Program:

24th Annual Kentucky Spinal Cord and Head Injury Research Trust Symposium

University of Kentucky
Lexington, KY

May 10, 2018
7:00-8:00 AM  Continental Breakfast, Registration, and Poster Set-Up  Lobby
8:00-8:10  Welcome and Opening Remarks  Room 124  
Kathy Saatman, Ph.D., Associate Director, Spinal Cord & Brain Injury Research Center (SCoBIRC), University of Kentucky

SESSION 1  Long-term consequences of Neurotrauma  Session 1  Room 124 Lee Todd
Session Chair: Adam Bachstetter, SCoBIRC, University of Kentucky

8:10-8:50  Mark Burns, Ph.D.  Georgetown University  
The effect of APOE genotype on outcome after experimental TBI

8:50-9:30  Warren Aliilain, Ph.D.  University of Kentucky  
Modeling cervical SCI to reflect the human population: Chronic injury, contusions, & genetic diversity

9:30-9:50  Chen Chen  Indiana University School of Med  
In vivo imaging of vascular injury following SCI

9:50-10:10  Victoria Jensen  University of Cincinnati  
Increasing the excitability of V2a neurons restores hemidiaphragm activity following SCI

10:10-10:30  Coffee break  Lee Todd Lobby

SESSION 2  Long-term consequences of Neurotrauma  Session 2  Room 124 Lee Todd
Session Chair: John Gensel, SCoBIRC, University of Kentucky

10:30-11:10  Junfang Wu, B.M., Ph.D.  University of Maryland  
Risk of dementia/depression and associated inflammatory mechanisms in spinal cord injury

11:10-11:50  Jon Godbout, Ph.D.  Ohio State University  
Consequences of microglial priming and immune reactivity in models of aging and TBI.

11:55-1:30  Lunch and Posters

SESSION 3  Neurotrauma Research along the Translational Spectrum  Room 124 Lee Todd
Session Chair: Brandon Miller SCoBIRC, Neurosurgery, U of Kentucky

1:30-2:10 PM  Steve Kirshblum, M.D.  Rutgers Med School  
Neurotrauma research along the translational spectrum: Clinical perspectives in SCI
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Introduction</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:10-2:50</td>
<td>Todd Kilbaugh, M.D.</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td></td>
<td>Biomechanical and age related effects on brain metabolism after a TBI</td>
<td></td>
</tr>
<tr>
<td>2:50-3:10</td>
<td>Coffee break</td>
<td>Lee Todd Lobby</td>
</tr>
<tr>
<td>SESSION 4</td>
<td>Scholars-in-Training</td>
<td>Room 124 Lee Todd</td>
</tr>
<tr>
<td></td>
<td>Session Chair: James Shaughnessy, DMD, Director, KSCHIRT Board</td>
<td></td>
</tr>
<tr>
<td>3:10-3:15</td>
<td>Session Introduction: James Shaughnessy, DMD</td>
<td></td>
</tr>
<tr>
<td>3:15-3:30</td>
<td>Scott Myers, Ph.D.</td>
<td>University of Louisville</td>
</tr>
<tr>
<td></td>
<td>Functional recovery after spinal cord injury is susceptible to Pde4b-mediated inflammatory responses driven by gut dysbiosis and endotoxemia</td>
<td></td>
</tr>
<tr>
<td>3:30-3:45</td>
<td>Michael Orr</td>
<td>University of Kentucky</td>
</tr>
<tr>
<td></td>
<td>Understanding the complexity of SCI through animal models</td>
<td></td>
</tr>
<tr>
<td>3:45-4:00</td>
<td>Tianci Chu, Ph.D.</td>
<td>University of Louisville</td>
</tr>
<tr>
<td></td>
<td>Complex signaling interplay in RAD-induced secondary hypoxic-ischemic brain injury in developing mice</td>
<td></td>
</tr>
<tr>
<td>4:00-4:15</td>
<td>W. Brad Hubbard, Ph.D.</td>
<td>University of Kentucky</td>
</tr>
<tr>
<td></td>
<td>Acute mitochondrial impairment underlies prolonged cellular dysfunction after repeated mild TBI</td>
<td></td>
</tr>
<tr>
<td>SESSION 5</td>
<td>Keynote</td>
<td>Room 124 Lee Todd</td>
</tr>
<tr>
<td></td>
<td>Session Chairs: W. Brad Hubbard &amp; Shelby Meier SCoBIRC, U of Kentucky</td>
<td></td>
</tr>
<tr>
<td>4:15-5:15</td>
<td>Patrick M. Kochanek, M.D., MCCM</td>
<td>University of Pittsburgh</td>
</tr>
<tr>
<td></td>
<td>Emerging targets and strategies for therapy development in acute brain injury</td>
<td></td>
</tr>
<tr>
<td>5:15</td>
<td>Closing Comments</td>
<td>Joe E. Springer, Ph.D. SCoBIRC Director, U. of Kentucky</td>
</tr>
</tbody>
</table>
The effect of APOE genotype on outcome after experimental TBI

Mark P. Burns, PhD
Georgetown University

The apoE protein is an important brain apolipoprotein whose primary function is to enable cholesterol transport. There are 3 apoE isoforms: apoE2, apoE3 and apoE4 – encoded for by polymorphisms in the APOE gene. The APOE4 allele, with a frequency of ~14%, is best known as a genetic risk factor for the development of Alzheimer’s disease, but is also associated with poor outcome after TBI in humans.

The Burns Lab has studied the mechanisms by which the APOE4 gene impairs recovery after experimental TBI. Using the mouse controlled cortical impact model of TBI, we have found that the APOE4 gene results in changes to the neuroinflammatory response to injury, slower clearance of amyloid-beta from the brain after TBI, and impaired pericyte migration/regeneration and blood brain barrier repair after TBI. Following a mild head impact, we also report that APOE4 mice activate different subcellular pathways involved in synapse stability – resulting in divergent synaptic responses to head impact in APOE4 mice compared to APOE3 or wildtype mice.

These data show that the apoE protein plays an important role in multiple repair pathways after TBI, and the presence of APOE4 allele prolongs a variety of deleterious cellular responses that results in the overall delay of spontaneous recovery and repair after injury.

Biography:
Mark has a BS in Physiology and a PhD in Neuropharmacology from the National University of Ireland in Galway, Ireland where his graduate thesis focused on animal models of depression. His postdoc research at both the Nathan Kline Institute in New York, and later at Georgetown University, examined how apoE genotypes and cholesterol levels impacted amyloid-beta production in mouse models of Alzheimer’s disease. He began the transition to traumatic brain injury research in collaboration with Alan Faden’s lab in 2006, studying the question of why TBI causes a rapid production and accumulation of amyloid-beta. In 2009 he established his own lab in Georgetown University to study the mechanisms by which TBI causes chronic neurodegenerative disease. His lab is funded by R01, UG3, and R21 grants from the NINDS.
Modeling cervical SCI to reflect the human population: Chronic injury, contusions, & genetic diversity

Warren J. Alilain, Ph.D.
Spinal Cord & Brain Injury Research Center
University of Kentucky

Spinal cord injury (SCI) most often occurs at the cervical level. At this level are the bulbospinal projections and motor neurons which mediate breathing. Therefore these injuries can result in respiratory motor insufficiency and a reliance on a mechanical ventilator in order to survive. The main goal of our laboratory is to restore breathing function after cervical SCI. Results from our lab have indicated that immediately after injury, there is a dramatic upregulation of inhibitory extracellular matrix molecules – the perineuronal net (PNN) and its main component, chondroitin sulfate proteoglycans (CSPGs). Digestion of these inhibitory factors with the bacterial enzyme, chondroitinase ABC (ChABC), can promote recovery. However, there is a differential response between administration of ChABC at acute and chronic timepoints after injury, with there being more recovery if treatment is delayed. Moreover, there are significant differences observed when moving from a lateral C2 hemisection model towards the more clinically relevant cervical SC contusion model. Lastly, preliminary results from our latest studies in the lab suggests that human genetic diversity which is overlooked in these pre-clinical animal models may influence the recovery process. Collectively, as we move forward towards human application of experimental approaches to restore function, a variety of factors still have to be considered in order to fully realize the maximal effectiveness of these therapeutic strategies.

Biography:
Warren J. Alilain received a BS in both Cell Biology and Biochemistry at the University of California, San Diego (UCSD). After graduation he was a research assistant at UCSD and the VA Medical Center. During his time there, he spent one summer with Dr. Mark Tuszynski where he was first exposed to SCI research. Those early experiences led him to focus his graduate and post doctoral research on SCI and the underlying plasticity which can help mediate the recovery process. For those years he was trained by some of the leaders in the field. Warren earned his Ph.D. in the laboratory of Dr. Harry G. Goshgarian, a pioneer and recognized expert in the field of respiratory motor deficits following SCI. As a post doc, he took the lateral C2 hemisection model of SCI to the laboratory of Dr. Jerry Silver where they applied his groundbreaking work on regeneration, inhibitory proteoglycans, and the perineuronal net. In his present position as an independent scientist he developed a novel cervical contusion injury model with a robust respiratory motor deficit and a way of accurately evaluating potential therapies aimed at repairing the injured cervical spinal cord. Warren is very interested in SCI, plasticity in both injury and learning models, the therapeutic potential of stem cells, and investigating new ways to restore function. His immediate career goals include acquiring and developing new technical skills and conceptual ideas in SCI research and conducting novel investigations with the ultimate aim of translating his work to an effective clinical treatment. Generous fellowships and grants from the Christopher and Dana Reeve Foundation, the Craig H. Neilsen Foundation, the International Spinal Research Trust, Conquer Paralysis Now, the National Institutes of Health, and the Department of Defense/Congressionally Directed Medical Research Programs have supported Warren’s lab.
In vivo imaging of vascular injury following spinal cord injury

Chen Chen 1,2, Yi Ping Zhang 3, Yan Sun 1, Christopher B. Shields 3, Wenhui Xiong 1, Xiaoming Jin 1, and Xiao-Ming Xu 1

1. Spinal Cord and Brain Injury Research Group, Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, IN, 46202, USA
2. Program in Medical Neuroscience, Paul and Carole Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, USA
3. Norton Neuroscience Institute, Norton Healthcare, Louisville, KY 40202, USA

The vascular network provides blood circulation and regulates the exchange of gasses and nutrients with the spinal cord through the blood-spinal cord barrier (BSCB), forming a pair of closely connected systems. Disruption of the vascular system, initiated by the primary mechanical impact of spinal cord injury (SCI), could break the supply-and-demand balance and exacerbate the progression of neuronal and glial cell damage originating from the injury epicenter. Compared to neurons and glial cells, the acute vascular dynamic changes following SCI have been understudied. Here we utilized our two-dye in vivo imaging method, using two-photon laser scanning microscopy (TPLSM), to characterize a temporospatial map of acute vascular dynamic changes following a contusive SCI. While both vascular effects and biochemical cellular alterations including excitotoxicity concurrently occurred in the direct injury site, we specifically found that vascular velocity, diameter, and permeability were all affected in the adjacent area, so-called “transitional zone”, as early as 0.5 hours after injury. In the transitional zone, the vessels were dilated and displayed increased permeability up to 4-hour post-injury, whereas our histological data showed the neurons and other types of cells were spared until 1 day after the injury. These data suggest that simultaneously with what happened in the epicenter, the vascular damage has occurred in the “transitional zone” prior to other cellular responses to the injury, which indicates a time window between vascular disruption and neuronal loss at the transitional zone. In addition, targeting towards these early vascular responses and protecting vascular integrity at an early phase of SCI could provide early neuronal protection and delay the expansion of damaged tissue in the spinal cord.

Biography:
Chen Chen received her undergraduate degree in biotechnology at Indiana University-Bloomington, College of arts and sciences in 2010. She went on to become a Ph.D. student under the supervision of Dr. Xiao-ming Xu, in Medical Neuroscience program in Paul and Carole Stark Neurosciences Research Institute (STARK) from Indiana University School of Medicine ever since 2013.

Her research interest is to study mechanisms underlying spinal cord injury (SCI) and to develop novel repair strategies to promote neural reorganization and functional recovery in experimental models of these injuries. More specifically, her current thesis work aims to address mechanisms of early vascular disruption after spinal cord injury and identify new targets for therapeutic intervention. Using in vivo two-photon microscopy, she is interested in capturing the dynamic vascular changes and the benefits of therapeutic drugs. This ongoing project is supported by Indiana Spinal Cord and Traumatic Brain Injury Research Fund Grant Program from Indiana State Department of Health. Another research interest of hers is focused on human immature astrocytes transplantation after spinal cord injury.
Increasing the excitability of V2a neurons restores hemidiaphragm activity following spinal cord injury.

Victoria Jensen  
University of Cincinnati

Here we investigate the therapeutic potential of glutamatergic V2a neurons to restore diaphragm activity following a C2 hemisection (C2hx). Our lab has shown that V2a neurons project to the preBotzinger complex and are required to maintain the frequency and regularity of breathing in neonatal mice [Crone et al., 2012]. Furthermore, V2a neurons in the brainstem and spinal cord are synaptically connected to phrenic motor neurons and accessory respiratory muscle motor (ARM) neuron pools. Interestingly, chronic electromyography (EMG) and plethysmography recordings from mice show that decreasing the excitability of V2a neurons activates ARMs and enhances ventilation without depressing diaphragm activity. Increasing the excitability of V2a neurons also activates ARMs and enhances ventilation, but disrupts diaphragm activity by causing tonic diaphragm EMG activity during interbreath intervals. It has recently been shown that V2a neuron connectivity to phrenic motor neurons is increased two weeks following a C2hx in mice [Zholudeva et al., 2017]. Therefore, we chronically recorded diaphragm EMG to determine whether activating V2a neurons can restore ipsilateral diaphragm activity following a C2hx in mice and if this ability changes as V2a connectivity to phrenic motor neurons is increased following injury. We show that as soon as one day following a C2hx, increasing the excitability of V2a neurons sporadically restores rhythmic diaphragm bursting activity.

**Biography:**  
Victoria Jensen received her Bachelor of Arts in Neuroscience from Carthage College. She is a 3rd year Ph.D. graduate student at the University of Cincinnati in Dr. Steven Crone’s lab, focusing her dissertation research on the role of glutamatergic V2a neurons in the control of respiration.
Risk of dementia/depression and associated inflammatory mechanisms in spinal cord injury

Junfang Wu, Ph.D.
University of Maryland School of Medicine

Spinal cord injury (SCI) research has focused on sensorimotor deficits, neuropathic pain and/or autonomic dysfunction. Although not well appreciated clinically, SCI can cause cognitive impairment including deficits in learning and memory, executive function, attention, and processing speed; it also commonly leads to depression. Although cognitive alterations have been discounted as likely reflecting undiagnosed concurrent head injury (TBI), studies clearly show that SCI patients who present without a history of TBI may develop cognitive decline and other neuropsychiatric abnormalities. Use of an anti-inflammatory drug in one recent clinical trial improved mood after SCI. Yet, little basic research has addressed potential mechanisms for cognitive or affective disorders after injury. We report that cognitive impairment in Y-maze, novel objective recognition, and step-down fear conditioning tasks were increased in moderate- and severe-injury mice that also displayed depressive-like behavior as quantified in the sucrose preference, tail suspension, and forced swim tests. The potent microglial activator cysteine-cysteine chemokine ligand 21 (CCL21) was elevated in the brain sites after SCI in association with increased microglial activation. Such inflammation is associated with greater neuronal endoplasmic reticulum (ER) stress and reduction in the number of newly-generated immature neurons in the hippocampal dentate gyrus. These findings indicate that SCI causes chronic neuroinflammation that contributes to neuronal loss, impaired hippocampal neurogenesis and increased neuronal ER stress in important brain regions associated with cognitive decline and physiological depression. Accumulation of CCL21 in brain may subserve a pathophysiological role in cognitive changes and depression after SCI.

Biography:
Dr. Wu is an Associate Professor in the Departments of Anesthesiology, Anatomy & Neurobiology, and Center for Shock, Trauma and Anesthesiology Research. Her areas of expertise include neuroinflammation, neuroprotection, neuropathic pain following spinal cord injury (SCI). She obtained her PhD from Nanjing Medical University, China and Post-Doctoral Training at NIH, USA. She joined the Georgetown University faculty in 2007 and then moved to University of Maryland where she rose to the rank of Associate Professor. Her ongoing NIH-funded research is directed at the understanding the cellular and molecular mechanism of functional recovery after SCI and developing potentially therapeutic strategies.
Consequences of microglial priming and immune reactivity in models of aging and TBI.

Jon Godbout, Ph.D.
Ohio State University

Microglia are important immune responders to traumatic brain injury (TBI) and these responses may be neuroprotective or maladaptive. There is evidence that TBI causes increased “priming” and immune reactivity of microglia that persists months after injury. In addition, TBI results in a unique population of elongated and rod-shaped microglia in the cerebral cortex. Overall the microglial contribution to long term inflammation and pathophysiology after TBI is unclear. Therefore, the purpose of this presentation is to discuss the concept of microglia priming with age and injury and to provide insight into longer term changes in microglial physiology after TBI. For example, we show that microglia maintained a primed (MHCII+) and pro-inflammatory profile 30 days after diffuse injury induced by midline fluid percussion injury (mFPI). This priming was associated with robust immune reactivity to a secondary immune challenge that resulted in significant neurobehavioral complications. Related to long-term changes in microglia, we also show that mFPI induced lba1⁺ rod microglia formation in the somatosensory cortex 7 days post-injury (dpi). This corresponded with increased and prolonged expression of genes related to neuronal injury and inflammation. Novel data show that TBI-induced rod microglia were in close proximity to axotomized (ATF3⁺) neurons, spatially overlapped with dense (GFAP⁺) astrogliosis, and aligned with apical pyramidal dendrites. To better understand the activation profile of microglia and rod microglia 7 dpi, microglia were eliminated prior to TBI by CSF1R antagonism (PLX5622). Microglial elimination did not affect neuronal axotomy induced by TBI, but attenuated rod microglial formation and astrogliosis. Furthermore, nanoString analysis of cortical gene expression 7 dpi showed prolonged neuroinflammation that was ablated by PLX5622. Taken together, microglia respond to neurons injured by TBI, align along their apical dendrites, and function to augment astrocyte activation and promote persistent neuroinflammation.

Biography:

Dr. Godbout is a Professor of Neuroscience at The Ohio State University Wexner Medical Center. He is also the Assistant Director of Basic Research for the Institute for Behavioral Medicine Research and he is a member of the Center for Brain and Spinal Cord Repair. In addition, Dr. Godbout is the Co-Director of the Neurosciences Graduate Program. Dr. Godbout has a B.S. (1996) and a Ph.D. (2001) from the University of Illinois-Urbana and was a post-doctoral fellow with Dr. Rodney Johnson. As a Principal Investigator, Dr. Godbout's research has been concentrated in the areas of aging, neuroimmunology, and neurotrauma. Overall, his primary research aim is to determine the degree to which the bi-directional communication between the brain and immune system is affected by age, stress, and traumatic brain injury (www.godboutlaboratory.com). Dr. Godbout is an active member of the neuroscience community and serves on committees for the National Neurotrauma Society, Psychoneuroimmunology Research Society (PNIRS), and Society for Neuroscience. He is a member of the NNRS-NIH study section, serves on the Editorial Board for J. Neuroscience, and is a managing editor for Brain Behavior and Immunity. Dr. Godbout is an author on over 75 publications and his research is/has been supported by grants from the NIA, NIMH, AFAR, Abbott Nutrition, and OSUMC. Dr. Godbout has received several awards including: PNIRS Ader New Investigator Award (2009), Siddens Award for Distinguished Faculty Advising (2012), Department of Neuroscience Faculty Research Award (2013 & 2017) and OSU Excellence in Research Award (2018).
Neurotrauma research along the translational spectrum: Clinical perspectives in SCI

Steven Kirshblum MD
Rutgers New Jersey Medical School
Kessler Institute for Rehabilitation

There has been tremendous progress in many aspects of the basic science understanding of spinal cord injury (SCI). Translational research studies have grown over the recent decades offering significant optimism to persons with SCI. However, this promise has not necessarily led to major changes in treatment nor neurological/functional recovery for those injured. In this presentation, current rehabilitation oriented projects will briefly be discussed, (e.g. intermittent hypoxia, spinal stimulation) followed by the importance of the perspectives of the clinical needs and wishes of persons with SCI. This includes the impact of chronic medical complications (e.g. neurogenic bowel/bladder, pain, and spasticity), and the importance of focusing future research in these areas. Recommendations for moving forward in translational research will be suggested.

Biography:
Steven Kirshblum, M.D. is the Senior Medical Officer and Director of Spinal Cord Injury (SCI) Program for Kessler Institute for Rehabilitation, Chief Medical Officer at Kessler Foundation and Project Co-Director of the Northern New Jersey Spinal Cord Injury System (NNJSCIS)—a NIDILRR-funded Spinal Cord Injury Model System of care. He is the Chair and Professor in the Department of Physical Medicine and Rehabilitation (PM&R) at Rutgers New Jersey Medical School. Dr. Kirshblum was the former President of the American Paraplegia Society and Academy of Spinal Cord Injury Professionals (ASCIP) and currently serves on the Board of Director for the American Spinal Injury Association (ASIA).
Biomechanical and age related effects on brain metabolism after a TBI

Todd Kilbaugh, MD
University of Pennsylvania
Children's Hospital of Philadelphia

Dysregulated metabolism and energy deficits are prominent characteristics of traumatic brain injury (TBI) [1, 2]. Indeed, alteration in mitochondrial function is a critical component of the secondary injury cascade initiated by TBI, with emerging evidence suggesting that mitochondria may be the switch between ongoing injury, or regeneration and repair [3, 4]. Mitochondria are fundamental to cellular bioenergetics by coupling substrate oxidation and phosphorylation to energy production. Mitochondria also generate intermediary metabolites and reactive oxygen species (mtROS) that in pathologic states propagate secondary injury [5]. We believe that mitochondria are the fulcrum of the secondary injury cascade in TBI. Thus, the next generation of therapeutic development should build on mechanistic insights of mitochondrial function tailored to specific TBI pathology, severity, and patient populations. Several pre-clinical investigations have established that mitochondrial-directed therapeutics can limit secondary injury cascades and improve neurologic outcome [6-12]. Hence, mitochondria are a critical convergence point for cell survival following acute brain injury and represents a key target for pharmacological approaches to neuro-resuscitation [13-17]. Rodent data, while critical to our mechanistic understanding, does not seem to follow a similar time course and magnitude of alterations in bioenergetic response as gyrancephalic animals [18-20]. Gender is also known to influence bioenergetic response following stress and injury [21, 22]. Age specific mitochondrial responses in the pre-myelinated brain versus the myelinated adult brain following TBI seems to vary in bioenergetic response but is not comprehensively studied [23-25]. Finally, the differences in mitochondrial response following diffuse closed head TBI and focal contusion TBI, at similar time-points in porcine models of the same age, emphasizes the heterogeneity of brain injury pathology. In conclusion, investigations of mitochondrial metabolic pathways following specific injury mechanisms and taking into account patient variability are essential to direct future mitochondrial intervention trials in TBI. Using a translational approach and outcomes that focus on pharmacodynamic outcome metrics that are directly translatable into human clinical trials including neuroimaging and behavior we have continued to build a platform as a bridge directly to human clinical trials with a focus on identifying and testing mitochondrial directed therapeutics

Biography:
Todd Kilbaugh, MD is an Associate Professor of Anesthesiology, Critical Care Medicine and Pediatrics. Dr. Kilbaugh has spent his career studying acute brain injury in pediatric large animal models as a bridge to understanding mechanisms and mitochondrial response to traumatic brain injury, cardiac arrest, and cardiopulmonary bypass.
Emerging targets and strategies for therapy development in acute brain injury

Patrick M. Kochanek, MD, MCCM
University of Pittsburgh School of Medicine

Clinical trials of potential new therapies across much of the field of acute brain injury, including diseases such as traumatic brain injury, spinal cord injury, and cardiac arrest, along with drug development in stroke have failed to show translational success from the bench to the bedside. In this presentation, several new targets and approaches that are being explored by investigators at the Safar Center for Resuscitation Research and its collaborators will be discussed. This includes new therapeutic approaches to the somewhat forgotten pathophysiological target of brain swelling, the potential role of novel neuronal death pathways, new approaches to enhance perfusion after acute brain injury, the use of combination therapy to enhance drug delivery, strategies to monitor therapeutic efficacy with cerebrospinal fluid metabolomics and the use of serum target engagement biomarkers of brain edema, axonal injury, and mitochondrial damage, new directions for the use of therapeutic hypothermia, use of novel translational studies in brain injury rehabilitation, and finally new and emerging study design strategies for both pre-clinical research, such as multi-center pre-clinical consortia, and clinical trials, such as comparative effectiveness trials and adaptive trial design.

Biography:
Patrick M. Kochanek, MD, is the Ake N. Grenvik Professor of Critical Care Medicine; Director of the Safar Center for Resuscitation Research; and Professor and Vice Chairman of Critical Care Medicine at the University of Pittsburgh School of Medicine. He is also Professor of Pediatrics, Anesthesiology, Bioengineering and Clinical and Translational Science. At the Safar Center, he has a long track-record of leading a translational and multi-departmental team of investigators studying traumatic and ischemic brain injury and neurointensive care, funded by the NIH, US Department of Defense, and the Laerdal Foundation. He has >500 citations on PubMed and was identified by ISI as the most prolific author in the field of TBI from 2001 to 2014. He is PI of Operation Brain Trauma Therapy for the United States Department of Defense and has been PI for 18 years of a T-32 titled “Pediatric Neurointensive Care and Resuscitation Research” funded by the NICHD. He has mentored numerous trainees, many of whom have gone on to receive independent funding and careers of national prominence. He is Editor-in-Chief of Pediatric Critical Care Medicine and is on the editorial board of numerous journals in the field of acute brain injury. Dr. Kochanek received the Distinguished Investigator Award from the American College of Critical Care Medicine in 2007, the Critical Care Distinguished Career Award from the American Academy of Pediatrics in 2008, was named Master of Critical Care Medicine by the American College of Critical Care Medicine in its inaugural class, and received the Lifetime Achievement Award from the Society of Critical Care Medicine in 2017.
Functional recovery after spinal cord injury is susceptible to Pde4b-mediated inflammatory responses driven by gut dysbiosis and endotoxemia

Scott Myers, Ph.D.
University of Louisville

Emerging evidence links changes in the gut microbiome and intestinal barrier function to alterations in CNS function. Using a contusive thoracic mouse model of SCI, we examined the role of endotoxin-responsive, cAMP-specific, Pde4 subfamily b (Pde4b) expression in neuro-inflammation and white matter loss post-injury. In C57Bl/6 wild type female mice, SCI led to significant shifts in the gut bacterial community including a significant increase in the phylum Proteobacteria, which consists of endotoxin harboring gram-negative bacteria. This was accompanied by increased peripheral marker of microbial translocation (sCD14) along with measures of the endoplasmic reticulum stress response (ERSR) and inflammation in the spinal cord injury epicenter. We also used Pde4b−/− mice to investigate the role of the Pde4b isoform in neuroinflammation and white matter loss post-injury. Deletion of Pde4b reduced epicenter expression of markers for the ERSR and inflammation, at both acute and chronic time points post-SCI. Correspondingly, expression of oligodendrocyte mRNAs increased. Within the injury penumbra, inflammatory protein markers of activated astrocytes (GFAP), macrophage/microglia (CD11b, Iba1), and the proinflammatory mediator Cox2, were decreased in Pde4b−/− mice. The absence of Pde4b improved white matter sparing and recovery of hindlimb locomotion following injury. Interestingly, SCI-induced gut dysbiosis, bacterial overgrowth and endotoxemia were also prevented in Pde4b−/− mice. The SCI-induced markers of the ERSR and inflammation were acutely attenuated acutely (48 hr) in germ-free (GF) mice indicating the relevance of gut-derived bacterial endotoxin. Taken together, these findings indicate that gut-derived endotoxemia and PDE4B play an important role in neuroinflammation following SCI.

Biography:
Scott Myers earned his PhD in Pharmacology from Vanderbilt University and is currently a research scientist in the laboratory of Scott R. Whittemore in the Kentucky Spinal Cord Injury Research Center at the University of Louisville. His current research interests include identifying the role of the endoplasmic reticulum stress response in mediating endothelial cell apoptosis following spinal cord injury and investigating the ability of SCI-induced gut dysbiosis in mediating post-injury CNS inflammatory responses.
Understanding the Complexity of SCI through Animal Models

Michael B. Orr
University of Kentucky

Spinal cord injury (SCI) researchers commonly use rodent models with contusion or compression injuries. Clinically, SCI is biomechanically heterogeneous, which can affect secondary injury progression and the efficacy of neuroprotective therapies. One common SCI is the burst fracture, where the spinal cord suffers a contusion with subsequent sustained compression. The specific effects of these independent injury biomechanics is poorly understood. To determine the effects of compression subsequent to spinal contusion, we studied in C57/BL6 mice after SCI with or without a 20 sec compression at two contusion impact forces (50 and 75kdyn). At both impact forces, compression caused decreases in functional and anatomical recovery. Interestingly, compression-dependent damage did not lead to differences in anatomical or functional recovery until 1 week after SCI, but then induces an early cessation in recovery. To determine if this recovery plateau is indicative of compression-specific changes in the SCI microenvironment, we examined macrophage activation state in histological spinal samples. We detected a compression-specific increase in macrophage expression of MARCO, a marker associated with pathological phenotypes, and a compression-specific decrease in macrophage expression of Arginase 1, a marker associated with reparative phenotypes. Collectively, these results indicate that the addition of compression to contusion SCI influences the macrophage response and the recovery progression following mouse SCI. Moving forward, SCI biomechanics and their influences on the subsequent microenvironment should be considered when evaluating the efficacy of therapies in the lab and in the clinic.

Biography:
Michael Orr is a third-year graduate student in Dr. John Gensel's lab at the University of Kentucky. Michael's research uses animal models to better understand the cellular and extracellular contributors to the spinal cord injury microenvironment. His overall objective is to contribute to the development and effective implementation of therapies promoting endogenous spinal repair.
Complex signaling interplay in RAD-induced secondary hypoxic-ischemic brain injury in developing mice

Tianci Chu, M.D.
University of Louisville

Abusive head trauma (AHT) is the leading cause of death from trauma in infants and young children. Unfortunately, modeling of AHT, especially for flexion-extension rotational acceleration-deceleration injury (RADi), has not been developed in mice. In this study, an AHT animal model was developed on 12-day-old mice subjected to 90° head extension-flexion sagittal shaking repeated 30, 60, 80, and 100 times. The mortality and return of consciousness were dependent on the number of repeats and the severity of injury. Under 60 times of repeated head shakings, the pups demonstrated apnea and/or bradycardia immediately after injury. Acute oxygen desaturation was observed by pulse oximetry during respiratory and cardiac suppression. The cerebral blood perfusion was dramatically reduced immediately after the trauma but not significantly improved within 24 hours. The injured mice began to experience reversible sensorimotor function at 9 days post-injury (dpi), which were completely recovered at 28 dpi. However, cognitive deficits and anxiety-like behavior remained. Subdural/subarachnoid hemorrhage, brain-blood barrier damage, and parenchymal edema were found in all pups subjected to 60 insults. Pro-inflammatory response and reactive gliosis were up-regulated at 3 dpi. Degenerated neurons were found in cerebral cortex and olfactory tubercles at 30 dpi. Intriguingly, HIF-1α protein accumulated in injured cortex at least during the first 3 days after injury, probably due to the inhibition of PHD1 expression. HIF-1 target molecules, Nrf2 and HO-1, were also significantly up-regulated. After 3 dpi, HIF-1α significantly decreased, followed by the increase of phosphorylation of p38 MAPK. This mouse model of repetitive brain injury by rotational head acceleration-deceleration partially mimics the major pathophysiological and behavioral events that occur in children with AHT. The resultant hypoxia/ischemia suggests a potential mechanism underlying the secondary brain injury in developing mice. Most importantly, HIF-1, p38 MAPK, and Nrf2 signaling pathways may comprise a complex interplay that underlies the pathology of RADi-induced secondary hypoxic-ischemia.

Biography:
Dr. Chu received her M.D. in Clinical Medicine from Tianjin Medical University, China in 2015. She works as a postdoc in Dr. Jun Cai’s lab in the University of Louisville after graduation. With a medical background in both clinic and research, she focuses on the pathological changes and signaling interplay in brain and spinal cord injury and diseases. As a young researcher, Dr. Chu has produced ten peer-reviewed publications (four as first-author and six as co-author) in the field of central nervous system injury and diseases.
Acute mitochondrial impairment underlies prolonged cellular dysfunction after repeated mild traumatic brain injuries

W. Brad Hubbard, PhD\textsuperscript{1,2,3}, Binoy Joseph, PhD\textsuperscript{1}, Malinda Spry, MS\textsuperscript{1}, Hemendra Vekaria, PhD\textsuperscript{1}, Kathryn Saatman, PhD\textsuperscript{1,2}, Patrick Sullivan, PhD\textsuperscript{1,3,4}

\textsuperscript{1}Spinal Cord and Brain Injury Research Center, University of Kentucky
\textsuperscript{2}Department of Physiology, University of Kentucky
\textsuperscript{3}Department of Neuroscience, University of Kentucky
\textsuperscript{4}Lexington Veterans’ Affairs Medical Center

Mild TBIs (mTBIs), accounting for over 80\% of the 1.7 million TBIs reported yearly in the U.S., can cause cognitive and behavioral impairments, the severity and duration of which increase in individuals that sustain additional mTBIs. 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 bilateral diffuse closed head injury (CHI) to examine mitochondrial function after mTBI. Previous data outlines the time course of mitochondrial respiration, utilizing the Seahorse XFe24 Flux Analyzer, from bilateral ventral cortex (including entorhinal cortex) and bilateral hippocampus homogenates collected at 6, 24, 48, and 96h post-injury. These results show that mitochondria exhibit a decrease in State III (ADP-mediated) oxygen consumption rate (OCR) in both regions compared to sham at 48h post-injury with recovery by 96h post-injury. To investigate if this impairment determines cellular vulnerability after mTBI, repeated CHIs (rCHI) were given at intervals of 48h and 96h to examine whether mitochondrial dysfunction is worsened and/or prolonged after rCHI. rCHI at 48h or 96h intervals did not notably worsen the depression in State III respiration compared to a single CHI, but rCHI repeated at a 48h interval resulted in more prolonged cortical mitochondrial dysfunction. Markers of oxidative stress, 4-hydroxynonenal (4-HNE), 3-nitrotyrosine (3-NT), and protein carbonyls (PC), were measured from mitochondrial homogenates. All markers in the hippocampus and PC in the cortex were significantly elevated at 48h after rCHI delivered 48h apart, but not after single CHI or two CHI delivered 96h apart. On-going studies show that synaptic and non-synaptic mitochondrial populations, derived from novel magnetic labeling, likely have differing regional and temporal respiration profiles after rCHI. This study establishes that mTBI results in early mitochondrial dysfunction that has region-specific temporal characteristics and this dysfunction may be a determinant for cellular vulnerability to repeated head impacts.

\textbf{Biography:}

Dr. Brad Hubbard is a postdoctoral scholar in the Spinal Cord and Brain Injury Research Center at the University of Kentucky under the mentorship of Dr. Kathryn Saatman and Dr. Patrick Sullivan. Brad received a B.S. from the University of North Carolina at Chapel Hill and a Ph.D. in Biomedical Engineering from Virginia Tech. His graduate work in blast-induced injuries resulted in national presentation awards and several first-author and co-author publications. Currently, his research is focused on examining mitochondrial dysfunction after mild traumatic brain injury (mTBI) and whether it underlies prolonged cellular dysfunction after repeated mTBIs. He has received a pilot grant through NSF EPSCoR for these studies in addition to multiple travel awards to present his research.
Poster #1

Mammalian target of rapamycin signaling pathway primes quiescent neural stem cells to promote neuroregeneration after traumatic brain injury.

Xiaoting Wang¹ • Xiang Gao, PhD² • Jinhui Chen, MD, PhD²
¹Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis • ²Neurological Surgery, Indiana University School of Medicine, Indianapolis

Student

Neural stem/progenitor cells (NSPC) in the adult hippocampus supports lifetime learning and memory function, as well as provide great promise for brain repair. It is widely shown that traumatic brain injury (TBI) stimulated NSPC proliferation in the adult hippocampus, indicating an innate repair mechanism. We previously reported that activation of mammalian target of rapamycin (mTOR) signaling is required for TBI-enhanced NSPC proliferation. However, it is unknown how mTOR mediates this neuroregeneration. In current study, by a nestin-enhanced green fluorescent protein (EGFP) transgenic mouse line, we were able to visualize while distinguish NSCs from NPCs. We demonstrated that TBI primarily promoted NSC rather than NPC proliferation, through activating mTOR signaling mainly in NSCs. Inhibition of mTOR activity by rapamycin administration largely diminished NSC proliferation post-injury, leaving NPC proliferation unaffected. Moreover, we captured a large amount of NSCs with mTOR activation but out of cell cycle at 24 h after TBI. At 48 h after TBI, only a small proportion enters cell cycle, while majority returns back to quiescence. Thus, we hypothesized that mTOR primes NSCs and facilitates their entrance into a reversible state, in which NSCs are able to either enter cell cycle or return back to quiescence, presenting a novel fine-tuning regulatory node of NSCs activity. Further investigations on the characteristics of the mTOR active primed NSCs and mechanisms determining NSC fate decision at primed state will help detailed understand the NSC-mediated neuroregeneration after TBI, as well as help develop potential therapeutic applications of NSCs for neuronal replacement post-trauma.
Poster #2

Flubendazole reduces α-tubulin acetylation, endosomal signaling, and pain behaviors after spinal cord injury

Colin Rogers, PhD • Hina Iqbal • Kate Davis • Savannah Young • Chen Guang Yu, MD, PhD

Neuroscience, University of Kentucky

Faculty

Microtubule hyper-stabilization and α-tubulin acetylation (a marker for microtubule hyper-stabilization) are strongly involved in endosomal/mitochondrial signaling transport and pain transmission. For instance, microtubule-stabilizing drug taxol frequently produces neuropathic pain (Li, Y. et al., 2015), while mice lacking the α-tubulin acetyltransferase Atat1, an inducer of α-tubulin acetylation, display profound reduction in mechanical pain (More, SJ., 2016). However, their roles in endosomal/mitochondrial signaling and pain transmission have not been previously investigated in pain caused by spinal cord injury (SCI). Microtubule-destabilizing agent Flubendazole (FluBZ) has been widely used in the treatment of intestinal and neural parasites in human. Here, we show a novel anti-nociceptive effect of FluBZ on pain behaviors after excitotoxic SCI through inhibition of acetyltransferase MEC-17, α-tubulin acetylation, and endosomal/mitochondrial signaling. The excitotoxic SCI was produced by intraspinal microinjection of AMPA/metabotropic receptor agonist quisqualic acid (QUIS). Intraperitoneal (IP) injection with 10 mg/kg/day (n=10) of FluBZ to Sprague-Dawley rats for 1 week was administered 3 hrs post-QUIS injury at T12 and L2. This resulted in a significant delay in the onset of grooming behavior, reduction in size of the grooming area, and decreased grooming severity after QUIS injury in rats, compared to vehicle-treated controls (n=10). FluBZ IP treatment also reduced the incidence of the pain behaviors following excitotoxic SCI. Immunofluorescence imaging data showed that excitotoxic injury significantly upregulated α-tubulin acetylation, acetyltransferase MEC-17 expression, endosomal EEA1-NR1 signaling, and mitochondrial cyclin B1 protein levels in the dorsal horn of rat spinal cord after QUIS injury. Mechanistic studies revealed that FluBZ IP treatment significantly reduced the excitotoxic injury-induced upregulation of α-tubulin acetylation, acetyltransferase MEC-17, endosomal-NMDA receptor (EEA1-NR1) signaling, and mitochondrial cyclin B1 in the dorsal horn of the spinal cord. Accumulation of cyclin B1 in mitochondria has been shown to mediate mitochondrial dysfunction in neurons under excitotoxic conditions. In conclusion, our results suggest that: 1) acetyltransferase MEC-17, α-tubulin acetylation, endosomal NR1 signaling, and mitochondrial cyclin B1 may be involved in pain transmission and pain behaviors after excitotoxic injury; and 2) FluBZ IP administration may attenuate pain behaviors through inhibiting α-tubulin acetylation and endosomal NR1 signaling after excitotoxic SCI.
Poster #3

**Selectively inhibiting reactive astrocyte proliferation aggravated synaptic degeneration following traumatic brain injury**

Xiang Gao, PhD

¹Neurological Surgery, SNRI

**Faculty**

Traumatic brain injury (TBI) causes drastic network disconnections in the brain, by affecting both neuronal and glial components, resulting in broad range of neurological complications. Among the glial responses, post-traumatic astrocyte reactivation has been extensively reported. However, the beneficial or deleterious functions of astrocyte reactivation are constantly under debate. In current study, we addressed the questions in mouse TBI model by detailed elaborating spatial- temporal pattern of astrocyte reactivation, and further elucidating signaling pathway mediating injury induced astrocyte proliferation. Our data demonstrated that astrocyte reactivation occurred at as early as 4 h after initial insult and reached a peak at 72 h, largely attributed to injury induced astrocyte proliferation. Further, platelet-derived growth factor receptor α (PDGFRα) expression, normally absent in resting astrocyte, was transiently detected in reactive astrocyte, at 72 h after injury. Conditional knockout of PDGFRα in reactive astrocyte extensively attenuated astrocyte proliferation after injury, as well as exacerbated synaptic loss. Taken together, our data proved that injury induced PDGFRα expression in astrocyte mediates astrocyte proliferation, which benefits synaptic preservation after trauma. Our result sheds light on potential therapeutic development targeting glial component to improve functional outcomes in TBI patients.
Establishing the utility of the African spiny mouse as a novel mammalian model of spinal cord injury

Michael Orr¹ • Kirsten Richards¹ • Chris Calulot² • Ashley Seifert, PhD³ • John Gensel, PhD¹
¹Physiology, University of Kentucky • ²Neuroscience, University of Kentucky • ³Biology, University of Kentucky

Student

Less than 1% of hospitalized victims of spinal cord injury (SCI) experience full recovery by the time of discharge, which reflects an inability to regenerate injured spinal tissues and an absence of SCI therapies. Researchers commonly use regenerating, non-mammalian models to identify targets for inducing regeneration, but test therapies on mammals that recapitulate human SCI. Unfortunately, the phylogenetic gap between animal models creates a barrier to translation. A mammalian model with enhanced regenerative capabilities would serve as a powerful tool for identifying translatable therapeutic targets for inducing regeneration after SCI. Spiny mice, mammals closely related to mice, exhibit scar-free regeneration from peripheral injuries, which coincides with a suite of pro-regenerative inflammatory and extracellular matrix (ECM) responses. The inflammatory and ECM response are key regulators of SCI progression, but spiny mouse SCI responses and regenerative capacity remain uninvestigated. We hypothesize that spiny mice will exhibit pro-regenerative inflammatory and ECM responses to SCI, which will lead to enhanced axonal regeneration compared to lab mice. Histological and in vitro techniques have been established for comparative SCI studies in spiny mouse and mouse. Initial results indicate spiny mice and mice have comparable gross spinal neuroanatomy and similar dorsal root ganglion neurite outgrowth inhibition by chondroitin sulfate proteoglycans; preliminary data also indicate potential differences in SCI responses. Future studies will more closely analyze SCI responses and the subsequent effects on axon regeneration following SCI. This study will determine the utility of the spiny mouse as a novel mammalian model of SCI and spinal regeneration.
Tissue Oxygenation Following Spinal Cord Injury

Allison Spears¹ • Francois Pomerleau, MS² • Jorge Quintero, MS³ • James Geddes⁴ • Greg Gerhardt, PhD⁴ • Chen-Guang Yu, MD, PhD⁴
¹Spinal Cord and Brain Injury Research Center, University of Kentucky • ²Neuroscience, University of Kentucky • ³Neuroscience - College of Medicine, University of Kentucky • ⁴College of Medicine, University of Kentucky

Student

Spinal cord injury damages not only the underlying tissue, but also the vascular supply to the spinal cord. This may result in reduced oxygenation of spinal cord tissue and a resultant impairment of neural activity. Maintenance of tissue oxygenation and cerebral blood flow is standard of care for traumatic brain injury. Surprisingly, however, there is a paucity of information regarding effects of spinal cord injury on tissue oxygenation and neural activity in the first few minutes to hours following the injury. Using a single Bioflex carbon-fiber based microelectrode array (Anal Chem 89: 12383-90,2017), combined in vivo amperometric oximetry and electrophysiology were measured pre- and post-spinal cord injury using a rat contusion injury model. Following anesthesia and laminectomy, the electrodes were inserted into the rat spinal cord at T10, caudal to the impact site. Recordings were obtained pre-injury, during contusive injury, and for 2h post-injury. The results demonstrate a decrease in the oxygenation of spinal cord tissue post-injury, accompanied by alterations in neural activity. The results suggest that monitoring and maintenance of tissue oxygenation following spinal cord injury should be incorporated into post-injury clinical care, similar to current standards for traumatic brain injury.
GF-1 Regulates Hippocampal Neurogenesis by Sox2 Phosphorylation

Sajad Mir, PhD¹ • Douglas A. Andres, PhD¹
¹Molecular and Cellular Biochemistry, University of Kentucky

Faculty

Insulin-like growth factor 1 (IGF-1) is known to have diverse effects on brain structure and function, including the promotion of stem cell proliferation and neurogenesis in the adult dentate gyrus. However, the intracellular pathways downstream of the IGF-1 receptor that contribute to these diverse physiological actions remain relatively uncharacterized. Here, we demonstrate that the Ras-related GTPase, RIT1, plays a critical role in IGF-1-dependent neurogenesis. Studies in hippocampal neuronal precursor cells (HNPCs) demonstrate that IGF-1 stimulates a RIT1-dependent increase in Sox2 levels, resulting in pro-neural gene expression and increased cellular proliferation. In this novel cascade, RIT1 stimulates Akt-dependent phosphorylation of Sox2 at T118, leading to its stabilization and transcriptional activation. When compared to wild-type HNPCs, RIT1-/- HNPCs show deficient IGF-1-dependent Akt signaling and neuronal differentiation, and accordingly, Sox2-dependent hippocampal neurogenesis is significantly blunted following IGF-1 infusion in knockout (RIT1-/-) mice. Consistent with a role for RIT1 in the modulation of activity-dependent plasticity, exercise-mediated potentiation of hippocampal neurogenesis is also diminished in RIT1-/- mice. Taken together, these data identify the previously uncharacterized IGF1-RIT1-Akt-Sox2 signaling pathway as a key component of neurogenic niche sensing, contributing to the regulation of neural stem cell homeostasis.
Poster #7

Dose-response relationship of transcutaneous spinal direct current stimulation in healthy humans: A proof of concept study

Elizabeth Powell, MS¹ • Radha Korupolu, MD² • Philip Westgate, PhD³ • Cheryl Carrico, MS¹ • Lakshmi Reddy¹ • Lumy Sawaki, MD, PhD¹
¹Physical Medicine and Rehabilitation, University of Kentucky • ²Physical Medicine and Rehabilitation, University of Texas Health Science Center • ³Biostatistics, University of Kentucky

Staff

Non-invasive transcranial direct current stimulation has been shown to modulate cortical excitability in various studies. Similarly, recent preliminary studies suggest that transcutaneous spinal direct current stimulation (tsDCS) may engender a modulation effect on spinal and cortical neurons. The purpose of this study was to evaluate the dose-response effects of tsDCS in healthy subjects and thereby lay groundwork for expanding treatment options for patients with spinal cord injury (SCI). Nine healthy subjects received each of the following 2 tsDCS conditions: Anodal and cathodal, in random order with at least 1 week washout period between each session. In order to test safety and dose response, various current intensities were used (2, 2.5, and 3 mA) for 20 minutes. The active electrode was placed vertically over T10-T11, and the reference electrode was placed over the left shoulder. To evaluate corticospinal excitability, motor evoked potentials over soleus muscle elicited by transcranial magnetic stimulation were measured. To assess spinal cord excitability, H- and M-wave over soleus muscle to calculate Hmax/ Mmax ratio were measured. Linear regression showed a dose response with cathodal tsDCS on motor evoked potentials measured from the left leg as well as with anodal tsDCS on Hmax/ Mmax ratio measured from the left leg. These findings indicate tsDCS effects are dose-dependent. These effects should be investigated in a larger sample.
Poster Abstracts

Poster #8

Characterizing the Endogenous Nrf2 Response after Controlled Cortical Impact Injury in Female and Male Mice

Jacob Dunkerson, MS¹ • Juan Wang, PhD¹ • Rachel Hill, PhD¹ • Edward Hall, PhD¹
¹Neuroscience, University of Kentucky

Student

Traumatic brain injury is a complex and chronic disease affecting nearly 2.8 million individuals in the U.S. each year. Free-radical-induced oxidative damage, arguably one of the most validated secondary injuries remains a sensible target for acute neuroprotective interventions. In that regard, understanding the endogenous antioxidant response, specifically the mechanisms of the redox-sensitive transcription factor Nrf2, has become of interest from a pharmacological standpoint. This study aimed to establish the time course of Nrf2 activity and a selection of its detoxifying enzymes.

Young adult CF-1 mice (n=30 females, n=30 males) received a severe unilateral controlled cortical impact injury centered over the left parietal cortex. Cortical tissue samples of the contusion site and the ipsilateral hippocampus were harvested at varying time points (24hr, 48hr, 72hr, 7 days) post-injury. Western blot analysis was performed using whole cell lysates on the following proteins: Nrf2, HO-1, NQO1, and GPx4.

Regarding cortical protein quantities, Nrf2 steadily decreased in females over 7 days, whereas males experienced a sudden and sustained drop until day 7. HO-1 spiked at 72hrs in both males and females and remained elevated by day 7 only in females. There were no significant differences in NQO1 in either sex. For both females and males, GPx4 quantities did not reach significance, though females did appear to have a trend towards a significant increase by day 7. Regarding hippocampal protein quantities, Nrf2 fluctuations did not reach significance at any of the recorded time points in either sex. HO-1 increased significantly at 48hrs and 72hrs in males. NQO1 increased significantly by 72hrs in females. The relative amount of GPx4 did not fluctuate significantly in either females or males.

In regards to protein quantification, the time course of Nrf2 activity and its downstream phase II proteins HO-1, NQO1, and GPx4, appears to be different between female and male mice. Further analysis including RT-qPCR will be conducted to draw a more complete conclusion.
Poster #9

Myelin modulates macrophage inflammatory responses after spinal cord injury

Timothy Kopper¹ • Bei Zhang, PhD¹ • John Gensel, PhD¹
¹Physiology, Spinal Cord and Brain Injury Research Center, University of Kentucky

Student

Spinal cord injury (SCI) produces chronic inflammation largely mediated by resident microglia and infiltrating monocytes (here, collectively referred to as macrophages). These activated SCI macrophages eventually adopt a pro-inflammatory, pathological state that continues long after the initial injury. Pro-inflammatory macrophages potentiate secondary damage and impair SCI recovery, yet the mechanisms driving chronic pathological SCI macrophage activation are poorly understood. After SCI, macrophages clear and accumulate extensive myelin debris. Published data demonstrates that myelin debris can directly stimulate macrophages to adopt different activation states. We hypothesize that myelin, in combination with inflammatory stimuli within the SCI lesion environment, increases pro-inflammatory macrophage activation. To test this hypothesis we stimulated bone marrow derived macrophage with pro-inflammatory stimuli (LPS+INF-gamma) in vitro in the presence or absence of myelin. Myelin co-stimulation significantly increased pro-inflammatory IL-12 cytokine production, decreased anti-inflammatory IL-10 production, and increased reactive oxygen species production relative to unstimulated or LPS+INF-gamma treated controls. One potential mechanism for the myelin-mediated pro-inflammatory potentiation is increased activation of the enzyme cytosolic phospholipase A2 (cPLA2) within macrophages. This enzyme has the potential to modify membrane lipids into direct and indirect pro-inflammatory stimuli. Indeed, through immunohistochemical analyses of spinal cord tissue sections after T9 contusion SCI in female C57BL/6 mice we observed cPLA2 activation in myelin-laden macrophages at both 7 and 28 days post injury. Ongoing studies aim to link this continued cPLA2 activity to potentiated pro-inflammatory macrophage activation and explore potential therapeutics to block these pathways after SCI.
Poster #10

Altered the Expression of Macrophage/microglia Phenotypic Markers using novel Peptide-based Nanoparticle microRNA Delivery in Traumatic Brain Injury

Paresh Prajapati, PhD¹ • Wang-Xia Wang, PhD² • Hemendra Vakaria, PhD¹ • Malinda Spry¹ • Patrick Sullivan, PhD¹ • Joe Springer, PhD¹
¹Spinal Cord and Brain Injury Research Center, University of Kentucky • ²Sanders-Brown Center on Aging, University of Kentucky

Fellow

Traumatic brain injury (TBI) is a leading cause of long-term impairments in higher cognitive function. At acute post-injury time points, functionally diverse subsets of pro-inflammatory M1 and reparative anti-inflammatory M2 microglia and macrophages contribute to secondary injury pathology and repair, respectively. It has been documented that M2-like macrophages/microglia peak at 5-7 days and then decline, while M1-like macrophages/microglia persist. Therefore, modulating the inflammatory environment to favor expression of the reparative M2 phenotype has potential to limit secondary injury. One approach is the use of specific microRNAs (miRNAs) that inhibit the pro-inflammatory M1 phenotype and/or promote anti-inflammatory M2 expression/activity. We recently found that miR-146a and miR-223 levels in the rat hippocampus are altered in TBI. Both miRNAs play a significant role in regulating microglia/macroglia polarization and/or expression of inflammatory cytokines. MiR-146a down-regulates pro-inflammatory NF-kB signaling by inhibiting IRAK1 and TRAF6 expression. We now report that peptide-based nanoparticle delivery of miR-146a inhibits IRAK1 and TRAF6 expression in LPS treated BV-2 microglia cells and in rat hippocampus 48 hr following TBI. TaqMan low-density array analysis revealed that miR-146a delivery resulted in significant down-regulation of the M1 genes IL-6 and NOS2 and up-regulation of the M2 genes IL-4 and Arg1. These initial experiments examining miR-146a are promising, however miR-223 may prove more effective as it targets a broader scope of pro-inflammatory signaling. Regardless, these results demonstrate that nanoparticle delivery of miRNAs targeting inflammatory signaling pathways may direct phenotypic expression of M1 and M2 microglia/macroglia states and limit pro-inflammatory signaling after TBI.
Pioglitazone promotes neuronal survival against pro-inflammatory responses in the rat nigrostriatal dopaminergic pathway after diffuse brain injury

Mei Liu, MD1 • Adam Bachstetter, PhD2 • Wayne Cass, PhD1 • Jonathan Lifshitz, PhD3 • Guoying Bing, MD, PhD1
1Neuroscience, University of Kentucky • 2Spinal Cord and Brain Injury Research Center, Neuroscience, University of Kentucky • 3Barrow Neurological Institute at Phoenix Children’s Hospital, University of Arizona

Staff

Traumatic brain injury (TBI) may raise the risk of developing idiopathic Parkinson’s disease (PD). Increasing evidence suggested that brain injury induced inflammatory responses and dopaminergic neurodegeneration in the substantia nigra of rodent models of TBI. Additionally, recent studies found peroxisome proliferation-activated receptor gamma (PPARγ) agonist pioglitazone have neuroprotective and anti-inflammatory actions in the animal models of neurodegenerative diseases. The present study investigates the vulnerability of dopaminergic nigrostriatal system via medial forebrain bundle (MFB) injuries and intervention with pioglitazone treatment after TBI. Adult male Sprague-Dawley rats were subjected to sham or moderate midline fluid percussion brain injury (mFPI), followed by an intraperitoneal injection of 10 mg/kg pioglitazone or vehicle beginning 30 min after the injury and subsequently every 24 h for 5 days. Following injury, pro-inflammatory cytokines/chemokine were acutely increased in the striatum and substantia nigra within 6 hours. Both of dopaminergic axonal damage and microglial activation were revealed in the MFB at 24h post-injury using immunohistochemistry. Microglial activation identified by either Iba1 or OX-6 immunostaining were persistently increased in the substantia nigra pars compacta at 7 and 28 days post-injury. Furthermore, mFPI induced significant dopaminergic neuronal loss, which was quantified by tyrosine hydroxylase immunostaining and retrograde fluorescent tracer fluorogold labelling, in the nigra at 28 days. Neuronal loss was accompanied by increased extracellular dopamine turnover in the striatum, indicating enhanced dopaminergic activity in functional compensation after nigrostriatal damage. Importantly, pioglitazone treatment attenuated microglial activation and promoted dopaminergic neuronal survival in the nigrostriatal system, which may contribute to locomotor recovery. These data suggest that targeting secondary inflammation could be a feasible therapeutic strategy to improve outcome of TBI.
Therapeutic Window of Intervention for Pioglitazone following TBI

Malinda Spry, MS¹ • Jenn Gooch, MS¹ • Amber Cloud, MS² • William Hubbard, PhD¹ • Hemendra Vekaria, PhD¹ • Patrick G Sullivan, PhD¹
¹SCOBIROC, University of Kentucky • ²College of Medicine, University of Kentucky

Staff

Traumatic brain injury, (TBI) is a serious health concern for which no pharmacological treatment is approved. Our group has demonstrated that Pioglitazone, an FDA approved compound used to treat diabetes, has neuroprotective properties following TBI and spinal cord injuries via interaction with the mitochondrial protein, mitoNEET. Recently, we determined the optimal dosing (20 mg/kg) of Pioglitazone and in this study we examine the therapeutic window of opportunity for Pioglitazone administration following TBI. Adult C57B/6 male mice received a severe TBI followed by initiation of Pioglitazone treatment at 1, 3, 6, 12, 18 or 24 hours post-injury. At 48 hours post-injury, animals were euthanized and mitochondria was isolated from the cortex, hippocampus and septal region and oxygen consumption rates (OCRs) were assessed. Results showed that initiating pioglitazone at 1 and 3 hours post-injury did not produce an increase in mitochondrial bioenergetics compared to vehicle treated animals. However, there was a significant increase in OCR in mitochondria extracted from ipsilateral cortex during State III and State V respiration when treatment was initiated at 6, 12, 18 and 24 hours post-injury. In both the Hippocampus and Septal regions there was an increase in respiration when the treatment was initiated at 12 hours that varied across respiration states. These results in conjunction with previous work in our lab where mitochondrial respiration was rescued when treatment was initiated 15 minutes post injury indicate that there is potentially a biphasic, extended treatment window in which pioglitazone can be administered to maintain mitochondrial homeostasis after TBI.
Poster #13

The Unique Phenotype of mitoNEET null mice, a Known Regulator of Mitochondrial Function and Target for Neuroprotection

Jennifer Gooch, MS¹ • Amber Cloud, MS¹ • Hemendra Vekaria, PhD¹ • Malinda Spry, MS¹ • Brad Hubbard, PhD¹ • Sara Ngo Tenlep² • Kevin Pearson, PhD² • Patrick Sullivan, PhD³
¹SCoBric, University of Kentucky • ²Pharmacology, University of Kentucky • ³SCoBIRC, University of Kentucky

Staff

The functional and cognitive deficits that manifest from traumatic brain injury (TBI) are the results of highly complex injury mechanisms. After primary injury or a mechanical insult to the brain, a secondary injury cascade occurs which encompasses increases in oxidative stress, changes in Ca2+ homeostasis, and mitochondrial dysfunction. mitoNEET, an outer mitochondrial membrane protein, has been shown to be critical to mitochondrial function after TBI and is a promising therapeutic target. Previous studies show that pioglitazone, an FDA-approved drug used to treat diabetes, interacts with mitoNEET to impact neuroprotection after TBI, independent of peroxisome proliferator activated receptor (PPARγ). Furthermore, NL-1, a pioglitazone derivative lacking PPARγ binding, increases cortical tissue sparing following TBI and improves cognitive outcome, an effect lost in mitoNEET knock-out (KO) mice. To develop a better understanding of baseline differences between mitoNEET KO mice and wildtype mice, we compared body composition and cognitive function. We hypothesize that mitoNEET KO mice will show cognitive and mitochondrial deficits compared to wildtype littersmates that will be exacerbated with age.

Body composition analysis, using magnetic resonance imaging, revealed mitoNEET KO mice had reduced fat mass compared to heterozygotes and WT littersmates, where as the lean mass composition and total body weight remained unchanged. Novel object recognition indicated a decrease in cognitive function in mitoNEET KO mice compared to heterozygous and WT littersmates. Results show unique mitochondrial-mediated phenotyping of mitoNEET KO mice, supporting the role of mitoNEET as a central modulator of mitochondrial bioenergetics and a novel target for intervention following CNS injury.
Fibrinogen Binding to Astrocyte Prion Protein is involved in Short-term Memory Reduction during Traumatic Brain Injury

Mariam Charkviani, MD • Nino Muradashvili, MD • David Lominadze, PhD
1Physiology, University of Louisville

Staff

Traumatic brain injury (TBI) is one of the most common neurological disorders leading to memory reduction, especially short-term memory. It has been demonstrated that during TBI an inflammatory marker, blood plasma protein fibrinogen (Fg) crosses blood-brain barrier and after deposition in vascular and astrocyte endfeet interface interacts with astrocytes and activates them. Increased Fg deposition in extravascular space is associated with reduction in short-term memory during TBI. However, molecular mechanisms of Fg actions are not clear. In this study, we aim to define Fg effects on astrocytes. Cultured mouse cortical astrocytes (MCAs) were treated with Fg (2 and 4 mg/ml) and vehicle. Binding of Fg to astrocytes was assessed with co-immunoprecipitation, expressions of cellular prion protein (PrPc), intercellular adhesion molecule 1 (ICAM-1) and phosphorylation of tyrosine kinase B (TrkB) were assessed with western blot analysis and immunohistochemistry. Astrocyte apoptosis was detected with TUNNEL assay.

Results show that Fg binds to astrocyte PrPc and increases expressions of PrPc and ICAM-1, enhances phosphorylation of TrkB and then it is associated with reduction of short-term memory. Fg binding to astrocytes dose-dependently increased MCAs apoptosis. Enhanced ICAM-1 expression can be considered as a marker of astrocyte activation as it is for the endothelial cells. Since it is known that PrPc and TrkB are involved in neuronal degeneration and memory loss, combined, these results show possible mechanisms of Fg effects on short-term memory during TBI.
Homocysteine and memory loss during TBI

Nino Muradashvili, MD1 • Mariam Charkviani, MD1 • Suresh C. Tyagi, PhD1 • David Lominadze, MD1
1Physiology, University of Louisville

Faculty

Elevated blood level of Homocysteine (Hcy), called hyperhomocysteinemia (HHcy) is considered as an independent and high risk factor for many cerebrovascular diseases. One of the important problems after an inflammatory disorder, traumatic brain injury (TBI) is a memory impairment, particularly loss of short-term memory. However, mechanisms involved are not clear. As a greater role of cellular prion protein (PrPC) in cognition is well known, we hypothesize that HHcy exacerbates the TBI-induced macromolecular protein extravasation resulting in enhanced Fg-PrPC complex formation leading to the short-term memory reduction.

Permeability of pial venules in pericontusional area 14 days after mild cortical contusion injury (CCI) was studied in wild-type (WT, C57BL/6J) and in mouse model of HHcy, cystathionine-β-synthase heterozygote (CBS+/-) mice and was assessed by measuring the extravasation of Alexa-flour 647-labeled bovine serum albumin (647-BSA) in mice with CCI or sham operation using an intravital fluorescence microscopy. Brain samples were tested with immunohistochemistry technique to assess deposition of Fg and Fg-PrPC complex formation in extravascular space. Short-term memory changes were evaluated by novel object recognition and Y maze (spontaneous alternation and two-trial recognition) tests.

Pial venular permeability to 647-BSA was greater in animals with CCI compared to that in sham-operated mice in all experimental groups. However, in CBS+/- mice protein extravasation was greater than that in WT animals. Deposition of Fg was increased during TBI, and formation of Fg-PrPC complex was greater in CBS+/- mice compared to that in WT animals. The cognitive deficiency was found in all animals with CCI and the greatest memory reduction appeared in CBS+/-mice during TBI.

A strong correlation of Fg-PrPC complex formation and memory reduction with development of HHcy reveals a novel effect of Hcy in TBI-induced memory impairment, which may be therapeutically targeted in future.
Neuroprotective strategies in severe experimental TBI: Lipid Peroxidation- Neurotoxic Aldehyde Scavenging & Inhibition of Mitochondrial Permeability Transition

Jacqueline Kulbe¹ • Indrapal Singh, PhD¹ • Jacob Dunkerson¹ • Juan Wang, MD¹ • Rebecca Smith, PhD¹ • Rachel Hill, PhD¹ • Peter Huettl² • Edward Hall, PhD¹
¹SCoBIRC, University of Kentucky • ²CenMeT, University of Kentucky

Student

Traumatic brain injury (TBI) represents a significant health crisis in the United States. Currently there are no neuroprotective FDA-approved pharmacotherapies for TBI. Due to the complex pathophysiology which occurs following TBI, more robust pharmacological approaches must be developed. Mitochondrial dysfunction and the formation of neurotoxic aldehydes contribute extensively to TBI pathology, making them promising therapeutic targets for prevention of cellular death and dysfunction following TBI. The following are evaluated. 1) The neuroprotective effect of cyclosporine A (CsA), on synaptic and non-synaptic mitochondrial respiration. Mitochondria are heterogeneous, consisting of both synaptic and non-synaptic populations, which have distinct properties. Our results indicate that compared to non-synaptic mitochondria, synaptic mitochondria sustain greater damage 24h following severe controlled cortical impact injury in young male rats, and are protected to a greater degree by CsA, an FDA-approved immunosuppressant, capable of inhibiting mitochondrial permeability transition. 2) The neuroprotective effects of a 72h subcutaneous continuous infusion of CsA combined with phenelzine (PZ), an FDA-approved monoamine oxidase inhibitor (MAOI) class anti-depressant capable of scavenging neurotoxic aldehydes. Our results indicate that individually CsA or PZ attenuate neurotoxic aldehyde formation, PZ maintains mitochondrial respiratory control ratio and cytoskeletal integrity, but together, PZ and CsA, do not maintain neuroprotective effects. 3) The ability of PZ (aldehyde scavenger and MAOI), to attenuate cognitive dysfunction following TBI compared to hydralazine (aldehyde scavenger) and pargyline (MAOI), in an attempt to further elucidate the role PZ's MAOI mechanism of action has in TBI pathophysiology. Additionally, HPLC is utilized to measure the monoamines dopamine, serotonin, and norepinephrine and their metabolites as an indicator of monoamine oxidase inhibition and monoamine turnover.
Poster #17

Continuous Gabapentin administration significantly alters cardiovascular hemodynamics after complete spinal cord injury

Khalid Eldahan\(^1\) • Dave Cox\(^2\) • Samir Patel, PhD\(^1\) • Alexander Rabchevsky, PhD\(^1\)

\(^1\)Physiology and SCoBIRC, University of Kentucky • \(^2\)SCoBIRC, University of Kentucky

Student

Spinal cord injury (SCI) can have profound effects on autonomic and cardiovascular systems, notably with injuries above high-thoracic levels frequently resulting in the development of autonomic dysreflexia (AD). AD is a potentially life-threatening condition characterized by volatile hypertension elicited by unopposed sympathetic reflexes that are typically triggered by noxious afferent stimulation below the injury level. We have reported that single daily injections of the neuropathic pain medication, gabapentin (GBP; 50 mg/kg i.p.) in rats with complete T4- transection SCI significantly reduced the magnitude of hypertension (AD) induced by noxious colorectal distension (CRD) shortly after administration, but insignificantly reduced the frequency of daily spontaneous AD events based on telemetric algorithm detections. Herein, we investigated the effects of more prolonged and higher GBP dose administration on the incidence of spontaneous AD and severity of CRD-induced AD, with the hypothesis that aberrant synaptic plasticity thought to underlie the development of AD would be mitigated based on GBP binding to a2d1 calcium channel subunit on glutamatergic terminals. Adult female Wistar rats under continuous telemetric hemodynamic monitoring underwent T4-transection SCI (n=21) and immediately received either saline vehicle (n=11) or 100 mg/kg GBP (n=10) every six hours (400 mg/kg/day, i.p.) for 4-weeks. In summary, GBP-treated rats showed impaired post-injury weight gain, and while GBP did not alter daily mean arterial pressure (MAP), it consistently elevated daily heart rate (HR). Unexpectedly, there were significantly more daily spontaneous AD events detected in chronic GBP-treated rats across time-post injury, but the extent of MAP increases during weekly CRD sessions relative to 30 second baselines was unaltered. However, GBP significantly reduced the absolute blood pressure reached during CRD, notably because it significantly lowered the baseline MAP versus vehicle in response to experimental handling prior to CRD recordings. Remarkably, chronic GBP further prevented characteristic baroreflex-mediated bradycardia during CRD, suggesting that it may impede vagal parasympathetic neurotransmission. In parallel, chronic GBP significantly reduced hindlimb spasticity evoked during weekly CRD versus vehicle, and these rats also had significantly increased spleen/body weight ratios compared to both vehicle-injured and naïve rats. Together, these results suggest that chronic GBP treatment modulates autonomic control of cardiovascular activity after SCI, perhaps acting as an anxiolytic agent associated with decreased sympathetic activation, and may have immunomodulatory effects related to increased spleen weights. Ongoing histological analyses are quantifying glutamatergic (VGluT2) and synaptophysin (pre-synaptic) colocalization in lumbosacral spinal cord sections to establish whether the cardiophysiological alterations observed with chronic GBP treatment correlate with modulation of synaptic plasticity within the dorsal spinal cord thought to contribute to the development of AD.
Poster #18

Mitochondrial biogenesis as a therapeutic target to restore mitochondrial function after traumatic brain injury

Hemendra Vekaria, PhD¹ • Natalie Scholpa, PhD² • Brad Hubbard, PhD¹ • Malinda Spry¹ • Jennifer Gooch¹ • Rick Schnellmann, PhD² • Patrick Sullivan, PhD¹
¹SCoBIRC, University of Kentucky • ²College of Pharmacy, University of Arizona

Fellow

Traumatic brain injury (TBI) leads to acute necrosis at the site of injury followed by a sequence of secondary events lasting from hours to weeks and leading to significant neuronal cell death. Mitochondria play a pivotal role in mediating these secondary events, including glutamate uptake by NMDA receptors, Ca²⁺ overload, opening of MPTP and apoptosis. Earlier studies from our laboratory and other groups strongly suggests that mitochondrial function measured in terms of bioenergetics predicts the loss or recovery in the neuronal function post-TBI. Mitochondrial biogenesis (MB) is an important mechanism to restore the mitochondrial function in many acute injury models like ischemic brain injury, spinal cord injury, acute kidney and liver disease. Here we hypothesized that promotion of MB after TBI will increase mitochondrial bioenergetics. We chose two compounds, formoterol and LY344864 ((N-[(3R)-3-(dimethylamino)-2,3,4,9-tetrahydro- 1H-carbazol-6yl]-4-fluorobenzamide), to induce MB to protect against secondary damage in the severe controlled cortical impact (CCI) model of TBI in C57BL/6 mice. Formoterol, a highly selective β₂-adrenergic receptor agonist, and LY344864, a serotonin (5-Hydroxytryptamine) 1F receptor selective agonist, and potent inducers of MB. The TBI mice were injected (i.p.) either with vehicle (normal saline) or formoterol (0.3mg/kg) / LY344864 (2mg/kg) at 15 min, 8h, 16h and 24h post injury. At 48h, the ipsilateral cortex, directly under the impact, was used to assess mitochondrial copy number and bioenergetic function. We observed that the mitochondrial respiration rate as assessed by oxygen consumption rate (OCR) was significantly improved with formoterol administration, but was not altered by LY344864. In addition, formoterol but not LY344864 increased mitochondrial DNA copy number, indicative of MB. These preliminary results indicate that MB may be an important target to restore mitochondrial function and aid brain recovery post-TBI.
Poster #19

Determining Effects of Exercise and Traumatic Brain Injury on Cognition and Adult Neurogenesis

Erika Correll¹ • Jennifer McGuire, PhD¹ • Laura Ngwenya, MD, PhD¹
¹Neurosurgery, University of Cincinnati

Staff

Background: Adult neurogenesis is the development of new neurons in the adult brain and it occurs in almost all mammalian species, including humans. Traumatic brain injury (TBI) and exercise are both known to produce increases in hippocampal neurogenesis, yet it remains unknown if these increases are similarly adaptive. Our study investigated effects of exercise and TBI on cognitive performance and new neuron maturation. We hypothesized exercise and TBI would result in increased hippocampal neurogenesis, but that TBI animals would display deficits in cognition and new neuron maturity.

Methods: Adult male Sprague-Dawley rats were randomly assigned to sham, sham with running wheel access, or TBI group. TBI was administered through a craniectomy over the right parietal cortex via lateral fluid percussion at an average pulse-pressure of two atmospheres. Sham and sham running groups received craniectomy but no injury pulse. We confirmed injury by measuring righting reflex time (RRT) immediately following injury or sham in all cohorts. To assess neurogenesis, animals received bromodeoxyuridine (BrdU), an S-phase marker, one week post injury. We tested locomotor activity and cognition using an open field test, a pattern separation task, and novel object recognition (NOR) from three to twelve days post injury. We examined histological features of neurogenesis via immunohistochemistry for Ki-67, BrdU, and markers of neuronal maturity.

Results: RRT after TBI (n=12) was significantly longer than in sham (n=11) or sham running (n=11) groups (p<0.01). TBI animals displayed significant increases in locomotor activity, travelling further distances at higher average velocities than both sham and sham runner groups (p<0.01). TBI animals also made more entries and spent more cumulative exploration time in the center of the open field apparatus compared to sham runners (p<0.01). All animals successfully completed the pattern separation task, but we found no differences between groups in percent of correct choices made. TBI animals displayed a tendency to perform worse on long-term NOR memory, but differences were insignificant (p>0.05). Histological analyses showed qualitative differences in neurogenesis between groups.

Conclusion: In these studies we demonstrated that although injured animals exhibited hyperactive behavior, they maintained the ability to perform memory and spatial discrimination tasks comparable to sham animals. We further hope to develop a maturation timeline of neurogenesis under these conditions using markers of different stages of neuronal maturity.
Poster #20

Bad to the bone: spinal cord injury impairs hematopoietic stem and progenitor cell function

Randall Carpenter¹ • Adrienne Dorrance, PhD² • Phillip Popovich, PhD¹
¹Department of Neuroscience, The Ohio State University • ²Division of Hematology, The Ohio State University

Student

Systemic immune system dysfunction is a major consequence of spinal cord injury (SCI). It is, fundamentally, a paralysis of the immune system due to disrupted communication from the brain to peripheral immune tissues. Clinically, it leaves patients susceptible to developing and dying from infections more than abled-bodied individuals. Yet, how SCI alters the generation of immune and red blood cells in bone marrow (i.e. hematopoiesis) has not been thoroughly examined. Here, an experimental mouse SCI model was used to determine how SCI alters the activity of hematopoietic stem/progenitor cells (HSPCs) in bone marrow, blood and spleen. In vivo imaging and flow cytometry showed a rapid increase in HSPC proliferation in systemic bone marrow during the first 7 days after SCI, with a peak at 3 days post-injury (dpi). We then used ex vivo culture and transplantation assays to determine impairments in bone marrow HSPC function. Interestingly, SCI improved the acute engraftment of bone marrow 8 weeks after transplantation into naïve mice relative to sham-injury. Preliminary PCR and flow cytometry of the transplanted SCI bone marrow suggested increased CXCL12-CXCR4 chemotactic ligand and receptor expression vs. sham-injury. Since CXCL12-CXCR4 signaling is critical for retaining HSPCs in bone marrow, we measured numbers of HSPCs in blood and spleen 3 days after SCI. Indeed, mobilization of HSPCs into blood and trafficking to spleen was completely impaired after SCI compared to sham-injury. Targeting CXCL12-CXCR4 signaling with Plerixafor (AMD3100) mobilized HSPCs into blood, facilitated HSPC trafficking to the spleen, and reversed SCI-induced leukopenia. These data are the first to demonstrate disrupted bone marrow chemotactic signaling in mediating hematopoietic/immune dysfunction after SCI. Ongoing experiments are targeting these mechanisms to improve immune system function, with the goal of preventing immune-related morbidity and mortality in SCI patients.
Poster #21

A Wearable Fiberless Optical Sensor for Continuous Monitoring of Cerebral Blood Flow in Mice

Chong Huang, PhD¹ • Yutong Gu, PhD² • Jing Chen, PhD¹ • Ahmed Bahrani, MS¹ • Elie G. Abu Jawdeh, MD³ • Henrietta Bada, MD³ • Guoqiang Yu, PhD¹ • Lei Chen, MD, PhD⁴
¹Department of Biomedical Engineering, University of Kentucky • ²Department of Electrical Engineering, University of Southern California • ³Department of Pediatrics, University of Kentucky • ⁴Department of Physiology, Spinal Cord and Brain Injury Research Center, University of Kentucky

Faculty

Introduction: Continuous and longitudinal monitoring of cerebral blood flow (CBF) in animal models provides information for studying fundamental mechanisms and interventions of versatile cerebral diseases. Since anesthesia may affect brain hemodynamics and function, researchers have been seeking wearable devices which can be installed on the head of conscious animals.

Materials and Methods: We present a wearable diffuse speckle contrast flowmeter (DSCF) for monitoring CBF variations in mice. The DSCF probe consists of a small low-power near-infrared laser diode as a point source and an ultra-small low-power camera as a 2D detector array, which can be installed on a mouse head. The movement of red blood cells in brain cortex (i.e., CBF) produces spatial fluctuations of laser speckles, which are captured by the camera. The DSCF system was calibrated using tissue phantoms and validated in a human forearm and mouse brains for continuous monitoring of blood flow increases and decreases against the established technologies.

Results: The DSCF system was first calibrated using tissue phantoms and then validated in a human forearm and mouse brains for continuous monitoring of blood flow increases and decreases against the established technologies. Significant correlations were observed among these measurements (R² ≥ 0.8, p < 10⁻⁵).

Discussion and Conclusions: The DSCF transmits photovoltaic conversion process to a small probe, which makes the signal transition between the probe and a control unit all electric. As a result, this small fiberless probe has the potential to be worn by a freely moving conscious rodent. Moreover, the flexible source-detector configuration allows for varied probing depths up to ~8 mm, which is sufficient for transcranially detecting CBF in the cortices of rodents and newborn infants.
Poster #22

Therapeutic potential of V2a neurons to restore diaphragm function following spinal cord injury

Victoria Jensen¹ • Kari Seedle² • Sarah Turner³ • Warren Alilain, PhD⁴ • Steven Crone, PhD³
¹Neuroscience Graduate Program, University of Cincinnati • ²Divisions of Neuorsurgery and Developmental Biology, Cincinnati Children's Hospital • ³Divisions of Neurosurgery and Developmental Biology, Cincinnati Children's Hospital • ⁴Neuroscience, University of Kentucky

Student

Here we investigate the therapeutic potential of glutamatergic V2a neurons to restore diaphragm activity following spinal cord injury. Our lab has previously shown that V2a neurons play important roles in respiratory rhythm generation in neonates, locomotor pattern generation, and recruitment of accessory respiratory muscles. Using chronic electromyography (EMG) recordings of respiratory muscles in adult transgenic mice in which the activity of V2a neurons can be altered by excitatory or inhibitory DREADDs, we now assess the roles of V2a neurons in respiratory rhythm and pattern generation in healthy adult animals as well as the potential of V2a neurons to promote activation of crossed phrenic pathways in the C2 hemisection model of spinal cord injury. We show that in healthy adult animals, V2a neurons regulate respiratory frequency, but are not required to maintain regularity of rhythm as they are in neonates. In addition, we find that either increasing or decreasing the excitability of V2a neurons activates accessory respiratory muscles, suggesting that a subset of V2a neurons are important for preventing accessory respiratory muscles from being activated at rest when they are not needed. Finally, we demonstrate that increasing the excitability of V2a neurons is able to restore rhythmic burst activity to a paralyzed hemidiaphragm ipsilateral to a C2 hemisection. These results indicate that targeting G-protein coupled receptors in V2a neurons has the potential to restore function to respiratory muscles following spinal cord injury without significant adverse side effects on respiratory rhythm generation.
Apolipoprotein E4 as a Barrier to Respiratory Motor Plasticity

Lydia E. Hager1 • Rachel S.J. Maggard1 • Daimen R. Stoltz1 • Kyle J. Ritter1 • Chris Calulot1 • Emily E. Huffman1 • Brittany N. Turba1 • Warren J. Alilain1

1Spinal Cord and Brain Injury Research Center • Department of Neuroscience • University of Kentucky • Lexington, KY

More than 50% of spinal cord injuries (SCI) occur at the cervical level. These injuries can disrupt axons that descend from medullary respiratory centers to the phrenic motor nucleus at C3-C6. Since diaphragmatic innervation originates from the phrenic motor nucleus, loss of these descending inputs leads to impairment of breathing function in cervical SCI patients. One approach to promote functional recovery is by enhancing plasticity through activation of spared but latent pathways or strengthening of synapses. One form of respiratory motor plasticity is long term facilitation (LTF), which is characterized by a prolonged increase in breathing activity. LTF can be induced by intermittent bouts of hypoxia (IH) or through intermittent doses of serotonin (5-HT) applied to the spinal cord. Increased signaling through 5-HT receptors and upregulation of glutamate receptor expression in excitatory synapses mediate LTF. In preclinical animal models of SCI, IH has been successful in promoting breathing recovery. However, human patients have exhibited varying degrees of response to IH treatment, indicating that there is an additional factor influencing plasticity that must be considered. We propose that genetic diversity among the human SCI population could be a key factor in determining an individual’s potential for plasticity. Apolipoprotein E (ApoE) is a promising gene of interest since the ApoE4 protein has been shown to reduce synaptic plasticity by decreasing the expression of glutamate receptors on the neuronal surface in vitro. In the present study, long term facilitation was induced in rats in the presence of human ApoE3 and E4 proteins to determine their influence on plasticity in vivo. In animals that received E4, diaphragmatic EMG recordings demonstrated that LTF was abolished and immunohistochemistry revealed that fewer glutamate receptors were localized in synapses. To investigate the effect of ApoE4 on respiratory motor plasticity after injury, C2 hemisections were performed on a second cohort of rats and LTF was induced at a 20-week post injury time point in the presence of human ApoE3 or E4. In ApoE3 treated animals, diaphragmatic activity ipsilateral to the injury increased in response to serotonin dosing, whereas this increase was abolished in Apo4 treated animals. Collectively, these experiments demonstrate ApoE4’s ability to inhibit plasticity, emphasizing that genetic diversity is an important factor to consider in the development of therapies for the human SCI population.
Human Apolipoprotein E Isoforms Differentially Influence Neurite Outgrowth and Regeneration

Rachel S.J. Maggard\textsuperscript{1} \textbullet\ Christopher M. Calulot\textsuperscript{1} \textbullet\ Lydia E. Hager\textsuperscript{1} \textbullet\ Kyle J. Ritter\textsuperscript{1} \textbullet\ Brittany N. Turba\textsuperscript{1} \textbullet\ Jared D. Hoffman\textsuperscript{2} \textbullet\ Ai-Ling Lin\textsuperscript{2,3} \textbullet\ Lance A. Johnson\textsuperscript{4} \textbullet\ Warren J. Alilain\textsuperscript{1}

\textsuperscript{1}Spinal Cord and Brain Injury Research Center, Department of Neuroscience, College of Medicine, University of Kentucky, Lexington, KY, USA.
\textsuperscript{2}Sanders-Brown Center on Aging, Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky, Lexington, KY, USA.
\textsuperscript{3}Department of Biomedical Engineering, University of Kentucky, Lexington, KY, USA.
\textsuperscript{4}Department of Physiology, College of Medicine, University of Kentucky, Lexington, KY, USA.

Student

Translating spinal cord injury (SCI) therapies from preclinical animal models into the human population is challenging. One potential explanation is that human genetic predispositions may limit the efficacy of treatments which enhance regeneration and sprouting. The clinically relevant ApoE4 (E4) allele, present in about 14\% of the human population, corresponds to an increased incidence of Alzheimer’s disease. Its role in recovery from SCI is poorly understood despite suggestive data implicating its involvement. Two clinical studies found that SCI individuals with the E4 allele had less motor recovery than individuals without the allele despite longer time in rehabilitation. ApoE4 may mediate this diminished recovery by limiting regeneration and sprouting. Robust regeneration is energy intensive and requires efficient mitochondria, and studies have shown that ApoE4 impairs mitochondrial function. Given these mitochondrial deficits, we hypothesize that ApoE4 can impair regeneration and sprouting. To test this hypothesis, we investigated the impact of ApoE4 on sprouting and neurite outgrowth. In our experiments, we cultured dorsal root ganglia neurons from mice expressing the human ApoE isoforms—ApoE2 (E2), ApoE3 (E3), or ApoE4—under the control of the mouse ApoE promoter. We then analyzed differences in 1) neurite complexity and 2) robustness of outgrowth between genotypes. Our results demonstrate that E3 neurons have more robust outgrowth than E4 neurons, as indicated by a higher total combined neurite length. Analysis of neurite branching indicates that E3 neurons also have higher neurite complexity than neurons expressing ApoE4. Preliminary data from the Spot Assay, an in vitro model of the glial scar and CNS regeneration, suggest that chondroitin sulfate proteoglycans may inhibit regeneration in E4 neurons to an even greater extent than in E3 neurons. Since outgrowth, sprouting, and regeneration all partially mediate recovery after CNS injury, impairments in these processes can adversely affect recovery. These foundational studies address not only the possible genetic influence of ApoE4 on recovery from CNS injury, but also a critical gap in knowledge—whether there is a genetic contribution underlying responses to treatment in SCI individuals.
Thank you to KSCHIRT!

The symposium is an annual event sponsored by the Kentucky Spinal Cord & Head Injury Research Trust (KSCHIRT). KSCHIRT, created in 1994 by the Kentucky Legislature and originally funded by a surcharge on speeding tickets, supports neurotrauma research at the University of Kentucky and University of Louisville.

Established in 1999, the mission of the University of Kentucky’s Spinal Cord and Brain Injury Research Center is to promote both individual and collaborative studies on injuries to the spinal cord and brain that result in paralysis or other loss of neurological function.

**2018 KSCHIRT Planning Committee**

John C. Gensel, Ph.D.
Adam Bachstetter, Ph.D.
Warren J. Alilain, Ph.D.
Joe Springer, Ph.D.
W. Brad Hubbard, Ph.D.
Ms. Shelby Meier
Ms. Zelneva Madison