochondrial dysfunction after TBI impacts a number of important cellular events. We demonstrated previously that specific hippocampal mitochondria-associated microRNAs (miRNAs) are altered following a controlled cortical impact (CCI) injury in rats. Our new studies reveal that the levels of several mitochondria-associated miRNAs (e.g. miR-142-3p, miR-142-5p, miR-146a, miR-155 and miR-223) are altered early on (3-24 hr), but start to normalize by 72 hr after TBI. Knowledge of the temporal changes in mitochondria associated miRNA levels after TBI provides an opportunity to target specific miRNA at specific time points. We have previously shown that miR-107 is significantly downregulated in hippocampal neurons at 24 hr following TBI. In the present study, we used a 21 amino acid peptide-based nanoparticle approach to test whether delivery of miR-107 mimic alters hippocampal levels of miR-107 and two miR-107 validated targets, granulin and beta secretase 1 (BACE1). At 48 hr following stereotaxic injections of peptide+miR-107 into the dorsal hippocampus, miR-107 level increased by 150% while granulin/BACE1 mRNA levels were downregulated by 20–40% depending upon the peptide+miR-107 ratio/concentration. The outcomes of this study suggest a temporally dynamic interaction of miRNA with mitochondria, and a potential role for mitochondria in dictating miRNAs' cellular function. The study also demonstrates the use of a peptide-based nanoparticle approach to effectively deliver miRNA mimics or inhibitors as a way to target specific miRNA activities following TBI.

Keywords: microRNA, mitochondria, traumatic brain injury, peptide-based nanoparticle, miR-146a, miR-155

B27-08

THE ATTENUATING EFFECTS OF A MAGNESIUM TREATMENT AFTER EXPERIMENTAL TBI ON PROPERTIES OF ANXIETY

Jennica Young, Marty Price, Eric Jacobs, Michael Hoane
Southern Illinois University, Department of Psychology: Brain and Cognitive Sciences, Carbondale, USA

The purpose of this study is to investigate an animal model of distress (conditioned suppression) to assess the effects of magnesium (MAG) on recovery of anxiety-like behavior following traumatic brain injury (TBI) in rats. TBI is a national health concern where victims experience cognitive impairments, such as post-traumatic stress disorder (PTSD). PTSD affects a TBI victim's ability to maintain daily activities and integrate back into society. MAG administered immediately after TBI may alleviate anxiety symptoms, helping the patient adjust to life after injury as MAD decreases brain swelling. An established method of conditioned suppression (CS), that generates fear responses in animals, will evaluate this intervention.

This study will compare the acquisition and extinction of CS in 3 groups of 10; sham-control, TBI/placebo, and TBI/MAG. Rats will be trained to lever-press for food to establish constant press-rates. This phase consists of 30-m sessions where lever-pressing is maintained by a variable-interval schedule of 30-sec. After a constant press-rate is reached, rats will receive a severe frontal cortex injury. MAG i.p. injections begin 4-hours after injury, then again at 24-hours, and at 72-hours. Post-injury testing consists of ten 30-m classical conditioning sessions where four 30-second tones will be presented and conclude with delivery of a shock (0.5-mA for 0.5-sec). Pairing tone with shock establishes the tone as a stimulus that elicits fear. Fear is measured by the extent to which lever-pressing is disrupted during the tone. Complete CS is achieved when lever-pressing during the tone decreases across successive conditioning trials, until the rat no-longer presses. The final phase presents the tone without the shock (extinction). Sessions will last 30-minutes with four presentations of a 30-sec tone. Extinction sessions conclude when rats have returned to baseline pressing.

Combination of magnesium is hypothesized to result in quicker extinction of lever-suppression than if one or no-intervention is given. Indicating, the treatment may be helpful as an intervention for decreasing PTSD symptoms after TBI.

Keywords: TBI

B28 STEM CELLS

B28-01

A COMBINATORIAL TREATMENT STRATEGY FOR REDUCING ASTROCYTE REACTIVITY USING NEURAL PROGENITOR CELLS AND HEPATOCYTE GROWTH FACTOR

Rachel Dragas^{1,2}, James Hong^{1,2}, Ahad Siddiqui, Michael Fehlings^{1,2,3}

¹Krembil Research Institute, Toronto Western Hospital, Genetics and Development, Toronto, Canada

²University of Toronto, Institute of Medical Science, Toronto, Canada

³University of Toronto, Department of Neurosurgery, Toronto, Canada

Reactive astrogliosis is a defining hallmark of secondary pathology following spinal cord injury (SCI), posing a significant challenge to endogenous reparative processes and stem-cell mediated regeneration. While neural progenitor cells (NPCs) are widely recognized for cell replacement and trophic support after injury, concurrent modification of the glial scar may further support tissue repair. Hepatocyte growth factor (HGF) is a potent multifunctional cytokine involved in cell and tissue regeneration that may synergistically complement NPC-mediated repair. The primary objectives of this study are to assess the effect of HGF on NPC cytokine secretion and the resultant combinatorial effect on astrocyte reactivity after injury. RNA-seq was used to quantify HGF expression after C6 SCI (23g 1 min clip compression) 3-56 days post-injury. Rat spinal cord-derived NPCs were pre-

treated with HGF for 4 days and conditioned media (CM) was characterized via Proteome Profiler cytokine array. Primary rat astrocytes were activated using TGF β 1 and cells were then washed and treated with either HGF protein (10-50 ng/mL), NPC-CM, or HGF-treated NPC-CM. Astrocytic GFAP, Vimentin, CSPG, and TGF β 1 production were assessed via Western blot and immunocytochemistry. RNA-seq revealed no significant change in endogenous HGF expression up to 56 days after SCI. HGF-treated NPCs demonstrated a decrease in IL-1 α secretion and an increase in TIMP-1 compared to untreated NPCs. Combinatorial HGF+NPC-CM treatment demonstrated a significant reduction in astrocytic GFAP, Vimentin, CSPG, and TGF β 1 production compared to HGF or NPC-CM alone. For the first time, we have shown reduced astrocyte reactivity with HGF+NPC-CM combinatorial treatment. This effect may be mediated via inhibition of the TGF β signaling pathway. As glial scarring remains a fundamental challenge to endogenous and stem cell mediated-regeneration after injury, combinatory treatment paradigms that strategically modify the scar and promote tissue repair should be further explored to increase therapeutic efficacy.

Keywords: neural stem cells, growth factors, astrogliosis, spinal cord injury

B28-02

ASSESSING THE REGENERATIVE POTENTIAL OF ADULT HUMAN SPINAL CORD NEURAL STEM/PROGENITOR CELLS

Ahmad Galuta¹, Krystal Walker², Catherine Smith², Diana Ghinda², Suzan Chen², Eve Tsai^{1,2}

¹University of Ottawa, Medicine, Ottawa, Canada

²Ottawa Hospital Research Institute, Ottawa, Canada

Background: Spinal cord (SC) injury is a debilitating disease during which pathophysiological mechanisms recruit endogenous neural stem and progenitor cells (NSPCs), but they fail to restore functionality of the SC. These NSPCs can be modulated towards beneficial fates to promote neurological recovery in animal models. However, it remains unclear how efficiently human SC NSPCs can be modulated towards similarly beneficial fates.

Methods: Using an in vitro assay, primary- and secondary-derived NSPCs (pd- and sdNSPCs) from adult human and rat thoracic SC were assessed identically for their proliferation and differentiation profile with 1 μ S, and their regenerative potential with the administration of exogenous factors in serum free media: RA, BMP4, or PDGF-AA to induce neural, astrocytic and oligodendrocytic fates, respectively. NSPCs had BrdU administered 24 hours prior to fixing, and cultures were characterized after seven or 14 days for differentiation (β -III tubulin, GFAP, O4), stemness (Sox2, Nestin), proliferation (BrdU), and death (TUNEL) using immunocytochemistry. NSPCs were then visualized by fluorescence microscopy and quantified as a percentage of immune-positive cells.

Results: Rat (n=3) pdNSPCs generated mostly astrocytes (71.8 \pm 5.6%) and to a lesser extent neurons (15.2 \pm 4.2%) and oligodendrocytes (2.82 \pm 1.3%) in 1 μ S, while human pdNSPCs (n=3) chiefly differentiated into neurons (68.5 \pm 16.9%) with little (<2%) gliogenesis. Similarly, human sdNSPCs (n=6) differentiated mostly into neurons (57.9 \pm 14.6%), which could be enhanced with RA treatment (2.2 \pm 0.8 for pdNSPCs and 2.1 \pm 0.8 for sdNSPCs). Finally, rat pdNSPCs proliferated at a greater rate than human pdNSPCs (2.3 \pm 0.8), but no differences in human and rat sdNSPC proliferation.

Conclusion: Our studies are the first to directly compare human and rat pd- and sd-NSPCs using the same culture conditions. Human NSPCs possess distinct proliferation qualities, tend to differentiate

more into neurons and respond differently to exogenous factor stimulation than rat NSPCs. This information is essential to successfully translate therapeutic strategies based on rat NSPC studies to humans.

Keywords: Neural stem cells, Adult human spinal cord, Proliferation / Differentiation, in vitro

B28-03

NEURONAL PRIMING MESENCHYMAL STEM CELL IMPROVES OUTCOMES OF ACUTE SPINAL CORD INJURY THROUGH THE MODULATION OF GLIOSIS

Choonghyo Kim^{1,3}, Byungju Park¹, Seung Tae Lee², Seungjin Lee^{1,3}, Jiha Kim^{1,3}, Eunhye Lee¹, Hyun Lee²

¹Kangwon national university hospital, Neurosurgery, Chuncheon, Korea South

²Kangwon national university, Department of animal biotechnology, Chuncheon, Korea South

³kangwon national university, Neurosurgery, Chuncheon, Koera South

Introduction: The mesenchymal stem cell(MSC) have a lot of advantages for human application. However MSC have a variation of cell quality, heterogeneity of cell characteristics, uneven reactivity, and sometime also unproven safety. We previously published the method for more homogenous neuronal differentiated MSC(NP-MSC) from short time incubation. Here we presented that transplantation of NP-MSC improve the outcome of rat acute spinal cord injury.

Methods: NP-MSC was prepared with hydrogel coated plate as prescribed previously. As for the comparison of behavioral outcome, twenty five rats were anesthetized and exposed to severe SCI using the clipping compression technique. NP-MSC was transplanted at 5mm above epicenter of acute injured spinal cord through the stereotactic microinfusor. Functional outcome was analyzed serially with the Basso, Beattie and Bresnahan (BBB) scale at postoperative one day and weekly for four weeks. Histopathologic analysis was undertaken at 28 days following injury. Hemaotylin-eosin and neurofilament double staining were performed for checking white mater sparing. The Cavalieri method was used to determine the relative mean percentage of spared white matter. NP-MSC survival was checked at postoperative one week, two weeks and four weeks after transplantation.

Results: Gliosis increased in NP-MSC transplanted group at 7day post SCI. After 21 days post SCI, statistically significant differences were observed in the BBB score of NP-MSC transplanted group versus control. NP-MSC transplantation increased white mater sparing at 28days post SCI. However survival of NP-MSC was decreased compared to other study.

Conclusions: NP-MSC transplantation promotes functional recovery of acute spinal cord injury of rat through modulation of acute glial response. However additional treatment for acute hostile environment should be considered for overcoming poor survival of NP-MSC.

Keywords: mesenchymal stem cell, Spinal cord injury, white mater sparing, gliosis

B28-04

MESENCHYMAL STEMS CELLS TO TREAT NEUROPATHIC PAIN IN SPINAL CORD INJURY

Katherine Ruppert¹, Daniel Kota¹, Karthik Prabhakara¹, Raymond Grill², Scott Olson¹

¹University of Texas Health Science Center Houston, Pediatric Surgery, Houston, USA

²University of Mississippi Medical Center, Neurobiology & Anatomical Science, Jackson, USA

Chronic neuropathic pain (NP) affects approximately 64-82% of all patients living with a spinal cord injury (SCI). Despite years of research, a thorough understanding of the underlying mechanisms involved in the development and maintenance of SCI-dependent NP remains elusive. Inflammation plays a dynamic role in the development of NP, and is a prime therapeutic target. With potent anti-inflammatory and immune-regulatory characteristics, mesenchymal stem cells (MSC) may provide a safe and effective method for modulating and attenuating NP. The objectives are to determine the efficacy of early MSC delivery to attenuate inflammation and NP development, and improve chronic NP and inflammation with delayed MSC treatment. Adult, male, Sprague-Dawley rats will receive a moderate, spinal contusion at T-10. All subjects and age-matched controls are subjected to neurosensory assessments of mechanical allodynia (von Frey filaments) and thermal hyperalgesia (Hargreave's test) in hind paws. Neurosensory assessments are performed prior to injury and 7, 14, 21 and 28 days post-injury for the acute study and again monthly for the chronic study. MSC (50×10^6 cells/ml) are delivered intravenously and intrathecally at 24 hrs (acute) or 7 months (chronic) post-injury. Spinal cord tissue is harvested at 28 days or 8 months post-injury, sectioned and stained for immunohistochemistry. MSC treatment after CNS injury is appealing due to their immunoregulatory and anti-inflammatory characteristics. The data generated in this study suggests that the administration of MSC after spinal contusion injury partially prevents NP associated with mechanical allodynia in the hind paws. The data produced in our chronic study reveal MSC treatment in the chronic phase of injury effectively improves pain and function scores, and indicate a decrease in neuroinflammation. These results support our hypothesis that MSC are an effective cellular therapy approach to treat SCI-dependent NP.

Funding provided by the UTHSC Bentsen Stroke Center Investigator Program.

Keywords: Animal model, mesenchymal stem cells, chronic SCI, neuroinflammation

B28-05

SPINAL CORD INJURY TREATMENT WITH MESENCHYMAL STEM CELL EXTRACELLULAR VESICLES IMPROVES NEUROINFLAMMATION AND LOCOMOTOR RECOVERY

Katherine Ruppert, Tin Nguyen, Karthik Prabhakara, Amit Srivastava, Scott Olson

University of Texas Health Science Center Houston, Pediatric Surgery, Houston, USA

Acute spinal cord injury continues to be a devastating problem worldwide with high morbidity and mortality. Long term morbidities include sensory, motor, and autonomic dysfunction. There is currently no consensus on pharmacological intervention to alter the clinical course of acute spinal cord injury and improve neurological outcome. Bone marrow-derived mesenchymal stem cells (MSC) have been shown to modulate the injury sequelae of SCI via paracrine effects, although the mechanisms are not completely clear. One potential modality is through secretion of extracellular vesicles (EV). EV are heterogeneous particles with lipid bilayer, containing growth factors, lipids, microRNAs, mRNAs, and proteins. We are testing the application of MSC-derived extracellular vesicles (MSCEv) to reduce neuroinflammation and improve sensory and motor recovery following SCI. Animals will be randomly assigned to three groups: sham laminectomy, or T10 contusion + vehicle or T10 contusion + MSCEv. 1, 2, 3, 5, 7, 10 and 14 days following injury, locomotor recovery is scored using the Basso, Beattie and Bresnahan (BBB) method. At

14 days post-injury, animals will be tested for mechanical allodynia via the Dixon Up-Down Von Frey method then sacrificed. Spleen will be harvested for neurotransmitter assays, and blood and spinal cord will be harvested for flow cytometry and immunohistochemistry. Preliminary data indicate significantly higher locomotor recovery scores in SCI + MSCEv animals when compared to SCI + vehicle animals on days 5, 7 and 14 post-injury ($p < 0.0001$, $p < 0.001$ and $p < 0.001$, respectively). Animals treated with MSCEv also demonstrate significantly higher force thresholds in the mechanical sensitivity test compared to vehicle treated animals at 14 days post-injury ($p < 0.05$). Flow cytometry analysis of spinal cord reveals increased activation of M1 and M2 microglia in MSCEv treated animals compared to vehicle. Spleen analysis indicates increased myeloid cells and MDSC with decreased NK cells and leukocytes in MSCEv treated animals. These results support our hypothesis that MSCEv are an effective therapeutic after SCI.

Funding provided by Glassell Family Pediatric Stem Cell Research Fund.

Keywords: Extracellular vesicles, locomotor recovery, neuroinflammation, secondary injury

B28-06

CO-ENCAPSULATION OF MESENCHYMAL STEM CELLS AND CORD BLOOD IMPROVE LOCOMOTOR RECOVERY AND NEUROINFLAMMATION IN SPINAL CORD INJURY

Katherine Ruppert, Daniel Kota, Karthik Prabhakara, Scott Olson
University of Texas Health Science Center Houston, Pediatric Surgery, Houston, USA

Over 17,000 new spinal cord injuries (SCI) occur each year in the United States, contributing to approximately 282,000 persons currently living with SCI. Currently, treatment consists of surgical stabilization, physical rehabilitation, and analgesia. There is an unmet need for new treatments to mitigate secondary damage that occurs after SCI. Pro-inflammatory cytokines and leukocyte infiltration play a significant role in the development of secondary injuries and present a specific target for cellular therapy. Bone marrow-derived mesenchymal stem cells (MSC) have been shown to modulate inflammation, reducing secondary injuries in SCI via paracrine effects. Our objective is to evaluate the efficacy of MSC co-encapsulated with umbilical cord blood (CB) effector cells and the potential synergistic effect on local neuroinflammation and behavior. MSC are encapsulated in alginate hydrogel beads that are implanted adjacent to the contused T-10 spinal cord 24hrs after injury. Animals are randomly assigned to one of five groups: Naïve, Sham + encapsulated MSC, Sham + co-encapsulated MSC + CB, SCI + encapsulated MSC, SCI + co-encapsulated MSC + CB, alginate beads alone, encapsulated MSC cells, encapsulated cord blood cells or MSC + cord blood encapsulated. Efficacy is measured by locomotor recovery, mechanical and thermal sensitivity, as well as immunohistochemical staining for leukocyte infiltration, demyelination, inflammatory mediators and blood-spinal cord barrier permeability. Animals are sacrificed 14 days after injury and spinal cords are harvested, sectioned and stained for immunoreactivity. Preliminary data indicates that local application of co-encapsulated MSC and cord blood cells significantly improves locomotor recovery 14 days post-injury when compared to systemic MSC (*, p value < 0.05). Interestingly, encapsulated MSC animals showed significantly improved locomotor recovery scores on days 2, 3, 5, 7, 10 and 14 when compared to all other groups. We anticipate histological changes will indicate that encapsulation of MSC and co-encapsulation of MSC+CB cells provides increased protection against secondary injury.

Funding provided by the UTHSC Bentsen Stroke Center Investigator Program and Glassell Family Pediatric Stem Cell Research Fund.

Keywords: Encapsulation, umbilical cord blood cells, mesenchymal stem cells, secondary injury

B29 SYNAPTIC FUNCTION

B29-01

SNARE COMPLEX DEFICITS ASSOCIATED WITH SYNAPTIC AND NEUROBEHAVIORAL DYSFUNCTION AFTER FLUID PERCUSSION BRAIN INJURY

Shaun Carlson¹, Jeremy Henchir¹, C. Edward Dixon^{1,2}
¹*University of Pittsburgh, Neurosurgery, Pittsburgh, USA*
²*VAPHS, Pittsburgh, USA*

Lasting cognitive impairments have been reported by patients afflicted with a traumatic brain injury (TBI) and replicated in experimental TBI models. Previous work from our lab and others implicates impaired neurotransmission as a contributor to neurobehavioral dysfunction after TBI, but little is known about the mechanisms underlying this pathology. In the synapse, formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex is a critical step in vesicle fusion and the release of neurotransmitters into the synaptic cleft. We recently showed severe TBI impairs SNARE complex formation; however, it is unknown if this contributes to synaptic dysfunction across the spectrum of injury severity. We hypothesized that lateral fluid percussion injury (IFPI) decreases SNARE complex formation in multiple brain regions and results in impaired function. To this end, 3 cohorts of male Sprague Dawley rats were subjected to sham or 2atm IFPI and assessed for 1) evoked neurotransmitter release, 2) SNARE protein and complex abundance by immunoblotting, and 3) histopathology and neurobehavioral performance. Preliminary findings from ongoing studies suggest deficits in potassium-evoked striatal dopamine release at 7d post-injury. Synaptosomal lysates were generated to focus on synaptic alterations. At 7d post-injury, IFPI significantly reduced hippocampal SNARE proteins VAMP2 and α -synuclein, and SNAP-25 immunoreactive SNARE complexes, but did not alter synaptophysin abundance. Additional alterations in SNARE protein abundance were observed in other brain regions. NeuN and cresyl violet staining revealed the formation of a characteristic cortical lesion at the white matter and neocortex interface, minor hilar neuron loss, and no overt CA1 or CA3 loss after IFPI. Beam balance and walking tasks revealed transient impairments in vestibulomotor function 1d post-injury. Spatial acquisition and memory, as assessed by the Morris water maze, was significantly reduced following IFPI 3 weeks post-injury, consistent with previously published literature. These data provide novel evidence for TBI-induced SNARE protein changes and synaptic dysfunction after brain injury.

Support: 1-F32NS090748, The Pittsburgh Foundation, NIH-NS40125, NIH-NS060672, VAI01RX001127.

Keywords: neurotransmission, synapse, hippocampus, fluid percussion injury

B29-02

SYNAPTIC FORMATION OF ALPHA SYNUCLEIN AGGREGATES ACUTELY FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY

Shaun Carlson¹, Youming Li¹, Xiecheng Ma¹, Jeremy Henchir¹, C. Edward Dixon^{1,2}

¹University of Pittsburgh, Neurosurgery, Pittsburgh, USA

²VAPHS, Pittsburgh, USA

Traumatic brain injury (TBI) has been identified as a risk factor for the development of chronic neurodegenerative diseases, including Parkinson's disease. Pathological protein misfolding is associated with disrupted cellular function and can contribute to subsequent neuronal loss. Current hypotheses suggest that synaptic dysfunction precedes neuronal loss in the pathological progression of neurodegenerative disease. Alpha synuclein (α -synuclein) has been well described for its role in contributing to the pathogenesis of Parkinson's disease. Pathological forms of α -synuclein have been observed at chronic time points following TBI. Emerging evidence highlights a central role for α -synuclein in the formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex responsible for neurotransmitter release. We recently reported that TBI results in an acute reduction in monomeric α -synuclein. We hypothesized that TBI results in acute formation of α -synuclein aggregates in synapses. To this end, male Sprague Dawley rats were subjected to sham or controlled cortical impact (CCI) injury (2.7 mm, 4 m/s, 150 msec) and sacrificed at 2, 7, or 14 days post-injury. High molecular weight α -synuclein aggregates, confirmed using two antibodies, were detected in hippocampal whole cell and synaptosomal-enriched lysates at all time points assessed. Immunohistochemical staining revealed aggregated α -synuclein immunoreactivity in pericontusional tissue and the dentate gyrus of the hippocampus at 2 weeks following CCI injury. These findings provide novel evidence for acute α -synuclein aggregates in the injured synapse that may contribute to impaired synaptic and neurobehavioral function after TBI.

Support: 1-F32NS090748, The Pittsburgh Foundation, NIH-NS40125, NIH-NS060672, VAI01RX001127.

Keywords: synapse, traumatic brain injury, alpha synuclein, hippocampus

B29-03

GREY MATTER INJURY AFTER MODERATE CLOSED-SKULL TRAUMATIC BRAIN INJURY

Andrew Sauerbeck, Richard Davenport, Grant Baxter, Sam Kim, Joong-Hee Kim, Adam Bauer, David Brody, Terrance Kummer
Washington University, Neurology, St. Louis, USA

Traumatic brain injury (TBI) is a highly-prevalent cause of morbidity and mortality worldwide with no effective therapy. The majority of TBI cases are mild or 'concussive.' These patients overwhelmingly recover well. Patients with complicated-mild or moderate TBI, however, suffer a dramatically higher burden of long-lasting deficits. These patients are therefore an attractive target for investigation. We developed a non-surgical, tunable, monitored model of mouse TBI based off of the closed-head impact model of engineered rotational acceleration (CHIMERA) platform, called modCHIMERA, that targets this TBI severity group. modCHIMERA is characterized by both impact and inertial loading (linear and rotational), with acceleration exceeding scaled human injury thresholds, and righting times consistent with a moderate injury. modCHIMERA induces diffuse damage characterized by multifocal white matter injury, cell death, neuroinflammation, and multidomain neurobehavioral deficits. Importantly, modCHIMERA does not generate a dominant focal, cavitary lesion as results from most moderate-severe TBI models. This approximates the diffuse injuries commonly observed in patients with complicated-mild and moderate TBI, and facilitates investigation of grey matter cellular and subcellular damage. Such grey matter injury pathways may drive lasting neurological impairment separate from

the white matter pathology that has been the primary focus of most studies on post-TBI neuronal damage. Consistent with this, modifications to modCHIMERA to limit axonal injury did not eliminate long-term neurobehavioral deficits. To characterize pathways leading to grey matter injury after TBI, cortical regions were assessed after modCHIMERA for neuronal cell death, dendrite length and arbor complexity, dendritic spine density, and synaptic density. Functional connectivity optical intrinsic signal imaging was used to evaluate local circuits vs. those dependent on projection axons, and demonstrates greater impact on ipsilateral node degree consistent with functionally-significant synaptic or dendritic injury. These studies will lead to a greater understanding of traumatic circuit disruption beyond axonal pathways, and aid in the development of novel diagnostic tools for characterization of grey matter injury after TBI.

Keywords: Grey matter, Synapse, Complicated-mild traumatic brain injury, Moderate traumatic brain injury, Functional connectivity, Optical intrinsic signal imaging

B30 TRANSPLANTATION

B30-01

TRANSIENT ELEVATION OF HYPOXIA INDUCIBLE FACTOR ALPHA PROTECTS AGAINST THE DETRIMENTAL EFFECT OF TRANSPLANTED CELL DEATH BUT DOES

Ying Dai^{1,2}, Brian David^{1,2}, Caitlin Hill^{1,2}

¹Burke Medical Research Institute, White Plains, USA

²Weill Cornell Medicine, Feil Family Brain and Mind Research Institute, New York, USA

Schwann cell (SC) transplants are beneficial in rodent models of spinal cord injury (SCI). In a Phase 1 clinical trial, SCs were demonstrated safe in humans. Previously, we demonstrated that transplanted cells die acutely, raising the concern that acute transplant death may counteract some of the beneficial effects of SC transplants. In the current study, we assessed the effect of transplanted cell death on spared spinal cord tissue and then examined the effects of pharmacologically increasing hypoxia-inducible factor alpha (HIF- α) in SCs as a strategy to promote transplanted cell survival. Following transplantation of 2×10^6 SCs one week post-SCI in the rat, nitrotyrosine (3-NT) was significantly increased within the injured spinal cord, suggesting a detrimental effect of cell transplants on spared spinal cord tissue. We then assessed the ability of HIF- α to enhance transplant survival. HIF- α levels were increased in SCs pharmacologically by manipulating the activity of HIF-prolyl-4-hydroxylases (PHD), enzymes that target HIF- α for proteasomal degradation. Treatment of SCs with 1% O₂ (24h), deferoxamine (DFO; 200 mM, 24h), or adaptaquin (AQ; 10 mM, 16h) increased the level of both HIF- α and its target genes. *In vitro*, elevation of HIF- α with DFO and AQ, but not 1% O₂, protected SCs against H₂O₂-mediated cell death. *In vivo*, HIF pretreatments attenuated the increase in 3-NT observed following SC transplantation, but pretreatment was insufficient to promote transplant survival or functional recovery. Examination of HIF- α and its target gene levels revealed that HIF protein and its targets were only transiently elevated within the spinal cord following pretreatment of the cells. Together, the results demonstrate that transplanted cell death can be deleterious and should be counteracted, and that while elevating HIF- α is protective, the effects of pretreatment are short-lived indicating prolonged treatment is required.

Support: NIH 1R01NS075375.

Keywords: Schwann cells, spinal cord injury, HIF, cell transplantation, cell death, cell survival

OVEREXPRESSION OF VP16-HIF-1 α IN SCHWANN CELLS IMPROVES TRANSPLANT SURVIVAL FOLLOWING SPINAL CORD INJURY

Brian David^{1,2}, Ying Dai^{1,2}, Jessica Curtin^{1,2}, David Goldberg^{1,2}, Caitlin Hill^{1,2}

¹Burke Medical Research Institute, White Plains, USA

²Weill Cornell Medicine, Feil Family Brain and Mind Research Institute, New York, USA

Cellular transplants provide replacement cells and a substrate for repair following spinal cord injury (SCI). Despite these benefits, the effects of cell transplants on functional recovery following SCI are modest. Previously, we determined that although cell transplants persist and integrate long-term, the majority of cells die acutely post-transplantation. We hypothesize that this acute death could attenuate the beneficial effects of cellular transplants. To address whether the acute death of transplanted cells is detrimental, we are developing approaches to enhance transplant survival. In the current study, we manipulated the expression of the transcription factor hypoxia inducible factor 1 alpha (HIF-1 α) in Schwann cells (SCs). Overexpression of VP16-HIF-1 α increased nuclear HIF-1 α protein levels four-fold and resulted in a one-fold increase in the protein levels of two HIF-1 α targets, VEGF and enolase. To test whether elevated levels of HIF-1 α in SCs could protect them from transplant-mediated cell death, 2×10^6 SCs (control, VP16, or VP16-HIF-1 α) were transplanted seven days post-SCI in the rat. Transplant survival was increased by 34% and 21% in VP16-HIF-1 α SCs relative to control and VP16 SCs, respectively. Stereological quantification of transplant survival is time-consuming. To determine if IVIS-based imaging could be used to rapidly screen transplant survival, we quantified light emitted from transplants using IVIS imaging for luciferase in live animals and for GFP fluorescence in *ex vivo* spinal cords. Luciferase-based IVIS imaging detected differences over time but not between treatment groups. GFP IVIS imaging of *ex vivo* spinal cords showed a trend for increased transplant survival. This suggests that GFP imaging may be useful for screening pro-survival strategies that lead to large differences in transplant survival. The increase in transplant survival with HIF-1 α suggests it may be a useful target for enhancing transplant survival. Additional studies that pharmacologically manipulate HIF levels in SCs are presented separately.

Keywords: Schwann cell, Spinal cord injury, transplantation, transcription factor, cell death

IMPROVING SCHWANN CELL TRANSPLANT SURVIVAL FOR SPINAL CORD REPAIR

Susana R. Cerqueira¹, Yee-Shuan Lee¹, Robert C. Cornelison³, Michaela W. Mertz⁴, Rebecca A. Wachs⁴, Christine E. Schmidt⁴, Mary Bartlett Bunge^{1,2}

¹Miller School of Medicine, University of Miami, Miami Project to Cure Paralysis, Miami, USA

²Miller School of Medicine, University of Miami, Department of Cell Biology, Department of Neurological Surgery, Miami, USA

³University of Texas at Austin, Department of Chemical Engineering, Austin, USA

⁴University of Florida, J. Crayton Pruitt Family Department of Biomedical Engineering, Gainesville, USA

Schwann cell (SC) transplantation has been extensively studied for spinal cord injury (SCI) repair. SCs are neuroprotective and promote axon regeneration and myelination in the implant site. However, substantial cell death occurs post-implantation, limiting its therapeutic potential. The use of injectable matrices improves transplanted SC survival, axon extension, and function in a rat contusion model. Yet, clinically relevant products are needed for translational use, as the currently available matrices have variable formulations and derive from animal tumors, as Matrigel. We tested an injectable acellular peripheral nerve (iPN) matrix to improve SC grafts in SCI. Acellular iPNs were processed to be injectable and thermally gelling. SCs in iPN were transplanted into the lesion epicenter of T8 contused rats and compared to SCs implanted in Matrigel. Our results showed a plethora of SC-myelinated axons within dense implants in both conditions. iPN grafts contained twice as many axons as Matrigel. Graft volume in iPN was twice as large compared to Matrigel. SC/iPN rats performed as well as SC/Matrigel rats in BBB testing, and showed a faster recovery in BBB subscores. On the grid walk, SC/iPN rats performed fewer errors at 4 weeks, equalizing at 8 weeks. Immune cell profiling by flow cytometry revealed that SC/Matrigel implants exhibited increased T cell and leukocyte infiltration into the lesion, whereas iPN implants were identical to SCI controls. The fact that this clinically relevant iPN matrix is immunologically tolerated and improves transplanted SC survival and axon growth within the graft offers a translational possibility for improving efficacy of SC treatment after SCI.

Keywords: Schwann Cell

Data Blitz Oral Presentations

DBA DATA BLITZ A: MINI PRESENTATIONS

DBA-01

NEUROIMAGING OF DIFFUSE AXONAL AND VASCULAR INJURY IN CHRONIC TRAUMATIC BRAIN INJURY

Margalit Haber¹, Franck Amyot^{2,3,4}, Kelly Flesher², Kimbra Kenney^{2,3,4}, Carol Moore³, Erika Silverman¹, Yi-yu Chou⁴, Dzung Pham⁴, Eric Wasserman⁴, Ramon Diaz-Arrastia¹

¹University of Pennsylvania, Neurology, Philadelphia, USA

²USUHS, Neurology, Bethesda, USA

³HJF, CNRM, Bethesda, USA

⁴NIH, NINDS, Bethesda, USA

Traumatic Brain Injury (TBI) results in diffuse axonal injury (DAI) and diffuse vascular injury (DVI). Both DAI and DVI result from inertial shearing forces, and the two terms are often used interchangeably, the spatial relationships between DAI and DVI have not been carefully studied. Multimodal magnetic resonance imaging (MRI) can help to distinguish these injury mechanisms: diffusion tensor imaging (DTI) provides information about axonal integrity, while arterial spin labeling (ASL) and functional Blood Oxygen Level Dependent imaging (BOLD) with hypercapnia challenge, reflect cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) respectively. Chronic TBI participants (n=27) and age- and education-matched healthy controls (n=15) underwent multimodality MRI. The Freesurfer image analysis suite (MGH, Harvard, MA) was used to segment each MP-RAGE image into regions of interest (ROIs). Mean values of mean diffusivity (MD), fractional anisotropy (FA), CBF, and CVR were extracted for each ROI. Additionally, maps were normalized into a common space (MNI Atlas) and z-score maps were generated based on a pool of healthy controls. Normality of an ROI/voxel was determined based on z-score (abnormal MD: z-score >2.5; abnormal FA, CBF, and CVR: z-score < -2.5). Abnormal ROIs in one modality were not predictive of abnormalities in another modality. Approximately 8–10% of abnormal voxels for CVR and CBF also show an abnormal voxel value for MD, while only 1% of abnormal CVR and CBF voxels show a concomitant abnormal FA value. These data indicate that chronic TBI patients display two distinct endophenotypes: microstructural tissue/axonal injury and vascular injury that are spatially independent.

Keywords: Diffusion Tensor Imaging, Cerebral Vascular Reactivity, Chronic TBI, MRI

DBA-02

CIRCULATING GFAP LEVELS TO MONITOR THERAPEUTIC RESPONSE TO GLIBENCLAMIDE IN CONTROLLED CORTICAL IMPACT: FINDINGS FROM OBTT

Stefania Mondello¹, Deborah Shear², Helen Bramlett³, C. Edward Dixon⁴, W. Dalton Dietrich³, Kevin Wang⁶, Ronald Hayes⁷, Audrey Lafrenaye⁸, John T. Povlishock⁸, Frank Tortella², Patrick M. Kochanek^{4,5}

¹Messina University, Messina, Italy

²WRAIR, Silver Spring, US

³Miami University, Miami, US

⁴Pittsburgh University, Pittsburgh, US

⁵Safar Center for Resuscitation Research, Pittsburgh, US

⁶Florida University, Gainesville, US

⁷Banyan Biomarkers, Alachua, US

⁸VCU, Richmond, US

Glibenclamide (GLI) is a sulfonylurea receptor antagonist that has shown promise as a therapy to prevent secondary injury following traumatic brain injury (TBI). The present work, part of the Operation Brain Trauma Therapy (OBTT) multi-center pre-clinical drug screening consortium, investigated the effects of GLI treatment on the levels of circulating glial fibrillary acidic protein (GFAP) and the relationships with histopathological and behavioral outcomes after controlled cortical impact (CCI). OBTT demonstrated that GLI treatment reduced contusion volume in CCI; thus, we sought to determine whether circulating GFAP in the initial 24h could inform theranostically on lesion volume at 21d post-injury. Adult male rats subjected to CCI received a bolus (10µg/kg IP) 15min after-injury, followed by a continuous SQ infusion (0.2µg/h) via osmotic pumps throughout 7d of GLI or vehicle. GFAP levels in blood were measured at 1, 4 and 24h after CCI. Vehicle-treated rats displayed distinct temporal profiles for GFAP vs. GLI-treated rats. GFAP in vehicle-treated animals demonstrated a sustained increase after-injury vs. shams (p<0.0001) peaking at 4h. Conversely, rats treated with GLI initially had high GFAP levels similar to vehicle. However, by 24h GFAP in GLI-treated rats did not differ from sham and were lower (p<0.05) than vehicle. Overall, GLI-treated rats had significantly lower GFAP release (area under the curve) throughout the study vs. vehicle. GFAP at 24h also strongly correlated with contusion volume at 21d (r=0.88, p<0.0001), hemispheric tissue loss (r=0.86, p<0.0001) and MWM latency (r=0.68, p=0.0001). Our findings support a role for GFAP in predicting tissue damage and as a marker of therapeutic response corroborating advantageous effects of GLI in CCI. Circulating GFAP may be useful for high-throughput screening of drugs in pre-clinical investigations. Support: USArmyW81XWH-10-1-0623.

Keywords: Biomarkers, GFAP, TBI, controlled cortical impact, rat, neuroprotection

DBA-03

DUAL TIME COURSE RNA-SEQ REVEALS LEVEL-SPECIFIC NEUROVASCULAR RESPONSE AFTER CERVICAL AND THORACIC SPINAL CORD INJURY

James Hong^{1,2}, Mahmood Chamankhah², Anna Badner^{1,2}, Dario Righelli³, Priscilla Chan¹, Claudia Angelini³, Michael Fehlings^{1,2}

¹University of Toronto, Institute of Medical Science, Toronto, Canada

²Toronto Western Hospital, Genetics and Development, Toronto, Canada

³Istituto Per le Applicazioni del Calcolo, BioinfoLab, Naples, Italy

Traumatic spinal cord injury (SCI) is a devastating neurological condition that occurs primarily at the cervical (cSCI, >65%) and thoracic (tSCI) levels. Despite the marked neurovascular distinctions of the two levels and strikingly positive response of cSCI to trial drugs such as cethrin compared to tSCI, the mechanisms driving level-specific heterogeneity between their respective milieu remains elusive. We posit that the increased vascularity and grey-white ratio of the cervical cord—relative to the thoracic—results in greater susceptibility to neurovascular disruption, ultimately manifesting a secondary injury of earlier onset, severity, and chronicity. A rat model of moderate clip compression injury was used to induce SCI at the C6-7 and T6-7 levels, with laminectomy-only animals serving as surgical controls. Following sacrifices at 3, 7, 14, and 56 days, samples were subject to RNA-seq, protein work, imaging, and immunohistochemistry. Results of RNA-sequencing revealed striking differences in the onset and temporal profile of astrocytic and pericytic neurovascular processes with canonic *stat3*-dependent gliotic markers—*lcn2*, *gfap* and *serpina3n*—being upregulated in the cervical cord across time. Further, 3D ultrasound and immunostaining revealed rapid tissue loss and hemorrhage starting as early as 3 days post-cSCI with increased *gfap* and *cspg4* staining in the cord. Finally, Western blotting confirmed an increase in *stat3*-dependent gliotic markers accompanied by a loss of key blood-brain-barrier proteins *tjp1* and *ocln* in cSCI across time. Taken together, this data demonstrates—for the first time—the level-specific heterogeneity of SCI, with cSCI having a quicker onset and chronicity compared to tSCI. Further, these results reconcile the potential reasons behind why preliminary tSCI-derived trial paradigms may not be suited—in both strategy and timing—to cSCI, and hopes to engage clinicians and scientists in the design and study of level-specific therapeutics.

Keywords: RNASeq, Neurovascular unit, Secondary injury, Pericyte

DBA-04

OPTICAL AND ELECTROGRAPHIC CHANGES IN THE BRAIN AFTER TRAUMA

Jenny Szu¹, Danielle Ornelas², Som Chaturvedi¹, Junze Liu², B. Hyle Park², Devin Binder¹

¹University of California, Riverside, of Biomedical Sciences, Division Riverside, USA

²University of California, Riverside, Department of Bioengineering, Riverside, USA

Traumatic brain injury (TBI) is a major cause of morbidity and mortality in persons under the age of 45 and is a primary public health concern in both the civilian and military populations. Posttraumatic epilepsy (PTE) is a long-term negative consequence of TBI in which one or more unprovoked seizures occur at least one week after the initial trauma and accounts for up to 20% of all symptomatic epilepsy. Severity of injury and early posttraumatic seizures are risk factors associated with PTE, however, predictors of PTE still remain highly variable. Current studies of PTE have focused on pharmacologically testing seizure threshold using pentylenetetrazole and imaging studies, while limited, have not identified a correlation between trauma and seizure susceptibility. In our study, we utilized optical coherence tomography (OCT) to detect functional and structural changes in the brain in a controlled cortical impact (CCI) injury mouse model of TBI. Additionally, animals underwent long-term video-electroencephalography (vEEG) recording to identify spontaneous seizures after TBI and intrahippocampal electrical stimulation to quantitatively define electrographic seizure threshold (EST) and duration (ESD). OCT imaging detected alterations in optical attenuation, a measure of both scattering and absorption of the biological sample, with and without vascular

contribution at different time points after TBI. Additionally, injured animals exhibited altered EST and ESD compared to naïve and sham controls. Altogether, our data suggest that seizure threshold can be quantitatively measured and OCT imaging can potentially offer useful information to identify predictors of PTE in our model.

Keywords: Optical coherence tomography, Seizure threshold, EEG

DBA-05

IN VIVO RETINAL IMAGING OF NEUROINFLAMMATION IN A MOUSE MODEL OF IMPACT CONCUSSION

Olga Minaeva^{1,2,3}, James D. Akula^{4,5}, Mark W. Wojnarowicz^{1,6}, R. Daniel Ferguson⁷, Mircea Mujat⁷, Erich S. Franz^{1,2}, Andrew M. Fisher^{1,2}, Juliet A. Moncaster^{1,3,6}, Bertrand R. Huber^{6,8}, Lee E. Goldstein^{1,2,3}

¹Boston University School of Medicine, Molecular Aging & Development Laboratory, Boston, USA

²Boston University, College of Engineering, Boston, USA

³Boston University Photonics Center, Boston, USA

⁴Boston Children's Hospital, Dept. of Ophthalmology, Boston, USA

⁵Harvard Medical School, Dept. of Ophthalmology, Boston, USA

⁶Boston University School of Medicine, Boston, USA

⁷Physical Sciences, Inc, Biomedical Optics, Andover, USA

⁸VA Boston Healthcare System, Boston, USA

Despite growing awareness of links between concussion, traumatic brain injury (TBI), and long-term effects of neurotrauma, the underlying mechanisms in the brain and possible changes in the retina are unknown. In this study, we used a new mouse model of concussion and a state-of-the-art retinal eye scanner to investigate post-traumatic inflammatory responses in the brain and retina. We utilized Ccr2RFP/Cx3cr1GFP mice (Jackson Laboratory) to enable immune cell visualization by class (monocyte, microglia), origin (peripheral, brain, retina), morphology, and location. We used a new closed-head impact injury mouse model (Tagge et al., submitted) that recapitulates key features of human concussion. We used a multimodal adaptive optics small-animal imager (MAOSI) (Physical Sciences, Inc.) for *in vivo* retinal studies. MAOSI has several channels: adaptive optics optical coherence tomography (AO-OCT) and adaptive optics scanning laser ophthalmoscopy (AOSLO) with reflectance and fluorescence channels. Before injury, Ccr2RFP/Cx3cr1GFP mice showed normal cellular distribution and morphological phenotype of microglia in retina and brain. We observed significant increase in microglia in retina and brain post-injury. Moreover, microgliosis in both retina and brain was notable for reactive cellular phenotype transformation with overlapping ramification fields. Closed-head impact injury is associated with similar reactive inflammatory responses and sequelae in both retina and brain. The retina can be used as a “brain proxy” for noninvasive diagnosis, prognosis, and monitoring of neuroinflammation after closed-head injuries.

Acknowledgment: DoD W81XH-14-1-0592.

Keywords: Impact Concussion, Mouse Model, Retinal Imaging, Neuroinflammation

DBA-06

EVIDENCE FOR A NEURAL-RESPIRATORY-INFLAMMATORY AXIS IN TRAUMATIC BRAIN INJURY-INDUCED ACUTE LUNG INJURY

Nadine Kerr¹, Juan Pablo de Rivero Vaccari¹, Ronald Zambrano¹, Sam Abbassi¹, Shu Wu¹, Dalton Dietrich¹, Robert Keane¹

¹University of Miami, Neurological Surgery, Miami, USA

²University of Miami, Department of Pediatrics, Miami, USA

³University of Miami, Department of Physiology and Biophysics, Miami, USA

Acute Lung Injury (ALI) is a common systematic complication of severe Traumatic Brain Injury (TBI). However, the pathophysiologic mechanisms of TBI-induced ALI are not well known. We have previously shown that the inflammasome plays a critical role in TBI-induced secondary pathophysiology and that inflammasome proteins are released in Extracellular Vesicles (EV) after TBI. Here we investigate whether EV-mediated inflammasome signaling contributes to the etiology of TBI-induced ALI. In this study, C57/BL6 mice were subjected to Controlled Cortical Impact Injury (CCI) and brains and lungs were examined for inflammasome activation, pyroptosome formation, and ALI. Also, an adoptive transfer experiment was performed by injecting serum-derived EV from sham and TBI injured mice into healthy mice and lungs were analyzed for inflammasome protein expression and ALI. Our findings indicate that brain and lungs of CCI-injured mice showed a significant increase in the expression of AIM2, IL-1b, caspase-1, IL-18 and HMGB1 acutely after TBI. Moreover, injured lungs also showed evidence of pyroptosis. Lungs of CCI-injured animals demonstrated evidence of ALI as determined by thickening of the alveolar septum, alveolar edema and inflammation, thus resulting in a higher ALI score. Adoptive transfer of serum-derived EV from TBI mice, but not sham mice, into healthy mice induced higher expression of inflammasome proteins in lungs and higher ALI scores. Interruption of this axis by administration of Enoxaparin or an anti-ASC significantly inhibited inflammasome activation and improved ALI scores. In conclusion, our data show that inflammasome activation plays a major role in TBI-induced ALI. These data provide strong evidence for activation of a Neural-Respiratory Inflammasome Axis, and demonstrate that targeting this axis with Enoxaparin or anti-ASC antibody may provide a novel therapeutic approach for neurotrauma-induced ALI.

Funding: Miami Project to Cure Paralysis, NIH grants: R42NS086274, F31 HL132425-01A1.

Keywords: Traumatic Brain Injury, Acute Lung Injury

DBA-07

EVALUATION OF MINOCYCLINE IN THE WRAIR PBBI MODEL: STUDIES FROM THE OPERATION BRAIN TRAUMA THERAPY (OBT) CONSORTIUM

Starlyn Okada-Rising, Jignesh Pandya, Krista Caudle, Jenny Browning, Rebecca Pedersen, Justin Sun, Deborah Shear
Walter Reed Army Institute of Research, Brain Trauma Neuroprotection, Silver Spring, USA

Minocycline was the 10th drug selected for testing by the OBT consortium. Minocycline is a broad-spectrum tetracycline antibiotic shown to be neuroprotective in neurodegenerative diseases and experimental models of stroke, spinal cord injury (SCI), and brain trauma. It has been reported to inhibit microglial activation, prevent oligodendrocyte and/or neuronal apoptosis, decrease oxidative damage, and reduce inflammation. A phase II clinical trial for acute SCI found Minocycline safe and tended to improve several motor recovery outcomes. The WRAIR site evaluated the effectiveness of Minocycline in the penetrating ballistic-like brain injury (PBBI) model. PBBI (10%) was performed unilaterally in the right hemisphere of anesthetized rats. A 30mg/kg Minocycline loading bolus was administered intravenously 15mins post-PBBI followed by continuous 72-hour infusion (2 mg/kg/hr). This protocol was shown in PK studies by our group to produce steady state blood levels

ranging between 5-10 mcg/mL, mimicking those in the successful phase II clinical trial. Groups consisted of TBI-MIN (n=14), TBI-Veh (n=19) or Sham (n=16). Motor and cognitive performance were assessed using rotarod (days 7 and 10 post-PBBI) and Morris water maze (MWM, days 13-17 post-PBBI). On day 21, brain tissue was processed for histology. Motor testing showed significant injury-induced deficits versus sham (p<.05). Overall rotarod latencies were reduced by 51.8±5.5% (TBI-Veh) and 47.4±8% (TBI-MIN) vs. sham. No significant therapeutic effects were detected on the rotarod task. MWM results revealed significant injury-induced deficits with latencies to locate the submerged platform increased by 56.8±14% (TBI-Veh) and 52.9±14.8% (TBI-MIN) versus sham. Positive trends towards improved memory retention (probe trial) and reduced lesion size were observed, but not significant. Current findings do not support further testing of Minocycline using this dosing regimen. However, additional testing may be warranted using higher doses and/or extended treatment durations in order to overcome peak microglial and inflammatory responses in the PBBI model. Supported by U.S. Army Grant W81XWH-10-1-0623.

Keywords: traumatic brain injury, OBT

DBA-08

IMPACT OF REPETITIVE MTBI ON FEAR MEMORY, DEPRESSIVE BEHAVIOR, AND MARKERS OF SYNAPTIC PLASTICITY IN A MOUSE MODEL OF CHRONIC PTSD

Moustafa Algama^{1,2}, Joseph O Ojo^{1,2}, Carlyn Lungmus¹, Philip Muza¹, Joan Owens¹, Benoit Mouzon^{1,3}, David Diamond³, Michael Mullan^{1,2}, Fiona Crawford^{1,2,3}

¹Roskamp Institute, Sarasota, USA

²The Open University, Milton Keynes, United Kingdom

³James A. Haley Veterans' Hospital, Tampa, USA

Background: Comorbid mTBI and PTSD can be clinically challenging to diagnose, primarily because of the heterogeneity and clinical overlap shared by both conditions. Clinical studies exploring comorbid mTBI and PTSD are often complicated by variances in type and severity of injury, time post-trauma, underlying comorbidities, and predisposing risk factors. These inherent limitations emphasize the urgent need to develop an etiologically relevant animal model whereby variables can be efficiently controlled. In this study, we use our refined mouse model of PTSD, and our established model of repetitive mTBI to assess the impact of mTBI on consolidation and/or extinction to a conditioned traumatic memory, including additional behavioral and neurobiological measures post-exposure.

Methods: C57BL/6J male mice were exposed to a 21-day stress paradigm at 3 and 5month of age followed by a battery of behavioral testing for fear-memory, anxiety, depression. Mice were euthanized 10days and 3month post-exposure, with brain and plasma samples collected for molecular profiling. The 21-day stress paradigm involved many randomized exposures to a danger-related predator odor (TMT) whilst immobilized, daily unstable social housing, and physical trauma in the form of five (separate) repeated inescapable footshocks. Animals receiving r-mTBI (x5) and stressors were exposed to a closed head injury 1hr after each conditioned footshock.

Results: Stressed mice showed significant weight loss, recall of traumatic memories, anxiety and depressive-like behavior when compared to control mice. Interestingly, repeated mTBI abrogated conditioned fear memory and depressive behavior in the forced swim test. Baseline TNF α plasma levels were elevated in stress only groups compared to controls, and repetitive mTBI mitigated this response in stressed mice. Biochemical analysis of hippocampal and amygdala homogenates revealed overlapping and unique changes to the HPA axis, glutamatergic and serotonergic signaling in stress and mTBI mice.

Conclusion: Our results demonstrates that our mouse model of PTSD develops persisting traits that capture critical aspects of PTSD symptomatology as defined by DSM-V. Unique traits were also observed with the comorbid presentation of mTBI and stress, and this was explained by some of our neurobiological measures. We anticipate that our model will be a useful platform to explore the neurobiology of PTSD and mTBI.

Keywords: PTSD, mTBI, Fear Memory, Depression, Mouse model, Closed Head Injury

DBA-09

OVERVIEW OF THE FIRST 12 THERAPIES EVALUATED BY OPERATION BRAIN TRAUMA THERAPY, A PRE-CLINICAL MULTI-CENTER CONSORTIUM FOR TBI

Patrick Kochanek¹, Helen Bramlett², C. Edward Dixon¹, Frank Tortella³, W. Dalton Dietrich², Stefania Mondello⁴, Ronald Hayes⁵, Kevin Wang⁶, John Povlishock⁷, Audrey Lafrenaye⁷, Samuel Poloyac¹, Philip Empey¹, Janice Gilsdorf³, Deborah Shear³

¹University of Pittsburgh, Safar Center, Pittsburgh, USA

²University of Miami, Miami, USA

³Walter Reed Army Institute of Research, Silver Spring, USA

⁴University of Messina, Messina, Italy

⁵Banyan Biomarkers, San Diego, USA

⁶University of Florida, Gainesville, USA

⁷Virginia Commonwealth University, Richmond, USA

Operation brain trauma therapy (OBTB) is a multi-center pre-clinical drug and biomarker screening consortium supported by the US DoD. OBTB screens therapies across three models in rats (parasagittal fluid percussion injury [FPI], controlled cortical impact [CCI] and penetrating ballistic-like brain injury [PBBI]) reflecting a range of phenotypes. Our goals include 1) to define therapies efficacious across models with the best chance for clinical translation, and 2) to define model-dependent effects to guide trials in targeted pathologies. The results of the first 5 therapies tested (nicotinamide, erythropoietin [EPO], cyclosporine [CsA], simvastatin, and levetiracetam) were published. OBTB has now screened 10 therapies, with ongoing testing of drugs 11-12. Drugs 6-12 include glibenclamide, kollidon-VA64, AER-271, amantadine, minocycline, E-64d and P7C3-A20. Levetiracetam showed benefit in multiple models. The second most successful drug, glibenclamide showed multiple benefits in CCI. Other therapies showed model dependent effects: tissue sparing by nicotinamide in CCI, benefit from CsA in FPI but toxicity in PBBI, benefit from amantadine in PBBI. Serum glial fibrillary acidic protein (GFAP) and Ubiquitin carboxy-terminal hydrolase-L1 were assessed at 4 and 24h. GFAP predicted 21d histology and drug effects. Levetiracetam merits additional testing and is being evaluated in our large animal model. Glibenclamide and Amantadine merit additional testing in contusion and penetrating brain injury, respectively. Our data support therapeutic use of serum GFAP.

Support: W81XWH-10-1-0623; W81XWH-14-2-0018.

Keywords: consortium, rat, reproducibility, rigor, pre-clinical, therapy

DBA-10

KCC2, A NOVEL THERAPEUTIC TARGET IN TRAUMATIC BRAIN INJURY

Pavel Lizhnyak, Andrew Riddler, John Povlishock, Andrew Ottens
Virginia Commonwealth University, Anatomy and Neurobiology,
Richmond, USA

There remains a need for more effective therapeutics in the treatment of traumatic brain injury (TBI). In this study, we tested whether an effective TBI intervention could be developed around a post-translational target occurring within an opportune treatment window as guided by temporal proteomics. Of interest were delayed-onset processes best managed after patient stabilization on the intensive care unit, focusing on events initiated a day or more after insult, out to two weeks following controlled cortical impact injury. We identified a subset of protein changes with the temporal profile of interest utilizing a self-organizing map approach. Enriched in this map were post-translational processes tied with ionic dysregulation, among which was a highly dynamic processing of neuron-specific K⁺-Cl⁻ cotransporter 2 (KCC2), an essential component for maintaining chloride homeostasis that is critical to inhibitory neurotransmission. We identified a potential therapeutic window of opportunity starting on day 1 preceding unique acetylation, phosphorylation and ubiquitination events guiding the functional loss of KCC2. To test this window, we administered the KCC2-targeting compound CLP290 daily (50 mg/kg, p.o.) before, at, and after the identified 1-day point of KCC2 post-translational processing. The therapy was most effective at 1-day, preserving plasmalemmal KCC2 within perilesional somatosensory neocortex needed to maintain chloride homeostasis and effective inhibitory neurotransmission. Furthermore, TBI-impacted sensorimotor integration improved significantly with the 1-day intervention on rotarod and whisker adhesive removal task assessments. Together, our findings demonstrate the therapeutic targeting of post-translational processes revealed with temporal proteomics to effectively preserve KCC2-mediated chloride homeostasis with improved functional recovery. Furthermore, the approach defines an effective administration window that can be extended for the intervention of other post-translational events. Lastly, KCC2-targeted therapy may be extended to other neurological insults known to involve chloride dysregulation such as epilepsy and stroke.

Supported by NINDS NIH 1F31NS100322-01, Department of Anatomy and Neurobiology, and VCU Neuroscience PhD Program.

Keywords: KCC2, Proteomics, Inhibition, CCI, Chloride, Post-translational

DBB DATA BLITZ B: MINI PRESENTATIONS

DBB-01

MILD TRAUMATIC BRAIN INJURY LEADS TO WIDE-SPREAD ACUTE BLOOD BRAIN BARRIER DISRUPTION

Maura Weber¹, William Stewart², D. Kacy Cullen¹, Douglas Smith¹, Victoria Johnson¹

¹University of Pennsylvania, Neurosurgery & Penn Center for Brain Injury and Repair, Philadelphia, USA

²University of Glasgow, Neuropathology, Queen Elizabeth University Hospital, Glasgow, UK

Mild traumatic brain injury (mTBI), or concussion, is a substantial public health problem that can result in persistent and debilitating symptoms in a subset of patients. Moreover, TBI, including repetitive concussion, has been linked to syndromes of progressive neurodegeneration such as chronic traumatic encephalopathy (CTE). However, the biology of mTBI and its link to late neurodegeneration remain poorly understood. A prominent feature of moderate or severe TBI at acute and late survival time-points is widespread disruption in blood brain barrier (BBB) integrity. However, it is unknown whether a single mTBI alone is sufficient to precipitate altered BBB integrity. Using an established swine model of pure rotational mTBI, animals were subjected to single mTBI or sham conditions (n=3-4 per group) and tissue was prepared for immunohis-

tochemical analyses at 6–72 hours post-injury. Following exposure to mTBI, widespread, abnormal extravasation of blood-borne proteins fibrinogen (FBG) and immunoglobulin-G (IgG) into the brain parenchyma was identified at all time points post-injury, despite an absence of hemorrhage. Typically, this appeared in a stereotyped distribution at structural interfaces, indicating a biomechanical etiology; although, notably, there was incomplete regional overlap between evidence of BBB disruption and axonal pathology. Triple immunofluorescence labeling revealed perivascular cellular uptake of both FBG and IgG in astrocytes (GFAP-positive), but not microglia (Iba1-positive) at these acute post-injury time-points. These data indicate that widespread BBB disruption can be demonstrated as a component of just a single mTBI using this swine model of pure rotational injury. The role of mTBI-induced BBB disruption in the acute presentation and outcomes of concussion, as well as its potential contribution to the late neurodegenerative pathologies of CTE, will be important to examine. Grant Support: DOD: PT110785, NIH: NS056202 and NS038104, The McCabe Fund (Univ. Pennsylvania).

Keywords: TBI model

DBB-02

SPINAL CORD INJURY-MEDIATED CPLA2 ACTIVATION CONTRIBUTES TO LYSOSOMAL DEFECTS LEADING TO IMPAIRMENT OF AUTOPHAGY

Yun Li¹, Jace Jones³, Chinmoy Sarkar¹, Shuo Liu¹, Maureen Kane³, Alan Faden¹, Eugene Koh², Marta Lipinski¹, Junfang Wu¹

¹University of Maryland, Baltimore, Department of Anesthesiology & Center for Shock, Trauma and Anesthesiology Research (STAR), Baltimore, USA

²University of Maryland, Baltimore, Department of Orthopedics, School of Medicine, Baltimore, USA

³University of Maryland, Baltimore, Department of Pharmaceutical Sciences, School of Pharmacy, Baltimore, USA

The autophagy-lysosomal pathway plays an essential role in cellular homeostasis and a protective function against a variety of diseases. However, under certain circumstances pathologically increased autophagy can contribute to cell death. This may occur particularly when lysosomal function is impaired and autophagic degradation is not able to proceed to completion, leading to pathological accumulation of dysfunctional autophagosomes. We have previously shown that autophagy is inhibited and contributed to injury after SCI. Here we examine mechanism of autophagy and lysosomal defects following SCI. Expression levels and processing of the lysosomal enzyme cathepsin D (CTSD) were decreased at 2 h after SCI. Enzymatic activity of CTSD and another lysosomal enzyme, alkaline phosphatase, were decreased 24 h post-injury, indicating lysosomal damage. Sub-cellular fractionation confirmed lysosomal membrane permeabilization (LMP) and leakage of lysosomal content into the cytosol. cPLA2 is an enzyme that cleaves fatty acyl linkage in the phospholipids of cellular membranes and increased activity of cPLA2 may be involved in membrane damage. cPLA2 was activated in the lysosomal fraction, accompanied by increased accumulation of the autophagosomal marker LC3-II and its substrate p62. To directly assess the extent and mechanism of damage to lysosomal membranes, mass spectrometry (MS)-based lipidomics was applied to compare the lipid composition of lysosomal membranes purified from sham or injured spinal cord at 2 h post-injury. Our data demonstrate increases in several classes of lysophospholipids- the products of phospholipases (PLAs), as well as accumulation of PLA activator, ceramide. Inhibition of cPLA2 decreased lysosomal damage, restored autophagic flux, and reduced neuronal cell damage. Taken together our data implicate lysosomal defects in the pathophysiology of SCI and further indicate that cPLA2

activation leads to lysosomal damage that causes neuronal autophagosome accumulation associated with neuronal cell death.

Keywords: spinal cord injury, autophagy, lysosomal damage, cPLA2

DBB-03

BIOMECHANICS OF CONCUSSION, TRAUMATIC BRAIN INJURY, AND CHRONIC TRAUMATIC ENCEPHALOPATHY IN A MOUSE MODEL OF CLOSED-HEAD IMPACT

Erich Franz¹, Chad Tagge¹, Andrew Fisher¹, Olga Minaeva¹, Mark Wojnarowicz², Juliet Moncaster², Andrew Anderson⁴, Robin Cleveland⁵, William Moss⁴, Lee Goldstein^{1,2,3}

¹Boston University, College of Engineering, Boston, USA

²Boston University, School of Medicine, Boston, USA

³Boston University, Alzheimer's Disease Center, CTE Program, Boston, USA

⁴Lawrence Livermore National Laboratory, Livermore, USA

⁵University of Oxford, Institute of Biomedical Engineering, Oxford, UK

The mechanisms underpinning concussion, traumatic brain injury (TBI), and chronic traumatic encephalopathy (CTE), and the relationships between these disorders, are poorly understood. To investigate causal mechanisms, we developed a mouse model of closed-head impact injury that uses momentum transfer to induce traumatic head acceleration. Unanesthetized mice subjected to unilateral impact exhibited abrupt onset, transient course, and rapid resolution of impaired arousal, contralateral hemiparesis, truncal ataxia, locomotor and balance impairments, and neurobehavioral deficits resembling human concussion. Acute neuropathological neuroexamination of brains from impact-injured mice demonstrated acute and chronic neuropathologies, including phosphorylated tau proteinopathy, that recapitulates early CTE pathologies in humans. Impact-induced CTE brain pathologies are also similar to brain pathology in blast-exposed mice and humans. Moreover, acute neurobehavioral signs of concussion were observed only after impact injury but not after blast exposure under conditions matched for head kinematics. Experimental and computational modeling showed that impact generated ipsilateral point loading on the head and seven-fold greater peak shear stress in the brain compared to blast. By comparison, blast induced distributed force loading on the head and diffuse, lower magnitude shear stress in the brain. We conclude that fast-acting, high-amplitude cortical shear stress triggers experimental concussion, whereas longer duration, lower amplitude shear stress associated with head motion induces structural brain damage and neuropathological sequelae. These results suggest that closed-head impact injuries, independent of concussive signs, can induce TBI as well as early pathologies and functional sequelae associated with CTE. These findings elucidate the origins of concussion and differentiate this condition from TBI and sequelae.

Funding: NIH, DoD, WWE, Concussion Legacy Foundation, private foundation.

Keywords: Impact, Chronic Traumatic Encephalopathy, Modeling, Blast, Neurobehavior

DBB-04

APPLICATION OF AN ACTIVITY TRACKER AND MOBILE APPLICATION TO TRACK ACTIVITY VERSUS REST FOLLOWING SPORT-RELATED CONCUSSION

Daniel Huber, Michael McCrea, Lindsay Nelson

Medical College of Wisconsin, Neurosurgery, Milwaukee, USA

Clinical management guidelines for sport-related concussion (SRC) emphasize initial rest followed by a gradual return to activities. How to

operationalize “rest,” however, is unclear, because the relationship between activity and SRC recovery is complex and dynamic. The aim of this pilot study was to develop and implement procedures for tracking activities after SRC and to explore associations between activities and recovery. High school and collegiate football players (25 concussed, 15 uninjured contact-controls) enrolled within 48 hours of injury and provided data daily for two weeks through a survey embedded into a smartphone application and a commercial fitness tracker (Fitbit). Metrics collected included magnitude and duration of physical activities, mental activities, sleep, and recovery. Completion rate of the daily data was 85%. As expected, concussed athletes reduced their activity levels (relative to controls) acutely after injury and gradually increased them to normal over the first two weeks post-injury, with somewhat different metrics for the Fitbit and mobile survey showing this pattern. Furthermore, there was significant variability across athletes in activities and recovery rates. For example, about 1/3 of athletes endorsed doing an activity that they believed would not be approved by their athletic trainers or doctors in the first week, and 3/4 avoided an activity that they believed could harm their recoveries. Endorsement rates of activity-related symptom exacerbation were extremely high in the first several days post-injury and diminished over time. However, endorsement of an unapproved activity or activity-related symptom exacerbation was not associated with recovery time. The results support the use of these technologies to remotely track athletes’ activities and suggest that activity that follows a graded exertion protocol or athletes’ judgment may be safe for clinical recovery after SRC. Future studies will evaluate the subset of athletes (nearly 1/3) who engaged in vigorous activity while symptomatic and will gather more information about the types of avoided and unapproved activities.

Keywords: concussion, sport, activity

DBB-05

PROLONGED KING-DEVICK TIME IN CONCUSSION PATIENTS

Briana Meyer, Meeryo Choe, Constance Johnson, Philip Rosenbaum, Sonal Singh, Zhe Wang, David McArthur, Christopher Giza
University of California, Los Angeles, Pediatric Neurology, Los Angeles, USA

The King-Devick (KD) test is a vision-based rapid number-naming task used to detect impairment in concussed individuals, primarily assessed on the sideline assessment. We tested the following hypotheses: (1) acute and chronic concussion will affect total KD time and (2) KD time will correlate with symptom recovery. KD was administered as recommended to three groups: High school student athletes (13–18 years, 234 females, 149 males) at preseason baseline evaluations during the 2016–2017 school year, patients diagnosed with an acute concussion ≤ 28 days (12–18 years, 21 females, 29 males) and patients diagnosed with a chronic concussion > 28 days (12–18 years, 26 females, 33 males). [GC1] Each participant completed Graded Symptom Checklist (GSC) providing a total score and four symptom subtypes: somatic, cognitive, sleep and emotional. In hypothesis 1, we examined group differences and found group (baseline, acute and chronic) had a significant effect on KD time ($F_{2, 489} = 18.91, p < 0.001$). KD time was significantly greater in the acute group than both the baseline and chronic groups ($p < 0.01$). Though not statistically significant, KD time was greater in the chronic group compared baseline ($p = 0.06$) [GC2]. In hypothesis 2, we evaluated the robust correlation between GSC total, GSC subtype and KD time in each group. No significant correlations were reported in the baseline or chronic groups ($r_{pb} = [GC3] 0.04$ and 0.08). KD time. In the acute group, GSC total and KD were significantly correlated ($r_{pb} = 0.51$). Somatic and cognitive symptom subsets were most strongly correlated to KD ($r_{pb} = 0.89$ and 0.80). Slower KD times in the acute concussion group show a

possible injury-related deficit in KD performance that correlates with concussion symptoms (GSC). Potentially, KD can be an objective measure of concussion and/or concussion related symptoms in the acute clinic setting. GSC and KD did not correlate in the chronic group, suggesting persistent symptoms may be due to other factors. High variability of KD time in all three groups supports the need for an individual baseline.

Supported by: UCLA BIRC, UCLA Steve Tisch BrainSPORT Program
Keywords: King-Devick, Vision, Clinical Setting, Recovery

DBB-06

ACTIVATION OF CANNABINOID RECEPTOR 2 ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED INFLAMMATION

Molly Braun¹, Zenab Khan^{1,2}, Nancy Saad³, M Badruzaman Khan⁵, Lin Yin³, David Hess⁵, Md Nasrul Hoda⁴, Babak Baban³, Krishnan Dhandapani¹, Kumar Vaibhav¹

¹Augusta University, Neurosurgery, Augusta, USA

²Augusta University, College of Nursing, Augusta, USA

³Augusta University, Oral Biology, Augusta, USA

⁴Augusta University, Department of Medical Laboratory, Imaging, and Radiological Sciences, Augusta, USA

⁵Augusta University, Neurology, Augusta, USA

Inflammation is an important component of secondary neurological injury after TBI. Immune cells, particularly macrophages, aid in clearance of cellular debris and tissue repair, but sustained release of pro-inflammatory mediators from infiltrating immune cells may exacerbate neuronal death, increase neurovascular injury, and contribute to long-term loss of white matter. Although the precise mechanisms underlying the dual beneficial and detrimental roles of macrophages after CNS injury remain poorly defined, it has been well established that macrophages polarize along a continuum from a classical pro-inflammatory (M1) state to an alternative anti-inflammatory (M2) state. Thus, modulation of macrophage polarization may identify novel opportunities for therapeutic intervention after TBI. Non-psychoactive cannabinoid receptor 2 (CB2R) is predominantly expressed on immune (lymphocytes, monocytes, macrophages) and endothelial cells in both rodents and humans. In the present study, we hypothesize that activation of CB2R attenuates inflammation post-TBI. Using a moderate controlled cortical impact (CCI) model of murine TBI, we assessed the endogenous upregulation of CB2R after TBI and the effects of CB2R agonist and antagonist post-injury. We observed acute upregulation of CB2R up to 3 days post TBI and moderate elevated expression persisted at 3 weeks. Furthermore, CB2R co-localized with infiltrated myeloid cells, specifically macrophages, after TBI. Finally, administration of selective CB2R agonist GP1a attenuated neuroinflammation, while treatment with antagonist AM630 worsened the injury. Interestingly, attenuation of inflammation by GP1a was associated with increased M2 polarization of macrophages. Our findings suggest that CB2R may play an important role in immune cell regulation; therefore, the development of selective CB2R agonists may provide clinical benefits for brain injury patients, without the psychoactive effects of CB1R activation.

Keywords: traumatic brain injury (TBI), cannabinoid receptor 2 (CB2), inflammation, macrophage

DBB-07

NADPH OXIDASE-2 REGULATES NLRP3 INFLAMMASOME ACTIVATION AFTER TRAUMATIC BRAIN INJURY

Merry Ma^{1,2}, Jing Wang^{1,2}, Krishnan Dhandapani^{2,3}, Darrell Brann^{1,2}

¹Medical College of Georgia - Augusta University, Neuroscience and Regenerative Medicine, Augusta, USA

²Charlie Norwood VA Medical Center, Augusta, USA

³Medical College of Georgia - Augusta University, Neurosurgery, Augusta, USA

Traumatic brain injury (TBI) contributes to over 30% of injury related deaths. A complex secondary injury cascade follows the initial mechanical injury and exacerbates the primary injury. Oxidative stress and inflammation play key roles in this TBI pathology. NADPH oxidase-2 (NOX2) and inflammasomes have been reported as major contributors to oxidative stress and inflammation in the injured brain, respectively, and deletion of NOX2 is neuroprotective. In particular, the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome can become activated in response to oxidative stress, but little is known about this mechanism following TBI. In this study we utilize NOX2 knockout mice to study the role of NOX2 in mediating NLRP3 inflammasome expression and activation in a controlled cortical impact model of focal TBI. Expression of NLRP3 inflammasome components (NLRP3, apoptosis speck-like protein containing a CARD (ASC), and caspase-1) was robustly increased in the injured cerebral cortex following TBI. Deletion of NOX2 attenuated the expression and assembly of the NLRP3 inflammasome components, which was coupled to reduced cleavage of the pro-inflammatory factor, interleukin-1 β (IL-1 β). Further work revealed that NOX2 regulation of NLRP3 inflammasome may be mediated by thioredoxin interacting protein (TXNIP), a sensor of oxidative stress. In conclusion, the results of the current study provide novel evidence that NOX2-dependent inflammasome activation contributes to TBI pathology.

Keywords: NADPH Oxidase 2, NLRP3, Inflammasome, Traumatic Brain Injury, Oxidative Stress, Controlled Cortical Impact

DBB-08

DIACYLGLYCEROL LIPASE- β KNOCKOUT MICE: SURVIVAL PROTECTIVE PHENOTYPE FROM TRAUMATIC BRAIN INJURY

Lesley Schurman (nee O'Brien)¹, Terry Smith², Linda Phillips², Thomas Reeves², Aron Lichtman¹

¹Virginia Commonwealth University, Pharmacology & Toxicology, Richmond, USA

²Virginia Commonwealth University, Anatomy and Neurobiology, Richmond, USA

The endogenous cannabinoid 2-arachidonyl glycerol (2-AG) serves as a rate-limiting precursor for arachidonic acid (AA), a precursor for the production of pro-inflammatory eicosanoids in brain. *In vitro* work shows that inhibiting the 2-AG biosynthetic enzyme diacylglycerol lipase- β (DAGL- β), which is highly expressed on CNS resident microglia and macrophages in the periphery, reduces inflammatory responses. Here, we examine whether DAGL- $\beta^{-/-}$ mice display a protection phenotype from the consequences of experimental traumatic brain injury. *Experiment 1*: Male DAGL- $\beta^{-/-}$ and $^{+/+}$ mice were subjected to a left lateral moderate Fluid Percussion injury (FPI) (1.94 ± 0.1 atm), and then assessed for spatial memory performance in a Morris water maze Fixed Platform task as well as for neurological motor impairments using the Neurological Severity Score (NSS) and the Rotarod assay. Unexpectedly, DAGL- $\beta^{-/-}$ mice demonstrated a significant survival protective phenotype (100% survival) in response to brain injury compared to DAGL- $\beta^{+/+}$ mice (77% survival), despite equal injury severities between groups. However, DAGL- $\beta^{-/-}$ mice displayed similar magnitudes of FPI-induced cognitive impairments in the Fixed Platform task and neurological motor deficits in the NSS and Rotarod assays as DAGL- $\beta^{+/+}$ mice. *Experiment 2*: We increased the magnitude of FPI to 2.0 ± 0.1 atm and 2.17 ± 0.1 atm in male and female DAGL- $\beta^{-/-}$ and $^{+/+}$ mice, and assessed mortality. Male DAGL- $\beta^{-/-}$ mice

demonstrated a significant survival protective phenotype at both 2.0 atm (100% survival) and 2.17 atm (90% survival) compared to DAGL- $\beta^{+/+}$ mice (75% survival at 2.0 atm, and 60% survival at 2.0 atm). In contrast, female DAGL- β mice generally survived both injury magnitudes regardless of phenotype, suggesting sex differences in survival from FPI. These findings suggest the provocative possibility that DAGL- β activity contributes to TBI-induced mortality, but not to the evolution of TBI cognitive or motor impairments in mice. Accordingly, DAGL- β inhibition represents a potential strategy to ameliorate post-traumatic fatality.

Keywords: Endogenous Cannabinoid, Mortality, Diacylglycerol lipase- β , 2-arachidonylglycerol (2-AG), Spatial Memory, Sex Differences

DBB-09

DEVELOP A NOVEL STRATEGY TO ENHANCE AXON REGENERATION AND NEUROPLASTICITY AFTER CNS INJURY

Shuxin Li¹, Yosuke Ohtake¹, Lena Ma¹, Makoto Horiuchi¹, Jason Berke¹, Shin-ichi Muramatsu²

¹Temple University School of Medicine, Dept. of Neurology, Philadelphia, USA

²Jichi Medical University, Division of Neurology, Shimotsuke, Japan

Axons in adult mammalian CNS fail to regenerate and there are no effective regenerative strategies to treat patients with CNS injuries. Several genes, including PTEN/mTor, Kruppel-like factors and insulin/insulin-like growth factor 1 receptor, partly regulate axon regeneration failure in adult CNS of mammals. Here we report the crucial role of liver kinase B1 (LKB1) in mediating CNS regeneration after CNS injury. LKB1 is a key serine/threonine kinase required for maintaining cell metabolism, energy homeostasis and cell polarity by activating a number of kinases. As the downstream effector of cAMP/PKA and PI3 kinase pathways, LKB1 is a major determinant for migration and differentiation of various cells, including neurons. We thus evaluate the role of LKB1 in regulating regenerative capacity of neurons in adult mammals using transgenic and adeno-associated virus (AAV) vector approaches. Upregulation of LKB1 significantly enhanced neurite extension of adult neurons cultured on inhibitory substrates CNS myelin and aggrecan. Transgenic overexpression of LKB1 stimulates robust regeneration of corticospinal tract (CST) axons in adult mice with mid-thoracic spinal cord injury. Local injections of AAV2-LKB1 into sensorimotor cortex or systemic application of mutant AAV9-LKB1 promote long distance regeneration of injured CST axons into the caudal spinal cord in adult mice. Systemic injection of mutant AAV9-LKB1 also enhances regrowth of descending serotonergic and tyrosine hydroxylase fibers. Importantly, LKB1 upregulation by either transgenic or viral approaches improves recovery of locomotor function after CNS injury. Therefore, LKB1 is critical for regulating growth capacity of mature neurons and may become an important target for designing highly effective therapies for CNS injury.

Keywords: Liver kinase B1, spinal cord injury, axon regeneration, Functional recovery, growth capacity

DBB-10

IN VIVO REPROGRAMMING OF NG2 GLIA INTO FUNCTIONAL NEURONS IN THE INJURED SPINAL CORD

Wenjiao Tai^{1,2}, Lei-Lei Wang^{1,2}, Chun-Li Zhang^{1,2}

¹University of Texas Southwestern Medical Center, Molecular Biology, Dallas, USA

²University of Texas Southwestern Medical Center, Hamon Center for Regenerative Science and Medicine, Dallas, USA

Spinal cord injury (SCI) results in irreversible loss of neurons and disruption of neural circuits, with a consequence of motor and sensory dys-

function. The goal of the present study is to regenerate functional neurons after SCI. A key SCI-induced pathological feature is the formation of glial scars surrounding the injury site. NG2 glia constitute a major component of the glial scars and their presence at the injury sites is inhibitory for axon regrowth. Reprogramming these cells into functional neurons may provide a regeneration-based therapeutic strategy for SCI repair. We established a lentiviral approach to specifically target endogenous NG2 glia by using a human NG2 promoter. SCI was introduced by contusion in the adult mice. Our preliminary data showed that neurogenesis can be induced through injection of SOX2-expressing lentivirus under this promoter in both healthy and injured spinal cord. Immunohistochemistry revealed that induced DCX+ new neurons

pass through an ASCL1+ neural progenitor and proliferation phase. Through genetic lineage mappings, we confirmed parenchymal NG2 glia as a cellular source for the newly reprogrammed neurons. We also excluded nestin+ neural stem cells or FOXJ1+ ependymal cells along the central canal as a cellular origin. Importantly, these NG2 glia-derived new neuron can mature into synapse-forming cells *in vivo*. Neuronal maturation can be further promoted by neurotrophic factors. Our results demonstrate that reactive NG2 glia can be robustly reprogrammed into neurons *in situ*, opening up the possibility of using endogenous cells for spinal cord regeneration after injury.

Keywords: *in vivo* reprogramming, neurogenesis, spinal cord injury, NG2 glia

Scientific Lectures

AANS02 TREATMENT OF THE INJURED BRAIN

AANS02-02

ARE WE GETTING ANY BETTER AT DETECTING CORTICAL SPREADING DEPRESSION/SPREADING DEPOLARIZATION?

Ryan Hirschi¹, Gregory Hawryluk¹, Jessica Nielson², J. Russell Huie², Lara Zimmermann³, Rajiv Saigal⁴, Quan Ding⁵, Adam Ferguson², Geoffrey Manley²

¹University of Utah, Neurosurgery, Salt Lake City, USA

²University of California San Francisco, Neurosurgery, San Francisco, USA

³University of California at Davis, Neurosurgery, Sacramento, USA

⁴University of Washington, Neurosurgery, Seattle, USA

⁵University of California at San Francisco, Physiologic Nursing, San Francisco, USA

Brain tissue hypoxia is common after traumatic brain injury (TBI). Technology exists to detect hypoxia and guide corrective therapy. Current guidelines for the management of severe TBI recommend maintaining $P_{btO_2} > 15\text{--}20\text{ mmHg}$, however uncertainty persists as to the optimal treatment threshold. P_{btO_2} measures were prospectively and automatically collected every minute from consecutive patients admitted to the San Francisco General Hospital (SFGH) intensive care unit over a 6-year period. We analyzed mean P_{btO_2} values in TBI patients and the proportion of P_{btO_2} values below each of 75 thresholds between 0 mmHg to 75 mmHg over various epochs up to 30 days from time of admission. Patient outcomes were calculated using the Glasgow Outcome Scale. We explored putative treatment thresholds by generating 675 separate receiver operator curves (ROC) and 675 generalized linear models (GLM) to examine each 1 mmHg threshold for various epochs. A total of 1,380,841 P_{btO_2} values were recorded from 190 patients recovering from TBI. A high proportion of P_{btO_2} measures were below 20 mmHg irrespective of examined epoch. Time below treatment thresholds was more strongly associated with outcome than mean P_{btO_2} . A treatment window was suggested; a threshold of 19 mmHg most robustly distinguished patients by outcome, especially from days 3–5. Benefit to maintaining values at least as high as 33 mmHg was suggested, however. Our “big data” analysis substantially informs the relationship between P_{btO_2} values and outcome. We were able to discern a therapeutic window for P_{btO_2} in TBI patients along with minimum and preferred P_{btO_2} treatment thresholds. Traditional treatment thresholds which have the strongest association with outcome may not be optimal.

Keywords: Brain oxygenation, P_{btO_2} , Outcome, Threshold, Treatment window

PL01 NEUROTRAUMA-INDUCED AUTOIMMUNITY

PL01-02

POST-TBI HYPOPITUITARISM AND ANTI-PITUITARY AND ANTI-HYPOTHALAMUS ANTIBODIES

Hasan Fahrettin Kelestemur

Erciyes University, Endocrinology, Kayseri, Turkey

Traumatic brain injury (TBI) which is a leading cause of death and disability is a significant public health problem worldwide. The causes of TBI are road traffic accidents, falls, violence, war accidents and sports-related head injuries including chronic repetitive head trauma seen in boxers and kickboxers. TBI is recognized as one of the most common causes of hypopituitarism. The pooled prevalence rate of any hormone deficiency is reported as 28%. GH is the most commonly lost hormone after TBI. The natural history of hypopituitarism after TBI is not well known. Pituitary function improves over time in a considerable number of patients, but it may also worsen rarely over the 1- and 3-year period after TBI. The mechanisms underlying the recovery and worsening of pituitary function have not been clarified yet. Anti-pituitary antibodies (APAs) are found in 50% of the TBI victims 3 years after TBI. The pituitary dysfunction development ratio is significantly higher in APA-positive patients when compared with APA-negative ones. According to the current data, a substantial number of patients with TBI develop permanent hypopituitarism, GH deficiency in particular, at the 5th year after TBI. There are strong associations between the presence of high titres of APAs and/or AHAs and hypopituitarism at the fifth year after TBI. Recent data demonstrated that sports-related chronic repetitive trauma including boxing and kickboxing may also result in pituitary dysfunction. Both APAs and AHAs are detected in boxers, but only AHA positive boxers may develop pituitary dysfunction. It seems that hypothalamic damage might be more important than pituitary damage in athletes exposed to chronic repetitive head trauma. Pituitary volume in retired boxers with GH deficiency has been found to be smaller than that in GH-sufficient boxers. Pituitary autoimmunity may be responsible for the shrinkage of pituitary volume over the years following TBI. Therefore, autoimmunity may play a role in the pathogenesis of TBI-induced pituitary dysfunction.

Keywords: Traumatic brain injury, hypopituitarism, anti-pituitary antibodies, anti-hypothalamus antibodies

PL01-04

GLUTAMATE RECEPTOR PEPTIDES AND AUTO-ANTIBODIES IN TBI AND ISCHEMIC BRAIN INJURIES

Svetlana Dambinova

DeKalb Medical Center, N.N. Burdenko Neurosurgery Institute, Brain Biomarkers Research Lab, Atlanta, USA

Brain impairments of traumatic and ischemic origin are connected to heterogenous profile of cerebral blood flow (CBF) deficit and neurovascular unit dysfunctions in cortical (grey matter), subcortical (white matter) and brainstem (cervical area). Ionotropic glutamate receptor (GluR) peptides are proposed as early indicators of vascular vasospasm associated with neurotoxicity and might serve as biomarkers of primary injury. It is hypothesized that neurotoxicity cascade after impact/onset is affecting to neuroplasticity and immune response in certain anatomical locations as well.

Our translational research of GluR biomarkers for concussions and mild TBI are presented in conjunction with neurological and radiological assessments. It was shown that ionotropic AMPAR subtypes are related to white matter denrite-axonal shearing and disconnections

causing the cognitive deficit and behavior changes after sport-related injury. Ionotropic kainate receptors are susceptible to carbon dioxide balance that potentially affects venous circulation in brainstem influencing involuntary life-sustaining functions and unconsciousness. At the same time NMDAR subtypes are involved in delayed cerebral ischemia and mental status decline.

Autoantibodies to AMPA, NMDA, and kainite receptor are neuroimmune tracers associated with severity of brain impairments radiologically defined in cortical, subcortical, and brainstem areas. The risk of delayed edema formation and microlesions development is associated with secondary injury and neurological complications.

Brain-derived biomarkers (GluR peptides and autoantibodies) detected in biological fluids by immunoassays may aid in stratification of the mild TBI/TIA/stroke assessments and in selection persons for advanced neuroimaging. Sideline or bedside testing of these biomarkers would assist to clinicians in triaging individuals with microstructural injuries for emergent therapeutic interventions.

Keywords: Translational research, Advanced neuroimaging, Neurological assessment

PL02 PAIN AFTER SCI: PLASTICITY, ACTIVITY, AND TREATMENT

PL02-01

PAIN AND LOCOMOTOR CIRCUITRY: COMPETITORS FOR ATTENTION

Catherine Mercier

Laval University, Quebec, Canada

Pain prevalence is particularly high in acute/subacute stage after a nervous system injury, and therefore a large proportion of patients have to undergo their motor rehabilitation in the context of pain. Surprisingly, very limited attention has been devoted to understanding the potential influence of pain on motor learning capacity and rehabilitation outcomes, despite a large body of evidence in animal models suggesting that central sensitization occurring in nociceptive pathways and plasticity related to motor learning interact with each other. In a series of recent studies, we assessed the effect of experimental nociceptive stimuli on the acquisition and next-day retention of a new locomotor task. Participants performed a locomotor adaptation task (perturbing force field applied to the ankle during swing using a robotized orthosis) on two consecutive days. Motor performance and motor strategies were assessed using kinematic measures and EMG activity. Across the different studies and experimental sessions, participants were either tested pain-free, or with cutaneous pain (topical application of capsaicin cream around the ankle). Pain had no significant effect on baseline gait parameters nor on motor performance (i.e. movement error reduction) on Day 1, but resulted in a different motor strategy (less anticipatory). A pain-free test performed on Day 2 showed that cutaneous pain can greatly interfere with retention, despite apparently normal performance during the acquisition. This finding appears to be explained more by the change in context between the motor acquisition and the retention test (i.e. pain vs. no pain) than to a direct interference of pain with the consolidation processes, as no retention deficit was observed when a similar pain was present on both days. The potential implications of these results for neurorehabilitation will be discussed.

Acknowledgments: C. Mercier is supported by a senior research scholar from Fonds de recherche Québec-Santé (FRQS), and the research was supported by a grant from the Canadian Institutes of Health Research (CIHR).

Keywords: motor learning, nociception, locomotion, spinal cord injury

S01 NOVEL APPROACHES TO TARGET MITOCHONDRIAL DYSFUNCTION FOLLOWING CNS INJURY: FROM BIOMARKER TO REPLACING THE DAMAGED POWERHOUSE

S01-01

CMX-2043 IMPROVES OUTCOMES AFTER FOCAL TBI

Todd Kilbaugh^{1,2}, Ross Plyler², Madeline Stowlow², Melissa Byro², Susan Margulies²

¹*Children's Hospital of Philadelphia, Philadelphia, USA*

²*University of Pennsylvania, Philadelphia, USA*

CMX-2043 (Ischemix) is a plasma stable Lipoic Acid-based small molecule that activates the Akt survival pathway through the PI3-Kinase. Additionally, CMX-2043 is a direct anti-oxidant with the added ability to upregulate endogenous antioxidants. Proof of concept trial for CMX-2043 in a porcine model of focal controlled cortical impact (CCI) traumatic brain injury (TBI) to determine effect on the mitochondrial electron transport chain (ETC), oxidative phosphorylation (OXPHOS), and mitochondrial reactive oxygen species (mtROS) generation. Piglets (N=16), 4-wk old approximately 8–9 kg, were randomized to 3 groups: Sham (N=5), CCI+Placebo (N=6), CCI+CMX-2043 (N=5). Treated animals received an intravenous loading, bolus of CMX-2043 (13.5 mg/kg) 1 hour post-CCI, and a 4.5 mg/kg bolus at 13 hours. At 24 hours post-TBI, fresh brain tissue was harvested and homogenized from the ipsilateral cortex (IPSI, penumbra zone) and mirrored contralateral region (CONTRA), and from corresponding cortex of shams. Real-time analysis of ETC function and simultaneous, mtROS was measured using a high resolution respirometry system (Oroboros) with an integrated fluorometer. mtROS generated with complex I (CI) and complex II (CII) substrates and the overall mitochondrial control ratio ([OXPHOS CI+CII]/[LEAKCI+CII]), during Oligomycin-induced respiration independent of ATP production were measured and compared across groups (ANOVA). The control ratio significantly decreased in both regions of CCI+placebo animals (IPSI: 6.0 ± 0.26 , $p < 0.0001$; CONTRA: 6.6 ± 0.32 , $p < 0.0001$) compared to sham cortex (19.44 ± 1.37). Treatment significantly improved the IPSI control ratio (CMX-2043 11.61 ± 2.28 , $p < 0.05$), but not the CONTRA (11.05 ± 1.71) compared to CCI+placebo. Treatment did not restore IPSI mitochondrial control ratio to sham levels. mtROS generation (in units of $\text{H}_2\text{O}_2/(\mu\text{mol O}_2/\text{s} \cdot \text{mg})$) was significantly increased in CCI+placebo on both the IPSI (18.77 ± 1.58 , $p < 0.0001$) and CONTRA (13.7 ± 0.58 , $p < 0.0001$) sides compared to sham (2.96 ± 0.19), with IPSI generation significantly greater than CONTRA. Treatment significantly lowered mtROS production on both sides (IPSI: 8.28 ± 1.47 , $p < 0.0001$; CONTRA: 5.13 ± 1.67 , $p < 0.0001$ versus CCI+placebo) to sham levels. CMX-2043 improves mitochondrial bioenergetics and limits mtROS generation following TBI.

Acknowledgments: Investigator-initiated study supported by Ischemix and University of Pennsylvania.

Keywords: mitochondria, reactive oxygen species, Preclinical Trial, porcine

S01-03

TRANSFER OF MITOCHONDRIA FROM ASTROCYTES TO NEURONS AFTER STROKE

Kazuhide Hayakawa¹, Elga Esposito¹, Xiaohua Wang^{1,2}, Yasukazu Terasaki¹, Yi Liu^{1,2}, Changhong Xing¹, Xunming Ji², Eng Lo¹

¹*MGH-Harvard, Neuroprotection Research Laboratory, Charlestown, USA*

²*Capital Medical University, Cerebrovascular Research Center, Beijing, China*

Recently, it was suggested that neurons can release and transfer damaged mitochondria to astrocytes for disposal and recycling¹. Here, we show that astrocytes can also release functional mitochondria that enter into neurons. Astrocytic release of extracellular mitochondria particles was mediated by a calcium-dependent mechanism involving CD38/cyclic ADP ribose signaling. Transient focal cerebral ischemia in mice induced astrocytic mitochondria entry to adjacent neurons that amplified cell survival signals. Suppression of CD38 signaling with siRNA reduced extracellular mitochondria transfer and worsened neurological outcomes. These findings suggest a new mitochondrial mechanism of neuroglial crosstalk that may contribute to endogenous neuroprotective and neurorecovery mechanisms after stroke.

Keywords: CD38, Extracellular mitochondria, ATP, Mitochondrial transfer

S02 PERIPHERAL ORGAN INVOLVEMENT IN CNS TRAUMA

S02-02

THE INFLAMMATORY RESPONSE FOLLOWING POLY-TRAUMA IN COMBINATION WITH MILD TRAUMATIC BRAIN INJURY

Fletcher White¹, Greg Gaski², Todd McKinley², Richard Rodgers³
¹Indiana University School of Medicine, Anesthesia, Indianapolis, USA

²Indiana University School of Medicine, Orthopediatric Surgery, Indianapolis, USA

³Indiana University School of Medicine, Neurosurgery, Indianapolis, USA

Background: Critically ill polytrauma patients frequently present with traumatic brain injury (TBI). Injury of this nature are accompanied by systemic release of proinflammatory cytokines. The objective of this study was to assess serum for the presence of cytokines and determine whether these inflammatory mediators correlate with the intensive care unit (ICU)/length of stay/morbidity in patients.

Methodology: The patient population consisted of 33 patients transported to a level I trauma center over 1 year. Descriptors meeting criteria for multiple organ dysfunction syndrome (MODS) criteria were identified for five of these seven systems on a daily basis. These included respiratory system (Po2/FIO2 ratio); the renal system (serum creatinine concentration); the hepatic system (serum bilirubin concentration); the hematologic system (platelet count); and the central nervous system (Glasgow Coma Scale). MODS scores from day 2 through day 5 were averaged and evaluated for criterion validity based on length of ICU stay and favorable/unfavorable outcome (< 5 days; patients with a favorable outcome or >5 days; patients with an unfavorable outcome). Serum samples were obtained on admission and at 8, 24 and 48 h after injury. Serum concentrations of 8 cytokines (HMGB1, sRAGE, IL-1b, IL-1RA, IL-6, IL-10, IL-17A and CCL2) were measured and compared.

Results: Discharge of patients with combined mild TBI and extracranial injury from ICU (< 5 days) exhibit diminished MODS scores and significantly lower serum cytokine levels for IL-1b, IL-1RA, IL-6, IL-10, IL-17A (n=5) within the first 48 hours than mild TBI/polytrauma patients with >5 days ICU (n=28). Serum concentrations of HMGB1 did not differ between the patient populations.

Conclusions: Cytokine secretion patterns appear to be different for patients developing complications when compared to patients with uneventful posttraumatic course. The development of immunomonitoring will help in the selection of the most appropriate treatment protocols for severely injured patients.

Keywords: DAMPs, MODS, polytrauma, extracranial

S02-03

INTESTINAL DYSFUNCTION FOLLOWING TBI: LESSONS FROM DROSOPHILA

David Wassarman, Barry Ganetzky, Rebecca Katzenberger
University of Wisconsin-Madison, Department of Medical Genetics, Madison, USA

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Unfavorable TBI outcomes result from primary mechanical injuries to the brain and ensuing secondary non-mechanical injuries that are not limited to the brain. To investigate the mechanisms underlying TBI pathologies, we developed a model of TBI in *Drosophila melanogaster*. Closed head TBI is inflicted with a mechanical device that subjects flies to rapid acceleration and deceleration. Similar to humans with TBI, flies with TBI exhibit temporary incapacitation, ataxia, activation of the innate immune response, neurodegeneration, and death. We demonstrate that age and diet activate distinct secondary injuries in a genotype-specific manner, and that concurrent activation of age- and diet-regulated secondary injuries synergistically increase mortality. Our Genome-wide Association study revealed that the probability of death following TBI is associated with single nucleotide polymorphisms in genes involved in tissue barrier function and glucose homeostasis. We found that TBI causes intestinal and blood-brain barrier dysfunction and that intestinal barrier dysfunction is highly correlated with the probability of death. Furthermore, we found that ingestion of glucose after a primary injury increases the probability of death through a secondary injury mechanism that exacerbates intestinal barrier dysfunction. Our results indicate that natural variation in the probability of death following TBI is due in part to genetic differences that affect intestinal barrier dysfunction.

Keywords: Intestine, *Drosophila*, GWAS, Neurodegeneration, Diet, Blood-brain barrier

S04 LATE EFFECTS OF TBI

S04-01

ASSOCIATION OF TRAUMATIC BRAIN INJURY WITH LATE-LIFE NEURODEGENERATIVE CONDITIONS AND NEUROPATHOLOGIC FINDINGS

Paul Crane
University of Washington, Medicine, Seattle, USA

Importance: The late effects of traumatic brain injury (TBI) are of great interest, but studies characterizing these effects are limited.

Objective: To determine whether TBI with loss of consciousness (LOC) is associated with an increased risk for clinical and neuropathologic findings of Alzheimer disease (AD), Parkinson disease (PD), and other dementias.

Design, Setting, and Participants: This study analyzed data from the Religious Orders Study (ROS), Memory and Aging Project (MAP), and Adult Changes in Thought study (ACT). All ROS and MAP participants and a subset of ACT participants consent to autopsy. Studies performed annual (ROS and MAP) or biennial (ACT) cognitive and clinical testing to identify incident cases of dementia and AD. The 7130 participants included members of a Seattle-area health care delivery system (ACT), priests and nuns living in orders across the United States (ROS), and Chicago-area adults in retirement communities (MAP). Of these, 1589 underwent autopsy. Data were accrued from 1994 to April 1, 2014.

Exposures: Self-reported TBI when the participant was free of dementia, categorized as ≤1 vs >1 hour of LOC.

Main Outcomes and Measures: Clinical outcomes included incident all-cause dementia, AD, and PD in all studies and incident mild

cognitive impairment and progression of parkinsonian signs in ROS and MAP. Neuropathologic outcomes included neurofibrillary tangles, neuritic plaques, microinfarcts, cystic infarcts, Lewy bodies, and hippocampal sclerosis in all studies.

Results: Of 7130 participants (2879 [40.4%] men; overall mean [SD] age, 79.9 [6.9] years), 865 reported a history of TBI with LOC. In 45,190 person-years of follow-up, 1537 incident cases of dementia and 117 of PD were identified. No association was found between TBI with LOC and incident dementia or AD. Associations were found for TBI with LOC and incident PD in ACT and progression of parkinsonian signs in ROS and MAP. TBI with LOC was associated with Lewy bodies and microinfarcts.

Conclusions and Relevance: Pooled clinical and neuropathologic data from 3 prospective cohort studies indicate that TBI with LOC is associated with risk for Lewy body accumulation, progression of parkinsonism, and PD, but not dementia, AD, neuritic plaques, or neurofibrillary tangles.

Keywords: Parkinson's disease, Lewy bodies, Alzheimer's disease, dementia, Alzheimer's pathology

S08 DAWN OF A NEW DAY: BRAIN EDEMA IS “BACK IN THE GAME” WITH NEW DISCOVERIES!

S08-01

WATER CHANNELS AND BRAIN EDEMA: SUCCESS AND FAILURE

Jerome Badaut

CNRS, UMR 5287-Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

Cerebral edema represents a major predictor for the patient outcome after several brain injuries. After the first description of water channel proteins, aquaporins (AQPs), in the brain led to intense research in understanding the underlying roles of each of them in the edema process. Here, the recent knowledge on the 3 main AQPs (AQP1, AQP4 and AQP9) will be reviewed with a look on the edema in TBI, SCI and stroke. Despite an evident crucial role of AQP4 in edema process, no specific therapeutic agents have been developed to either inhibit or enhance water flux through these channels.

Keywords: astrocyte, aquaporin, TBI, Edema

WS05 HUMAN TBI NEUROPATHOLOGY

WS05-01

HUMAN TBI NEUROPATHOLOGY: CTE AND THE LATE EFFECTS OF BRAIN INJURY

Willie Stewart^{1,2,3}

¹*NHS GGC, Dept. of Neuropathology, Glasgow, United Kingdom*

²*University of Glasgow, Institute of Neuroscience and Psychology, Glasgow, United Kingdom*

³*University of Pennsylvania, Dept. of Neurosurgery, Philadelphia, USA*

Few issues in research can have attracted as much attention as the association between traumatic brain injury and late neurodegenerative disease. Hollywood movies, endless news articles, billion-dollar litigation and polarised opinions in sport and research have contributed to the, quite literally, game-changing phenomenon that is currently discussed as chronic traumatic encephalopathy (CTE).

However, despite this attention, current understanding of the neuropathology of CTE is based on observations on remarkably few cases; reflecting the many challenges in research in human TBI

neuropathology, not least of which is case accrual from autopsy. Perhaps a consequence of the rarity of access to human tissues and experience in their interpretation, there are inevitable limitations in insight into the complex neuropathology of late survival from TBI.

Through a combination of pre-circulated case material, directed reading and case-based discussions this short interactive workshop is designed to provide attendees with insight into current understanding of the neuropathology of late survival from TBI in humans and the continued challenges to research in this field.

In advance of the workshop attendees will be provided with a link to access scanned tissues sections from exemplar cases, which will provide the context for discussions at NNS.

References:

Hay, J et al (2016). Chronic traumatic encephalopathy: the neuropathological legacy of traumatic brain injury. *Ann Rev Pathol*, 11, 21–45.

McKee, A, et al (2016). The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathol*, 131, 75–86.

Keywords: chronic traumatic encephalopathy, neuropathology, human studies, diffuse axonal injury, neuroinflammation, blood-brain barrier

WS06 PRECLINICAL COMMON DATA ELEMENTS: A COMMON LANGUAGE FOR THE COMMUNITY

WS06-03

PRECLINICAL COMMON DATA ELEMENTS: A COMMON LANGUAGE FOR THE TBI COMMUNITY

Leonie Misquitta¹, Michelle LaPlaca², Douglas Smith³, Edward Dixon⁴, Candace Floyd⁵, Edward Hall⁶, Lindsey Hulbert⁷

¹*Valencia College, Orlando, USA*

²*Georgia Tech, Atlanta, USA*

³*University of Pennsylvania, Philadelphia, USA*

⁴*University of Pittsburgh, Pittsburgh, USA*

⁵*University of Alabama at Birmingham, Birmingham, USA*

⁶*University of Kentucky, Lexington, USA*

⁷*Kansas State University, Manhattan, USA*

Multiple factors contribute to the challenge of reproducibility in pre-clinical research including, but not limited to, inconsistent naming conventions of relevant experimental factors. The goal of the TBI Preclinical Working Group effort was to identify experimental factors, variables, and conditions (data elements) that are critical for describing and thus reproducing the most frequently used preclinical outcome measures. Importantly, this effort is not meant to standardize methodology or limit innovation but rather to create a dictionary of common data elements for preclinical outcome measures with the goal of enhancing intra- and inter-laboratory data harmonization. The long-term goal of the TBI Preclinical Working Groups is to address the translational challenge in TBI through rigor and methodological transparency allowing for improved and accelerated therapeutic development that ultimately improve outcomes for TBI patients. The general working group has divided into Sub-Working Groups to create common data elements (CDEs) associated with: (1) General Health and Affective Disturbance tests, (2) Cognition and Motor tests, or (3) Large Animal Models. The resulting CDEs will be available for public comment and will eventually be accessible within the Federal Interagency Traumatic Brain Injury Research (FITBIR) Informatics System platform. These meetings explored the relationship between clinical phenotypes of TBI and animal models, discussing what constitutes useful translational model systems and CDEs for data standardization, particularly in the context of Research and Development decision making.

Keywords: Preclinical, Data, Common Data Elements, Traumatic Brain Injury

